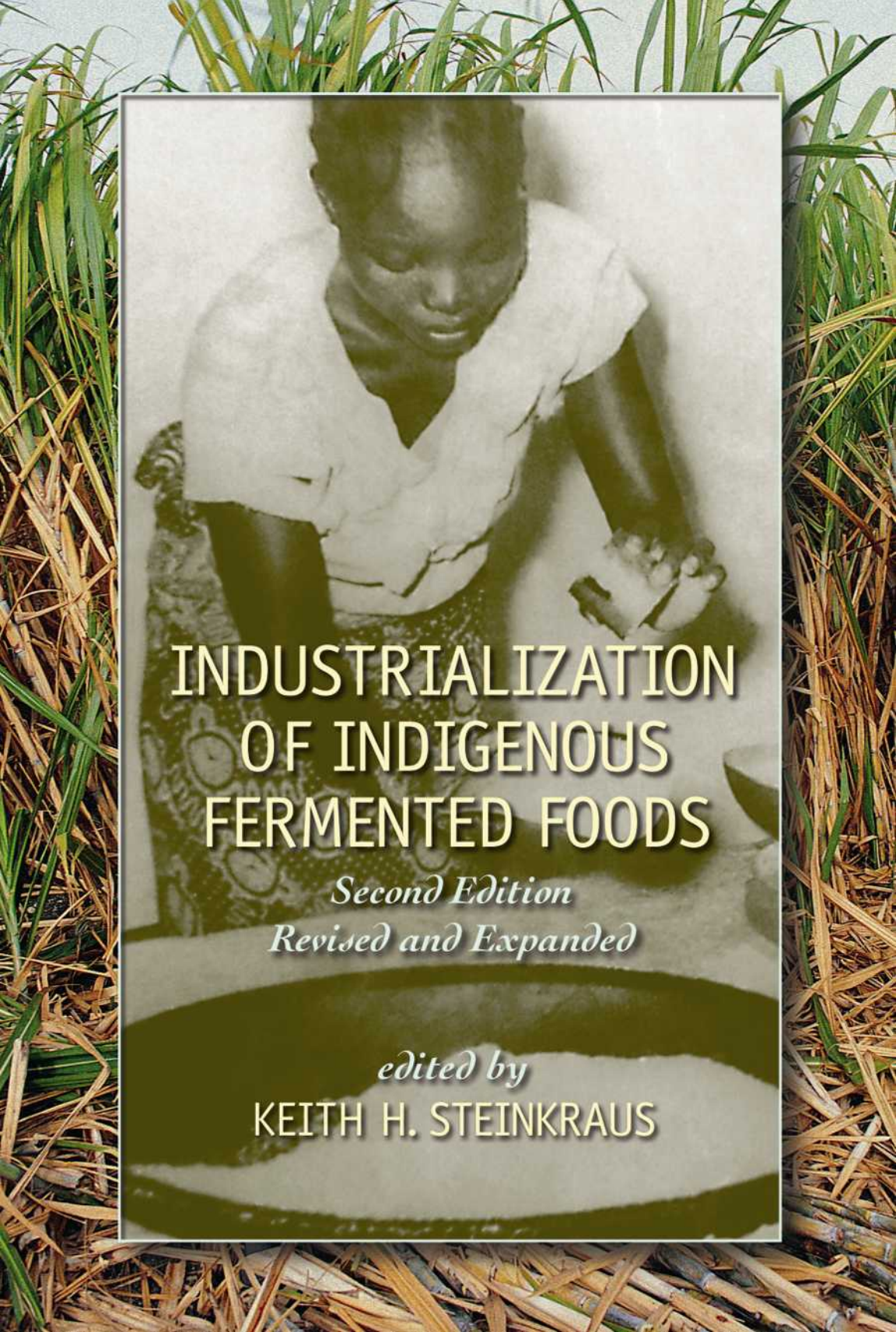
INDUSTRIALIZATION
OF INDIGENOUS
FERMENTED FOODS
Second Edition
Revised and Expanded
edited by
KEITH H. STEINKRAUS

Industrialization of Indigenous Fermented Foods

Second Edition, Revised and Expanded

edited by

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Cornell University
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Preface to the Second Edition

It has been 13 years since publication of the first edition of *Industrialization of Indigenous Fermented Foods*. In this new edition, we include new developments in the processing of soy sauce, Japanese shoyu, Japanese miso, Japanese sake, Malaysian tapai, African indigenous millet, sorghum and maize beers, African mageu, and African ogi and gari. Over the past decade, a number of indigenous fermented foods have been further industrialized. Among these are tempe, which was originally Indonesian and Malaysian but has been adopted by consumers in the United States, the Netherlands, and Japan. This edition covers the industrial development of tempe manufacture in Indonesia and Japan, comparing the practices in these countries. Industrialization of Japanese natto fermentation is included in this edition, along with the closely related African dawadawa and Indian kenima production. Pulque is the most ancient alcoholic beverage in Mexico, and it is a major source of low-cost vitamins for Mexican children. Its industrial production is also covered in this volume.

Fish sauces and pastes are very important, and widely consumed, savory foods and condiments in Asia, particularly in Myanmar, Thailand, Malaysia, and the Philippines. These products are also becoming important foods and condiments in the United States and Europe. Large-scale processing of fish sauces and pastes is of considerable interest to producers

of savory flavors in the developed and developing world. The subject is added to this new edition.

This newly revised and expanded edition provides food scientists, microbiologists, nutritionists, anthropologists, and biologists with an authoritative, comprehensive review of the processing of these important foods and how their production has varied from prehistoric to industrialized production to satisfy the food and nutritional needs of consumers around the world. Indigenous fermented foods have played an essential role in feeding humans since they evolved on earth, and these foods promise to provide nutritious food in a wide diversity of flavors, aromas, and textures to an ever-growing world population that will likely reach 8 to 12 billion people in the twenty-first century.

Keith H. Steinkraus

Preface to the First Edition

If you know the history of food, you know the history of man. Fossil microorganisms have been found in rocks 3.5 billion years old. Plants were likely the next forms of life to evolve as, until plants developed, there was no oxygen in the atmosphere. Plants had to survive in a sea of microorganisms that served a major purpose of recycling organic matter. Even today a seed germinating in the soil has to be able to survive the onslaught of billions of microorganisms that, given the opportunity, would destroy and recycle the developing plant. Plants, including their leaves, roots, berries, fruits, nuts, and seeds, particularly wheat, rice, maize, barley, rye, oats, millet, sorghum, beans, and peas, are major staples in the diet today.

Millions of years before man, all the chemical reactions needed for food fermentations, such as souring of milk, fermentation of fruits and fruit juices to wine, and germination of grains as the first step in brewing, were already in existence. When man evolved, he had to consume his food supply before microorganisms consumed the raw materials. When microbes produced unpleasant aromas or flavors in food or produced toxins that caused illness or death, the food was spoiled and man had to learn to avoid it. In other cases, the microbes produced attractive aromas, flavors, and textures, and man learned to appreciate and desire such foods. These were food fermentations; the important ones, for example, yogurt, sour milk, cheeses, wines and beers, vinegar, lactic producers, sauerkraut, Korean kimchi,

Nigerian ogi, Nigerian gari, Kenyan uji, and a multitude of others still survive today.

Some anthropologists hypothesize that it was the alcoholic fermentation and the desire for alcohol that motivated humans to settle down and become agriculturists.

It would have been impossible for man to survive without indigenous fermented foods over the millennia as fermentations are part of nature; they also preserve food, improve digestibility and enrich substrates with essential vitamins, proteins, and amino acids. They provide methods of converting vegetable proteins to meatlike flavors and textures. They are also responsible for many of the diverse flavors and aromas that enrich the human diet.

Most of the indigenous fermented foods will probably eventually be industrialized and commercialized. It was extensive studies of the flavor components in Japanese shoyu and miso that led to development of the huge monosodium glutamate and flavor-enhancing nucleotide industries of today. And it was research on Japanese sake, shoyu, and miso that led to development of the first commercial enzyme, taka-diastase, and eventually led to the present giant international enzyme industry.

One cannot predict what new industries will develop from further study of the indigenous fermented foods, but it is highly likely with the new emphasis on biotechnology and genetic engineering that detailed study of indigenous fermented foods will offer many benefits for mankind, insuring the extensive supplies of food needed by millions of consumers in various parts of the world as the Earth's population reaches 8 to 12 billion in the twenty-first century.

The editor acknowledges the excellent technical assistance of Catherine Cigna of the Institute of Food Science as associate editor of this volume.

Keith H. Steinkraus

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1

Industrialization of Fermented Soy Sauce Production Centering Around Japanese Shoyu

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I. INTRODUCTION

In the Orient, there are many kinds of indigenous fermented food that involve soybeans. The main ones are soy sauce, miso (fermented soy paste), natto (fermented whole soybeans), sufu (fermented soybean protein curd), tempeh (fermented whole soybeans), and the like, which have been reviewed and discussed from various historical, technological, microbiological, biochemical, toxicological, biotechnological, and genetic engineering standpoints (1–14). Among these, soy sauce, which is consumed in many Oriental countries, is the most popular product.

Soy sauce originated in China and was introduced into Japan some time before the 7th century. It is called "chiang-yu" in China and "shoyu" in Japan. Large-scale commercial production in Japan began in the early 17th century and grew enormously during the Edo Period (1603–1867) because of a dramatic increase in its market, primarily among urban residents. According to Kyoho-Yosetsu published in the early 18th century, Edo (modern Tokyo) already had a population of more than one million at that time (15).

The fermentation technology of soy sauce has been improved through experience over many years. However, as recently as the 1950s, a new look was taken at the production of soy sauce from a scientific point of view. As a result, the manufacturing techniques and facilities changed entirely. Today,

fermented soy sauce is manufactured by the most modern and sophisticated methods, in terms of both facilities and technology.

This chapter deals with historical aspects, fermentation technologies and facilities on both traditional and modern ways, microbiology and biochemistry, the application of new biotechnologies, and the future outlook for fermented soy sauce.

II. HISTORY

A. Historical Development of Soy Sauce in China

1. Chiang

In 1979, Kinichiro Sakaguchi proposed a unique hypothesis regarding the origin of soy sauce and miso, based on his historical and biochemical investigations. This hypothesis was introduced by Fukushima in English (9,12). However, new literature on the origin of soy sauce and miso appeared, based on more detailed historical evidence (16–19). According to these articles, soy sauce was derived from a Chinese food called "chiang."

Chiang is a tasty mash product with salt and is not a liquid like soy sauce. Therefore, chiang belongs in the category of "miso" in Japan. The first record on chiang can be found in the book entitled *Chou-li* by Chou-kung, which was published around 1,100 BC in the Chou dynasty (1122 BC to 249 BC). This book covers the matters on the early years of the Chou dynasty in ancient China (about 3000 years ago). According to this literature, chiang was made by the following procedure. First, the millet, on which yellow aspergilli grow, is made. Such mold-grown cereals are called "koji". The millet koji thus prepared is mixed with the meat of fish, flesh, or fowl, put into a bottle with salt and a good liquor, and kept for 100 days. This chiang was called fish-, flesh-, or fowl-chiang, depending on the kind of meat, but soybeans were not used yet in this period. The first literature, in which the description of soybeans as a substitute for meat in chiang appeared, was *Chi-min Yao-shu* by Chia Ssu-hsieh, the world's oldest encyclopedia of agriculture, published in AD 535 in China. This indicates that the period in which soybeans was used for chiang was sometime between the Chou and Han dynasties, when the cultivation of soybeans prevailed. The meats used in the chiang in *Chou-li* were gradually replaced by soybeans in the course of time and further cereals such as wheat, barley, and rice came to be used instead of millet, resulting in the production of many types of chiang. In the process of making chiang during these periods, soybeans were not used as the material of koji, but they were added to the koji prepared from the cereals other than soybeans. As a result, the soybeans are digested by the enzymes of the koji made by the

cereals, except soybeans. All of the chiang manufactured before the later Han dynasty (about AD 25–220) was in a state of mash as a final product. The liquid products which belong to the category of soy sauce do not appear in the literature of these periods.

There is a description of the liquid product which was made by separating the liquid portion from the chiang in *Ssu-ming Yueh-ling*, published by Ts'ui Shih in the later 2nd century in the later Han dynasty. This liquid was called chiang ch'ing which means "clear chiang." The manufacturing processes of chiang and chiang ch'ing are shown in Figs. 1 and 2. Chiang ch'ing is a prototype of soy sauce, but it differs from "chian'yu," which literally means shoyu or soy sauce in the Chinese character. The first appearance of the name of chiang-yu was in Shan-chia Ch'ing-kung by Lin Hung in the Sung dynasty (AD 960–1127).

The first record of soybean chiang, in which all of the cereal materials (i.e., both soybean and wheat) are used for preparing koji, was *Nung-sung I-shin Ts'o-yao* by Lu Ming-Shan, published in the Yuan dynasty (AD 1271–1368). The manufacturing process of this type of soybean chiang is shown in Fig. 3. The chiang-yu described in *Pen-ts'ao Kang-mu*, published in 1590 by Li Shin-chen in the Ming dynasty, was also made with koji manufactured by using both soybeans and cereals (Fig. 4). The ratio of soybeans to wheat in making koji was 3:2. This ratio is very close to the regular type of Japanese shoyu, which is made by using soybeans and wheat half and half, as will be described later. The general manufacturing methods of soy sauce in the Ch'ing dynasty (AD 1616–1912) are recorded in *Ch'ing-yuan Lu*, written by Li Hua-nan. Both cooked soybeans and uncooked wheat were the materials used in making koji. The resultant koji was mixed with brine. After aging, the soy sauce was collected by pressing a deep bamboo colander into the aged mash and ladling out the liquid which had accumulated.

The original chiang was a mash-type product and was made using a koji that was prepared from cereals other than soybeans, such as wheat, barley, rice, and so forth. Therefore, the soybean constituents were only changed through the in vitro biochemical reaction by the enzymes from the mold grown on the cereals. Accordingly, the degree of change of the soybean constituents was not large and most of the soybean proteins were partially hydrolyzed into polypeptides through the in vitro enzyme action. The degree of liquefaction was not very large and the flavor was not so strong. In the case of chiang-yu, however, mold is grown on both the soybeans and cereals, and as a result, the soybean constituents are changed largely through the biochemical reaction both in vivo and in vitro by the mold throughout the entire process of manufacturing. Accordingly, much of the soybean constituents can be liquified. The soybean proteins are hydrolyzed to single amino acids and, therefore, the flavor is sharp and strong in chiang-yu. Thus, it can be

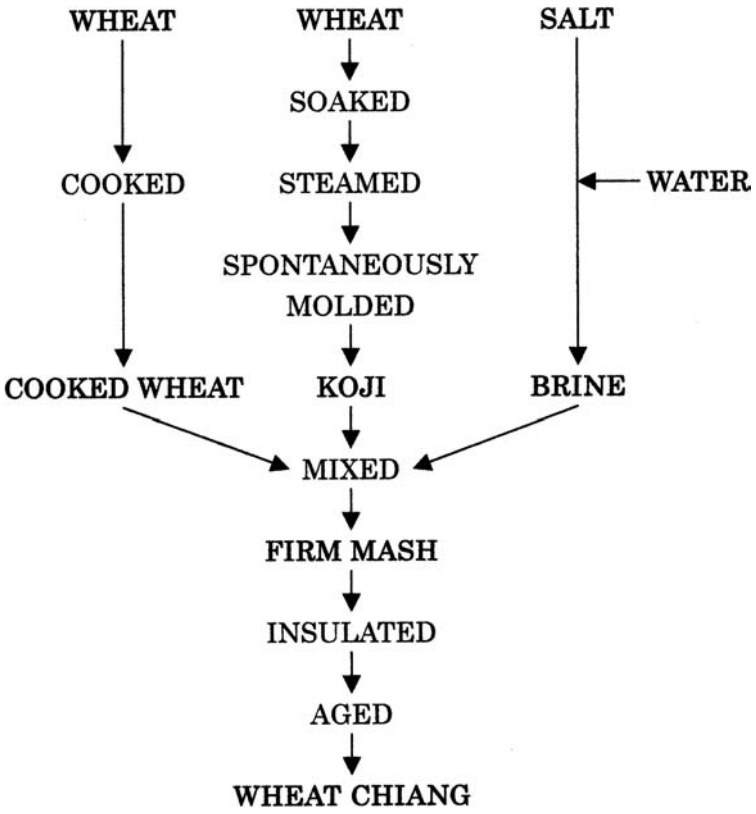

Figure 1 Manufacturing process of wheat chiang, appearing in *Shi-ching* (*Shokkei* in Japanese) by Hsie Feng (Sha Fu in Japanese), published in the Han dynasty (206 BC to AD 220). *Shi-ching* has not survived to the present day, but the original *Shi-ching* process of the wheat chiang is cited in *Chi'i-ming Yao-shu* (*Saimin-Yojutsu* in Japanese) by Chia Ssu-hsieh (Ka Shikyo in Japanese), published in AD 535. (From Ref. 17.)

concluded that (a) the progenitor of miso is chiang, originated in China about 3000 years ago, (b) the progenitor of soy sauce is chiang ch'ing, originated in China about 2000 years ago, and (c) chiang ch'ing had developed into chiang-yu in China at least 1000 years ago, which is the progenitor of the regular type of shoyu called koikuchi in Japan.

It is an amazing fact that the Chinese had utilized the enzyme action of mold in food manufacturing as early as 3000 years ago. They deliberately selected yellow aspergilli from many types of aspergilli, because they best

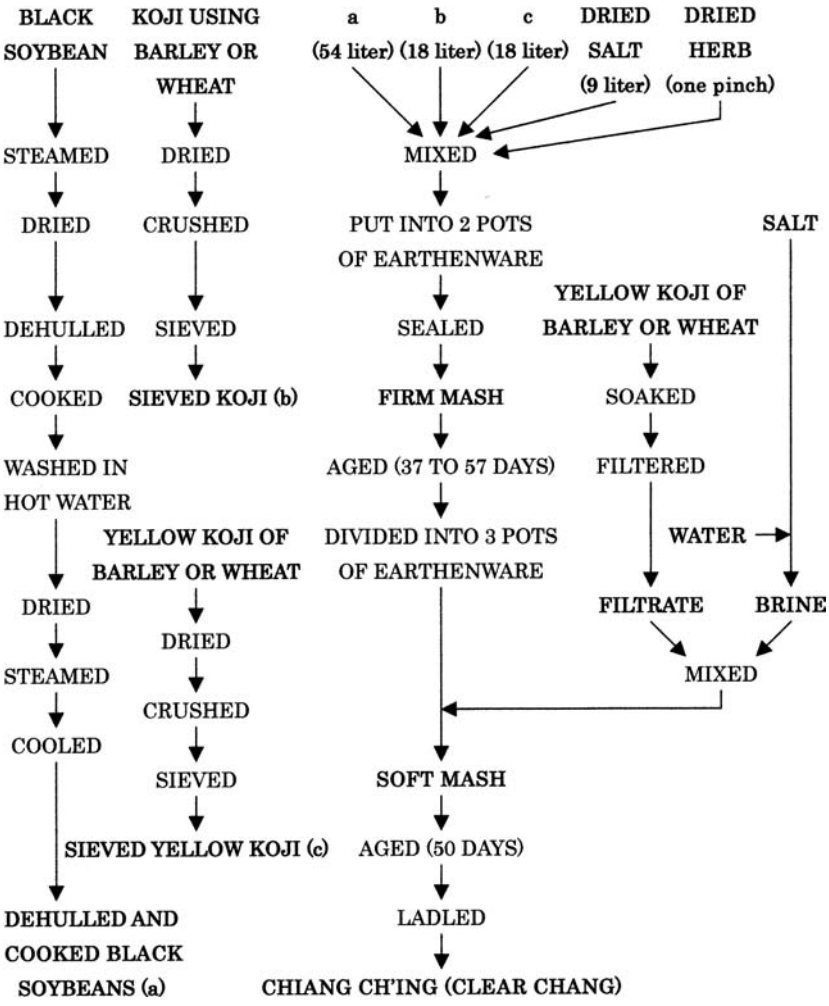

Figure 2 Manufacturing process of chiang ch'ing (clear chiang) appearing in *Chtiming Yao-shu* (*Saimin-Yojutsu* in Japanese) by Chia Ssu-hsieh (Ka Shikyo in Japanese), published in AD 535. (From Ref. 17.)

facilitated the manufacture of chiang. If the definition of "biotechnology" is to make the products necessary for the welfare of humans by using life phenomena, it can be said that people in ancient China had already produced foods by biotechnology as early as several thousand years ago. In this sense, it is not an exaggeration to say that soy sauce was a pioneer of the actual application of biotechnology.

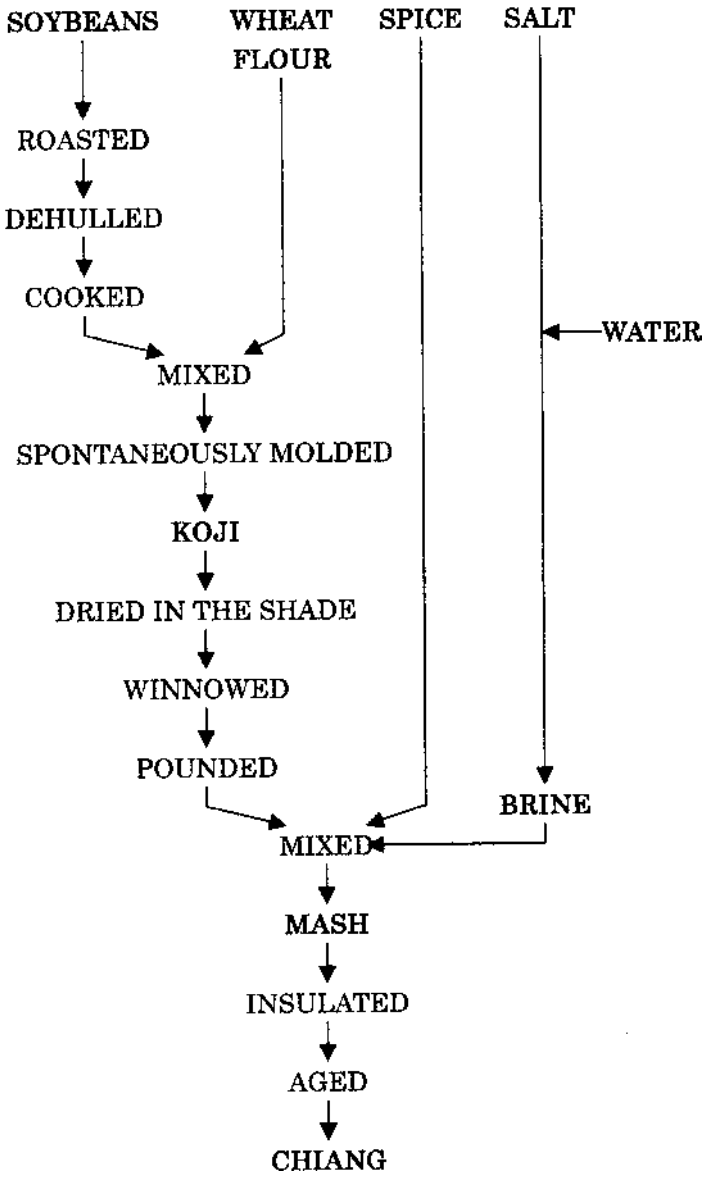

Figure 3 Manufacturing process of soybean chiang appearing in *Nung-sang I-shih Ts'o-yao* (*Noso-Ishoku-Satsuyo* in Japanese) by Lu Ming-Shan (Ro Meizen in Japanese), published in the Yuan dynasty (AD 1271–1368). (From Ref. 17.)

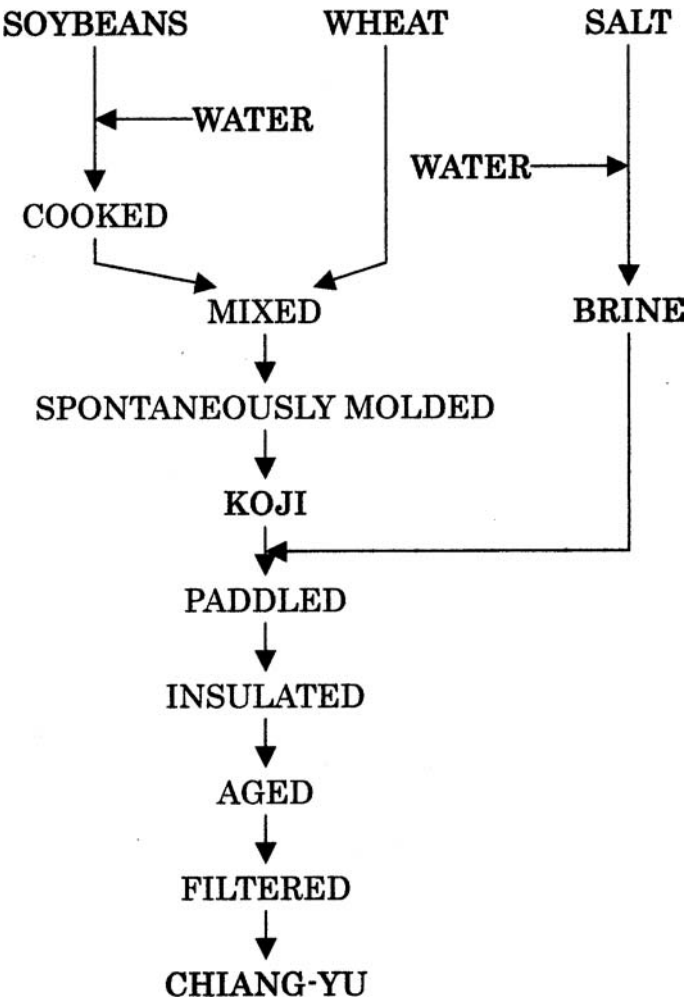

Figure 4 Manufacturing process of chiang-yu appearing in *Pen-ts'ao Kang-mu* (*Honso-Komoku* in Japanese) by Li Shin-chen (Ri Jichin in Japanese) published AD 1590. (From Ref. 17.)

2. Shih

Shih is a tasty nugget with or without salt, which was made from soybeans by fermentation. Shih is classified into five types by the kind of micro-organism used in its manufacture. They are *aspergillus* type (shih in the areas of Pei-ching, Hu-nan sheng, and Taiwan; hamanatto in Japan), *Mucor* type (shih in the area of Ssu-ch, nan sheng), *Rhizopus* type (tempeh in Indonesia), *Bacillus* type (shih in the area of Shan-tung sheng and natto in Japan), and *Neurospora* type (oncom in Indonesia). The shih described here is the shih of *Aspergillus* type, which relates to soy sauce.

The earliest literature in which shih appeared is *Shih-chi* by Ssu-ma Ch'ien, which was published in 85 BC. Shih is also described along with chiang in *Shuo-wen Chie-tzu* by Hsu Shen, the oldest dictionary in China published in AD 121 in the later Han dynasty. The material of the shih is soybeans, as shown in Fig. 5. Therefore, shih as soybean nuggets contains a high amount of protein. In shih, much of the soybean constituents are present in a soluble state. The soybean proteins are hydrolyzed to single amino acids, and therefore, the flavor is sharp and strong. The flavor constituents of shih can be extracted easily by a salt solution. The original shih was served as nuggets, but the brine extract gradually came to be used as a seasoning. In Chi-min Yao-shu (AD 535), there is a description of about 70 kinds of cookery using shih extracts. It should be mentioned that shih and its brine extract developed into today's tamari-shoyu in Japan.

B. Introduction of Soy Sauce in Japan

There is no literature on the exact time when chiang and shih were introduced into Japan. In AD 702, however, the Hishiotsukasa, the Bureau for the Regulation of Production, Trade and Taxation of Hishio, was established by the Taiho-Ritsuryo, one of Japan's earliest constitutions. This bureau was located at the imperial palace and produced various kinds of hishio (chiang) which was consumed by the imperial household. Among these products, the word "misho" is found with the words "chiang" and "shih." It should be noted that the word "misho," which is very close to the present word "miso," is found as one of the products in the bureau. Misho is also found in Engishiki, the enforcement regulation of the statutes, which was completed in AD 927 and became effective in 967 in Japan. The first appearance of the present word "miso" was in *Nihon-Sandai-Jutsuroku*, a history book by Sugiwara, Minamoto, Fujiwara, and Okura, published in AD 901 in Japan. On the other hand, the word "shoyu" first appeared in *Ekirinbon-Setsuyoshu* (anonymous), a Japanese dictionary published in 1474 during the Muromachi period. In the Edo period (1603–1867), several pieces of literature describing miso and

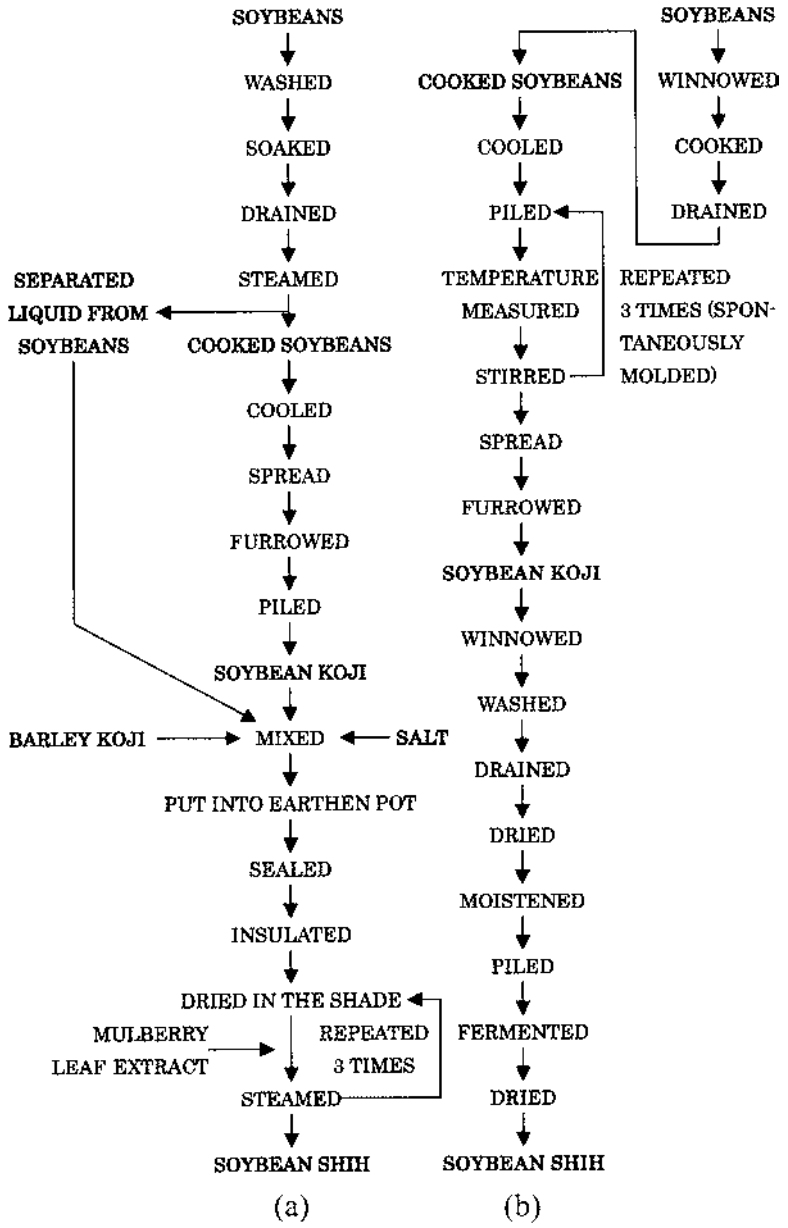

Figure 5 Manufacturing process of soybean shih. (a) Process appearing in *Shi-ching* (*Shokkei* in Japanese) by Hsie Feng (Sha Fu in Japanese), published in the Han dynasty (206 BC to AD 220). *Shi-ching* has not survived to the present day, but the original *Shi-ching* process of the soybean shih is cited in *Ch'i-ming Yao-shu* (*Saimin-Yojutsu* in Japanese) by Chia Ssu-hsieh (Ka Shikyo in Japanese), published in AD 535. (b) Process appearing in *Ch'i-ming Yao-shu* (*Saimin-Yojutsu* in Japanese), published in AD 535. (From Ref. 19.)

shoyu were written, such as *Wakan-Sansai-Zue* by Narushima (1712), *Honcho-Shokkan* by Hitomi (1695), *Mankin-Sangyotai* by Miake (1732), *Yoshu-Fushi* by Kurokawa (1682), and the like. The manufacturing processes described in these works are close to the present methods for producing miso and koikuchi-shoyu. Thus, it is presumed that the basic manufacturing processes of today's Japanese soy sauce had been completed by the early 17th century.

In the second half of the 17th century, the large-scale industrial production of soy sauce occurred, backed by a huge consumption in large cities such as Edo (now Tokyo). Surprisingly enough, there are written records that soy sauce had been exported to India, southeast Asia, and Europe as early as the middle of the Edo period (15). According to documents of the Dutch East India Company stored in the archives in the Hague, Holland, soy sauce was exported from Nagasaki in the Kyushu district of Japan to several places of India, namely to Coromandel in 1668 and 1716, Ceylon in 1670 and 1699, Surat in 1717, Bengal in 1699, and Nagappattinam in 1699. Carl Thunberg, a ship's doctor for the Dutch East India Company who stayed in Japan for a year and a half from 1775 to 1776, published a book of travels in 1796 (20). According to his book, a large quantity of soy sauce was shipped to Batavia (the former name for Djakarta), India, and European countries (Europeans used it by mixing with sauces). Further, it is described in this book that they bottled soy sauce in ceramic containers after boiling in an iron pot, stoppered, and then sealed with pitch, in order to prevent soy sauce from deteriorating in quality during transportation. Thus, a method for protecting soy sauce from decay using thermal sterilization was developed. This invention preceded the invention of canned foods by Nicolas Appert by 50–100 years and the success of the famous low-temperature sterilization method in 1862 by Louis Pasteur by 10 decades. Figure 6 shows the ceramic bottles of soy sauce with Dutch lettering exported from the port of Nagasaki.

C. Origin of Fermented Soy Sauce in the United States

According to historical investigations by Kenzaburo Mogi, the heir of the Kashiwaya family in Kikkoman Corporation, the manufacturing plant of fermented soy sauce was opened in 1907 by Shinzaburo Mogi. This was the first plant built in the United States for fermented soy sauce, but it was unsuccessful. After that, another soy sauce company named Oriental Show-You Company was promoted in 1917 in Columbia City, Indiana. Shinzaburo Mogi also took part in this company as one of the stockholders. In the Oriental Show-You Company, Shinzo Ohki began to make koikuchi-shoyu in the same manufacturing way as in Japan. The amount of soy sauce produced here was about 30,000 gal (about 114 kL) per year. The Oriental Show-

Figure 6 Ceramic bottles of soy sauce with Dutch letters exported from Japan to southeast Asia, India, and Europe during the 17th and 18th centuries. (Courtesy of Noda City Museum, Chiba, Japan.)

You Company was sold to Beatrice Foods, Inc. in 1961 and was subsequently turned over to LaChoy Food Products.

III. PRESENT SOY SAUCE SITUATION

A. Japan

According to statistical data of the Japan Soy Sauce Association, there were about 2500 soy sauce producers in 1986 in Japan, but they decreased to 1600 in 2000. However, half of the Japanese domestic market is still controlled by the five largest manufacturers Kikkoman, Yamasa, Higashimaru, Higeta, and Marukin. Their brands are called the big five brands and their market shares was 30.5%, 9.0%, 4.5%, 3.0%, and 2.5% in 1986, respectively. The total market share for these five big brands were 49.5% in 1986 and 49.4% in 2001, almost unchanged for the past 15 years. The total amount of soy sauce produced in Japan was about 1.2 million kL/year in 1986, but it has decreased to about 1.0 million kL/year in 2001.

1. Types of Soy Sauce

Generally, soy sauce is divided into two categories: fermented soy sauce and chemical soy sauce. In Japan, however, 100% chemical soy sauce is not recognized as soy sauce by the Japanese government. Chemical soy sauce is used only as an extender of soy sauce in Japan. In fermented soy sauce, the proteins and carbohydrates contained in the raw materials are hydrolyzed very slowly below 30°C for over 5 mo, whereas in chemical soy sauce, they are hydrolyzed very quickly by hydrochloric acid at over 80°C for 8–10 h. The chemical hydrolysis is a very inexpensive and quick process, but during the hydrolysis, various secondary reactions occur and produce undesirable compounds such as dark humins, furfural, dimethyl sulfide, hydrogen sulfide, levulinic acid, formic acid, and the like, which are not present in fermented soy sauce. Most recently, it was reported that carcinogenic substances such as 1,3-dichloropropane-2-ol, 2,3-dichloropropane-1-ol, and 3-monochloropropane-1,2-diol are produced during the hydrolysis by HCl (21). In addition, tryptophan, one of the nutritionally important amino acids, is destroyed almost completely. As shown in Fig. 7, the main organic acid of fermented soy sauce is lactic acid, whereas the main organic acid of chemical soy sauce is formic acid. Levulinic acid, present in chemical soy sauce, does not exist in the natural world. Therefore, the quantity of chemical soy sauce mixed with fermented soy sauce can be determined by measuring the levulinic acid content. In order to improve the odor of chemical soy sauce, semichemical soy sauce called "shinshiki-shoyu" was devised. It is made by hydrolyzing raw soybeans with a lower concentration of hydrochloric acid (7–8%) as the first step, followed by fermenting the resultant hydrolyzate with salt-tolerant yeasts in the presence of wheat koji. The odor and flavor of the resultant semichemical soy sauce are improved partly by the fermentation procedure, but it is essentially the chemical soy sauce which has the undesirable compounds previously described.

Five varieties of soy sauce exist in Japan: koikuchi-shoyu (regular soy sauce), usukuchi-shoyu (light-colored soy sauce), tamari-shoyu, saishikomi-shoyu, and shiro-shoyu. These five types of soy sauce are recognized by the Japanese government and each type is classified into three grades: special, upper, and standard. Each grade is determined by organoleptic evaluation, contents of total nitrogen, alcohol, and soluble solids other than sodium chloride, and color. "Special grade" is assigned to high-quality soy sauce made only by the action of micro-organisms. The use of chemical and/or enzymatic hydrolyzates for special-grade soy sauce is not permitted. Among the soy sauce products produced in Japan in 1986, about 74.8% are purely fermented products, about 21.8% contain semichemical soy sauce, and the remaining 3.4% are mixture products with chemical soy sauce. The annual

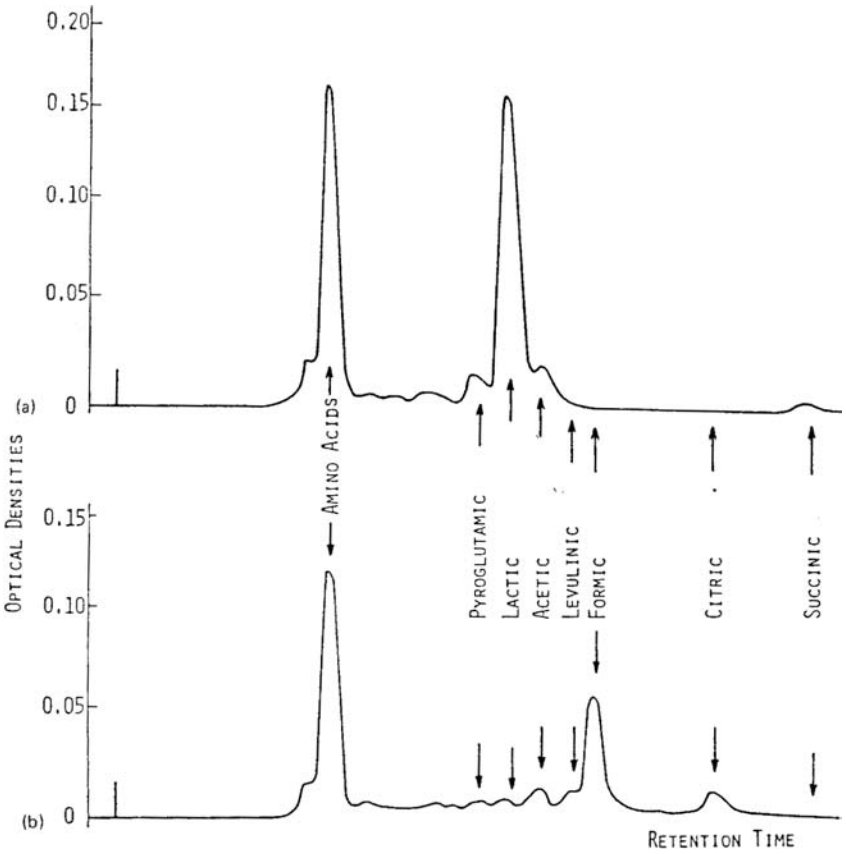

Figure 7 Chromatograms of organic acids on fermented (a) and chemical (b) soy sauce. (Data from Refs. 4 and 9.)

production of soy sauce marked by the Japanese Agricultural Standard (JAS) in 1986 and 2001 is shown in Table 1.

The typical composition and annual production of the five varieties of soy sauce are shown in Tables 2 and 3. Among these, koikuchi-shoyu, typical Japanese-style fermented soy sauce, accounts for over 80% of the total soy sauce production in Japan. It is an all-purpose seasoning characterized by a strong aroma, myriad flavors, and deep reddish brown color. These properties of koikuchi-shoyu are mainly derived from the use of equal amounts of wheat and soybeans as the materials of the koji and vigorous yeast fermentation under proper temperature controls.

Table 1 Annual Production of Soy Sauce Marked and Nonmarked by JAS in 1986 and 2001 in Japan

	1986		2001	
JAS grades	Kiloliters	Percent	Kiloliters	Percent
Special[a]	675,894	56.3	533,740	52.0
Upper	285,059	23.8	190,835	18.6
Standard	92,279	7.7	46,711	4.5
Non-JAS	145,962	12.2	256,067	24.9
Total	1,199,194	100.0	1,027,353	100.0

[a] Special grade is granted for the soy sauce which consists of fermented soy sauce only.
Source: Statistical data from the Japan Soy Sauce Association.

Table 2 Typical Compositions of Five Varieties of Soy Sauce Recognized by the Japanese Government

	Varieties of soy sauce				
Analytical item	Koikuchi-shoyu	Usukuchi-shoyu	Tamari-shoyu	Saishikomi-shoyu	Siro-shoyu
Extract[a]	18.70	15.92	26.95	23.95	24.25
NaCl (g/100 mL)	16.40	18.57	17.35	15.70	16.85
Total nitrogen (g/100 mL)	1.582	1.196	2.620	1.948	0.543
Glutamic acid (g/100 mL)	1.20	0.91	1.70	1.20	0.31
Reducing sugar (g/100 mL)	2.55	4.05	4.36	4.70	21.04
Alcohol (ml/100 mL)	2.68	3.13	0.15[b]	Trace[b]	Trace[b]
pH	4.74	4.82	4.93	4.83	4.48
Color	Deep brown	Light brown	Dark brown	Dark brown	Yellow to tan

[a] Soluble solids other than NaCl.
[b] The values of alcohol are variable because alcohol is often added.
Source: Ref. 22.

Table 3 Annual Production of Each Variety of Soy Sauce Marked by the JAS in 1986 and 2001 in Japan

	1986		2001	
Varieties of soy sauce	Kiloliters	Percent	Kiloliters	Percent
Koikuchi-shoyu	886,329	84.2	637,765	82.7
Usukuchi-shoyu	138,821	13.2	108,496	14.1
Tamari-shoyu	18,962	1.8	14,179	1.8
Saishikomi-shoyu	5,765	0.5	6,148	0.8
Siro-shoyu	3,355	0.3	4,698	0.6
Total JAS soy sauce	1,053,232	100.0	771,286	100.0

Source: Statistical data from the Japan Soy Sauce Association.

Usukuchi-shoyu is characterized by a lighter reddish brown color and milder flavor and aroma. It is used mainly for cooking when one wishes to preserve the original flavor and color of the foodstuff itself. It is said that this product originated in the Tatsuno area of Japan in 1666 and is used mainly in the western area of Japan. The ratio of soybeans and wheat in this type of soy sauce is the same as in koikuchi-shoyu, but the fermentation is done under such conditions that color development can be prevented.

The third variety of soy sauce is tamari-shoyu, which is characterized by a higher amino acid content, although it lacks aroma. The distinguishing feature of this product is that the main material is soybeans and that wheat is not used or is only used in small amounts. The yeast fermentation does not occur or only slightly, because of the lack of wheat as a source of carbohydrates, resulting in the lack of the aroma based on alcoholic fermentation. Tamari-shoyu is consumed locally only around the Nagoya region of Japan.

Saishikomi-shoyu is characterized by using unheated soy sauce instead of the salt solution. In this case, equal amounts of wheat and soybeans are used in making koji. This type of soy has both aroma and full-bodied taste.

Shiro-shoyu is produced by using a very high ratio of wheat to soybeans as the material of koji and its moromi mash is controlled so as to prevent color development. It is characterized by a very light yellow to tan color, but its amino acid content is very low.

Saishikomi-shoyu and shiro-shoyu are produced and consumed only in isolated localities or for special industrial uses in Japan. The tamari type of soy sauce is produced widely in southeast Asia as a traditional Chinese type of soy sauce, as will be described later. In mainland China, however, a modified type of soy sauce is produced at present by a new method quite different from the traditional one. This method will be described later.

2. Manufacturing

The manufacturing process of fermented soy sauce consists of three major processes: koji making, brine fermentation, and refining, as shown in Figs. 8–10.

Making koji is a characteristic technique of the Orient through the history of 3000 years (23). This unique process is used in the manufacture of such fermented foods as soy sauce, miso, sake (rice wine), shochu (rice wine spirits), yonezu or komezu (a rice vinegar), and the like. Koji is a source of enzymes for converting the carbohydrates and proteins of the raw materials into sugars, peptides, and amino acids. The substances converted by the enzymes of koji become the nutrients of lactic acid bacteria and yeasts in the subsequent brine fermentation. In making koji, soybeans or, more commonly, defatted soybean flakes or grits are moistened and cooked under pressure. Previously, the soybeans were cooked at 0.9–1.0 kg/cm^2 gauge pressure for 40–45 min in a batch-type cooker. Currently, the soybeans are cooked at about 7 kg/cm^2 gauge pressure for 0.25 min or less in a continuous cooker, which allows high-pressure, short-term cooking. The relationship between the heating conditions of soybeans and their ability to be hydrolyzed by koji enzymes is shown in Table 4.

The wheat component of koji is roasted by a continuous roaster at 170–180°C for a few minutes and then cracked into four or five pieces. In koikuchi-shoyu, usukuchi-shoyu or saishikomi-shoyu, the cooked soybeans or defatted soybeans are mixed with equal amounts of the roasted wheat and then inoculated with a pure starter culture of *Aspergillus oryzae* or *Aspergillus sojae*. This culture is called the "koji starter" or "seed mold." Generally, koji becomes a greenish yellow mass as a result of the sporulation. In tamari-shoyu the ratio of wheat to soybeans must be 10% or less. In shiro-shoyu, on the other hand, a very high ratio of wheat to soybeans must be used. In the usukuchi-shoyu and shiro-shoyu, species of *Aspergillus*, which do not cause strong color development during brine fermentation, are used.

The second step in the manufacture of fermented soy sauce is brine fermentation. This fermentation is unique in that it utilizes halophilic lactic acid bacteria and salt-tolerant yeasts. The presence of NaCl in brine (16–19 g salt/100 mL) effectively excludes undesirable micro-organisms. The harvested koji is transferred to deep fermentation vessels containing a salt solution of 22–25% (w/v) by means of a snake pump. In saishikomi-shoyu, however, unheated soy sauce is used instead of a fresh salt solution to obtain a thicker taste of soy sauce. The resultant mixture is called moromi or moromi mash. The moromi mash is held for 5–8 months under appropriate temperature controls with occasional brief aeration to mix the contents and to stimulate microbial growth. In usukuchi-shoyu and shiro-shoyu, the holding time is

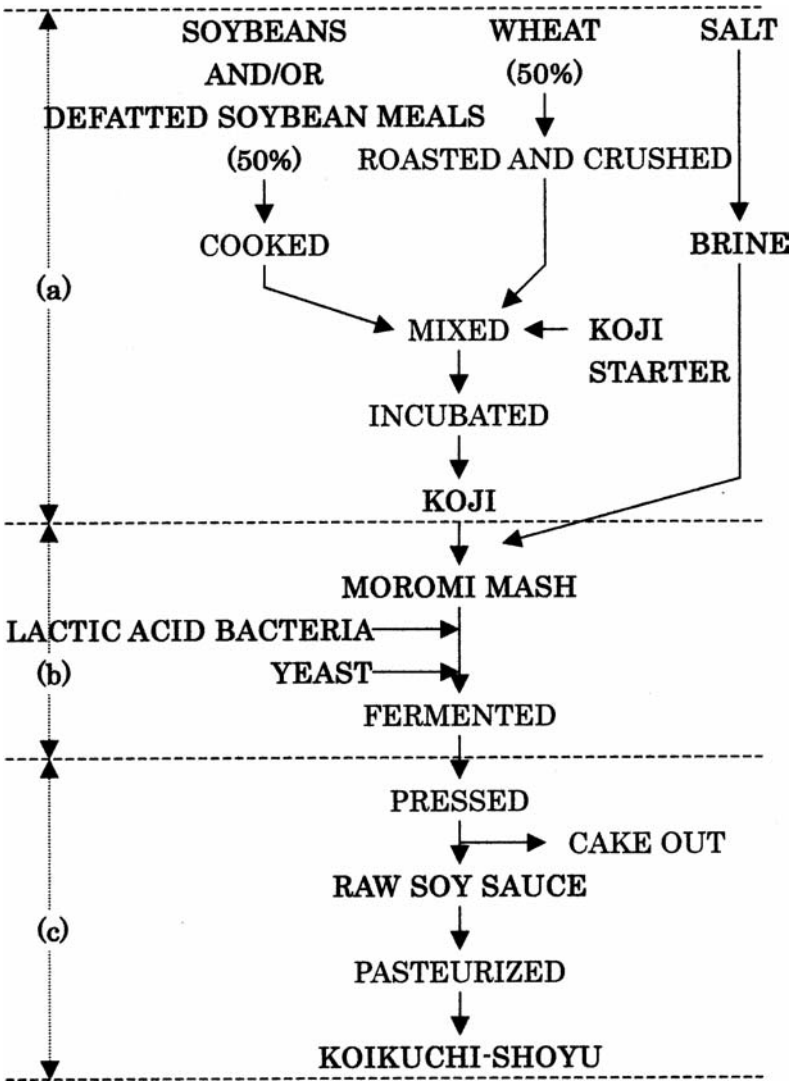

Figure 8 Manufacturing process for koikuchi-shoyu, which is a representative fermented soy sauce in Japan: (a) koji-making process; (b) brine fermentation process; (c) refining process. (From Ref. 9).

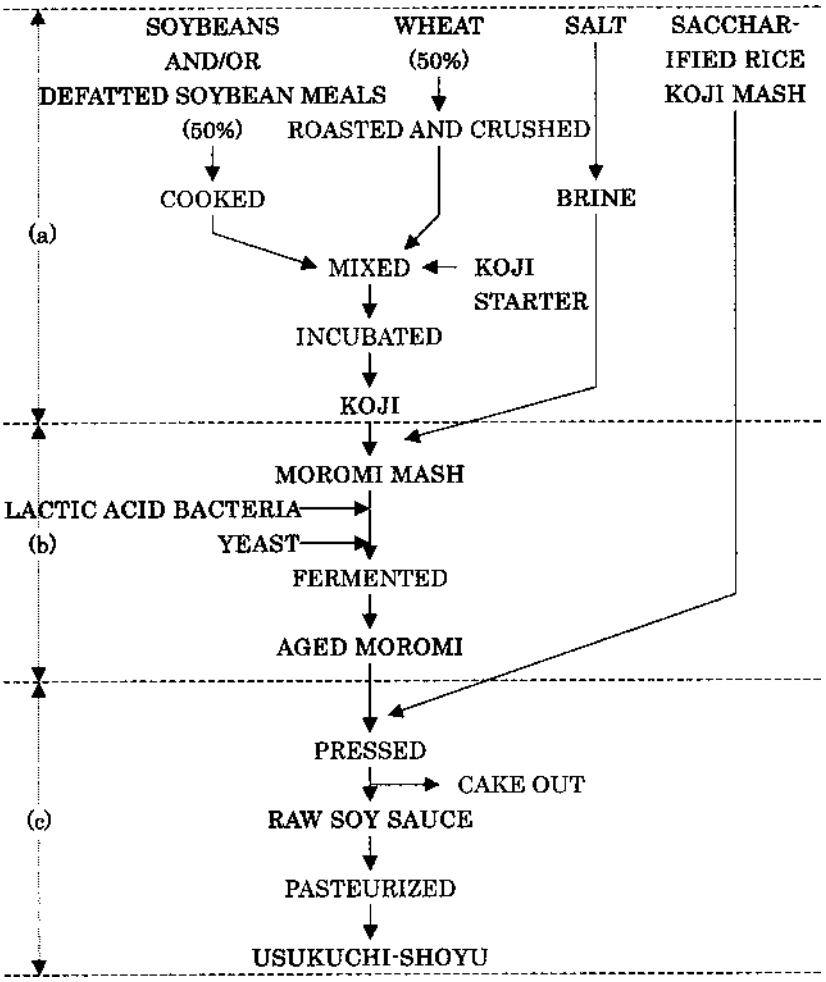

Figure 9 Manufacturing process for usukuchi-shoyu: (a) koji-making process; (b) brine fermentation process; (c) refining process. (From Ref. 9.)

shorter to prevent color development. During the fermentation period, the enzymes from koji hydrolyze most of the proteins in the materials to amino acids and low-molecular-weight peptides. Much of the starch is converted to simple sugars, which are fermented primarily to lactic acid, alcohol, and carbon dioxide. The pH drops from an initial value of 6.5–7.0 down to 4.7–4.8. The high salt concentration effectively limits growth to a few desirable osmophilic types of micro-organisms. In the first stage of moromi mash,

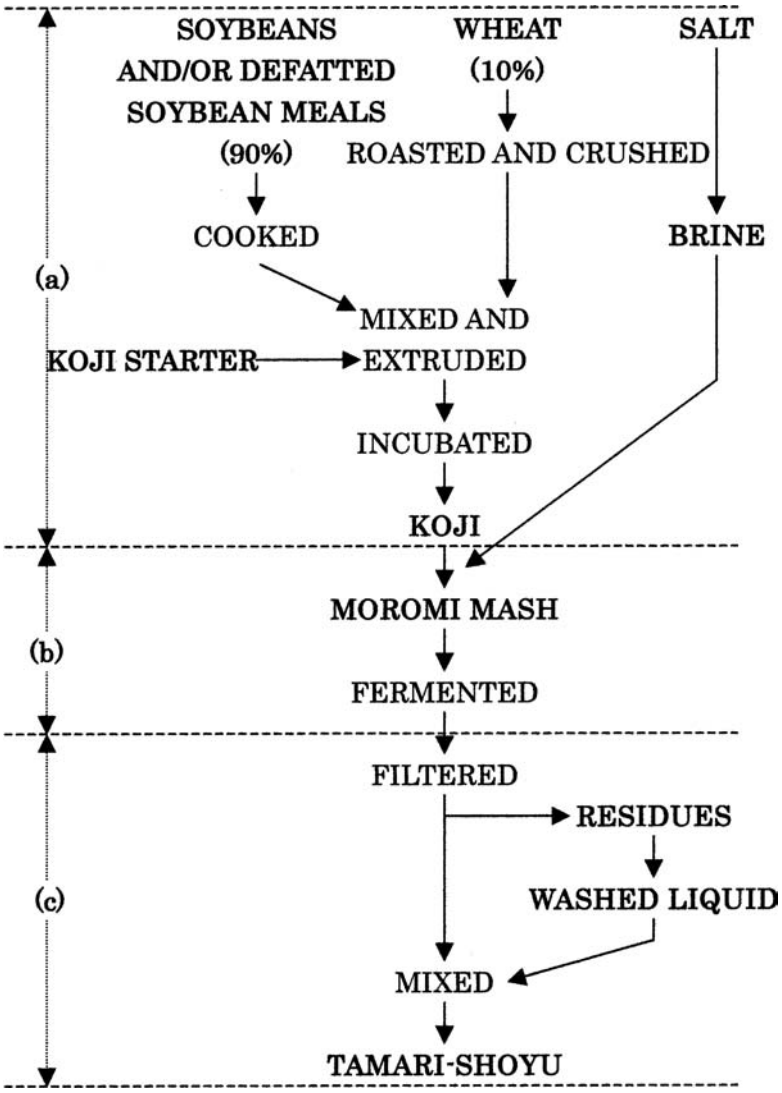

Figure 10 Manufacturing process for tamari-shoyu: (a) koji-making process; (b) brine fermentation process; (c) refining process. (From Ref. 9.)

Table 4 Relationship Between Cooking Conditions and Yields of Soy Sauce

Cooking conditions			Chemical analysis of resultant soy sauce			
Pressure (kg/cm)	Temp. (°C)	Time (min)	NaCl (g/100 mL)	Total nitrogen (g/100 mL)	Amino nitrogen (g/100 mL)	Yield[a] (%)
0.9	118	45	17.40	1.653	0.817	82.05
2.0	132	5	17.40	1.697	0.837	84.24
6.7	151	2	17.40	1.752	0.839	86.87
7.0	170	0.25	17.40	1.778	0.878	88.26

[a] The yields are expressed by the percent of hydrolyzed nitrogen versus the total nitrogen contained in the raw materials.
Source: Ref. 24.

Tetragenococcus halophilus (halophilic lactic acid bacteria) grows and produces lactic acid, which causes a drop in the moromi pH. In koikuchi-shoyu and usukuchi-shoyu, *Zygosaccharomyces rouxii* (salt-tolerant yeasts) grow rapidly along with the decrease of the moromi pH. As a result, a vigorous yeast fermentation occurs and the quantities of ethanol in the juice of moromi mash reach 2–4% (v/v). At the same time, *Z. rouxii* produces many compounds related to flavor, such as esters, organic acids, alcohols, aldehydes, franones, and so on. In tamari-shoyu, however, *Z. rouxii* does not grow much because of its sugar shortage and the inhibiting effects due to high nitrogen content. Therefore, tamari-shoyu lacks the good aroma based upon the yeast fermentation. In koikuchi-shoyu and usukuchi-shoyu, *Candida* strains, which are another group of salt-resistant yeasts, sometimes grow during the middle and last stage of moromi fermentation. These strains produce phenolic compounds such as 4-ethylguaiacol and this compound adds some unique aroma to soy sauce (25). The techniques to control the micro-organisms during brine fermentation are established in koikuchi-shoyu and usukuchi-shoyu. This was accomplished by adding pure cultures of *T. halophilis* and *Z. rouxii* to moromi mash. The technique allows for the constant production of a desirable quality koikuchi-shoyu and usukuchi-shoyu.

The final process of making soy sauce is refining, which includes filtering and pasteurizing by heat. In koikuchi-shoyu, the aged moromi mash is put into a cloth and then pressed with a hydraulic press machine until the water content of the residue is less than 25%. After pressing, the filtered soy sauce is heated at 70–80°C by a plate heater. Heating is very important for (a) developing large numbers of compounds such as aldehydes, acetals, mercaptans, mercaptols, organic acids, pyrazines, furfurals, and others which

contribute to the sharp flavor and aroma characteristic of koikuchi-shoyu, (b) developing a reddish brown color characteristic of koikuchi-shoyu, (c) improving clarity by precipitating heat-coagulable substances, (d) increasing resistance to growth of undesirable film yeasts by production of organic acids and phenolic compounds, and (e) inactivating most of the enzymes. After clarifying the resultant soy sauce by sedimentation and filter membranes, the clear supernatant is bottled and packed. In usukuchi-shoyu, however, a digestion mixture of rice koji (saccharified autolyzates) is added to the aged moromi and then the pressing of the moromi mash is carried out, as shown in Fig. 9. The purpose of adding the digestion mixture to usukuchi-shoyu is to make the flavor bland. The heating and clarification of usukuchi-shoyu are carried out in the same way as that of koikuchi-shoyu, except that the heating temperature is lower. In tamari-shoyu, the separation of soy sauce from moromi mash is done with natural gravity filtration, followed by the leaching of the residue with a salt solution. Usually, a preservative such as sodium benzoate is added to the resultant filtrate and/or leaching solution before bottling. In tamari-shoyu, pasteurization is not done in principle. In general, preservatives such as sodium benzoate had been widely used in most types of soy sauce. In Japan, however, most soy sauce is bottled without using any preservative other than alcohol.

The consumer's interest in the excessive intake of salt from a nutritional point of view has become stronger and stronger. As a result, the consumption of salt-reduced soy sauce has been increasing rapidly. There are two methods for preparing salt-reduced fermented soy sauce which are quite different. One is prepared by removing some quantities of salt from ordinarily made soy sauce (method A). The other is produced by adjusting the salt content by the addition of water to specially made low-salt, high-nitrogen soy sauce (method B). In method A, the reduction of the salt is done either by dialysis through membranes, such as electrodialysis through ion-exchange membranes or dialysis through dialyzing membranes, or by salt precipitation which occurs by concentrating regular soy sauce by vacuum evaporation. In method B, salt reduction is accomplished through the following basic principle. In the regular methods of soy sauce making, the salt content of moromi mash liquid during brine fermentation is kept at approximately 17% (w/v) to keep the normal activity of osmophilic micro-organisms and to prevent the growth of undesirable micro-organisms. However, the salt content can be decreased to around 13% (w/v) when the total nitrogen concentration of moromi mash liquid is elevated to 2.3–2.5% (w/v), because the decrease of the antimicrobial power of the soy sauce due to the decrease of the salt content is compensated for by the increase of the total nitrogen content. The contribution of the increased nitrogen compounds to the decrease of the water activity compensates for the increase of the water activity which occurs as a result of the

decrease of the salt content. Soy sauce with a high total nitrogen content can be produced by increasing the ratio of soybeans to wheat. Soy sauce of various salt concentrations can be made by adding water to the resultant soy sauce, because the contents of total nitrogen and other flavor constituents in the original soy sauce are 20–50% higher than those of regular soy sauce. Soy sauce with a salt content below 9 g (3550 mg as sodium) per 100 ml is designated as "genen-shoyu." The salt content of genen-shoyu is 50% lower than that of regular soy sauce, and it is recognized as a low-sodium food under the regulation of the Ministry of Health and Welfare of Japan. On the other hand, soy sauce with a salt content 20% lower than that of regular soy sauce is designated as "usujio-shoyu" under the regulation of the JAS. The production of usujio-shoyu was 17,228 kL in 1986, but it decreased to 9236 kL in 1996. On the other hand, genen-shoyu increased to 16,007 kL in 1996 from 11,067 kL in 1987 (26). Most of the genen-shoyu is produced by electrodialysis through ion exchange membranes by method A; usujio-shoyu is mainly produced by method B.

3. Flavor Components and Quality Evaluation

In general, good quality Japanese-style soy sauce (koikuchi-shoyu) has a pH of 4.7–4.8 and contains 1.50–1.65% (w/v) as total nitrogen (simple peptides: 45%; amino acids: 45%; ammonium: l0%), 2–5% (v/v) as reducing sugars (glucose: about 85%), 1–2% (w/v) as organic acids (lactic acid: about 70%), 1.0–1.5% (w/v) as polyalcohols (glycerol: 80–90%.), 2.0–2.5% (v/v) as ethanol, and 16–18% (w/v) as sodium chloride. The typical analytical data on amino acids in koikuchi-shoyu and its materials are shown in Table 5. The amino acid composition of fermented soy sauce is almost the same as that of the original mixture of soybeans and wheat with the following exceptions: arginine is converted to ornithine by some of the lactic acid bacteria; tryptophan and cystine are unstable in moromi mash; and tyrosine is partly precipitated from moromi mash liquid because of its low solubility or it is changed to tyramine by lactic acid bacteria. It has been found that there exist special lactic acid bacteria in moromi mash which can decompose aspartic acid, histidine, phenylalanine, and threonine (27). However, these bacteria can be eliminated by the addition of other lactic acid bacteria to the moromi mash of soy sauce.

The composition of flavor constituents in soy sauce is very complicated and about 300 compounds have been detected (28–31). As shown in Fig. 11, the gas chromatographic (GC) profiles of flavor volatiles of soy sauce show more than 200 peaks (32). The flavor and aroma components contained in koikuchi-shoyu are arranged in Table 6 in order of the concentrations (33). The components contained in large amounts are ethanol, lactic acid,

Table 5 Ratio of Each Amino Acid to Total Amino Acids in Koikuchi-shoyu and Its Raw Materials

Amino acid	Soybean (%)	Wheat (%)	Raw materials used for koikuchi-shoyu (%)	Koikuchi-shoyu (%)
Arginine	8.42	4.71	7.58	2.6
Histidine	2.55	2.12	2.45	2.5
Lysine	6.86	2.67	5.90	6.5
Tyrosine	3.90	3.19	3.74	1.0
Tryptophan	1.28	1.13	1.25	—
Phenylalanine	5.01	4.43	4.88	4.2
Cystine	1.58	1.80	1.63	0.9
Methionine	1.56	1.74	1.60	1.4
Serine	5.57	5.22	5.49	5.3
Threonine	4.31	2.76	3.96	4.2
Leucine	7.72	6.52	7.45	7.3
Isoleucine	5.10	3.78	4.80	4.8
Valine	5.38	4.69	5.22	5.5
Glutamic acid	21.00	29.30	22.89	22.5
Aspartic acid	12.01	4.85	10.38	10.5
Glycine	4.52	3.94	4.39	3.9
Alanine	4.51	3.37	4.25	4.4
Proline	6.28	9.94	7.11	6.5
Ornithine	—	—	—	5.7
Total	106.56	96.16	104.97	100.1

Source: Ref. 9.

glycerol, acetic acid, 4-hydroxy-5-methyl-3(2H)-furanone (HMMF), 2,3-butanediol, isovaleraldehyde, and, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF). Among these, HEMF is a very unique compound (34), which exists only in shoyu (100–200 ppm) and miso (a small amount) and not in other foods. HEMF has an intense and caramel like sweet aroma, which resembles that of moromi mash of koikuchi-shoyu (35). Further, it has the lowest odor threshold value (below 0.04 in water) among these koikuti-shoyu flavor compounds and its odor value is calculated to be greater than five million (36). HEMF significantly contributes to the flavor of koikuchi-shoyu and, accordingly it can be mentioned that HEMF is definitely a character-impact compound of Japanese-style fermented soy sauce (koikuchi-shoyu and usukuchi-shoyu). In addition, it has been elucidated that HEMF possesses quite a strong anticarcinogenic activity (37), as explained later. The precursor for HEMF is D-xylulose 5-phosphate, which is present in the

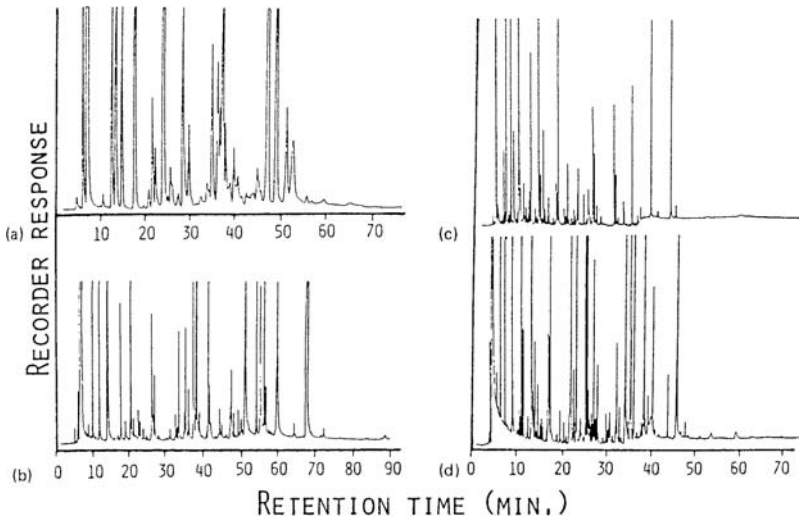

Figure 11 Gas chromatographic profiles of soy sauce aroma. The aroma was concentrated by the distillation–extraction technique in (a), (b), and (d) and by the headspace technique in (c). The columns used were a packed column in (a), a glass capillary column in (b), and a fused-silica capillary column in (c) and (d). (From Ref. 32.)

moromi mash before the growth of yeast (36,38). Therefore, there is no production of HEMF without yeast fermentation. Japanese-style soy sauce, such as koikuchi-shoyu and usukuchi-shoyu, is characterized by vigorous yeast fermentation. This is the reason why Japanese-style soy sauce contains a great deal of HEMF. On the contrary, tamari-shoyu does not contain HEMF, because yeast fermentation does not occur substantially in the moromi mash of tamari which lacks wheat.

Nunomura and Sasaki (33) analyzed the content of the flavor components contained in soy sauce which was collected in the U.S. market in 1983. They were 6 samples from China, 5 samples from Singapore, 6 samples from Hong Kong, 4 samples from Korea, 4 samples from Taiwan, 2 samples from Japan, and 12 samples from the United States. Analyzed flavor components were alcohols, aldehydes, dimethyl sulfide, *n*-valeric acid, ethyl formate, isovaleric acid, furfuryl alcohol, and HEMF. From these data, they classified the world soy sauce into four categories. They are (1) a group of pure chemical soy sauce, (2) a group of chemical soy sauce which underwent fermentation, (3) a group of fermented soy sauce using soybeans and wheat, which underwent only very weak yeast fermentation, and (4) a group of fermented soy sauce using soybeans and wheat, which underwent very strong yeast fermen-

Table 6 Flavor and Aroma Components in Japanese-style Koikuchi-shoyu

Flavor and aroma component	Concentration (ppm)
Ethanol	31,501.00
Lactic acid	14,346.57
Glycerol	10,208.95
Acetic acid	2,107.74
4-Hydroxy-5-methyl-3(2*H*)-furanone (HMMF)	256.36
2,3-Butanediol	238.59
Isovaleraldehyde	233.10
4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF)	232.04
Methanol	62.37
Acetol	24.60
Ethyl lactate	24.29
2,6-Dimethoxyphenol	16.21
Ethyl acetate	15.13
Isobutylaldehyde	14.64
Methyl acetate	13.84
Isobutyl alcohol	11.95
Furfuryl alcohol	11.93
Isoamyl alcohol	10.01
Acetoin	9.78
n-Butyl alcohol	8.69
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF)	4.83
Acetaldehyde	4.63
2-Phenylethanol	4.28
n-Propyl alcohol	3.96
Acetone	3.88
Methionol	3.65
2-Acetylpyrrole	2.86
4-Ethylguaiacol	2.77
Ethyl formate	2.63
4-Butanolide	2.02
Methional	1.42
4-Ethylphenol	0.34
Dimethyl sulfide	0.04

Source: Ref. 33.

tation. Among 39 samples, 35 samples contained no HEMF. Only two samples from Japan contained a great deal of HEMF (250.0 and 162.2 ppm). One sample from Korea and one sample from the United States contained a small amount of HEMF (31.6 and 1.6 ppm, respectively). Thus, it can be mentioned that Japanese-style soy sauce is characterized by the presence of large quantities of HEMF.

Indispensable information for quality control, production control, and product design can be extracted from multivariate analysis of the GC profiles. Aishima analyzed GC data of many soy sauce samples using multiple-regression analysis, discriminant analysis, principal-component analysis, and cluster analysis (32). As a result, he established an objective evaluation system for the odor of soy sauce by GC profiles. Further, his group (39) compared aroma characteristics among koikuchi-shoyu, tamari-shoyu, sinsiki-shoyu (semi-chemical soy sauce), and Chinese soy sauce, by applying quantitative descriptive analysis (QDA®)). As a result, large differences were found among these four types of soy sauce for the flavor characteristics. This suggests that there is no key and character-impact compounds common to these four types of soy sauce. HEMF is a character-impact compound found only in Japanese-style fermented soy sauce. When the headspace volatiles of soy sauce were analyzed using a gas sensor array, it was proved through multiple linear regression analysis and partial least square regression analysis that the sensor responses have a good relationship with sensory attributes.

B. China

The soy sauce manufacturing method used in mainland China at present is quite different from those already described. The following method has been adopted as the manufacturing process for soy sauce in large cities in China. Defatted soybean meal and wheat bran are used in a ratio of 60:40, respectively. Koji manufacturing time has been reduced from 48 h to 24 h by improvement of the mold strain. Brine fermentation time has also been shortened to about 3 weeks by using a special fermentation method, namely the koji is mixed with a smaller volume of the brine (about half the usual amount) having a lower content of salt (about three-fourths). The resultant very firm moromi mash is kept for 3 weeks at 40–45°C. After 3 weeks, the moromi mash is transferred to a separation tank, mixed with brine, and then heated to over 80°C, followed by the separation of the liquid from the bottom of the tank without any forced pressure. The resultant soy sauce is mixed with benzoic acid, clarified, bottled, and shipped. This method is very economical, but the ratio of amino nitrogen to total nitrogen is not high enough because the length of both the koji and brine fermentations is very short. Furthermore, the lack of yeast fermentation due to both the use of wheat bran instead of

wheat and the shorter brine fermentation under the higher temperature gives the soy sauce a different flavor from that of koikuchi-shoyu. Most recently, however, the production of Japanese-style fermented soy sauce (koikuchi-shoyu) started in 2001 in China by the joint cooperation of Japan (Kikkoman Corporation) and Taiwan (Uni-President Enterprizes Corporation). The annual production of soy sauce in China is not clear, but it is said that in 1986, about 1.7 million tons of soy sauce were produced in about 4000 factories which belong to the Vegetable Processing Main Corporation. According to some recent estimation, however, it is said that five million kiloliters of soy sauce are produced annually, although it remains to be confirmed.

C. Southeast Asia

Tamari-type soy sauce is widely produced in the countries of Southeast Asia such as Indonesia, Malaysia, the Philippines, Singapore, Thailand, and so forth. The manufacture of soy sauce in these areas is a very old industry that originated with the Chinese. According to the reports presented at the ASEAN Workshop on Soy Sauce held in Singapore in 1978, most of the soy sauce in these areas is manufactured in small-scale factories following traditional Chinese techniques handed down from generation to generation. Production is generally carried out as follows. Soybeans are boiled in a large cauldron, drained in bamboo baskets, and mixed with a small amount of wheat flour. The mixture is then spread on a bamboo tray and kept in the incubation room for 1 week to make koji. The inoculation of the mold is usually done spontaneously in the incubation room or by mixing with a previous batch of koji. Because a pure starter is not used, the harvested koji usually consists of more than one kind of mold. The koji thus prepared is placed in large earthenware jars of 45–70 L with a brine solution. The jars are left outside and the moromi mash is exposed to the sun for a period of 1–6 months depending on the factory. After the brine fermentation period, the bamboo baskets are dipped in the moromi mash and the liquid accumulated in the baskets is transferred to another earthenware jar and exposed to the sun for several weeks. The first-grade soy sauce is made from this liquid. Fresh brine is added to the residue two more times to extract the second- and third-grade soy sauce, followed by exposure to the sun for a few weeks. Caramel and monosodium glutamate are added to the second and third extracts. The soy sauce is bottled after the addition of sodium benzoate. Pasteurization is carried out before bottling in some factories. In Taiwan, however, there are modern soy sauce factories in which Japanese style fermented soy sauce is made, using equipment imported from Japan. It is said that about 100,000 kL of soy sauce are produced annually in Taiwan at present, of which half is fermented soy sauce.

Kikkoman Corporation opened a plant producing Japanese-style fermented soy sauce (koikuchi-shoyu) in Singapore in 1988. The annual production was 3000 kL at the beginning, but it has grown into 10,000 kL in 2001. This soy sauce is exported mostly to countries in Southeast Asia and Australia. Further, the joint corporation between Kikkoman and Uni-President Enterprizes Corporation was established in Taiwan in 1990 and fermented soy sauce of the Kikkoman brand is produced. The annual production of Kikkoman and Uni-President brand soy sauce in Taiwan is about 10,000 and 16,000 kL, respectively.

D. United States

The market for soy sauce in the United State underwent a complete change since Kikkoman opened an integrated plant of Japanese-style fermented soy sauce in Wisconsin in 1973. Prior to that, the non-Oriental market for soy sauce had been dominated by chemical soy sauce produced by LaChoy and Chun King, whereas fermented soy sauce had been imported mostly from Japan for the Oriental market. Therefore, fermented soy sauce was not fami-

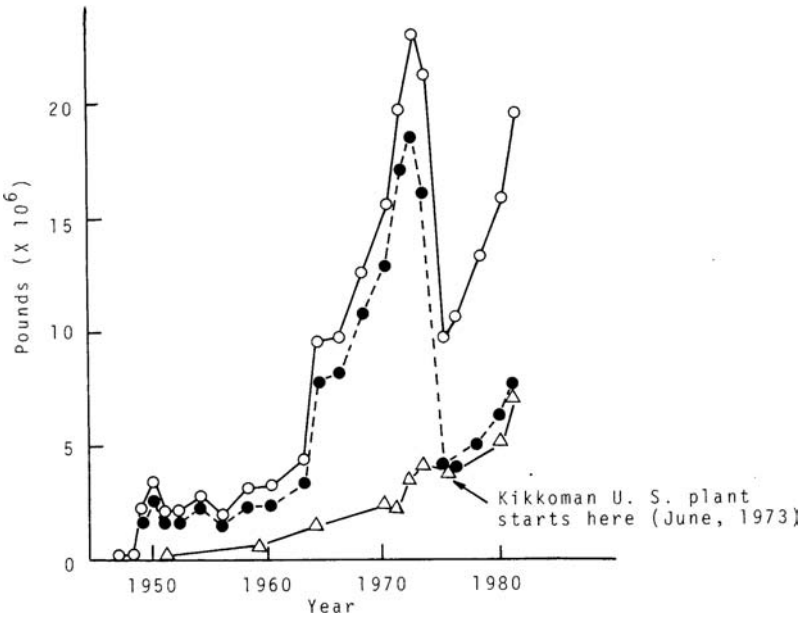

Figure 12 Time course of the amount of soy sauce imported in the United States until 1982: ○, imported products total; ●, imported products from Japan; Δ, imported products from Hong Kong.

liar to non-Oriental people. However, the improved aroma and flavor of Japanese-style koikuchi-shoyu began to be recognized gradually by the non-Oriental people through the production of fermented soy sauce by the Kikkoman U.S. plant, and koikuchi-shoyu penetrated steadily into the U.S. non-Oriental market. The annual consumption of soy sauce in 1982 in the United States was estimated as 50,000 kL. Of the 50,000 kL, 20,000 kL were fermented soy sauce produced by Kikkoman, 21,000 kL were chemical soy sauce produced by LaChoy and Chun King, and the residual 9000 kL were soy sauce imported from Japan (3400–3500 kL), Hong Kong (3200–3300 kl), and other countries including Canada, Taiwan, Mainland China, Korea, Thailand, Singapore, West Germany, and so on (2300 kL). In Fig. 12, the time course of the amount of the imported soy sauce from the end of 1940s to the beginning of 1980s is shown (40). The imported soy sauce contains various types of soy sauce. Soy sauce samples of 91 brands from 76 companies were collected from the U.S. market in 1982 and the values of total nitrogen, reducing sugar, alcohol, lactic acid, NaCl, acidity, aminonitrogen, and color were analyzed. Among these values, the relationship between alcohol and lactic acid in each sample is plotted in Fig. 13. In this figure, the brands of soy

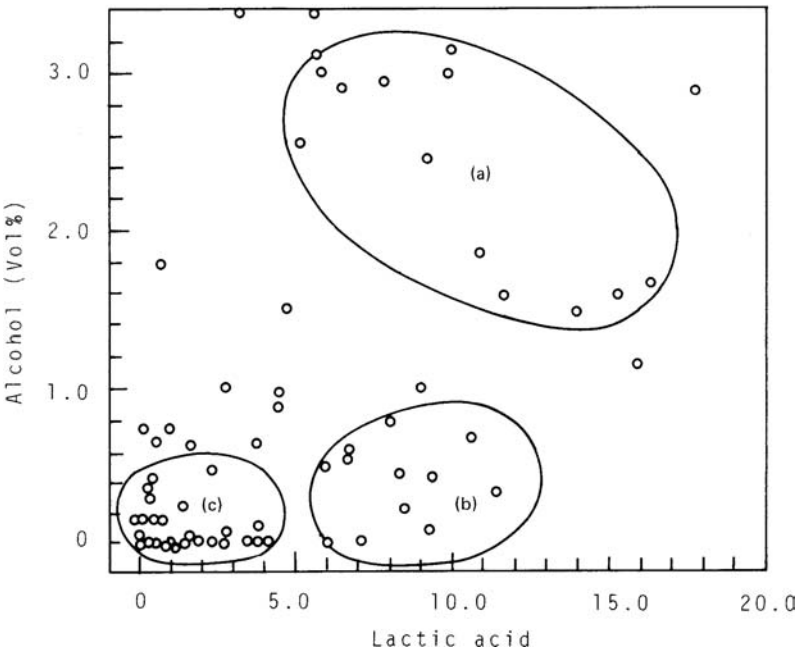

Figure 13 Relationship between alcohol and lactic acid in each of 91 brands of soy sauce from 76 companies, collected from the U.S. market in 1982. (From Ref. 40.)

sauce are divided into three groups. Group a shows a high value in both lactic acid and alcohol. Soy sauce of koikuchi-shoyu and usukuchi-shoyu types belong to this group. Group b is low in alcohol and high in lactic acid. Tamari-shoyu belongs to this group. Group c is low in both alcohol and lactic acid and this is the category of chemical soy sauce. Currently, however, alcohol is often added to the soy sauce after pressing in order to enhance the aroma and/or preservation of the product. Therefore, the high-alcohol and low-lactic-acid samples seen in Fig. 13 should be considered as alcohol-added soy sauce. From these data, it can be seen that many brands of chemical soy sauce are sold on the U.S. market, although the sales of each brand are very small.

Figure 14 shows the time course of the total consumption of soy sauce in the United States. As seen in this figure, the curve of the consumption of soy sauce rises suddenly with the turning point in 1973, when Kikkoman opened the first plant of soy sauce in Wisconsin. Kikkoman opened the second plant in California in 1998. In 2001, the fermented soy sauce production of Kik-

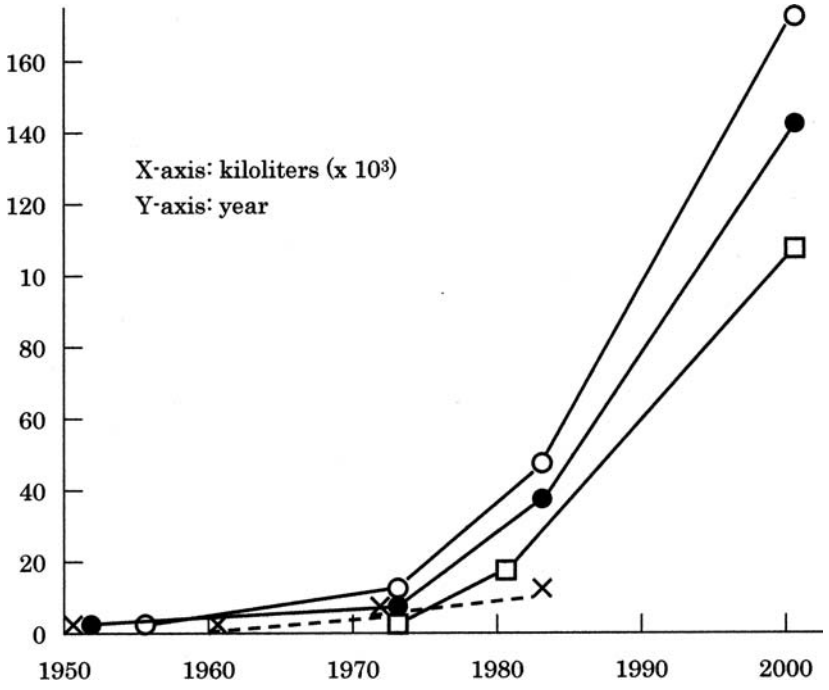

Figure 14 Time course of the total consumption of soy sauce in the United States: ○, consumption total; ●, domestic production total; □, Kikkoman U.S. production total; ×, Imported products total.

koman plants reached 85,000 kL in the Wisconsin plant and 20,000 kL in the California plant. With the rapid expansion of the soy sauce market, several foreign makers opened plants for fermented soy sauce production. Wan Ja Shan (Taiwan) opened a koikuchi type plant around 1980, San-J (Japan) opened a tamari-type plant in 1991, and Yamasa (Japan) opened a koikuch-type plant in California in 1994. The total of the annual production of these three makers is estimated as about 20,000 kL in 2001. In addition to these, chemical soy sauce such as Chun King, La Choy, and Aloha is also produced. The total annual production of these chemical soy sauce makers are estimated as about 15,000 kL in 2001.

IV. CHANGE OF SOY SAUCE MANUFACTURING METHODS FROM INDIGENOUS TO MODERN PROCESSING

The manufacturing method in the late 19th century is shown in Fig. 15. In the first half of the 20th century, many mechanical devices were introduced, but it was not until the 1950s that a new look was taken at the process of soy sauce manufacturing from a scientific point of view. After that time, remarkable progress was made. Useful strains of induced mutants of yellow *Aspergilli* were created for koji manufacturing and innovative methods for processing soybean materials were developed. The role of each group of the microorganisms which are involved in brine fermentation was elucidated and this made the rational control of the brine fermentation possible. Further, each production process was mechanized, automated, and made continuous. This modernization enabled a stable supply of a high-quality product all year round. The consequence was a great leap in yield from 60–63% in the past to more than 90%. These percentages indicate the hydrolysis ratio of the

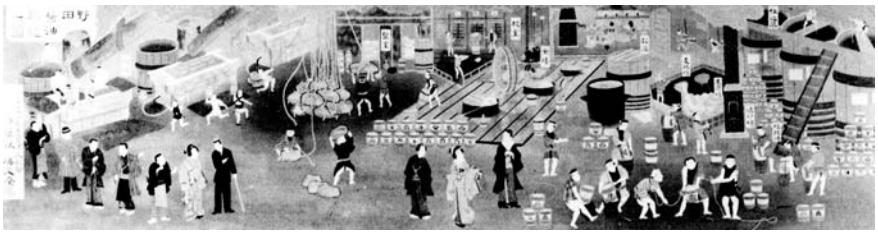

Figure 15 Painting depicting the manufacture of soy sauce in the late 19th century. Painted for the exposition held in Tokyo in 1878. (Courtesy of the Kikkoman Corporation.)

proteins contained in the materials to the amino acids and small peptides liberated into the soy sauce liquid.

A. Treatment of Soybeans and Wheat as Materials

Before 1910, soybeans were soaked and then steamed in a large steam cooker under atmospheric pressure, which is shown in Fig. 16 (41). Around 1910, however, a large pressure cooker was devised, as shown in Fig. 17 (left). The soaked soybeans were autoclaved at a gauge pressure of 1 kg/cm^2 for about 1 h. Traditionally, the cooked soybeans were not taken out from the cooker, immediately after the steam was stopped, but they were left in the cooker until the next day. As a result, the soybeans became very soft in texture and dark brown in color, due to the residual heat in the cooker. In this period, the yield of soy sauce was very low, ranging from 60% to 63%. Here, the yield of soy sauce indicates the percent of the nitrogen solubilized into soy sauce liquid to the nitrogen contained in the materials, (i.e., soybeans and wheat). Around 1955, however, it was discovered that the yield of soy sauce increased from

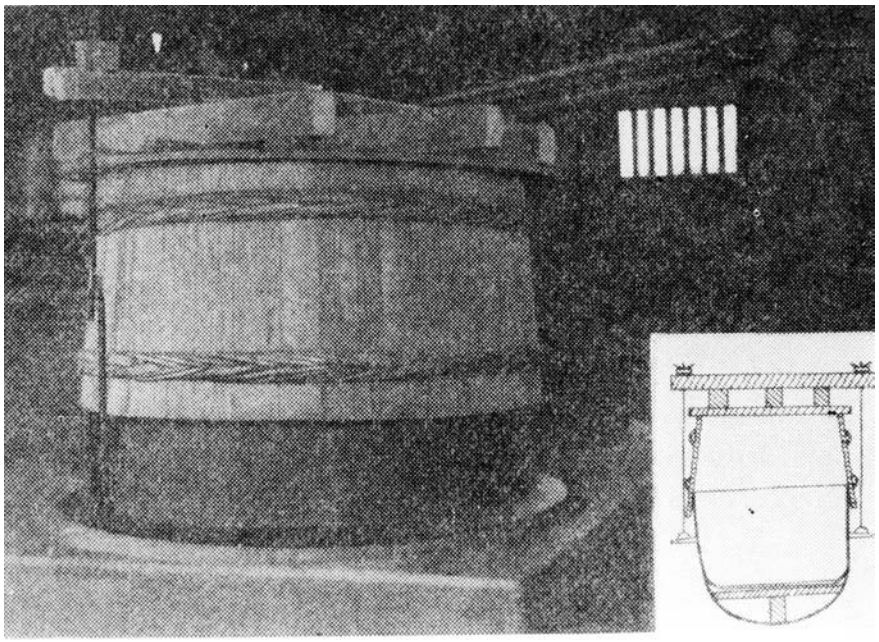

Figure 16 Large steam cooker which was used before 1910. (Courtesy of the Kikkoman Shoyu Co., 1968.)

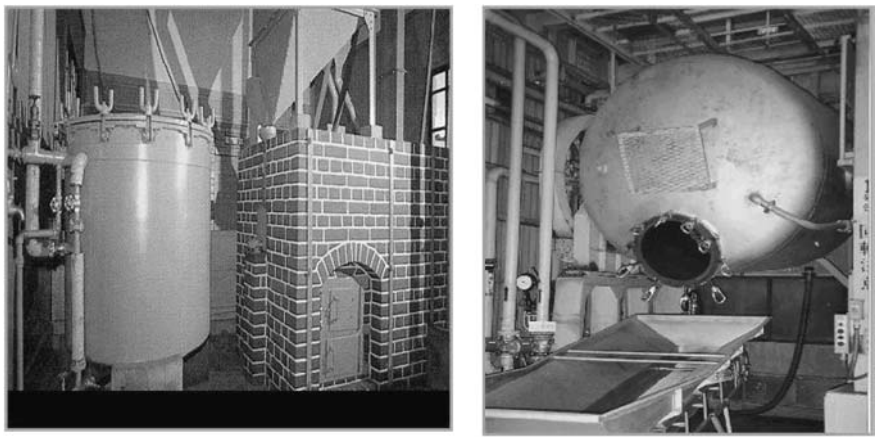

Figure 17 Batch-type pressure cookers for soybeans. Left: Standing type used from 1910 to 1955. Right: Rotating cooker with a quick cooling system, called the NK-cooker, which was used from 1955 to 1970. (Courtesy of the Kikkoman Corporation.)

60–63% to 70–73%, when the cooked soybeans are taken out from the cooker immediately after 1 h autoclaving (41). Further, an improved cooker was devised, called the NK-cooker, which is shown in Fig. 17 (right) (42). The NK-cooker consist, of both a rotary pressure cooker and a jet ejector. Soaked soybeans or soybean meal are autoclaved in this rotating cooker for 30–45 min at about 120°C (gauge pressure: around 1 kg/cm^2), cooled by a jet ejector, and then taken out. As a result, the yield of soy sauce was improved from 70–73% to nearly 78%. The cooking of soybeans was carried out by the NK-cooker for 15 years (from 1955 to 1970), until a new continuous cooker of soybeans was developed. During this period, the heating conditions of soybeans were investigated in detail, under the concept that the excessive heating greatly lowers the degree of the hydrolysis of the proteins by the enzymes (43,44). The relationship between the cooking conditions and the yields of soy sauce is given in Table 4. As shown in this table, the higher the pressure and temperature, the greater the yield, whereas the longer the time, the lower the yield. The new concept was that the soybeans must be processed at a higher temperature in the shortest period possible.

To realize this concept, Kikkoman Corporation developed two types of continuous soybean puffing system. One is a wet puffing system capable of processing water-absorbed soybeans or defatted soybean flakes (water content around 60%) within 60 s under a gauge pressure of 5–7 kg and at a temperature of 160–170°C. The other is a dry puffing system which processes soybeans without adding water (water content of 8–15%) for several seconds

under a gauge pressure of 4–8 kg and at a temperature of 200–280°C. The flow diagrams and photographs on the former system are shown in Figs. 18 and 19, whereas those on the latter system are shown in Figs. 20 and 21. When one uses the soybeans or defatted soybeans treated by these systems, the decomposition ratio of soybean proteins is very much increased and, as a result, the yield of soy sauce has increased to over 90%. This means nearly 30% higher than the yield in the days before 1955. These wet and dry puffing systems were put into practical use in 1970 and 1971 by the Kikkoman Corporation, respectively. At present, the Kikkoman Corporation uses these puffing systems for the treatment of soybeans. One of the continuous soybean cooking devices now available on the market is shown in Fig. 22.

Unlike soybeans, wheat is roasted without adding water. The indigenous processing of wheat was carried out in a shallow pan in the period before the early 20th century, as shown in Fig. 23. In 1951, however, a continuous-sand-mixing, hot-air wheat raoster was developed (Fig. 24). Wheat grains preheated by hot air are mixed with hot circulating sands and the resultant mixture is transferred into the outlet through a heated screw conveyer. During this process, wheat grains are roasted. The roasted wheat is separated from sand at the wire net near the outlet and discharged. The separated hot sand is returned to the screw conveyer near the inlet to be mixed with raw wheat. Figure 24 is a photograph of the outlet of a continuous-sand-mixing, hot-air wheat roaster. Because this type of roaster is simple in structure and high in performance, it was in use until lately. In the early 1970s, however, a fluidized

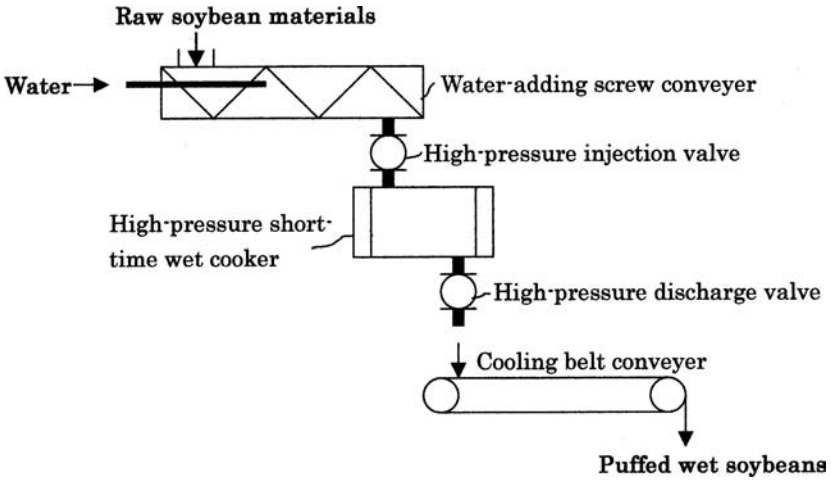

Figure 18 Flow diagram of continuous puff cooking process for water-added soybeans under high-pressure steam. (Courtesy of the Kikkoman Corporation.)

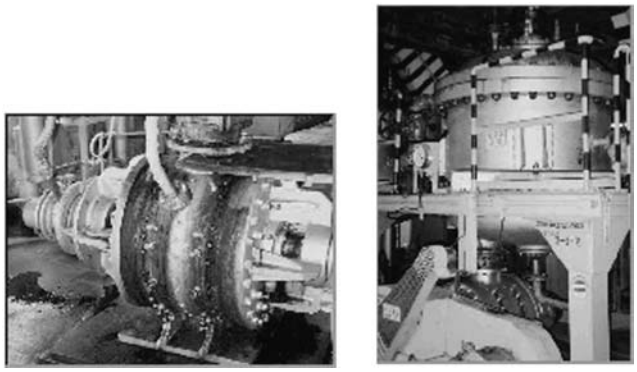

Figure 19 Charging rotary valve (left) and cooker (right) in continuous puff cooking process of water-added soybeans (used after 1970 by the Kikkoman Corporation).

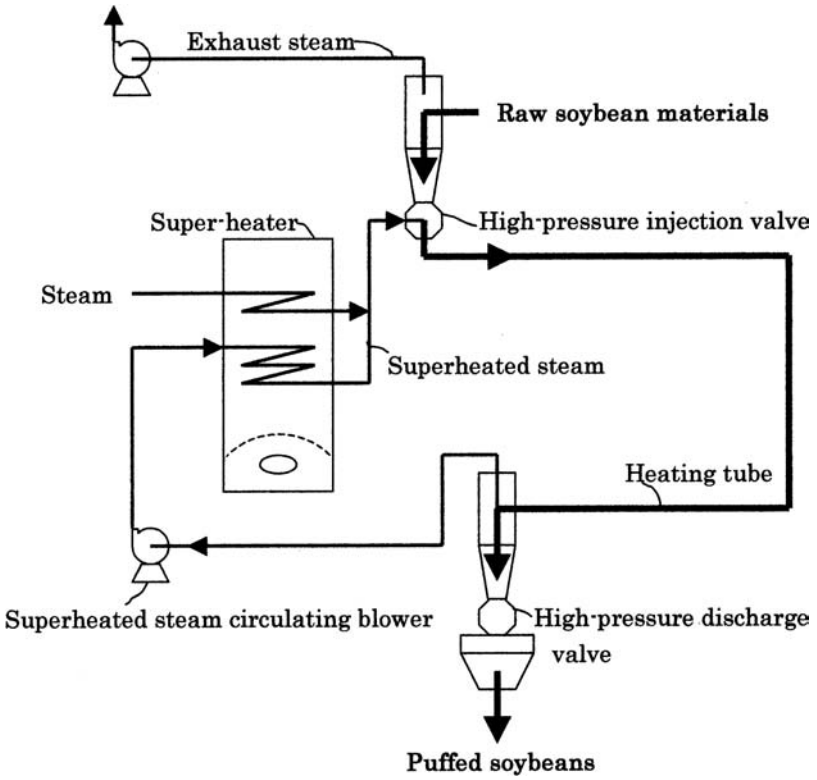

Figure 20 Flow diagram of pneumatic puffing process of non-water-added soybeans using pressurized superheated steam.

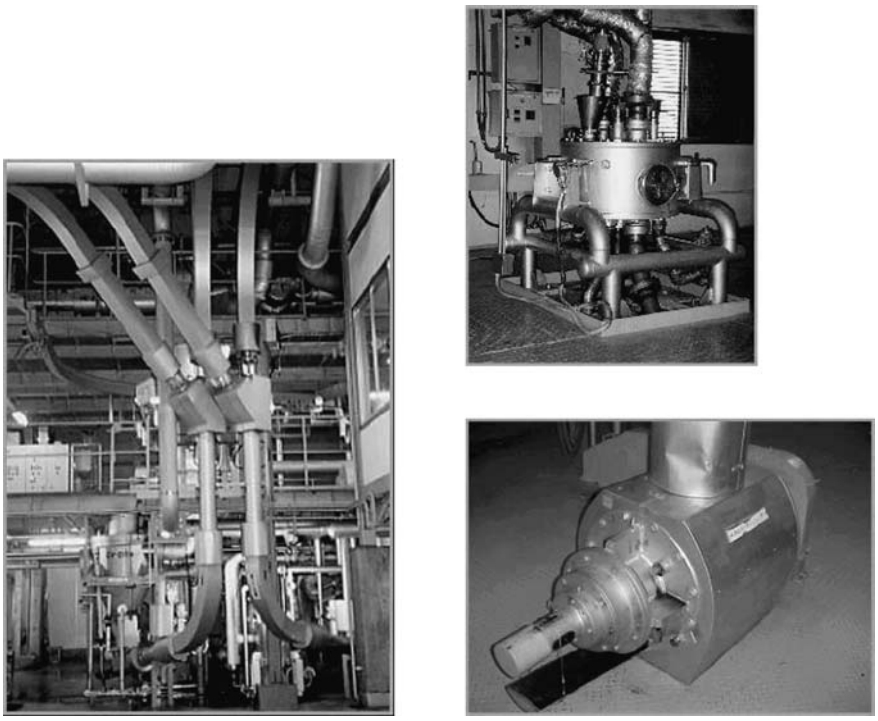

Figure 21 Pneumatic puffing process of non-water-added soybeans using pressurized superheated steam (used after 1971 by the Kikkoman Corporation): right, the external appearance of the system; left, the charging rotary valve (upper) and discharging rotary valve (lower).

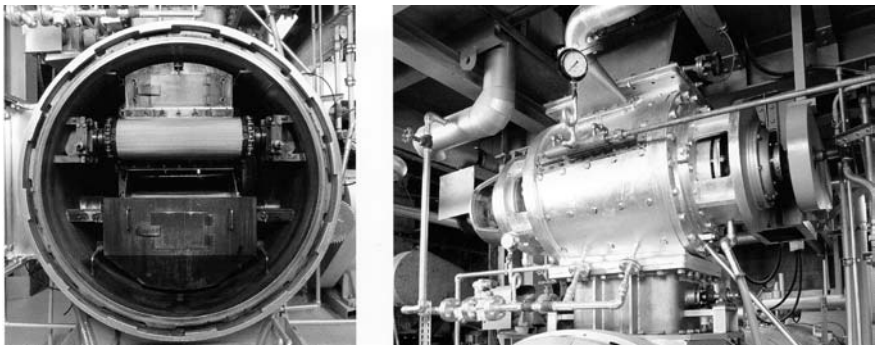

Figure 22 Continuous soybean cooker under high-pressure steam commercially available in 2002: left, the inner part of the cooker; right, the charging rotary valve. (Courtesy of the Fujiwara Techno Art Corporation.)

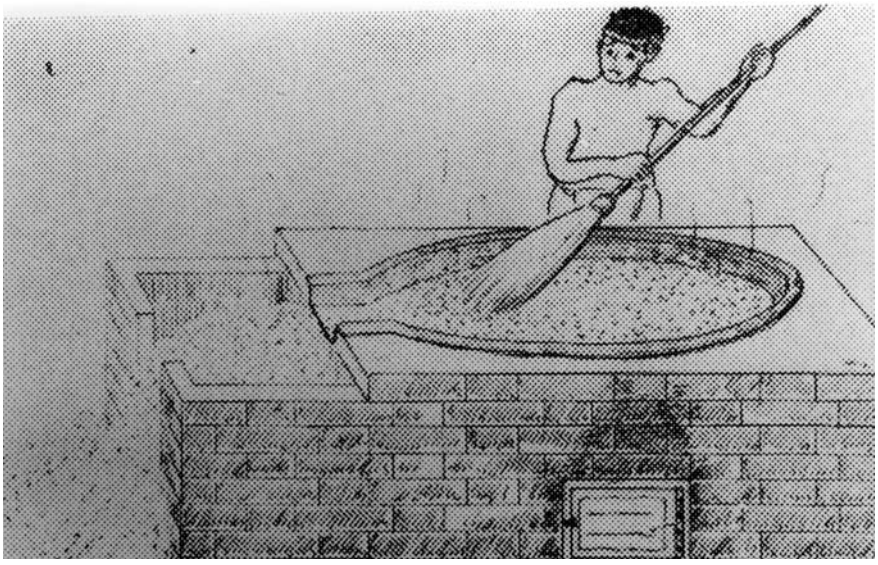

Figure 23 A shallow pan for roasting wheat, which was used before the early 20th century. (From Ref. 41.)

hot-air wheat roaster was developed. Recently, Fujiwara Techno Art Corporation has developed a continous through-flow hot-air wheat roaster, as shown in Fig. 25. At present, the through-flow or fluidized wheat roaster is in use, instead of a sand-mixing, hot-air wheat roaster.

B. Koji Fermentation

In the traditional way of preparing koji, the mixtures of soybeans and wheat, which were inoculated with a koji starter, were put into small wooden trays and kept for 3–4 days in a koji-making room, as shown on the left side of the Fig. 26. During the growth of the aspergilli, the temperature and moisture of the cultured mixture were controlled manually by stirring the mixture (Fig. 27). The first stirring was performed at about 20 h and the second at about 25 h after inoculation. When the stirring was done too late, the temperature of the koji became too high, leading to the decrease of enzyme production and sometimes to the death of the koji mold.

Before the mid-1950s, koji manufacturing had been carried out manually, as described earlier. In 1962, however, the automatic koji-making processes with a forced through-flow air system with temperature and humidity controls have been developed to replace the traditional wooden tray method. The new equipment includes a continuous cooker (already described), an

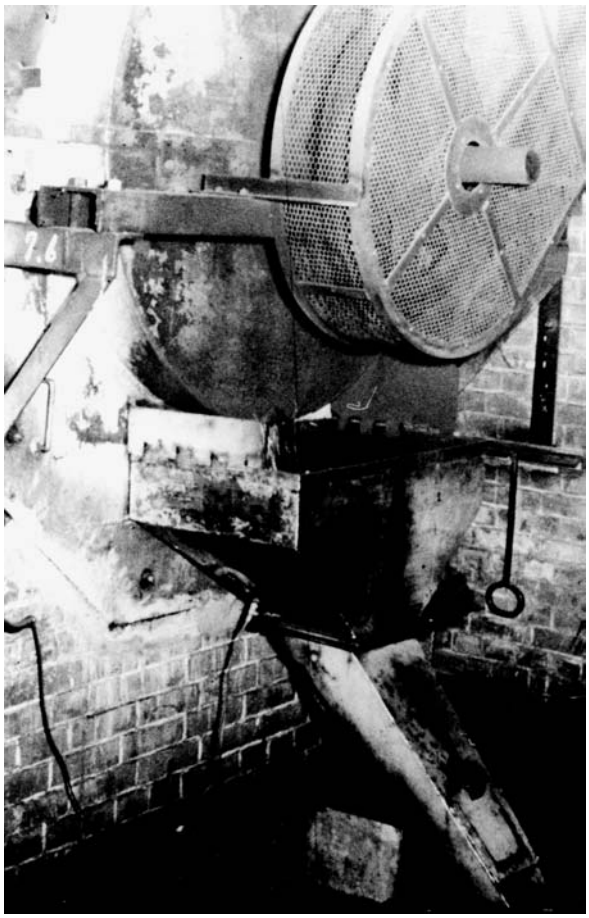

Figure 24 Continuous-sand-mixing wheat roaster. (Courtesy of the Kikkoman Corporation.)

automatic inoculator, an automatic mixer, large perforated shallow vats in closed chambers equipped with forced-air devices, temperature controls, and mechanical devices for turning the substrates during incubation. The inoculated mixture is put into a shallow perforated vat and forced air is circulated through the mass, as shown in the right side of the Fig. 26. A large amount of heat is generated during the growth of the mold, but the temperature of the koji bed is controlled by the air which flows through the koji bed. The culture mixture of *Aspergillus oryzae* or *Aspergillus sojae* becomes a green-yellow as a result of sporulation after 3 days. Table 7 shows the comparison of the

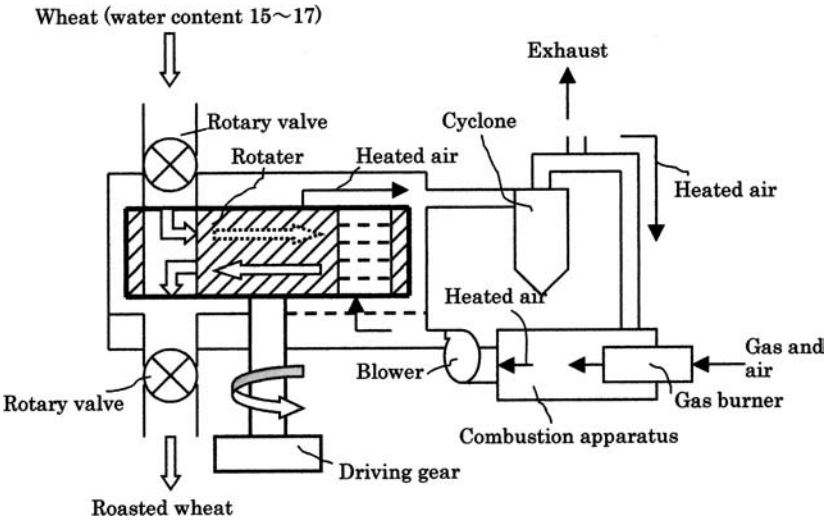

Figure 25 Flow diagram of a continous-through-flow hot-air wheat roaster. (Courtesy of the Fujiwara Techno Art Corporation.)

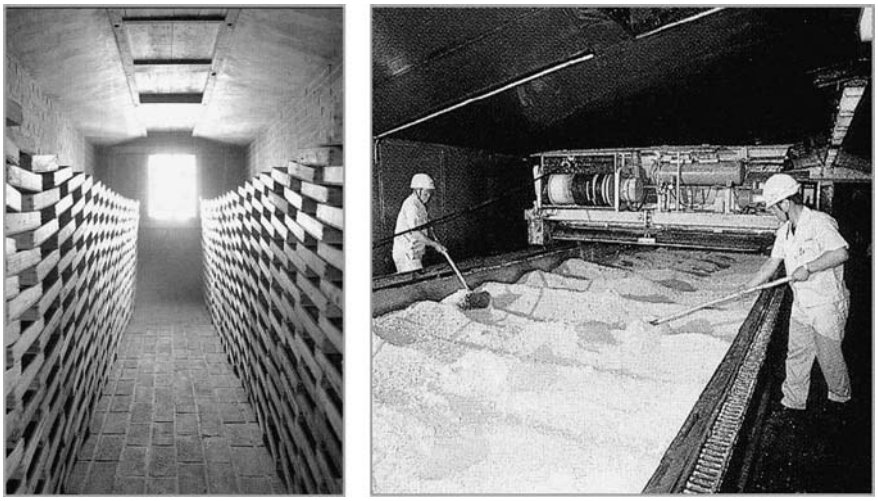

Figure 26 Preparation of koji: left, traditional koji making using a wooden tray; right, a new koji-making system which is composed of the forced through-flow air system with temperature and humidity controls (used from 1962 to 1990). (Courtesy of Kikkoman Corporation.)

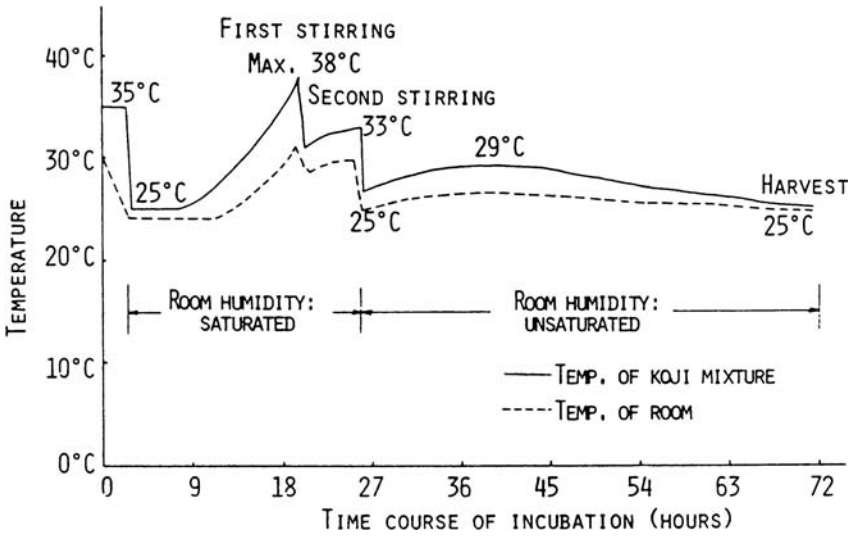

Figure 27 Temperature control of koji by a traditional method using a small tray. (From Ref. 45.)

protease activities of koji and the yield of soy sauce between the wooden tray and automatic koji-making equipment. The application of the automatic koji-making system to soy sauce production brought the increase of the yield of soy sauce through that of the protease activities of koji. In addition, it protected koji from infection by undesirable organisms which give soy sauce an unpleasant odor. Thus, this type of koji-making equipment (Fig. 26, right) was made in 1962 and in use until 1990, although it was not a continuous one. Figure 28 shows a typical batch-type koji-making machine, which is coming into wide use (47). The development of the completely continuous koji-making equipment was also done by Kikkoman Corporation in 1986. Figure 29 depicts the outside and inside of that equipment. This continuous equip-

Table 7 Comparison of Protease Activity of Koji and Yields of Resultant Soy Sauce Between the Wooden Tray and Automatic Equipment Methods

Method	Activity of proteases of koji (unit)	Yield of soy sauce (%)
Wooden tray	28.40	75.0
Automatic equipment	40.53	81.0

Source: Ref. 46.

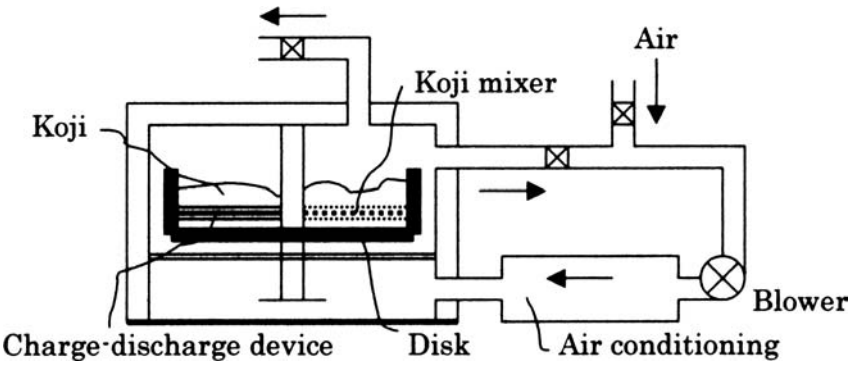

Figure 28 Disk-type automatic koji-making machine coming into wide use at present. (From Ref. 47.)

Figure 29 Completely continuous huge, circular koji-making equipment having the through-flow forced- air system with temperature and humidity controls (developed by Kikkoman Corporation). Hourly capacity of koji production is 4.150 tons. Original soybeans and wheat used are 1.650 and 1.775 tons/h, respectively. Upper: External appearance; lower: rotary koji bed (left) and mixing device (right).

ment is very large and the outside diameter of the koji bed with a doughnut shape is 38 m long, as shown in Fig. 30. The koji bed circulates very slowly. The mixture of 1650 metric tons of soybeans or defatted soybeans and 1775 metric tons of wheat is fed per hour and 4150 metric tons of koji is harvested per hour.

A concern subsequent to the development of the koji equipments was the improvement of koji mold. Many of experiments were carried out to obtain induced mutants having stronger protease activities from yellow aspergilli. Ultraviolet rays, methylnitronitrosoguanidine (MNNG) and ethylmethanesulfonate (EMS) were used as mutagenic agents. As a result, mutants with four times as much proteolytic activities as those of the original strains were obtained (48). These mutants of yellow aspergilli were put to practical use by the Kikkoman Corporation. The contribution to the yield of soy sauce, (i.e., the hydrolysis ratio of the proteins in the raw materials) is at least 5% when these mutants are used.

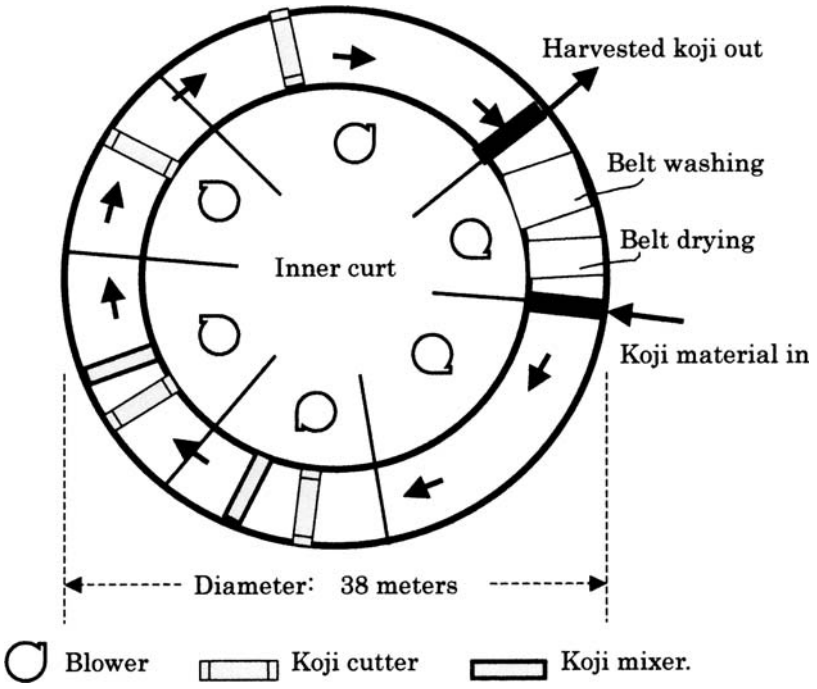

Figure 30 Flow diagram of the huge, continuous koji-making equipment with a circular rotary koji bed (see Fig. 29).

C. Brine Fermentation

The harvested koji is mixed with the brine, containing at least 20% salt (weight/volume). Brine was made by dissolving solid salt into boiling water in a large cauldron until 1890, but boiling water proved to be unnecessary for this purpose and cold water eventually came to be used. Figure 31 shows a recent facility for making salt solution from solid salt. Salt is piled in a concrete tank and water is run to the bottom of accumulated salt. Salt-saturated solution is taken out from the other side of the bottom, at the same time, salt is supplied continuously. The saturated salt solution is filtered, diluted to a given concentration around 20–23%, and then mixed with koji for brine fermentation.

In earlier times, koji was put into 4–13-kL wooden tubs with brine for brine fermentation, as shown in Fig. 32 (upper). In 1920, however, 18–34-kl concrete tanks came into use (Fig. 32, lower). Iron tanks lined with epoxy were installed in 1965 (Fig. 33), the large scale outdoor tanks in 1970 (Fig. 34, left side), and the indoor tanks made by FRP in 1975 (Fig. 34, right side). It became clear through the many investigations after 1950 that three groups of micro-organisms, *Tetragenococcus halophilus*, *Zygosaccharomyces rouxii*, and *Candida* sp., play an important role in brine fermentation. What is important in this step is how the growth of these three groups of micro-organisms is controlled. In the initial lactic acid fermentation in stage, it is necessary to prevent the pH from declining too quickly. The rapid decrease of the pH results from a sudden growth of the lactic acid bacteria. Such a drop in pH obstructs not only the action of the proteolytic enzymes derived from koji but also that of the glutaminase from koji, the enzyme which converts glutamine to glutamic acid. Consequently, the decomposition ratio of soy-

Figure 31 Brine-making facilities: left, solid salt; right, brine. (Courtesy of the Kikkoman Corporation.)

Figure 32 Brine fermentation tanks of moromi mash: upper, large wooden tubs traditionally used; lower, concrete tanks used after 1920.

bean proteins drops and so does the concentration of glutamic acid in the final product. To avoid this, the following two methods have been adopted to control brine fermentation: (a) Specially selected lactic acid bacteria are cultured and added as starter to moromi mash and (b) the temperature of moromi mash is kept relatively low (15–20°C) for the first month or so, in order to prevent too rapid growth of lactic acid bacteria. Thus, the pH of moromi mash falls gradually. When it reaches pH 5, *Z. rouxii* cultured in advance is added to moromi mash as starter. Then, the temperature of moromi is raised

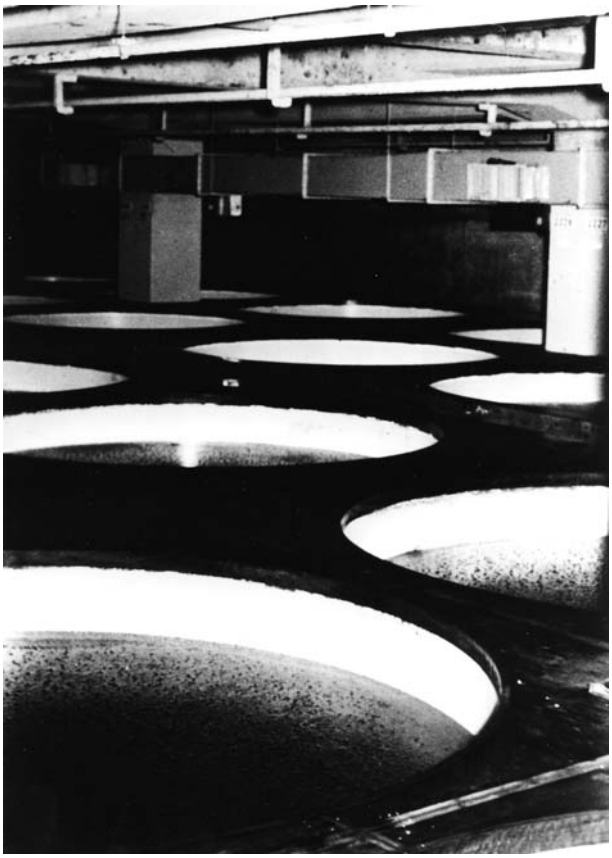

Figure 33 Iron fermentation tanks lined with epoxy resin, which was installed in 1965. (Courtesy of the Kikkoman Corporation.)

slowly up to nearly 30°C until vigorous alcoholic fermentation occurs. After alcoholic fermentation ends, the temperature is again dropped and kept at around 25°C during the last 2 months.

D. Pressing

In earlier times, three kinds of moromi mash aged for 1, 2, and 3 years were mixed, followed by pressing. The traditional pressing apparatus is shown in Fig. 35 (41). The pressing was done by a wooden lever, from the end of which 10–24 stones (75–100 kg each) were hung. 1.5 to 2.0 liters of the aged moromi

Figure 34 Modern fermentation tanks: left, outdoor large tanks used after 1970; right, indoor fermentation tanks made from glass fiber, used after 1975. (Courtesy of the Kikkoman Corporation.)

mash was put into cotton bags (83 x 26 cm) which were then piled into the pressing box. The bags were pressed by gravity for the first day, followed by stone hanging. Pressing was completed in 8 days.

The hydraulic press machine was invented to replace stone pressing in 1906. The filter cloths were changed from cotton bags to square cloths. Moromi mash is put into nylon square cloths, wrapped, piled up in a large wooden box, and pressed (Fig. 36). Another type of hydraulic press machine was devised in 1930 (Fig. 37). The moromi sheets pressed in the wooden box were piled up and pressed again by this hydraulic press machine (Fig. 37). Pressing required much labor compared with the other processes of soy sauce production, but, today, very efficient semiautomatic press machines are in use, as shown in Figs. 38 and 39. The machine in the left-hand side of Fig. 38 is a continuous moromi wrapping feeder. This is located at the top of the preliminary pressing tower shown on the right-hand side of Fig. 38. After 1 day, moromi sheets piled up inside the tower are taken out and pressed again by the hydraulic press shown on the left-hand side of Fig. 39. The pressed cakes in the filter cloths are removed by the machine shown on the right-hand side of Figure 39. Figure 40 depicts a vertical-type pressing machine which is available commercially. Formerly, the cake of soy sauce was used as feed. After 1990, however, it has been used as boiler fuel after drying. Figure 41 is a special boiler using soy sauce cake as fuel.

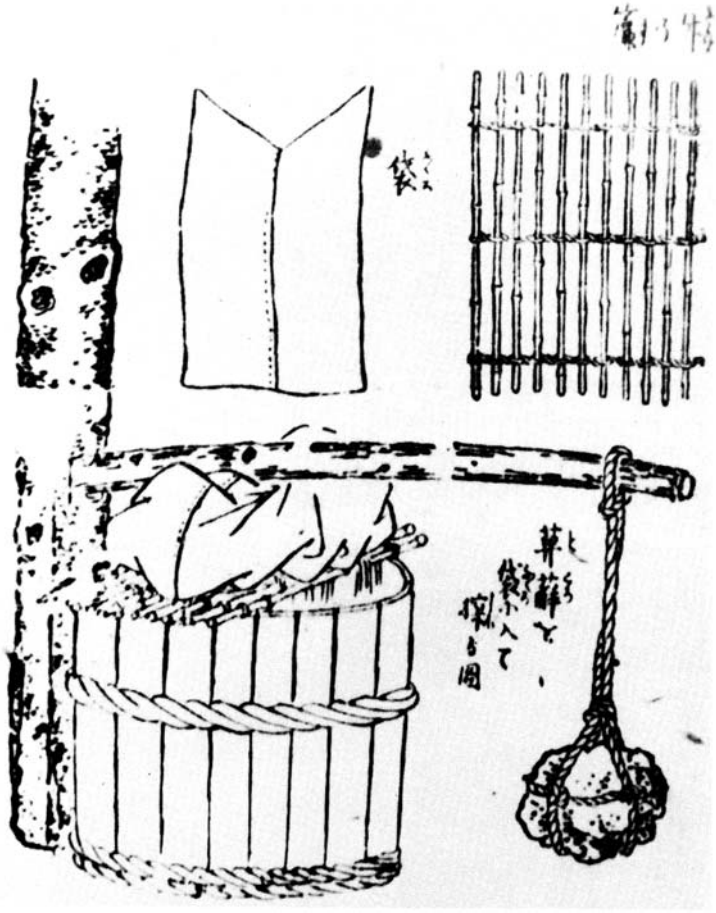

Figure 35 The traditional way on pressing moromi mash. (From Ref. 41.)

E. Pasteurization, Refining, and Bottling

In the old days, the pasteurization of soy sauce was carried out by boiling in a large iron pot. The heat exchanger shown in Fig. 42 has been in use since 1929, but now a plate heater has been used for pasteurization (Fig. 43). Some sediments are produced during the pasteurization of unheated soy sauce. Formerly, the sediments were removed through the sedimentation by gravity during the storage of heated soy sauce. At present, however, sediments are removed through ultra-filtration and micro-filtration membranes, as shown in Fig. 44.

Figure 36 Box-type hydraulic machine. (Courtesy of the Kikkoman Corporation.)

Soy sauce containers have changed from kegs (16 or 18 L) to glass bottles (1.8 or 2 L), and then to plastic bottles (1 L), as shown in Figs. 45, 46, and 47, respectively. The shipping by containers for industrial use has been increasing recently (Fig. 48).

V. MICROBIOLOGY AND BIOCHEMISTRY

A. Role of Koji as an Enzyme Source

1. Unique Source of Enzymes

Just as "malt" is widely used in the West as an enzyme source in the preparation of foods such as beer and whiskey, "koji" has been widely used for centuries in the Orient as an enzyme source not only in soy sauce and miso, but also in sake, a traditional Japanese alcoholic beverage made from rice by fermentation (49). The major purpose of koji is in the utilization of its

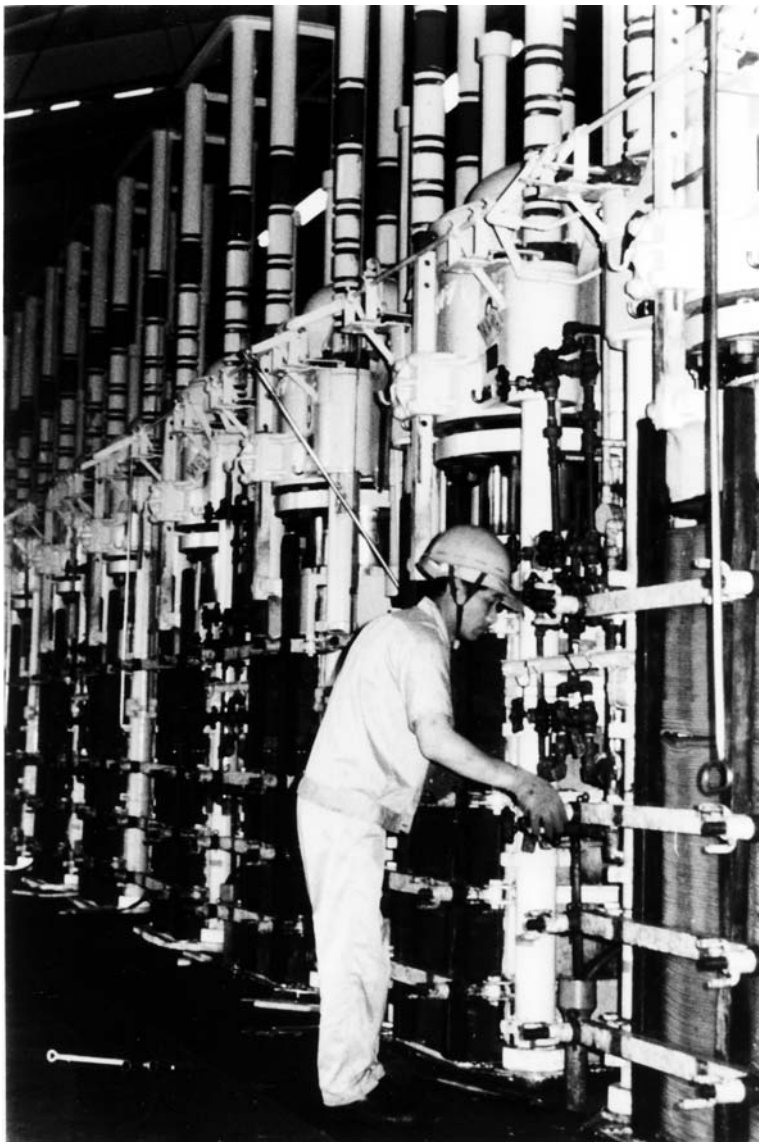

Figure 37 Vertical-type hydraulic press machine devised in 1930. (Courtesy of the Kikkoman Corporation.)

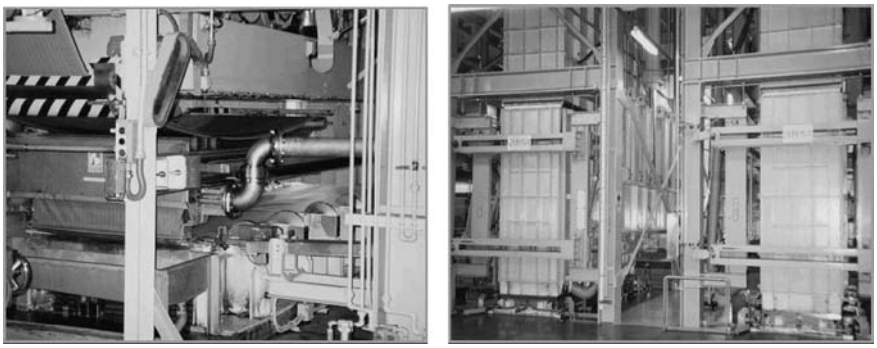

Figure 38 Moromi wrapping feeder (left) and preliminary pressing tower in or L6-type modern moromi pressing machine. (Courtesy of the Kikkoman Corporation.)

amylolytic and/or proteolytic enzymes. In the brewing of sake, the koji is used just as malt in beer or whiskey making, to convert starch into fermentable sugars. In miso and soy sauce making, however, the koji is used as an enzyme source necessary for the breakdown of the polypeptides of the proteins into amino acid as well as for that of starch into glucose.

The most unique features of koji as an enzyme source are that (a) koji is the solid culture of green-yellow aspergilli and (b) the proteins can be hydro-

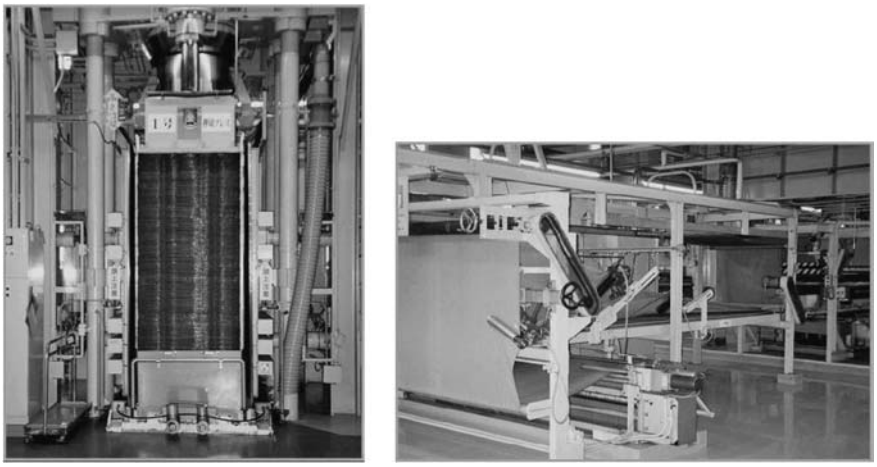

Figure 39 Final pressing equipment (left) and the machine tearing the residual cake from filter cloths (right), in an L6-type modern moromi pressing machine. (Courtesy of the Kikkoman Corporation.)

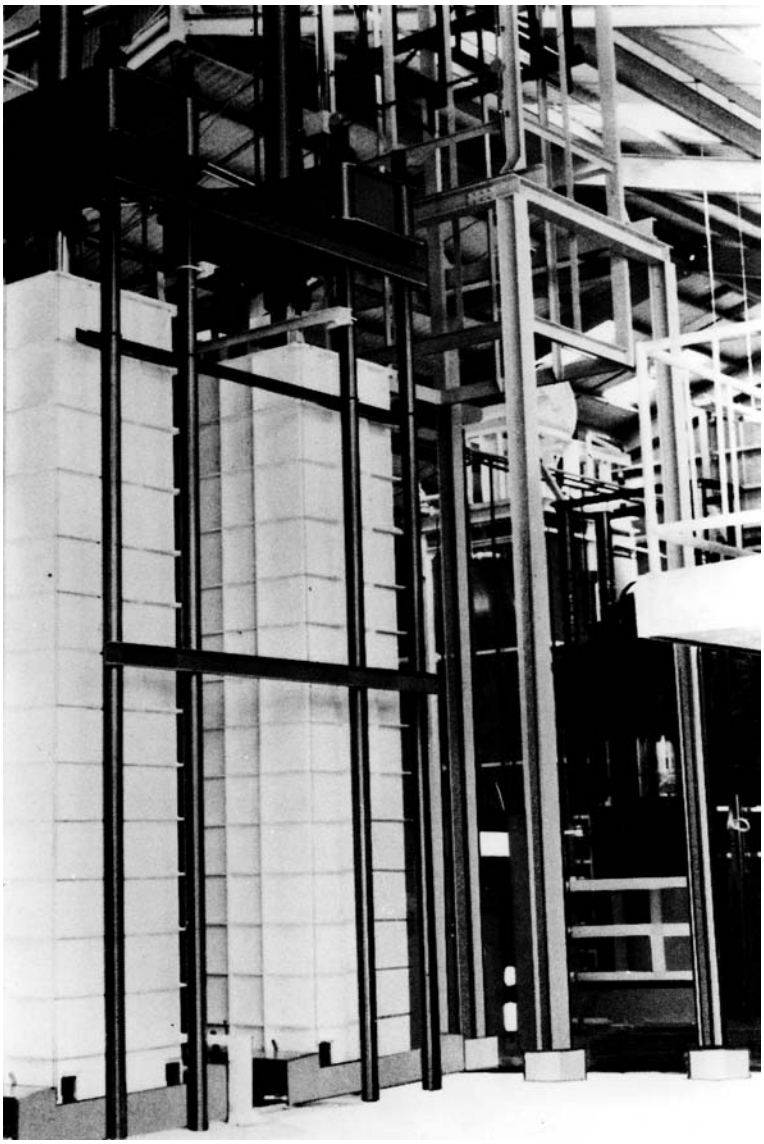

Figure 40 Automatic pressing tower of moromi mash. (From a pamphlet from Yamazaki Tekkojo, Ltd., 1986.)

Figure 41 Special boiler using a soy sauce cake. (Courtesy of the Kikkoman Corporation.)

lyzed almost completely into free amino acids with a high yield. In fermented soy sauce, for instance, 90–92% of the proteins contained in the materials are hydrolyzed into the liquid phase mostly as free amino acids by the enzymes of the koji, as shown in Fig. 49. One of the reasons why the solid culture is advantageous is because of its enzyme composition. In liquid culture, the production of the proteolytic enzymes is very large, but other enzymes are produced only in small quantities, as shown in Table 8 (51). It is clear from this table that the liquid culture is better for producing proteolytic enzyme preparations which do not contain other enzymes. However, the liquid culture is not suitable for the production of fermented foods which need the cooperation of various kinds of enzyme. For instance, amylases are indispensable for the production of glucose, which is the substrate of the subsequent lactic acid fermentation or alcoholic fermentation. Macerating enzymes are

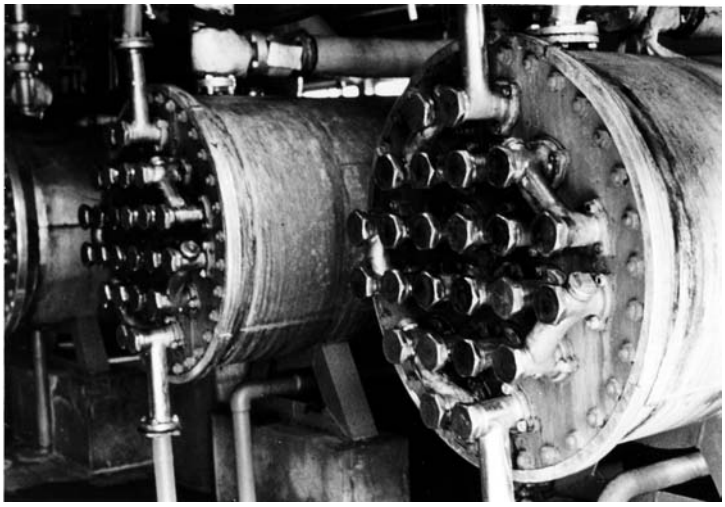

Figure 42 Old-type heat exchanger first equipped in 1929 for pasteurization of soy sauce. (Courtesy of the Kikkoman Corporation.)

very important for the solid–liquid separation. In the experiments to manufacture soy sauce by enzymatic hydrolysis, Nakadai compared the result of using the enzyme solution from liquid culture with that from a solid culture. He found that the solid–liquid separation of the digestion mixture was almost impossible in using the enzyme solution from the liquid culture, because of the strong cohesiveness due to the lack of macerating enzymes (51). In addition,

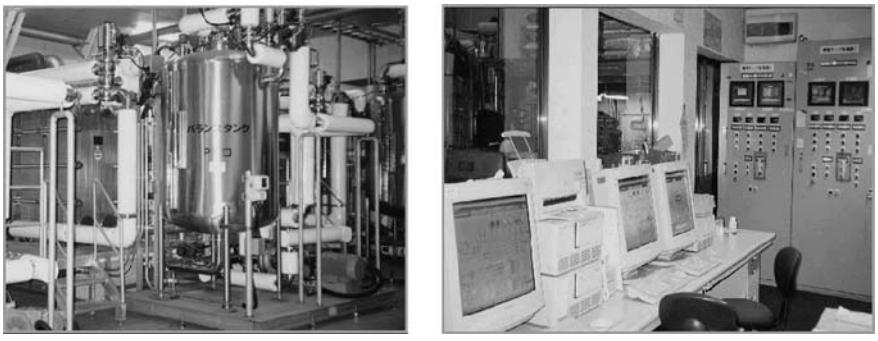

Figure 43 Pasteurization of soy sauce: left, plate heater; right, operating room. (Courtesy of the Kikkoman Corporation.)

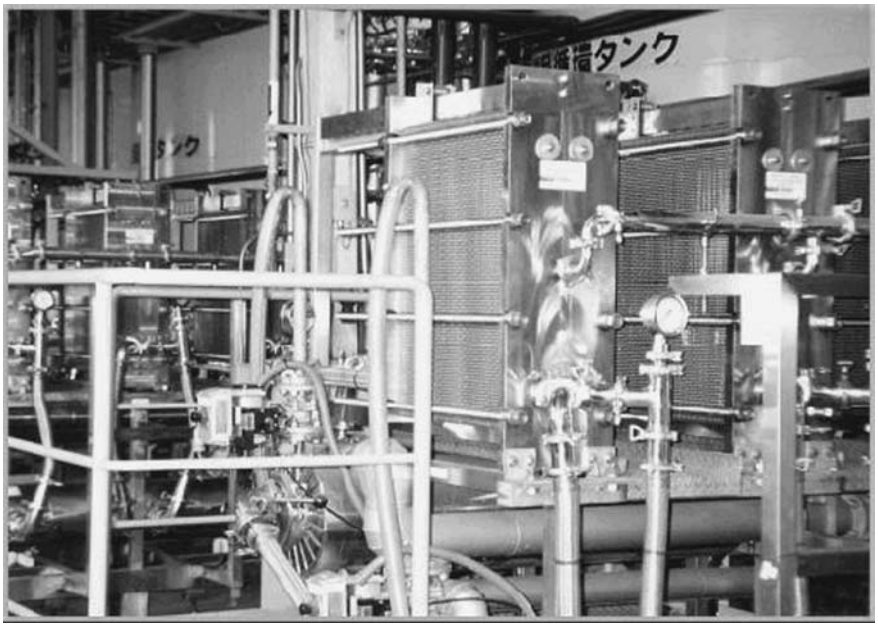

Figure 44 Separation of the sediments made through pasteurization from soy sauce by ultra-filtration and microfiltration membranes. (Courtesy of the Kikkoman Corporation.)

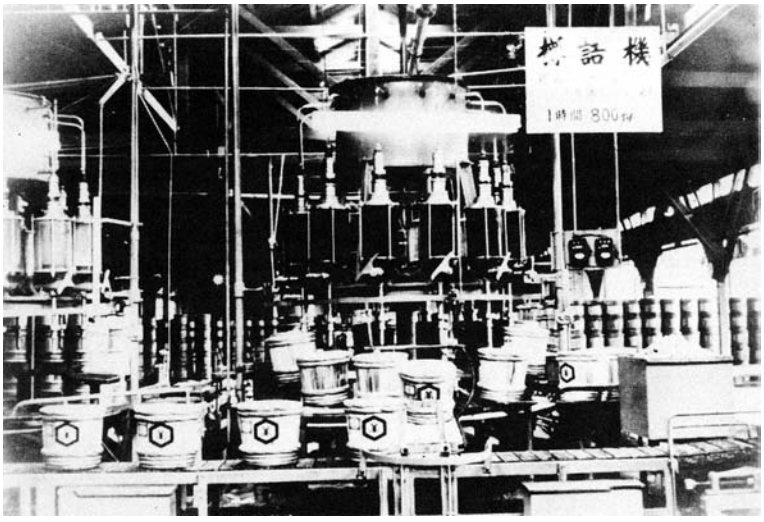

Figure 45 Filling of soy sauce into kegs, which were very popular vessels for soy sauce before 1965. (Courtesy of the Kikkoman Corporation.)

Figure 46 Filling of soy sauce into 2-L glass bottles. (Courtesy of the Kikkoman Corporation.)

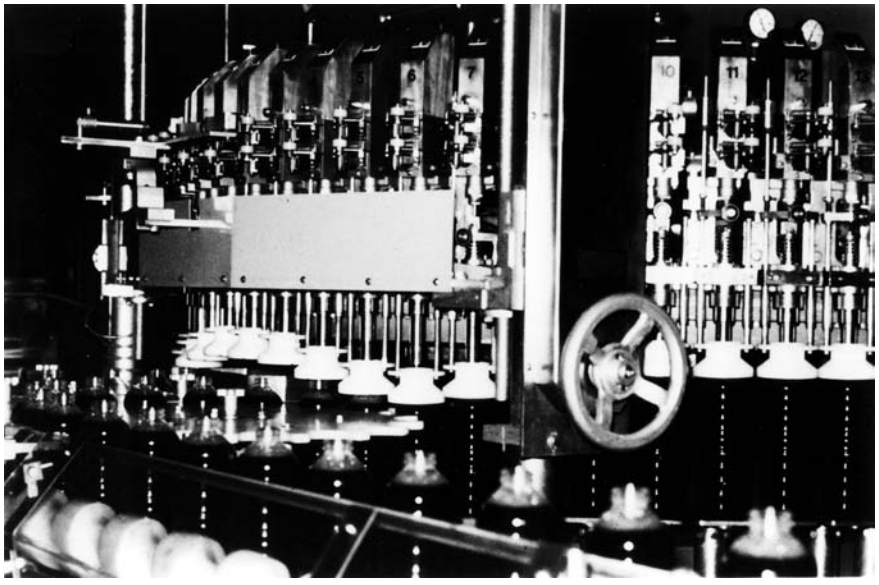

Figure 47 Filling of soy sauce into 1-L plastic bottles. (Courtesy of the Kikkoman Corporation.)

Figure 48 Filling of soy sauce into containers (from Kikkoman Corporation.)

the macerating enzymes enhance the contact between the proteolytic enzymes and proteins through the disruption of the cell wall structure in soybeans and wheat, leading to an increase in the degree of protein hydrolysis. Another advantage of the solid culture is that it is simpler and more economical than the liquid culture.

It has been shown that the proteinases of *A. oryzae* or *A. sojae* include seven kinds of proteinase with four different optimum pH values, as shown in Table 9 (52). Of these proteinases, alkaline proteinase has been studied in most detail (53–64). It is a serine enzyme which is inhibited by diisopropylfluorophosphate. This proteinase is active in a broad pH range (between 6 and 11). The detailed properties of semialkaline proteinase are not known (65). Neutral proteinases I and II are both zinc proteinases which are inhibited by chelating agents (66,67). Neutral proteinase I has specificities which are common to the metal proteinases of micro-organisms. Neutral proteinase II has an exceptionally high specificity to basic nuclear proteins, such as protamine, histone, salmine, clupeine, and the like (68–74). Among the acid proteinases investigated so far, acid proteinase I has been studied most thoroughly (75, 76). Acid proteinase I is a sugar protein containing 12.5% sugar and neutral proteinase II contains 4% sugar (77). The above-described proteinases are all endo types which do not possess any aminopeptidase or carboxypeptidase action. Accordingly, they hydrolyze proteins only into peptides. Free amino acids are not liberated substantially by these proteinases.

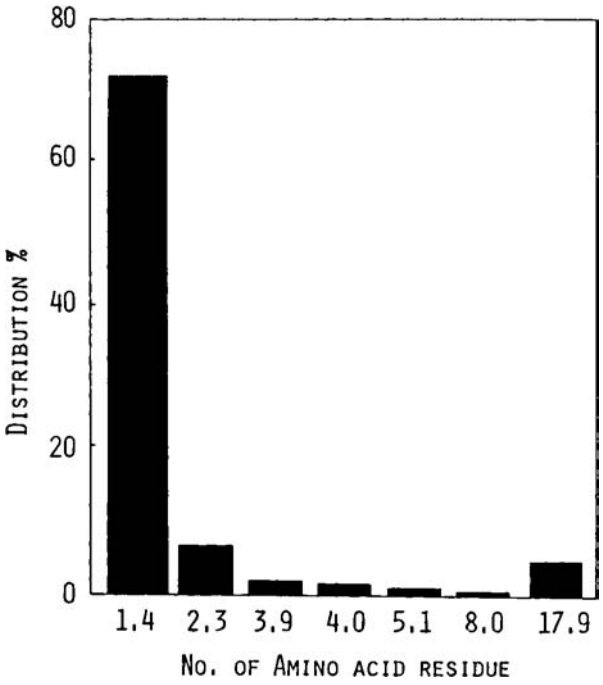

Figure 49 Distribution pattern of peptide chain length in regular fermented soy sauce (koikuchi-shoyu). (From Ref. 50.)

Table 8 Comparison of Enzyme Activities Between Solid and Submerged Cultures

Enzymes		Solid culture[a] (1)	Submerged culture[a] (2)	(2)/(1)
Peptidase[b]	(a)	6	91	15.2
	(b)	70	314	4.5
	(c)	37	89	2.4
Proteinase[c] (× 100)	(a)	102	780	7.6
	(b)	194	635	3.3
Amylase (× 100)		206	48	0.2
Pectinase		167	0	0.0
Cellulase		411	199	0.5

[a] Unit against casein per gram of enzyme preparation.
[b] Substrate: (a), Leu-Gly-Gly, (b) Cbz-Glu-Try, and (c) Leu-*p*-NA.
[c] Measured at pH 7.0 in (a) and pH 3.0 in (b).
Source: Ref. 51.

Table 9 Proteases in Koji

Proteinase	Molecular Weight ($\times 10^3$)	Optimum pH	Activity[a]	Enzyme weight[b]
Alkaline	23	10.5	929	418
Semialkaline	32	8.3	55	—
Neutral I	41	7.0	80	131
Neutral II	19	6.0	9	152
Acid I	39	3.2	44	617
Acid II	100	3.0	10	—
Acid III	31	3.0	5	—

[a] Unit against casein per gram of koji.
[b] Microgram per gram of koji.
Source: Ref. 52.

2. Peptidases in Koji

Koji contains various kinds of exo-type peptidases which liberate the amino acids from the carboxy or amino terminal of peptides successively. So far, four kinds of carboxypeptidase and seven kinds of aminopeptidase have been separated from koji up to 1977 (Table 10 and 11). Because the optimum pH of all of the carboxypeptidases is in the acidic range, they should be called acid carboxypeptidases (78–81). All of the aminopeptidases have a high specificity for the amino terminal leucine and, therefore, they should be called leucine aminopeptidases. The effects of pH, temperature, metals, chelating agents, and other variables on these peptidases have been investigated in detail (52,82–85). Further, another kind of aminopeptidase was discovered in 1992 and is called dipeptidyl-peptidase IV (86).

Table 10 Acid Carboxypeptidases in Koji

		Acid carboxypeptidases in koji			
Properties		I	II	III	IV
Molecular Weight ($\times$ 1000)		120	105	61	43
Optimum pH		3–4	3–4	3	3–4
Activity[a]	(a)	—	0.18	0.05	0.11
	(b)	0.25	—	0.01	0.02
Enzyme weight[b]		10	19	62	8

[a] Substrate: (a) Cbz-Glu-Try and (b) Cbz-Ala-Glu. Activity: unit against casein per gram of koji.
[b] Microgram per gram of koji.
Source: Ref. 52.

Table 11 Leucine Aminopeptidases in Koji

Enzyme	Molecular Weight ($\times 10^3$)	Optimum pH	Activity[a]	Enzyme weight[b]
I	27	8.5	0.10	319
II	61	5–8	0.25	54
III	55	8.0	0.15	301
IV	130	7.0	0.15	200
V	100	—	0.11	—
VI	39	—	0.01	—
VII	170	—	0.03	—
Arylamidase	130	8.5	0	—

[a] Substrate: Leu-Gly-Gly. Activity: unit against casein per gram of koji.
[b] Microgram per gram of koji.
Source: Ref. 52.

3. Role of Proteinases and Peptidases in Koji During Protein Digestion

In order to clarify the role of each koji enzyme during soybean protein digestion, the effect of the purifed proteinase preparations on protein digestion was investigated first. Table 12 (87) outlines the individual and combined effects of the proteinases on the digestion of soybean proteins at pH 5.0. Here, total

Table 12 Soybean Protein Hydrolysis by Purified Proteinases

Enzyme[a]	Enzyme used (mg)	Total N (mg %)	Formol N (mg %)	FN/TN[b]	Glutamic acid (mg %)
Crude extract	69.5	174	76	44.0	102
Alp	4.5	137	11	8.0	3
Alp	9.0	148	12	8.0	2
NP-I	1.3	97	8	8.3	0
NP-I	2.6	107	9	8.5	0
NP-I	3.9	109	10	9.0	0
NP-II	2.6	114	9	7.5	0
NP-II	5.3	125	10	7.9	0
Alp + NP-I	9.0 + 1.3	161	19	11.6	8
Alp + NP-II	9.0 + 2.6	159	16	9.9	6

[a] Alp = alkaline proteinase; NP = neutral proteinase.
[b] See text.
Source: Ref. 87.

and formol nitrogens mean nonproteinous and α-amino nitrogen contents in the filtrate of the digestion mixture, respectively. The ratio of formol to total nitrogen (FN/TN) relates to the length of the peptides in the hydrolyzate. It is quite clear from Table 12 that each proteinase contributes a great deal to the liberation of nonproteinous nitrogen, but the resultant nonproteinous nitrogen substances (i.e, polypeptides) are not broken down into small peptides or amino acids, even by the combination of these proteinases. On the contrary, the crude extract of koji hydrolyzes the proteins into small peptides as shown by the fact that the percent of FN/TN is 44%. This indicates the participation of enzymes other than proteinases in the crude extract of koji. Table 13 (87) shows the results of the hydrolysis of soybean proteins by alkaline proteinase to which peptidases were added. As can be seen, formol nitrogen and glutamic acids were very much increased by the addition of the peptidase preparations, indicating that the peptides produced through the action of proteinases were hydrolyzed into amino acids by the added peptidases. The same results are also obtained in the combination experiment with acid carboxypeptidase IV. In order to know the degree of the contribution of each peptidase to the liberation of formol nitrogen and glutamic acid, two experimental designs with an L16-type orthogonal arrangement were drawn up as shown in Table 14 (88). According to the result of the variance analysis in this table, all of the peptidases isolated from koji contributed to the liberation of the formol nitrogen and glutamic acid. Leucine aminopeptidase

Table 13 Soybean Protein Hydrolysis by Peptidases Added to Alkaline Proteinase

Enzyme[a]	Enzyme added (mg)	Total N (mg %)	Formol N (mg %)	Glutamic acid (mg %)
Crude extract	(69.5)	174	76	102
Control	(9.0)	148	12	2
AcCP-I	0.8	158	27	23
AcCP-II	2.3	147	31	20
AcCP-III	0.9	156	27	30
LAP-I	4.9	168	45	27
LAP-II	2.4	163	49	76
AcCP-I + LAP-II	0.3 + 2.4	173	53	82
AcCP-II + LAP-II	0.9 + 2.4	162	54	82
AcCP-IV + LAP-II	0.3 + 2.4	164	51	79
AcCP-I + AcCP-III	0.3 + 0.9	163	43	60

[a] AcCP = acid carboxypeptidase; LAP = leucine aminopeptidase.
Source: Ref. 87.

Table 14 Variance Analysis

Design	Factor	Enzyme (mg)	Row	Contribution (%)	
				Formol N[a]	Glutamic acid[a]
2	NP-I	0.3	1	1.0	—
	AcCP-II	0.4	2	3.4	3.6
	LAP-II	1.6	4	43.4	75.0
	LAP-I	5.0	8	22.6	3.9
	AcCP-III	0.4	15	6.2	10.2
1	NP-II	3.6	1	1.7	—
	AcCP-I	0.3	2	4.7	2.6
	LAP-III	0.6	4	9.8	13.1
	LAP-I	2.7	8	16.4	2.2
	AcCP-IV	0.8	15	62.3	75.5

[a] Significant at the 1% level.
Source: Ref. 88.

II and acid carboxypeptidase IV were especially significant in the hydrolysis of the peptides produced by the proteinases.

4. Role of Glutaminase in Koji in Formation of Glutamic Acid During Protein Digestion

In general, vegetable proteins such as soybeans and wheat contain quite a lot of glutamines as well as glutamic acid as residues of the polypeptide chains. Part of the glutamines liberated by the peptidases are modified into glutamic acids through the action of the glutaminases present in koji. However, the rest of the glutamines change easily into pyroglutamic acids, which are meaningless as flavor, as illustrated in Fig. 50 (8). Therefore, glutaminase is indispensable for the enzymatic digestion of cereal proteins, because it increases the contents of the glutamic acid, which is one of the most important flavor components of the hydrolyzates (89–91).

As shown in Table 15 (92), the production of glutaminase by yellow-green aspergilli is much larger in a solid culture than in a liquid one. Therefore, it can be said that koji is the most adequate enzyme source for the hydrolysis of cereal proteins because it has a high content of glutaminase as well as the composite peptidases. Because glutaminase is fairly sensitive to acidic pH as well as to salt (93), the brine fermentation in soy sauce making is carried out at a lower temperature (15–20°C) for the first month to prevent the loss of glutaminase activity. The role of each enzyme contained in koji is illustrated in Fig. 50 (8).

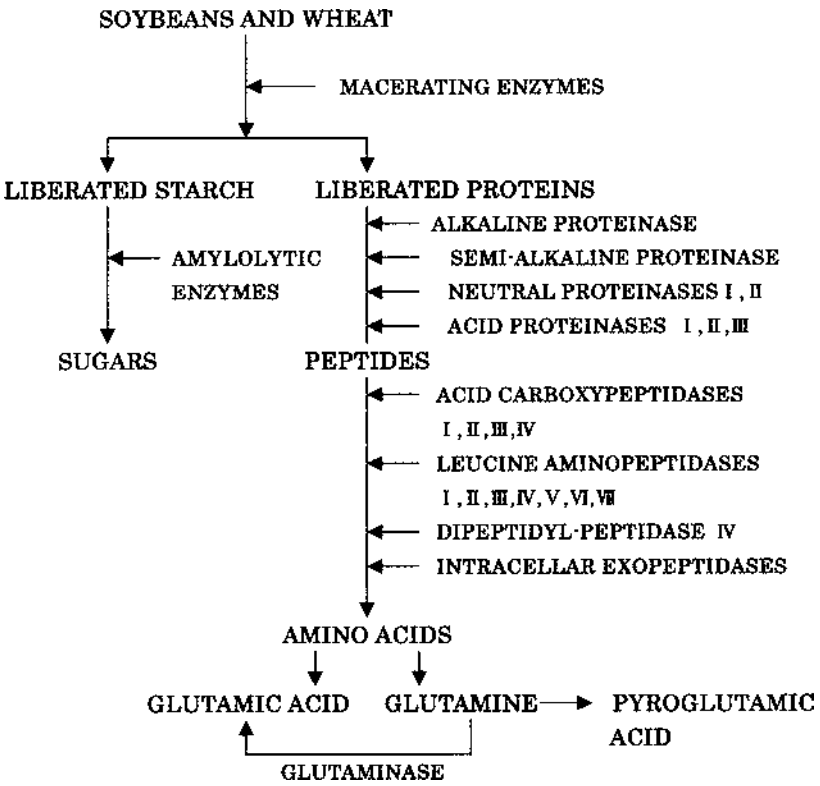

Figure 50 Role of each enzyme in koji during hydrolysis of cereal components in moromi mash. (From Ref. 8.)

Table 15 Comparison of Glutaminase Activities Between Solid and Submerged Culture

	Solid culture[a]		Submerged culture[b]	
Aspergillus species	Extracellular	Intracellular	Extracellular	Intracellular
A. sojae K.S.	1.2	7.2	0.7	0.5
A. sojae X-816	0.4	5.7	0.5	0.6
A. sojae X-74	0.8	3.3	0.6	0.8
A. oryzae 460	0.9	2.9	1.0	0.4
Asp. sp. Y-PH	0.9	13.5	0.4	0.5
Asp. sp. Y-D	0.7	12.7	0.4	0.7

[a] Units of glutaminase activities contained in 1 mL of fivefold water extract of solid culture koji.
[b] Units of glutaminase activities contained in 1 mL of submerged culture medium.
Source: Ref. 92.

B. Effect of Heat Treatment of Soybean Proteins on Their Digestibility and Nutritive Value

1. Enzyme Digestibility and Yield of Soy Sauce

The digestibility of soybean and wheat proteins by enzymes is markedly influenced by the heat treatment of the soybeans. Native soybean protein is quite resistant to proteolysis because of its compact conformation (94). The rate of proteolysis is dependent on the degree of unfolding of the substrate protein molecules, as shown in Fig. 51 (94). Accordingly, when soybean proteins are used as substrates for proteases, the protein molecules must be unfolded by some treatment such as heating (6) (Fig. 52). However, extended heat treatment decreases the maximum extent of proteolysis, as shown in Fig. 53 (44). Therefore, the denaturation of proteins leads to better proteolysis, but too much heat treatment decreases the degree of proteolysis through some chemical modifications of the protein other than denaturation; namely it is known that some amino acid residues of proteins are modified during heating through the reaction with other compounds or through cross-linking under conditions around the pH (95,96). For instance, α- and ε-amino groups may be modified by reaction with aldehyde compounds such as glucose, which is produced during the heat treatment of soybeans (44). In addition, the N-terminal glutamine residues of the proteins may become dehydrated into pyrolidone-carboxylic acid (pyroglutamic acid) (97), and the tryptophan

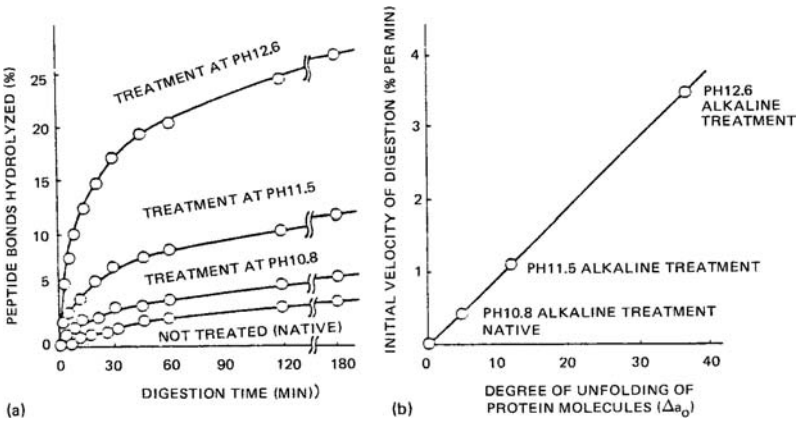

Figure 51 Relationship between the degree of unfolding of 11S globulin molecules and their susceptibility to proteolysis. In (b), Δa_o is calculated as $(a_o^{\text{sample}} - a_o^{\text{sample}})/(a_o^{\text{urea}} - a_o^{\text{native}})$ in the Moffitt–Young equation for optical rotary dispersion. The samples were treated at 20°C for 90 min at the indicated pH and then neutralized. (From Ref. 94.)

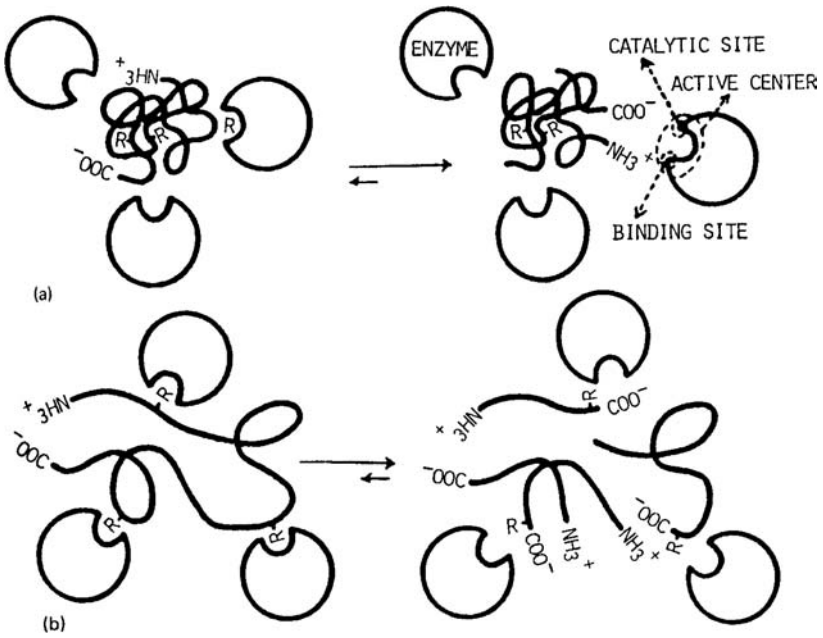

Figure 52 Schematic explanation for enzymatic hydrolysis of denatured proteins: (a) enzymatic hydrolysis of folded protein molecule (native protein); (b) enzymatic hydrolysis of unfolded protein molecule (denatured protein). (From Ref. 6.)

residues are transformed into mutagens during pyrolysis (98). During severe heat treatments, lysine can react with glutaminyl and asparaginyl residues to give isopeptide bonds, which are ε-(γ-glutamyl) lysine and ε-(β-aspartyl) lysine, respectively (99). The alkaline proteases of *Aspergillus* species used in soy sauce manufacture are specific for tyrosine, phenylalanine, leucine, lysine, and arginine residues in proteins. It has been shown that lysine, arginine, and cystine are partially destroyed or modified during heat treatment of defatted soybean flour in the presence of water (Table 16) (100). Their destruction or modification will result in a decrease in the degree of maximum hydrolysis. Further, the new intermolecular or intramolecular interactions among the hydrophobic residues of the unfolded proteins (101), which may occur during prolonged heating, will also result in a decrease of enzymatic hydrolysis. With due consideration to the effect of heating on the digestibility of soybean protein, various investigations were carried out on the conditions of the high-temperature, short-term treatment for denaturation of the soybean proteins in making soy sauce, as already described. High-temperature treatment

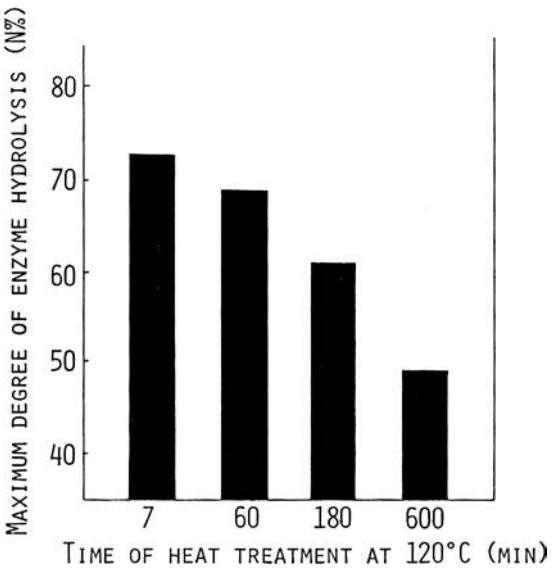

Figure 53 The effect of heat treatment of soybean protein on the maximum extent of enzymatic hydrolysis by proteases of *Aspergillus* species. (From Ref. 44.)

achieved maximum unfolding of the proteins. A very short-term treatment minimized the other deteriorative changes. The yield of soy sauce, based on the total nitrogen of the starting materials, has increased from 65% 20 years ago to a little over 90% today.

2. Enzyme Digestibility and Nutritive Value of Protein

Decreased digestibility of soybean protein with an increase of heat treatment time is also observed for trypsin and pepsin. This decrease gives decreased nutritive values. The action of trypsin on unheated soybean protein preparations is particularly low in comparison with other enzymes due to trypsin inhibitors present in soybeans. Therefore, the increase of trypsin digestibility by heating is attributed to inactivation of trypsin inhibitors as well as unfolding of the native protein molecules.

The digestion of heated or unheated soybean proteins by various enzymes is schematically compared with the nutritive values in Fig. 54 (9). In the figure, pattern a is typical of acid proteinases such as pepsin, which acts under a strong acidity. Because substrate proteins are denatured by a strong acidic condition, the proteins do not have to be denatured in advance. Pattern b is typical of enzymes such as papain, bacterial neutral protease, and the like.

Table 16 Destruction of Amino Acids During the Heating of Defatted Soybean Flour Protein Under the Presence of Water

		Amino acid residue in 100 g soybean protein (%)							
		126°C (hs)				115°C (hs)			
Amino acids	No heat	0.5	1	2	4	0.5	1	2	4
Gly	4.1	4.1	4.1	4.2	4.0	4.2	4.1	4.0	4.1
Ala	4.4	4.4	4.5	4.6	4.5	4.4	4.5	4.3	4.2
Val	5.4	5.5	5.4	5.5	5.5	5.7	5.5	5.5	5.1
Ile	5.2	5.1	5.2	5.0	5.0	5.1	5.2	5.1	4.9
Leu	8.4	8.5	8.4	8.5	8.5	8.4	8.5	8.4	8.3
Asp	12.2	12.0	12.2	12.0	12.1	12.1	12.1	12.1	12.2
Glu	19.7	19.2	19.5	19.4	19.8	19.5	19.5	19.6	19.5
Lys	6.3	6.0	5.9	5.8	5.4	5.6	5.6	5.1	4.0
Arg	7.6	7.5	6.8	6.3	6.1	6.2	6.3	5.9	4.5
His	2.4	2.6	2.3	2.3	2.3	2.5	2.3	2.5	2.4
Phe	5.1	4.9	5.3	5.1	5.1	5.2	5.2	5.0	5.0
Tyr	3.3	3.2	3.1	3.2	3.3	3.2	3.3	3.2	3.1
Pro	5.5	5.4	5.5	5.4	5.6	5.3	5.4	5.5	5.5
Trp	1.1	1.1	1.1	1.1	1.1	1.0	1.1	1.0	1.0
Met	0.98	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0
Half Cys	1.3	1.3	1.2	1.1	0.9	1.2	1.0	1.0	0.8
Ser	6.4	6.3	6.2	6.1	6.2	6.0	6.2	6.0	5.7
Thr	4.6	4.5	4.6	4.5	4.4	4.5	4.6	4.6	4.5

Source: Ref. 100.

Soybeans do not contain any inhibitors for these proteinases, but prior denaturation of the proteins is required for molecular unfolding. Pattern c is typical of trypsin and the prior heat treatment of the substrate proteins is required to destroy the trypsin inhibitors in soybeans as well as for molecular unfolding. The decrease in digestibility with prolonged heating, seen in pattern a, b, and c, is due to the modification of substrate proteins, as described previously.

C. Micro-organisms During Brine Fermentation in Soy Sauce

1. Change of Microflora During Brine Fermentation

Generally speaking, there are several contaminating micro-organisms in koji, such as some species of yeasts, as well as *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Bacillus*, and the like, because of the nonsterile operation of koji

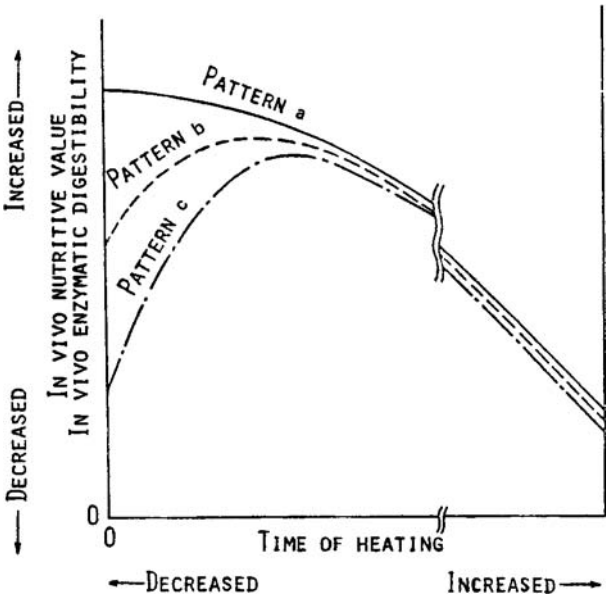

Figure 54 Schematic representation of effect of heat treatment on soybean protein and its hydrolysis patterns by various enzymes: pattern a, pepsin and other acid proteinases; pattern b, the proteinases having an optimum near neutrality, such as papain, bacteria neutral proteinase, *Aspergillus* alkaline proteinase, *Aspergillus* neutral proteinase; pattern c, trypsin and in vivo nutritional values. (From Ref. 9.)

making. These contaminants are not resistant to the high concentration of salt and therefore die within 1 or 2 months, as shown in Fig. 55. Only the spores of *Bacillus* survive the brine without germinating, but these spores are killed by high-temperature/short-term pasteurization before bottling. The micro-organisms which grow in moromi mash are limited to special lactic acid bacteria and yeasts, which are resistant to high concentrations of salt. They are *Tetragenococcus halophilus* (halophilic lactic acid bacteria), *Zygosaccharomyces rouxii* (salt-tolerant yeasts), and some species of *Candida* (halophilic yeasts). The changes of these micro-organisms during the fermentation of moromi mash are also shown in Fig. 55.

The most important micro-organism of Japanese-style soy sauce is *Z. rouxii*, a salt-tolerant yeast, and it can grow best at a pH around 5.0. Because this yeast cannot grow in a pH over 6.0 in the presence of around 18% (w/v) salt, it is not present at the initial stage of brine fermentation. When the moromi pH decreases through the lactic acid fermentation, the growth of *Z. rouxii* starts and reaches a maximum at the pH around 5.0. Thus, the fermentation of mororni mash is switched from lactic acid fermentation to the

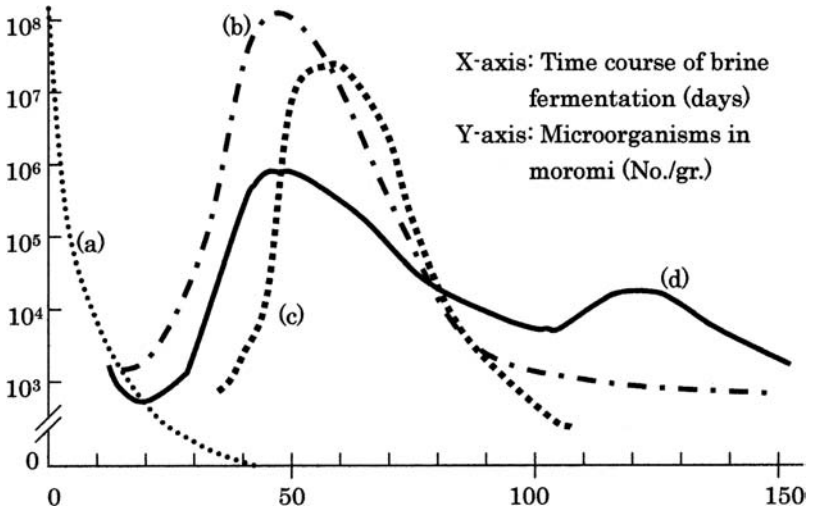

Figure 55 Microflora in soy sauce moromi mash: (a) salt nonresistant micro-organisms of koji; (b) *T. halophilus*; (c) *Z. rouxii*; (d) *C. versatilis*. (From Ref. 102.)

principal fermentation of alcohol and HEMF, for which *Z. rouxii* is responsible. This fermentation of *Z. rouxii* becomes a maximum at around 2 months after the brine fermentation starts.

Other micro-organisms found in the brine fermentation are *Candida* species such as *C. versatilis* and *C. etchellsii*, which are in the halophilic yeast groups. The growth of these *Candida* species starts at an early stage, but it is much slower than that of *Z. rouxii*. This is due to the fact that *Z. rouxii* is more anaerobic than the *Candida* species (103). In the last stage, however, the growth of the *Candida* species is promoted and they produce alkylphenols and aromatic alcohols (104). The content of total nitrogen in moromi mash liquid increases gradually to the final stage. *Candida* species are more resistant than *Z. rouxii*, to the high concentration of total nitrogen, and to the presence of these alkylphenols and aromatic alcohols. This is the reason why fermentation by *Z. rouxii* in the middle stage is taken over by fermentation by *Candida* species in the last stage (105,106). It is very interesting that there are three typical changes of microflora during the brine fermentation. Various kinds of ester are made by the reaction between the organic acids from the first lactic acid fermentation by *T. halophilus* and the alcohols from the second alcoholic fermentation by *Z. rouxii*. Alcohols and HEMF are also made during the third fermentation by the *Candida* species.

The fermentation by *Candida* species is characterized by the production of 4-ethylguaiacol (4-EG), the compound which has some influence on the character of soy sauce aroma (107). An optimum concentration of 4-EG is 4.5 ppm. An excessive presence of 4-EG over 4.5 ppm deteriorates soy sauce aroma. *Candida* fermentation is not essential for the production of Japanese-style soy sauce, because a high-quality koikuchi-shoyu with good flavor and aroma can be produced without the fermentation by *Candida*.

2. Properties of *Tetragenococcus Halophilus*

Tetragenococcus halophilus, the lactic acid bacteria of soy sauce and miso, is a gram-positive micrococcus with a diameter of 0.6–0.9 μm. It is halophilic and facultatively anaerobic. The growth curve of this microbe relative to the water activities of the medium is shown in Fig. 56 (108). The optimum water activities are 0.99–0.94, which correspond to 5–l0% (w/v) salt content. The lowest water activity in which *T. halophilus* can grow is 0.808, as shown in Table 17 (108). This water activity corresponds to 24% (w/v) salt content. This bacterium can grow in a pH range of 5.5–9.0 and a temperature range of 20–42°C. Its optimum growth occurs at 25–30°C.

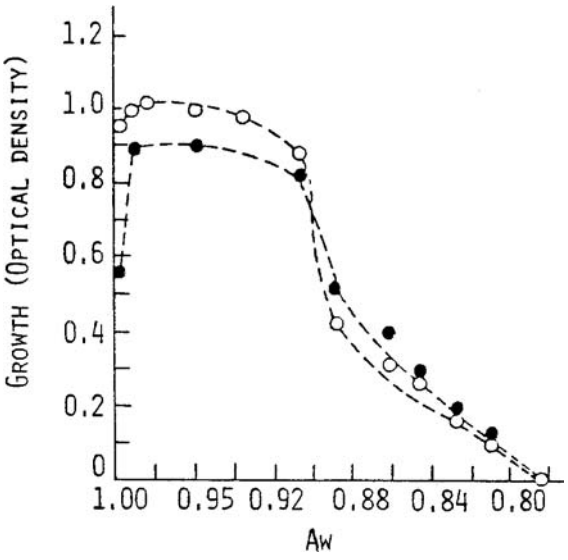

Figure 56 Effect of water activities on the growth of lactic acid bacteria of soy sauce and miso. (○) *T. halophilus* I; (●) *T. halophilus* II. (From Ref. 108.)

Table 17 Minimum Value of Water Activity (A_w) on Growth of Lactic Acid Bacteria and Yeasts of Soy Sauce and Miso

		NaCl	
Micro-organisms	Minimum A_w	w/v %	w/w %
T. halophilus (I, II)	0.808	24	20.43
Z. rouxii (I, II)	0.787	26	22.22
Z. rouxii (III)	0.787	24	20.69
C. etchellsii	0.787	26	22.22
C. versatilis	0.810	26	22.22

Source: Ref. 108.

The essential amino acids for these bacteria are leucine, isoleucine, valine, glutamic acid, arginine, histidine, tryptophan, and phenylalanine. Some strains also require uracil. The vitamin requirements are listed in Table 18 (109).

In moromi mash, large quantities of glucose are liberated into the brine from the starch contained in the materials through the action of koji enzymes. Galactose, arabinose, xylose, and the like are also liberated through the same action from the carbohydrates of the materials. *T. halophilus* converts 1 mol of glucose to 1.71 mol of L-lactic acid, 0.28 mol of acetic acid, and 0.17 mol of formic acid. Galactose, arabinose, and xylose are also converted mainly to lactic acid. Citric acid is also contained in the soybeans. One mole of citric acid is converted to 0.16 mol of L-lactic acid, 1.86 mol of acetic acid, and 0.59 mol of formic acid by *T. halophilus*.

3. Properties of *Zygosaccharomyces Rouxii*

Zygosaccharomyces rouxii is the yeast responsible for the principal alcoholic and HEMF fermentation during the brine fermentation of soy sauce and miso. It is one of the salt-tolerant yeasts and can grow in either a salt-free or salt-containing medium. It has a strong resistance to salt, as shown in Fig. 57 and Table 17, and can grow in medium with a salt content as high as 24–26% (w/v) or water activities of 0.787–0.810. In salt-free medium, both glucose and maltose can be fermented to alcohol, whereas in a medium with a concentration of salt, only glucose can be fermented by this yeast.* One of the most important features of salt-resistant *Z. rouxii* as well as *Candida* species

*Sucrose fermenting strains of *Z. rouxii*, (e.g., NRRL strain 2547) are known.

Table 18 Vitamin Requirements of Lactic Acid Bacteria and Yeasts of Soy Sauce and Miso

	T. halophilus[a]		*Z. rouxii*[a]		*C. etchllsii*[a]		*C. versatilis*[a]	
	A	B	A	B	A	B	A	B
Biotin	E, S	E, S	E, S	E	E, S	E	E, S	E
Thiamine (B_1)	N	N	N, (S)	S	S	E	E, S	E, S
Riboflavin (B_2)	E	S	N	N	N	N	N	N
Vitamin B_6	E	E	N	N	N	N	N	N
Nicotinic acid	E	E	N	N	N	N	N	N
Pantothenic acid	E	E	S, E	E	N	S, E	N	N, (S)
Inositol	N	N	N, (S)	S	N	S	N, (S)	S, E

[a] A = salt-free medium; B = salt-containing medium. E, S, and N are essential, stimulated, and not required, respectively. In addition to these vitamins, folic acid (only in salt-containing medium), betaine or choline, and leucoverin are required.

Source: Ref. 109.

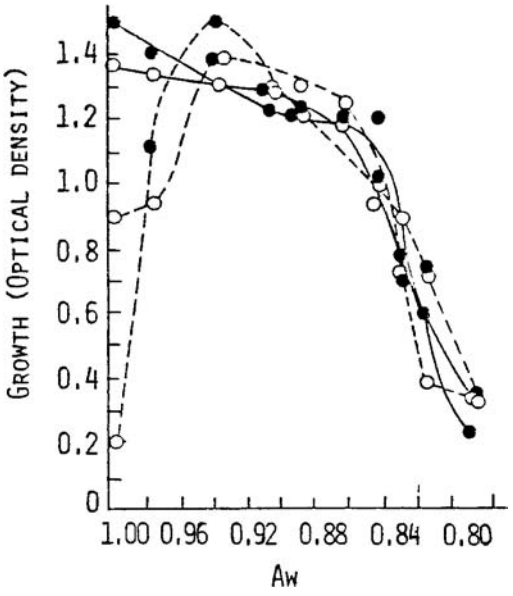

Figure 57 Effect of water activities on the growth of yeasts of soy sauce and miso: (○–○) *Z. rouxii* I; (●–●) *Z. rouxii* II; (○---○) *C. etchellsii*. (●---●): *C. versatilis*. (From Ref. 108.)

(described later) is the fact that the polyalcohol fermentation is accelerated under a high concentration of salt because it is assumed that polyalcohols, such as glycerol and the like, act as a compatible solute to obtain a strong resistance to salt (110).

The pH at which *Z. rouxii* can grow is between 3 and 7 in a salt-free medium. In the presence of 18% (w/v) salt, however, it can grow only in the pH range 4–5 (111). The range of the temperature for growth is 20–35°C in a salt-free medium, but the upper limit of the temperature is elevated to 40°C in 18% (w/v) salt. *Z. rouxii* requires biotin, thiamine, pantothenic acid, and inositol for growth as shown in Table 18. The inositol requirement increases in media with a high salt concentration. This inositol is supplied to the brine largely through the decomposition of the phytin and phosphatidyl inositol contained in soybeans by the koji enzymes.

4. Properties of *Candida* Species

The typical species of this group observed in soy sauce moromi mash or miso are *C. versatilis*, *C. etchellsii*, and the like. These yeasts are not the ones responsible for the principal alcoholic fermentation, but they convert glucose or other sugars into alcohols. The alcoholic fermentation characteristic of *Candida* species results from the fermentation of sugars other than glucose, such as maltose, in the presence of 18% (w/v) salt in the medium. As shown in Fig. 57, *Candida* species are halophilic and they grow well in media having water activities between 0.975 and 0.840. They also have a strong salt tolerance in that they are able to grow in media with up to 26% (w/v) salt content (Table 17). The growth curve in relation to the pH of the medium with or without salt is shown in Fig. 58. The range of the growth temperature is 20 to 30°C in a salt-free medium, but the upper limit of the temperature is elevated to 35°C in the presence of 18% (w/v) salt. The vitamin requirements for these species are listed in Table 18. *Candida* produce glycerol and erythritol, which are considered to be responsible for their tolerance to salt (110).

Among the compounds *Candida* species produce, 4-EG is important, because it has some influence on the aroma characters of soy sauce and is a compound not produced by *Z. rouxii*. The presence of 4-EG in a concentration over 8 ppm has a minus effect on the aroma of soy sauce, although it has a plus effect on the aroma below 8 ppm, as shown already. However, the control of the growth of *Candida* species in moromi mash is very difficult. As a matter of fact, the fermentation of moromi mash can be done without the growth of *Candida* species and fermented soy sauce with a satisfactory aroma quality can be produced.

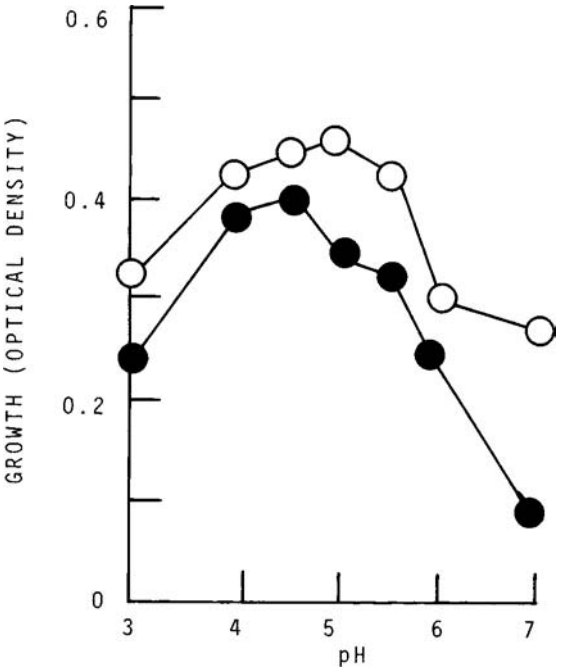

Figure 58 Effect of pH on the growth of *C. versatilis*: (○) salt-free medium; (●) 18% salt medium. (From Ref. 112.)

D. Safety of Soy Sauce and Physiologically Functional Properties

1. Studies of Mycotoxins and Safety of Soy Sauce

The micro-onganisms used in soy sauce and miso are *A. oryzae* or *A. soiae*, which are closely related to *A. flavus* and *A. parasiticus*. Because *A. flavus* and *A. parasiticus* are well known as strong aflatoxin-producing fungi, extensive investigations were carried out to determine whether or not *A. sojae* or *A. oryzae* produces aflatoxin or other mycotoxins. Hesseltine et al. (113) examined samples of soy sauce, miso, toushih, and tempeh made from soybean, rice, or wheat, and they found no aflatoxin.

Yokotsuka et al. found that one-third of 64 industrial strains of *Aspergillii* produced compounds similar to aflatoxin B compounds in fluorescence spectra and R_f values in thin-layer chromatograms, but those compounds were flavicol and six kinds of new pyrazine compounds which proved to be

nontoxic in animal tests (114–119). There were also compounds similar to aflatoxin G compounds in fluorescence spectra and R_f values, but they were not aflatoxin G compounds (120). Murakami et al. (121,122) likewise examined 214 industrial strains of *A. oryzae* and *A. sojae* and found all of them to be negative. He maintained that (a) all of the industrial strains which have been used so far belong to *A. oryzae*, *A. sojae*, and *A. tamari* and they are all aflatoxin negative; (b) aflatoxin-positive strains all belong to *A. flavus*, *A. parasiticus*, and *A. toxicarius* Murakami; and (c) there are some definite taxonomic differences between the (a) and (b) groups described earlier (123–130). Kinoshita et al. (131) failed to isolate aflatoxin-positive strains from 24 fermented foods from several regions of Japan known to have a high death rate caused by cardiovascular disease, stomach cancer, and hepatoma. Matsuura (132) investigated 238 koji mold strains used for manufacturing miso and soy sauce obtained from the All Japan Koji Starter Association, but none produced aflatoxin. In his study, he found a number of chloroform-soluble fluorescent compounds in 52 strains of *A. oryzae* and *A. sojae*. However, these compounds proved to be nontoxic (133–135).

There are 68 strains of *Aspergillus* which have been widely used in Japanese food industries. Of these, 26 strains produced aspergillic acid after more than 10 days in the surface culture of 30°C on a modified Mayer's medium and after 4–l0 days of soy sauce koji fermentation on a solid medium (136, 137). However, this is not a problem because the koji is usually harvested within 2–3 days. A small amount of -nitropropionic acid was produced by one of the tested strains at the beginning of culturing, but it rapidly disappeared. Kojic, oxalic, or formic acids were formed by some molds during koji making, but only in trace amounts. Considering the low toxicity of these compounds, they create no problems.

At present, it is generally accepted that *A. oryzae* and *A. sojae* which have been used for fermented food production never produce aflatoxin under any culture conditions (138). However, these *Aspergillus* species belong to the same flavi section as *A. flavus* and *A. parasiticus*, which produce aflatoxin (139). Because of the taxonomical similarity of these *Aspergillus* species used for food manufacturing to aflatoxin-producing fungi, it is essential to elucidate the reason why *A. oryzae* and *A. sojae* used for fermented foods do not produce aflatoxin at the genetic level. Aflatoxin B_1 and aflatoxin G_1 are enzymatically produced through the following pathway: acetate/malonate→ nolsolorinic acid (NA)→averantin (AVN)→ 5′-hydroxyaverantin (HAVN)→ averufin (AVR)→ versiconal hemiacetal acetate (VHA)→ versiconal (VHOH)→ versicolorin B (VB)→ versicolorin A (VA)→ demethylsterigmatocystin (DMST)→ sterigmatocystin (ST)→ o-methylsterigmatocystin (OMST)→ aflatoxin B_1 (AFB_1), aflatoxin G_1 (AFG_1) (140). In this aflatoxin biosynthesis, more than 20 genes are involved, which constitute a gene cluster

in the genomic DNA of *A. parasiticus* and *A. flavus* (141). In aflatoxin-producing fungi, the transcription of these genes is activated by aflR, which is the main transcriptional regulator of aflatoxin-related genes (142–145). Further, it is kown that the aflatoxin-producing ability is lost by the disruption of aflR (146). As for the industrial strains of *A. oryzae* (147) and *A. sojae* (148), which do not produce aflatoxin, they also have the genes of the above-described aflatoxin biosynthetic pathway. Matsushima et al analyzed the enzymatic activities in various steps of aflatoxin biosynthesis on *A. sojae* and *A. parasiticus* (Table 19). As seen in Table 19 (149), the cell-free extract from *A. parasiticus* NIAH-26 cultured in aflatoxin-conductive YES medium showed the enzymatic activities for all the steps in the aflatoxin biosynthetic pathway which were tested. On the other hand, the cell-free extract from *A. sojae* strain 477 had no detectable activities, except for the esterase activity converting VOAc to VOH and this esterase activity was concluded to be nonspecific enzymatic activity, different from the enzyme of *A. parasiticus* NIAH-26, which was induced by being cultured in YES medium. Further, all enzymatic activities associated with the aflatoxin biosynthesis were not detected or neg-

Table 19 Enzymatic Conversion of Various Intermediates During the Aflatoxin Biosynthetic Pathways by Extract of *A. sojae* Strain 477 and *A. parasiticus* NIAH-26 Grown on Aflatoxin-Conductive YES Media

Substrate	Product	*A. sojae* 477 (pmol/mg/min)	*A. parasiticus* NIAH-26 (pmol/mg/min)
NA	AVN	—[a]	47.2
AVN	HAVN	—	77.2
HAVN	AVR	—	281.0
VOAc	VOH	38.0	131.0
VHA[b]	VOAc	—	281.0
VHOH[c]	VB	—	140.0
DMST	ST	—	14.5
ST	OMST	—	46.7
OMST	AFB_1	—	2.88
	AFG	—	0.0117

[a] Not detected.

[b] For reason given in footnote c,VHA was used as the substrate for the cyclase reaction converting VHOH to VB. VHA was converted to VHOH with the addition of excess porcine esterase and successively converted to VB by the cyclase.

[c] VHOH is too unstable to quantify. Therefore, instead of measuring VHA→VHOH conversion, versiconol acetate (VOAc)→VOH conversion was measured, which is catalyzed by the same esterase.

Source: Ref. 149.

ligible in *A. sojae* strain 477. In contrast, all industrial strains lacked detectable transcripts of aflR, the main regulatory gene for aflatoxin biosynthesis, under the aflatoxin-inducing condition. This suggests that the defects in aflR expression cause the lack of expression of aflatoxin-relating genes which results in the absence of aflatoxin biosynthesis in these industrial strains (149,150). It is concluded that the lack of aflatoxin production by *A. sojae* can be attributed, at least partially, to the premature termination defect in aflRs, which deletes the C-terminal transcription activation domain that is critical for the expression of aflatoxin biosynthetic genes (151).

2. Studies of Mutagens and Safety of Soy Sauce

Recently, Wakabayashi et al. (152) and Nagao (153) separated two fractions from soy sauce, which showed mutagenic activities when mixed with a high concentration of nitrite (approximately 3500 ppm) at pH 3.0. They identified these compounds as (a) (−)-(1*S*,3*S*)-1-methyl-1,2,3,4- tetrahydrocarboline-3-carboxylic acid [(−)-(l*S*,3*S*)- MTCA] and its stereoisomer (−)-(1*R*,3*S*)-MTCA and (b) tyramine, respectively. However, soy sauce itself shows a mutagenic activity only in mixing with more than 100 ppm nitrite and it did not show any mutagenicity in mixing with nitrite less than 100 ppm, as shown in Fig. 59 (153).

Nagahara et al. (154) reported that (a) the two stereoisomers of MTCA contained in soy sauce cannot react with the nitrite at pH 3.0, when the concentration of the nitrite is below 250 ppm, as shown in Fig. 60, (b) the

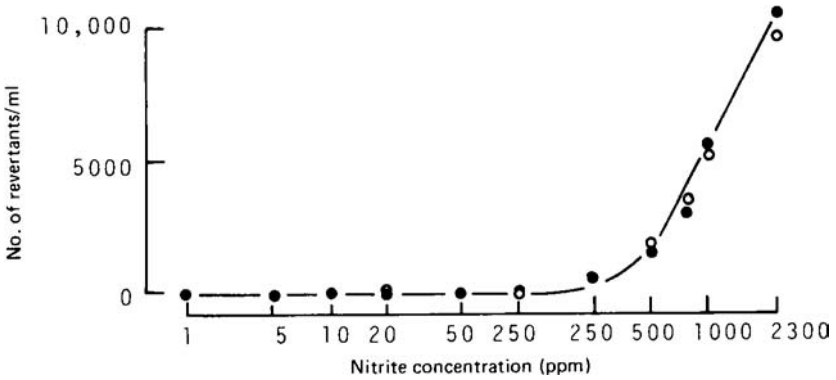

Figure 59 Mutagenicity of soy sauce treated with various concentrations of sodium nitrite at pH 1.0 (○) or pH 3.0 (●), demonstrated in *Salmonella typhimurum* TA 100 without S-9 mix and expressed as number of revertants/mL of soy sauce. (From Ref. 154.)

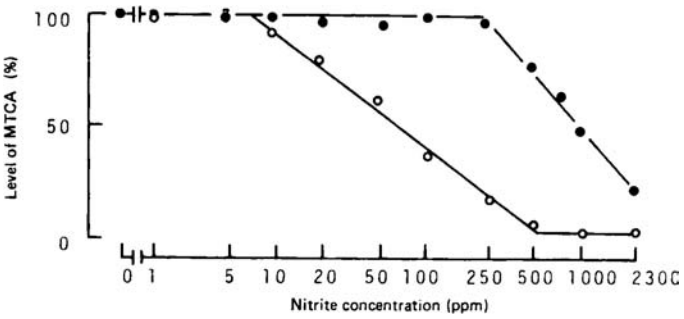

Figure 60 Levels of MTCA remaining in soy sauce (●) and remaining in a solution of authentic MTCA (○) after treatment with sodium nitrite at various concentrations. The amounts of MTCA in the samples without nitrite treatment were taken as 100%. (From Ref. 154.)

tyramine contained in soy sauce cannot react with the nitrite at pH 3.0, even when the concentration is 2300 ppm or more, as shown in Fig. 61, and (c) the pure MTCA isomers or tyramine separated from soy sauce react easily with the nitrite in the pure systems of the reaction, even though its concentration is less than 250 ppm (Fig. 61). Nagahori et al. (155) reported that the reaction between nitrite and dimethylamine at pH 3.6 was inhibited by the addition of soy sauce and concluded that this suppression of the nitrosation is due to the competitive inhibition through the Van Slyke reaction between the nitrite and the amino acids contained in the soy sauce. Therefore, the lack of a reaction to the MTCA or tyramine to nitrite in a low concentration of nitrite

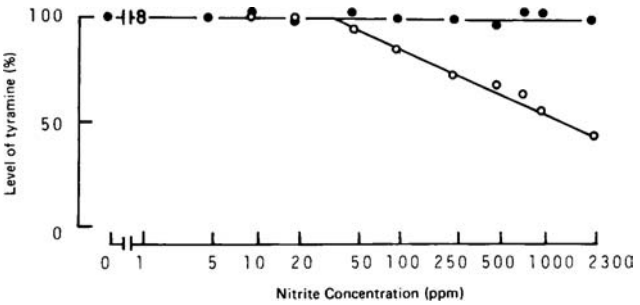

Figure 61 Levels of tyramine remaining in soy sauce (●) and remaining in pure tyramine solution (○) after treatment with sodium nitrite at various concentrations. The amounts of tyramine in the samples without nitrite treatment were taken as 100%. (From Ref. 154.)

may be ascribed to competitive inhibition by the amino acids contained in the soy sauce. The concentration of nitrite in a human stomach is usually only 6–10 ppm (156). It is reported that the nitrite content of human saliva varies widely, generally ranging from 0.1 to 30 ppm (157,158). According to Ishiwata et al. (159), the concentration of nitrite in human saliva does not exceed 50 ppm, even after the ingestion of 100 g of salted Chinese cabbage which contains 0.2 mg nitrite and 152 mg nitrate. No more than 50 ppm nitrite has been reported in normal or diseased human stomachs so far (160). Therefore the presence of MTCA isomers or tyramine in soy sauce creates no problem under these physiological conditions.

3. Long-Term Animal Tests and Anticarcinogenecity of Soy Sauce

Long-term animal tests using rats to determine the carcinogenicity of Japanese soy sauce were carried out by MacDonald and Dueck (161). They divided 100 rats into 4 groups: intact and fundusectomy rats on a control diet and intact and fundusectomy rats on the same diet saturated with soy sauce (Kikkoman soy sauce was used as the sample). The rats on soy sauce were clearly smaller than those on the control diet. The longest lived, most active, and apparently most healthy rats were those with an intact stomach which were fed soy sauce. Fifteen rats in this group were apparently healthy at 33 months compared with only 7 of the control rats with intact stomachs. Only 9 operated rats lived 33 months. Mammary tumors were found in 8 of 24 intact control rats and in 2 of 21 operated control rats, whereas they were found in none of the rats fed soy sauce, as shown in Table 20 (161).

According to the data presented by Kikkoman Foods Inc. in the SCOGS (the Select Committee on GRAS Substances) Hearing, held in 1977, shoyu showed the same acute toxicity to rats and mice as that of a saline solution with an equivalent sodium chloride concentration, as shown in Table 21 (162). In a long-term (1.5 years) feeding test on mice, there was no

Table 20 Distribution of Tumors Among the Four Groups of Rats

Group No.	Diet	Stomach	No. of rats at risk	Tumor site			
				Breast	Uterus	Lymphoma	Other
1	Control	Intact	24	8	3	1	0
2	Control	Operated	21	2	1	2	1
3	Soy sauce	Intact	24	0	2	0	0
4	Soy sauce	Operated	20	0	0	3	0

Source: Ref. 161.

Table 21 Acute Toxicity of Liquid and Powdered Shoyu Compared with Saline Solution of the Same Salt Concentrations

Sample	NaCl content (% w/v)	Acute toxicity (LD_{50}/kg)	
		Rats (mL)	Mice (mL)
Liquid shoyu	17.4	20.6	27.3
Saline solution	17.4	18.5	28.3
Powdered shoyu solution (52.3%)	23.5	16.4	19.9
Saline solution	23.5	16.5	20.4

Source: Ref. 162.

difference in mortality between the tests and controls (162). In addition to these experiments, many investigations on the safety of soy sauce have been done, such as experiments for 2 years on rats, for 1.5 years on mice, and for 1 year on dogs in the Kikkoman Research Laboratories. All of these experiments showed that soy sauce is safe (Kikkoman Corporation, unpublished data).

In 1988, Benjamin et al. found that a refined diet supplemented with Japanese-style fermented soy sauce inhibits benzopyrene-induced forestomach neoplasia in mice and they suggested that the anticarcinogenic effect is due to antioxidants in soy sauce (163). The substantial component of the antioxidant activities of Japanese-style fermented soy sauce is HEMF, which is the character-impact substance on its flavor and aroma, as already described in detail in Section III.A.3. The molecular formula of HEMF is shown in Fig. 62. In 1992, Nagahara et al. fed HEMF to mice following benzopyrene administration and found that it inhibited the subsequent development of

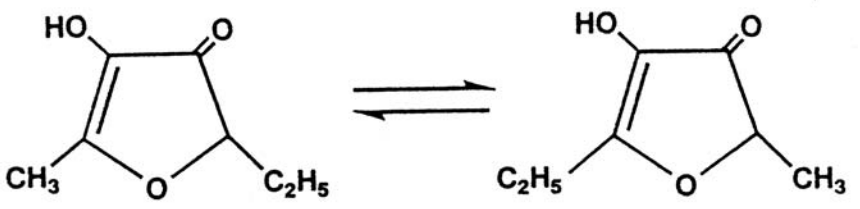

Figure 62 Chemical structure of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)furanone (HEMF), which is the compound with a strong anticarcinogenic activity as well as the character-impact compound in the flavor and aroma of Japanese-style fermented soy sauce.

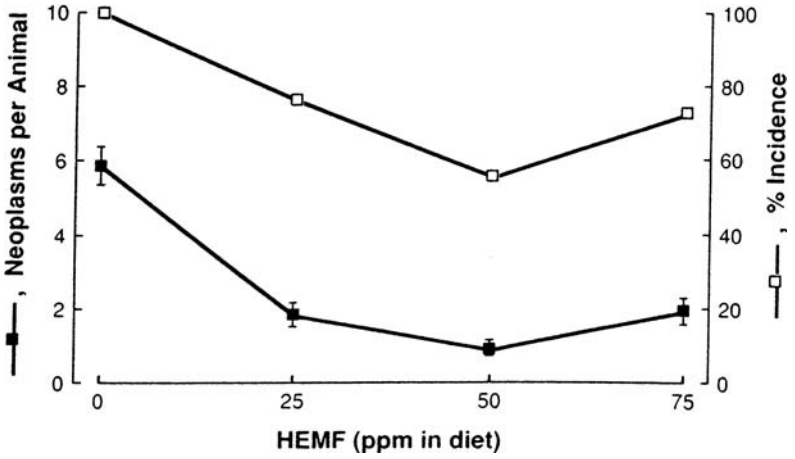

Figure 63 Inhibition of benzopyrene-induced forestomach neoplasia by HEMF. For neoplasms per animal, all treatment groups were significantly different from the untreated control group ($p \leq 0.05$). Data are expressed as means with SEM shown where they exceed the size of the symbols. $n = 25$–27 mice/group. (From Ref. 37.)

forestomach neoplasia, as shown in Fig. 63 (37). HEMF was effective when fed at 4 mg/kg body weight/day, indicating that it is a potent anticarcinogen.

4. Other Physiologically Functional Properties of Soy Sauce

Kinoshita et al. found that fermented soy sauce has a blood-pressure-lowering effect and they isolated the substance which inhibits angiotensin-converting enzymes as a substance responsible for it (164,165). This compound was identified as nicotinamine. Nicotinamine is not the substance produced by micro-organisms, but it is the one originally contained in soybeans. In addition to these, there is the report that melanoidens derived from soy sauce possess the strong protective activity against NO-induced DNA damage (166).

VI. APPLICATION OF NEW TECHNOLOGY FOR SOY SAUCE MANUFACTURE

A. Manufacture of Soy Sauce Through Fermentation by Bioreactor with Immobilized Whole Cells

The brine fermentation and aging of moromi mash of soy sauce needs more than 5 months, as already described. However, this process can be shortened

considerably through fermentation by a bioreactor (167,168). Figure 64 shows the manufacturing process of soy sauce through the fermentation by the bioreactors with the immobilized whole cells of *T. halophilus*, *Z. rouxii*, and *C. versatilis*. The first step of this process is the preparation of the hydrolyzate, which is accomplished through the digestion of defatted soybeans by the koji enzymes at 55°C in a salt concentration of 10% (w/v). The resultant hydrolyzate is fermented through the bioreactors with the microbial cells entrapped in calcium alginate beads (167) or in the mixture gel beads of alginate and silica (169). Further, ceramics have been developed for the immobilization of microbial cells and tested for the fermentation of soy sauce through bioreactors (170).

In the experiments using the pilot-plant bioreactors of Fig. 65, the time course of the continuous soy sauce fermentation through the bioreactors with the immobilized whole cells entrapped in calcium alginate gel beads is shown

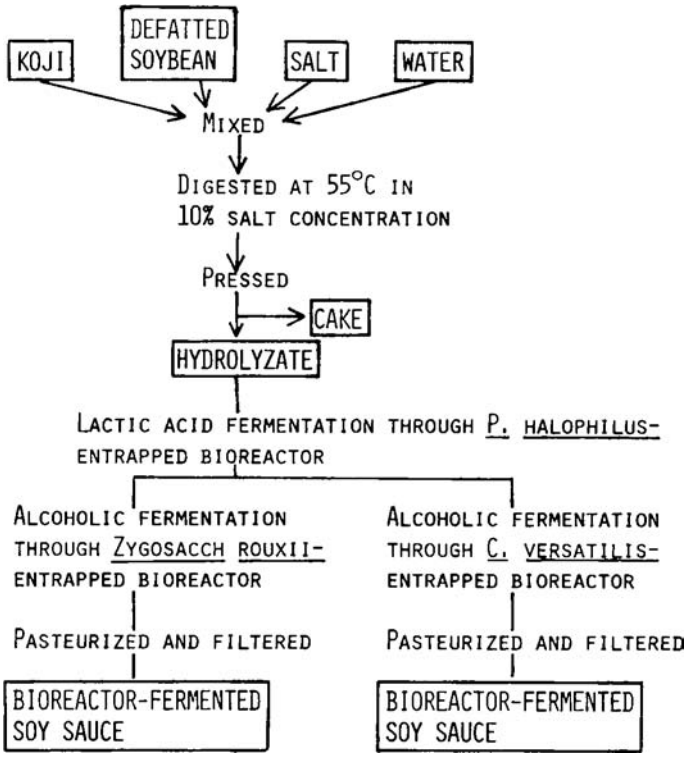

Figure 64 Manufacturing process of soy sauce through fermentation by bioreactors with immobilized whole cells. (From Ref. 167.)

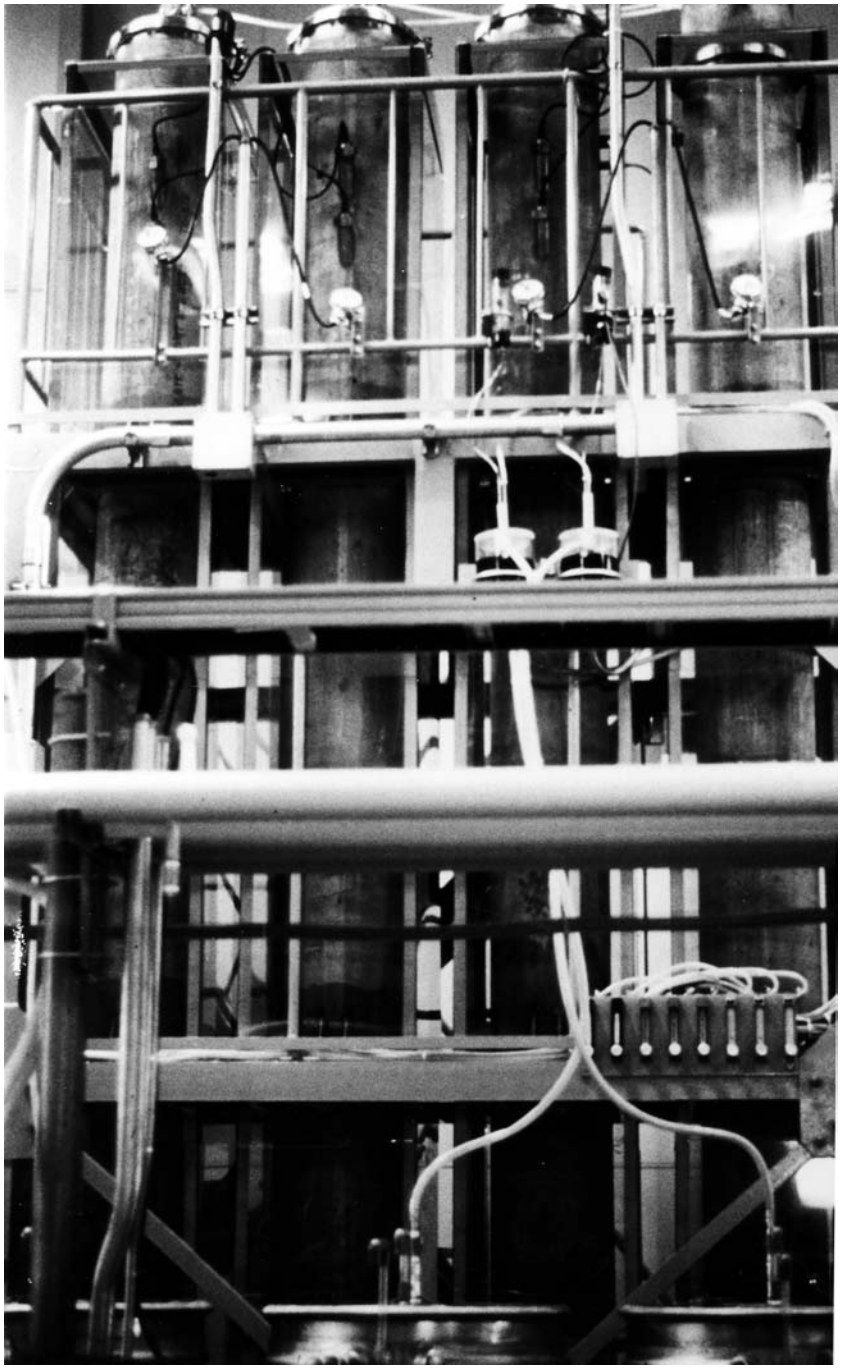

Figure 65 The pilot-plant equipment of 280-L column-type bioreactors for the manufacture of soy sauce. (Courtesy of the Kikkoman Corporation.)

in Fig. 66 and the analytical data of the aroma in the fermentation fluid is given in Table 22. In the reactor with immobilized *T. halophilus* (Fig. 66, top), the viable cells in gels at steady state showed a decimal increase of 2.5 over that of the initial state of immobilization. The number of viable cells in gels became 1,000–10,000 times higher than that of the effluent with a holding time of 40 h. The production of lactic acid achieved a satisfactory level of 10 gr/L after

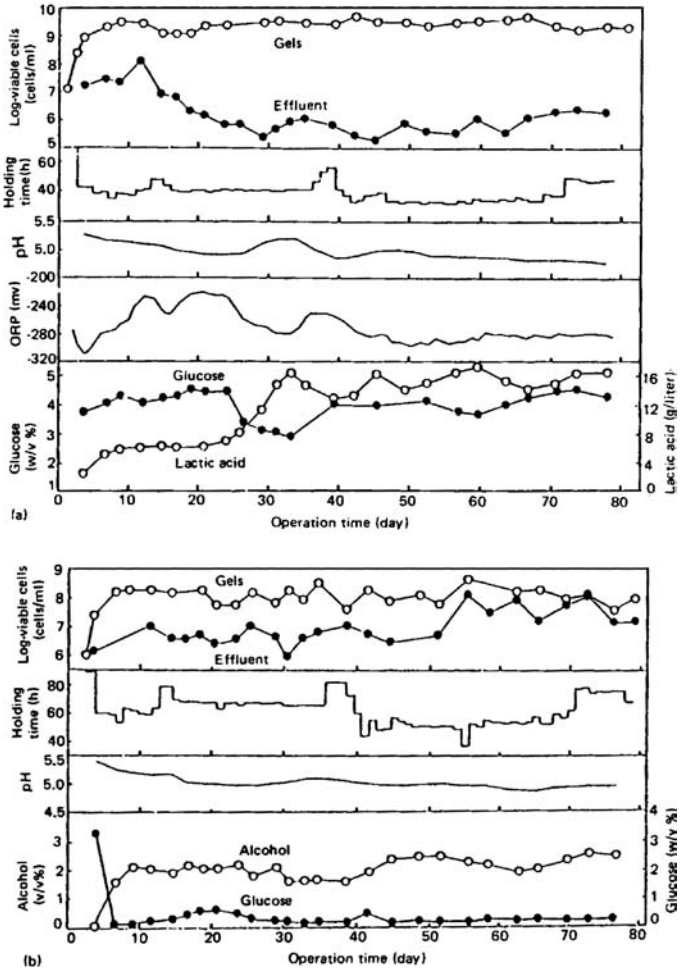

Figure 66 Time course of continuous soy sauce fermentation in a 280-L column-type reactor containing immobilized microbial cells: (a) lactic acid fermentation by *T. halophilus* IMA 1693; (b) alcoholic fermentation by *Z. rouxii* ATCC 13356. (From Ref. 167.)

Table 22 Aroma Components[a] of Extraction Concentates from Refined Products Fermented in the Column-Type Reactors Containing Immobilized Whole Cells

	Peak number[c]											
Sample[b]	1	2	3	4	5	6	7	8	9	10	11	12
24S	35.3	4.6	63.2	9.4	8.3	5.2	4.6	21.6	1.0	0.5	162.6	81.9
25T	25.1	5.8	46.4	11.6	10.2	4.2	3.8	24.9	0.0	8.6	160.6	66.7
71S	43.2	4.2	89.3	17.2	16.8	3.4	3.9	29.7	0.3	0.4	180.3	95.8
71T	45.5	5.3	86.6	17.8	16.5	3.6	3.8	27.9	0.0	5.7	165.6	71.2
Control[d]	12.6	10.7	11.3	5.6	13.1	11.4	4.1	6.2	0.6	1.9	241.1	120.1

[a] Concentrations of aroma compounds are reported as mg/L.
[b] Numerals indicate the days lapsed; S: *Z. rouxii*; T: *C. versatilis*.
[c] Peak number: (1) isobutyl alcohol; (2) *n*-butyl alcohol; (3) isoamyl alcohol; (4) acetoin; (5) ethyl lactate; (6) furfuryl alcohol; (7) methionol; (8) 2-phenylethanol; (9) 4-hydroxy-2,5- dimethyl- 3- (2H)-furanone (HDMF); (10) 4-ethyl-2-methoxyphenol (4-ethylguaiacol); (11) 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF); and (12) 4-hydroxy-5- methyl-3-(2*H*)-furanone (HMMF).
[d] Commercial soy sauce.
Source: Ref. 167.

28 days. In the subsequent alcoholic fermentation by *Z. rouxii* (Fig. 66, bottom) or *C. versatilis*, using a bioreactor, the difference of the number of viable cells between gels and effluents decreased gradually with time. About 2% alcohol was produced continuously by 40–80 h and the pH level reached 4.8–5.2. These results indicate that both lactic acid and alcoholic fermentations are achieved in a very short time at a satisfactory level. However, the aroma pattern of the samples is slightly different from that of commercial soy sauce (Table 22). The samples are higher in isobutyl alcohol, isoamyl alcohol, and 2-phenylethanol and lower in HEMF, which is one of the most important aroma components characteristic of Japanese-style koikuchi shoyu, as described previously. The aroma pattern of these samples may be improved by alteration of fermenting conditions such as temperature, aeration, holding time, and so on.

In addition to these, there are some other problems to be solved. For instance, the filtration of the digestion mixture by the method of Fig. 64 using koji is very difficult in industrial operations. Furthermore, soy sauce manufacturing without having any aging processes of moromi mash is not recognized as "fermented soy sauce" by the Japanese government.

The total manufacturing time of soy sauce made through fermentation by the bioreactors with immobilized whole cells is only 2 weeks. It is much shorter in comparison to the 5 months for the usual process consisting of the koji making, brine fermentation, and aging of moromi mash. Therefore, this

method is effective in preparing liquid seasonings like soy sauce with a smaller investment in numbers of fermenting tanks. However, the production of soy sauce or other liquid seasonings using a bioreactor has not yet been carried out as of 2002.

B. Enzymatically Hydrolyzed Vegetable Protein

In the 1990s, it was reported in Europe that carcinogenic substances are produced through the hydrolysis of vegetable proteins by HCl, such as 1,3-dichloropropane-2-ol, 2,3-dichloropropane-1-ol, and 3-monochloropropane-1,2-diol (21). Vegetable protein products hydrolized by HCl (HVP) are very popular products, but since then a strong demand for the vegetable protein products hydrolyzed by enzymes has occured. At present, there are various commercial products hydrolyzed through enzymes. Figure 67 and Table 23 show the manufacturing procedure and analytical data of the commercial product of enzymatically hydrolyzed vegetable protein (EHVP) developed by the Kikkoman Corporation (171). First, *A. sojae* or *A. oryzae* is cultured by a submerged culture. Then, wheat gluten is hydrolyzed by the enzymes contained in the culture mixture of *A. sojae* or *A. oryzae* under the presence of NaCl. As described previously, the production of proteinases and peptidases is much higher in submerged culture than in solid culture, (i.e., solid koji). In contrast with these enzymes, the production of amylases, pectinases, and cellulases is much lower in submerged one than in solid koji. Because wheat gluten does not contain starch, pectin, and cellulose, the submerged culture mixture is suitable as an enzyme sourse. Recently, EHVP was used instead of acid-hydrolyzed vegetable protein and the consumption of EHVP has increased rapidly. This product can also be used as a base hydrolyzate for soy sauce fermentation through a bioreactor.

C. Breeding of Koji Mold Through Protoplast Fusion

So far, the breeding of koji mold (i.e., *A. oryzae* or *A. sojae*), has been carried out (a) by mutation with mutagenic agents such as x-rays, ultraviolet light, MNNG, and so on (172) and (b) by crossing (173). Recently, however, the techniques of intraspecific and interspecific protoplast fusion among the filamentous fungi have been developed using *Aspergillus* (174,175), *Penicillium* (176), and *Mucor* (177).

Among the koji molds used for the production of shoyu, sake, and miso, protease hyperproducers generally show low glutaminase activities, whereas glutaminase hyperproducers usually have insufficient protease activities. In order to obtain the strains having high activities of both proteases and glutaminases, a breeding experiment by protoplast fusion was done, using

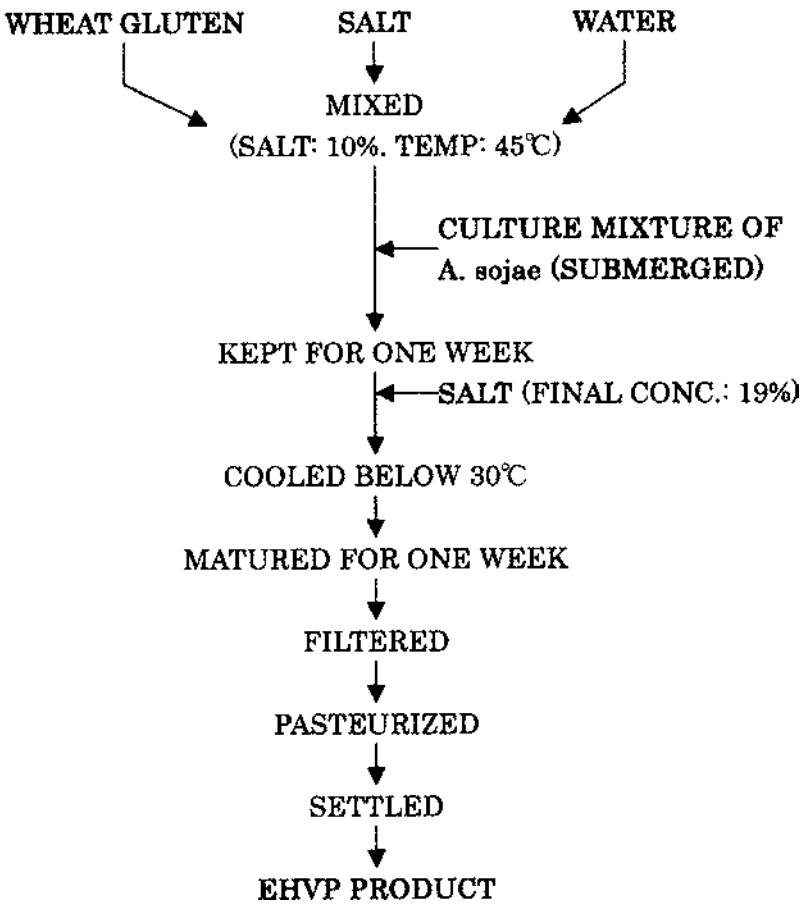

Figure 67 Manufacturing process of EHVP. (From Ref. 171.)

the double-marker mutants of conidial color and nutritional requirement among genealogically unrelated *A. sojae* cultures (178). Because many of the fused products were heterokaryons, ultraviolet treatment was applied to obtain stable heterozygous diploids. As a result, 130 diploid strains with green conidia were induced and their enzyme activities are shown in Fig. 68. As can be seen in the figure, the activities of both protease and glutaminase were distributed within the range of those of the parent strains, and in most strains, the sum of the two activities did not exceed those of the parents. Occasionally, however, a few diploids with well-balanced high productivities of both enzymes were obtained. The next step of the experiment was to

Table 23 Analytical Data[a] of Enzyme-Hydrolized Vegetable Protein

Analytical item	EHVP[b]	HVP[c]	Soy sauce (koikuchi)	Soy sauce (usukuchi)
Total nitrogen	3.0	2.3	1.7	1.2
Total amino acid	11.8	12.8	7.0	5.5
Glutamic acid	4.2	4.1	1.3	1.0
Color	Light brown	Light brown	Deep brown	Light brown
NaCl	19.0	19.0	16.0	18.5

[a] Percent (w/v).
[b] Hydrolyzed vegetable protein by enzymes (Kikkoman product).
[c] Hydrolyzed vegetable protein by HCl (commercially available product).
Source: Ref. 171.

improve the well-balanced strains thus obtained (179). Two approaches were tried. One was the usual mutation of the heterozygous diploid. Both protease and glutaminase activities were simultaneously improved to a certain degree. The other approach was the haploidization of the heterozygous diploid with benomyl or *p*-fluorophenylalanine. The strains suitable for this purpose were screened among the haploid recombinants. These strains produced as much

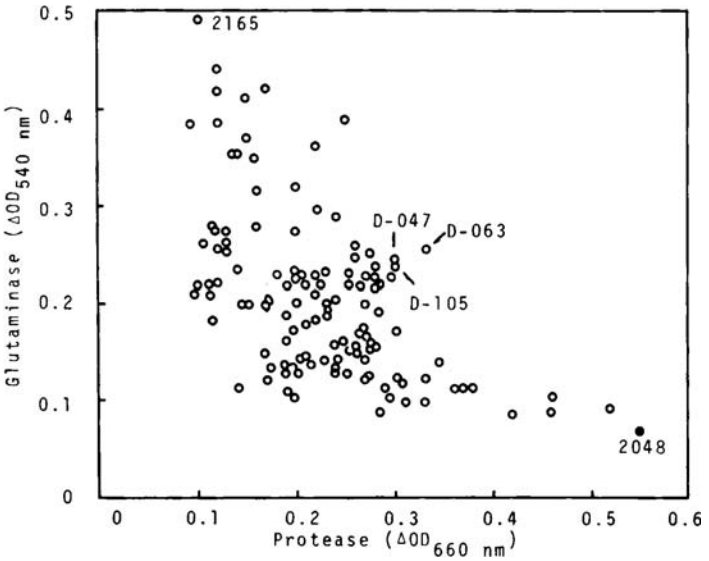

Figure 68 Distribution of the protease and glutaminase activities of 130 fused green strains. The fused green strains (D-047, D-063, and D-l05) showing excellent activities with a good balance are indicated by arrows. (From Ref. 178.)

proteases as the hyperprotease producer and almost as much glutaminases as the hyperglutaminase-producing parent. Thus, it was shown that mutation and especially haploidization of heterozygous diploids are useful techniques for the breeding of industrial koji molds.

VII. FORECAST

Annual per capita use of soy sauce in Japan had been comparatively unchanged between 10.0 and 10.9 L through the 30 years from 1955 to 1984. After that, however, it decreased gradually from 9.8 L in 1985 to 8.1 L in 2001 (26). This decrease perhaps depends on the change of the dietary life of young people to Western cuisine. Therefore, growth of fermented soy sauce in Japan is not to be expected.

In Western countries, however, fermented soy sauce, especially Japanese-style shoyu, began to enter the area of Western cuisine recently and is acquiring a new market as a very promising commodity. Particularly, the consumption of shoyu is growing dramatically in the United States. Before 1960s, chemical soy sauce had dominated the non-oriental market in the United States. However, shoyu has been penetrating the non-oriental market very rapidly, with the construction of an integrated shoyu plant by the Kikkoman Corporation in Wisconsin in 1973. At present, fermented soy sauce production manufactured in the United States domestically reached 160,000 kL, as shown in Fig. 14, indicating that shoyu has becoming a new growing commodity in Western countries. Shoyu is emerging from being just a seasoning peculiar to the East to becoming a global one. Annual per capita use of shoyu in the United States is still very low. Therefore, it can be said that the future of fermented soy sauce is very bright.

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2

Industrialization of Japanese Miso Fermentation

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I. INTRODUCTION

Miso is a semisolid fermented food made from soybeans, rice or barley, and salt (1,2). Similar foods are also produced in other parts of East and Southeast Asia. They are referred to as "jang" in China, "doenjang" in Korea, "taucho" in Indonesia, and "tao-tsi" in the Philipines. Ninety percent or more of miso is used as a soup ingredient in Japan, whereas most misolike products are generally used as seasonings in most Southeast Asian countries. Miso soup is simply prepared by dissolving 1 part of chopped miso in 10 parts of hot water in which other ingredients such as vegetables, tofu, fried tofu, mushrooms, meat, fish, shellfish, and so forth have been cooked previously.

Although many varieties of miso exist, they can be classified into three major types on the basis of raw materials employed: (1) rice miso made from rice, soybeans, and salt, (2) barley miso made from barley, soybeans, and salt, and (3) soybean miso made from soybeans and salt. They are further divided into sweet medium, sweet, and salty groups and each group is further divided by color (white, light, yellow, and red varieties) (Table 1). Blended miso is also described in the Miso Quality Standard (Food Agency, Ministry of Agriculture Forestry and Fishery, Japan). Miso is often identified by its place of origin, such as Sendai miso, Sado miso, and Edo miso. The ratio of rice miso, barley miso, soybean miso, and blended miso is 78 : 7 : 5 : 10. The amount of blended miso is increasing gradually.

There were about 1300 miso factories in Japan in 1999 using 162,000 tons of soybeans, 103,000 tons of rice, 22,000 tons of barley, and 63,000 tons

Table 1 Varieties of Miso

Varieties	Taste	Color	Principal areas of production in Japan
Rice miso	Sweet	White	Kyoto, Kagawa,
	Sweet	Red	Hiroshima, Tokyo
	Medium salty	Light yellow	Shizuoka
	Salty	Light yellow	Nagano
	Salty	Red	Tohoku province, Hokkaido, Niigata, Kanto province, Hokuriku province, Sanin province
Barley miso	Sweet	Light yellow	Kyushu province, Chugoku province, Ehime
	Salty	Red	Kyushu province, Kanto province
Soybean miso	Salty	Red	Aichi, Mie, Gifu

of salt; 554,000 tons of miso were produced in 1998. In addition, the amount of homemade miso is estimated at roughly 16,000 tons. The amount of miso supplied per capita was 4.5 kg in 1998. Daily per capita consumption was 28–30 g from 1951 to 1959. From that time until 1998, it gradually decreased to 12.3 g. The cost for 1 kg of packaged miso ranges from 300 to 1300 yen (US$2.30–10) (3). The amount of miso exported in 2001 was 6182 tons, 223% of the quantity exported in 1991 (Japan Trade Monthly Statistics, Ministry of Finance)

II. HISTORY AND EARLIEST KNOWN REFERENCES TO MISO (4)

The forerunner of miso originated with jang, the oldest seasoning cited in the *Analets of Confucius* (Scroll 2, Chapter 10) in China. However, jang at that time was produced mainly from fish or meat in a mixture of salt, water, and/or rice wine. Later, soybeans were used in place of animal protein as an ingredient of jang.

The earliest reference on fermented soybeans or beans was found in the Chinese dictionary *Shuo-wen Chiehtzu* by Hsu Shen (Japanese: *Setsumon Kaiji* by Kyoshin) about AD 121 during the Kokan dynasty (AD 25–220). It defines "shi," soybean nuggets ("kuki" in Japanese), as a soybean or bean

product made by holding the beans in a dark room for natural fermentation and ripening.

There are detailed descriptions on the manufacturing methods of the two major fermented soybean foods, jang and shi, in *Chimin Yaushu* (AD 535–550), the world's oldest encyclopedia of agriculture. For making fine shi, the preparation of soybean koji is the most important process. The manual describes the following essential items: koji chamber (koji incubation chamber), suitable seasonings for preparation, sufficient amount of soybeans for one batch, selection of suitable soybeans, new or old crop, cooking method to obtain well-cooked soybeans, checkpoints for evaluation of the cooked soybeans, koji-making method in the koji chamber, including the stacking and form of the cooked soybeans for fermentation on the floor in the chamber, stirring frequency during preparation, and proper temperature and its measurement.

Most of these descriptions agree with modern scientific knowledge. For example, it was recommended that the stacked materials be stirred up and down when the inner temperature felt by hand inserted into the material goes up to that of the armpit. This was at a time when there were no thermometers. Also, it is quite remarkable that even so long ago the Chinese were consciously utilizing the yellow molds, probably *Aspergillus oryzae*, to produce fermented foods such as jang and shi (5,6).

Although the origins of miso are not clear, they are believed to have been introduced from China directly or via Korea. The earliest written records on Japanese fermented soybean products are found in the record of Hishio Tsukasa (Shoin) dating from AD 701 [Bureau for the Regulation, Production, Trade and Taxation of Hishio (jang and its related products)]. An unknown new produce named "misho" is listed along with jang and shi in the record. However, there is no description of the preparation or properties of misho.

In the northeast district of China and Korea, there was a fermented soybean product which was called "misun" in northeast China and "mirujo" in Korai, northern Korea (AD 939–1392) and "mijo" in south Korea. The characteristic method for making this product (not seen in the *Chimin Yaushu*) includes pounding and mashing of cooked soybeans, shaping them into balls, wrapping these in rice straw, hanging the wrapped materials under eaves for natural fermentation, washing out the molds grown over the surface of the balls, and mixing with brine for a second fermentation. This process was very popular at farmhouses, especially in the northern districts in pre-World War II Japan. A modified production process employing a motor-driven extruder for soybean ball making, mold starter, and koji fermenter is now widely used for making soybean miso in the prefectures of Aichi, Mie, and Gifu located in the central part of Japan.

The new Japanese name, "miso," first appeared in the *Sandai Jitsuroku*, a history book published in AD 901; thereafter it was often used instead of "misho." The earliest Japanese document containing information on the ingredients of misho was the *Engishiki* written in AD 927 dealing with ceremonies and manners in Court. It described that 1 koku (100 shous) (180 L) soybeans, 5.4 shous (9.72 L) rice, 5.4 shous (9.72 L) koji wheat, 8 shous (14.4 L) sake (rice wine), and 40 shous (72 L) salt are used to obtain 1

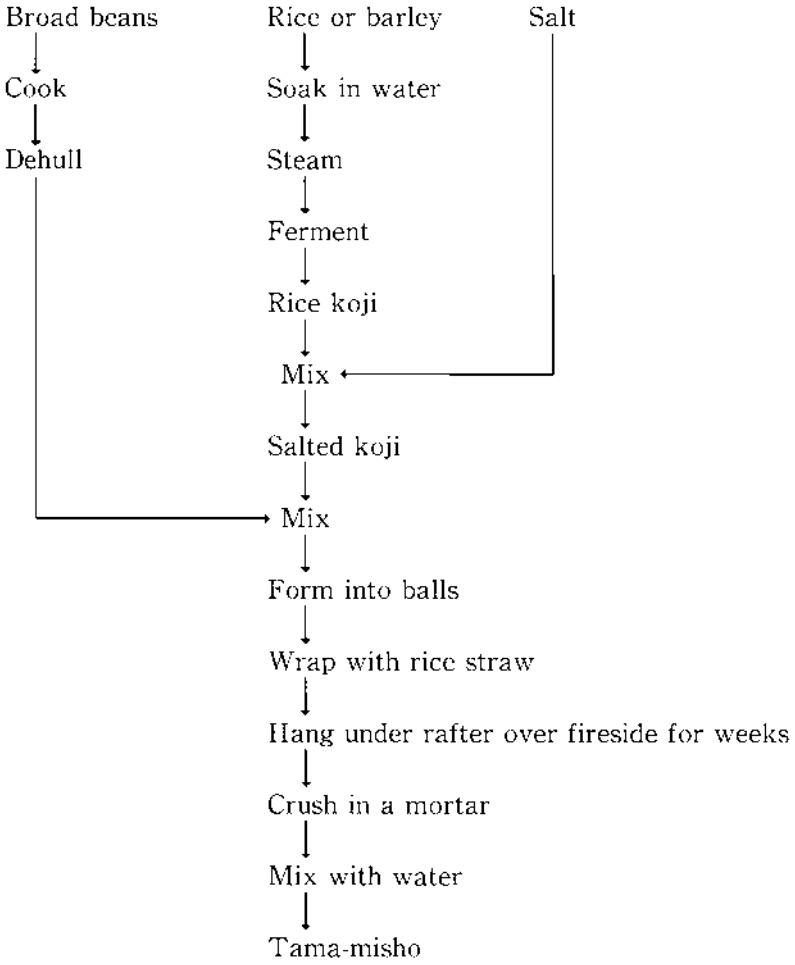

Figure 1 Flowchart for preparation of tama-misho. (From *Wakansansaizue*, Vol. 105, 1712.)

koku (180 L) misho (1 shou = 1.8 L and 1 koku = 180 L in Japan today) (see Fig. 1).

Miso was originally made at Buddhist monasteries for their own use and for aristocratic circles. The industrial production of soybean miso was started in Aichi prefecture in 1625 and that of rice miso was started in Sendai by craftsman in 1645; Sendai miso remains the most typical variety today. Thereafter, the number of miso factories increased rapidly in the Edo period (1600–1867). During this period, almost varieties today were produced as attractive foods for town people.

III. INDIGENOUS PROCESS: RAW MATERIALS USED IN ANCIENT TIMES

According to the *Engishiki* (927) which noted the ingredients for the first time, soybeans, rice, wheat or barley, salt, and sake were the main ingredients for making misho without details on the quality. *Wakansansaizue* (dictionary with line drawing illustrations published in 1712) (Figs. 1 and 2) indicated that whitish soybeans, polished rice koji, and salt are the major components for misho of higher grade and broad beans in place of soybeans are used for misho of lower grade.

Conventionally, the amount of ingredients and their ratio in the miso production depend on the amount of agricultural products where the miso was produced. This has resulted in the creation of many varieties characteristic of the province, such as barley miso produced in Kyushu, where naked barley was harvested in large quantity and the ordinary ratio of soybeans to barley is 10 : 20. On the contrary, soybeans are suitable to produce miso in northern provinces such as Akita prefecture, 40 N., and the ratio of soybean to rice is 10 : 10 or 10 : 5 when used for miso making.

According to *Wakansansaizue*, traditional tama-misho (or powdered misho) is prepared employing broad beans in place of soybeans. Sorted broad beans are cooked in water and dehulled, on the other hand, sorted rice or barley is soaked in water and cooked in steam after draining the water, and then fermented with koji starter to make rice koji, or barley koji, which are mixed with salt. Thus, salted koji is then mixed well with cooked beans and formed into balls and wrapped with rice straw. The wrapped balls are hung under a rafter over a fireside for weeks and finally crushed in a mortar and mixed with a small amount of water to prepare misho soup. This tama-misho is impossible to find in Japan now, but it is noteworthy that it remained the traditional process introduced from the Chinese continent over 1000 ago.

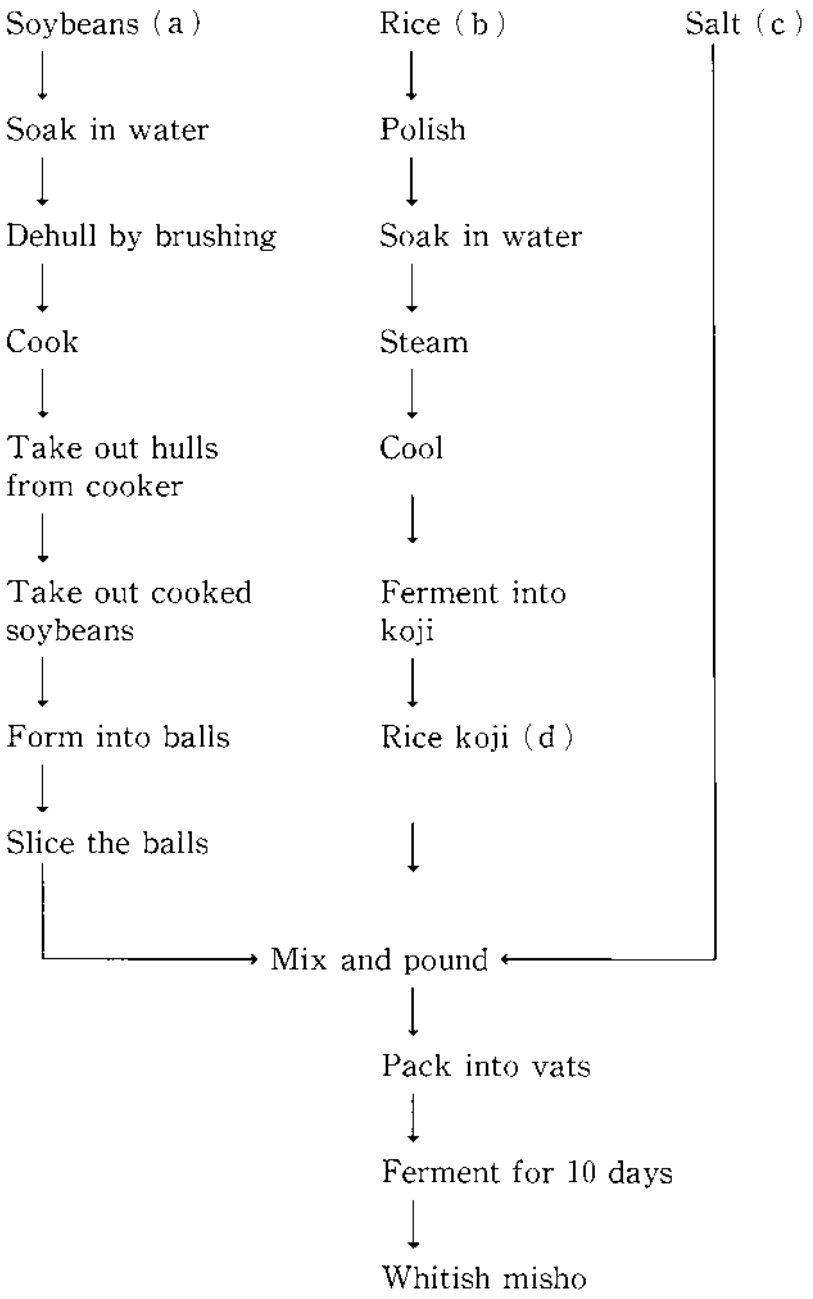

Figure 2 Flowchart for preparation of whitish misho: (a) 10 shous, (b) 14 shous, (c) 1.3 shous, (d) 16 shous. (From *Wakansansaizue*. Vol. 105, 1712.)

As seen in Fig. 2, the process of making whitish misho is as follows: Ten shou (18 L) of soybeans are soaked in water, brushed to take off the hulls, and cooked in three volumes of water. The floated hulls are removed and cooked soybeans are pounded and made into large balls and sliced. The sliced materials are mixed with 16 shous (28.8 L) of rice koji and 13 shous (23.4 L) of salt for brine ripening for 10 days in winter and spring, whereas 15 shous (27.0 L) of salt is necessary for 4–5 days ripening in summer and autumn.

IV. INDUSTRIAL/COMMERCIAL PRODUCTION TODAY

A. Ingredients (7)

1. Soybeans

Yellow soybeans rich in protein are generally used. Japanese domestic soybeans are better suited for making miso than imported soybeans. Suitable soybeans are soft, smooth, and sweet when cooked. Soybeans with a seed coat and hilum of light yellow are particularly ideal for making white or light yellow miso (8). Soybean varieties of higher-water-absorbing and higher-water-holding capacity, higher carbohydrate, and lower oil and calcium content can also be used (Fig. 3).

Dehulled soybeans or soybean grits are often used for making white or light-colored miso (9). Defatted soybeans are not suitable for miso preparation because they give poor color and texture to the final product (10).

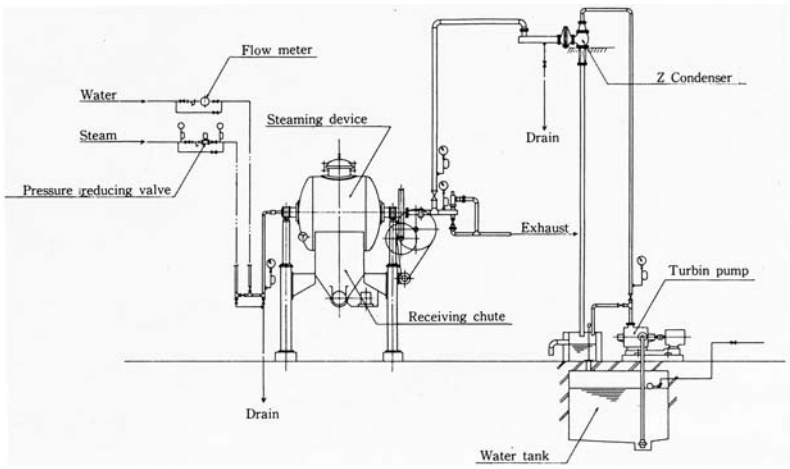

Figure 3 Soybean cooker with vacuum cooling equipment. (Courtesy of Fujiwara Techno-art Co. Ltd.)

2. Rice

Although there are many varieties of rice, Japonica type, irrigated type, and nonglutinous type are generally suitable for miso making. Nonglutinous polished rice (milling ratio 92% or less) is used for making rice koji. The adaptable conditions as ingredient of miso are (a) the sizes of granules are uniform, (b) containing less broken, damaged, unripend rice and foreign matter, (c) non-off-flavored rice, (d) pH value of more than 5.5, (e) easy koji making, and (f) high-quality koji with strong proteolytic and amylolytic enzymatic activity.

3. Barley

Barley is traditionally used for making miso at the farmhouse for personal use. This is often called "rural miso." Domestic barley is generally more suitable for making miso than imported barley. The milling ratio of barley is 65–70%.

Because barley is rich in glutamic acid and aromatic compounds such as ferulic acid and vanillic acid, barley miso is generally characteristic in flavor. Barley of glossy pale yellow and attractive aroma with less seed coat is suitable as an ingredient.

4. Salt

Although crude salt was used conventionally, most factories at present are employing semirefined salt (nami-en, more than 95% purity) produced from seawater by an ion-exchange membrane method. Because refined salt gives strong saltiness, processed salt rich in minerals other than sodium chloride is available for miso making. This special salt often reduces the saltiness to some extent and activates the miso fermentation. However, the effects of special or crude salt have not yet been clarified for all varieties of miso (11).

5. Water

Water of low calcium and iron content is preferable, because calcium often causes hard-cooked beans. The daily use of water at large factories is more than 500 tons and the supply from wells is not always enough. Municipal water is often used in place of well water without any problem.

6. Tane-koji

Tane-koji is olive green in color and consists of the spores of *Aspergillus oryzae* grown on cooked slightly milled brown rice with a small amount of wooden ash. In olden times, a part of well-prepared koji was used as the mold

starter for the next inoculation. This method, however, did not give good results with repeated cultivation. Today, tane-koji is prepared under strict quality control to prevent microbial contamination.

One kilogram of tane-koji is enough to inoculate 1000 kg of rice or barley. There are many varieties of commercial tane-koji, each having different capabilities of hydrolyzing proteins, carbohydrates, and lipids in the raw materials. It is very important to select an appropriate variety for miso production.

Although large soy sauce producers make tane-koji themselves to yield the characteristic flavor and aroma of their own products, miso makers do not produce tane-koji for their use. For salty rice miso rich in protein, tane-koji of high proteolytic activity is suitable, whereas for sweet rice miso rich in starch, tane-koji of high amylolytic activity is preferable. Koji making without the present level of tane-koji was one of the most difficult processes of miso making in ancient times. Currently, it is rare to make koji without tane-koji even on the village scale or in farmhouse production.

7. Starter of Yeasts and Lactic Acid Bacteria

Well-fermented miso requires the growth of favorable micro-organisms such as halophilic yeasts and lactic acid bacteria. In the traditional or indigenous method, soundly fermented miso (tane-miso) was often added at a concentration of 10% of the young miso as the source of these micro-organisms. Since 1955, inocula of yeasts and lactic acid bacteria separately or in combination have been used as starter to accelerate the fermentation. Halophilic yeasts, *Zygosaccharomyces rouxii* and *Candida versatilis*, and halophilic lactic acid bacteria, *Tetragenococcus halophilus*, are used. One liter of the culture is added for 1 ton of young miso. These starters are prepared by large-scale miso factories or supplied by laboratories under miso industrial cooperatives or tane-koji makers.

B. Soybean Processing

1. Screening and Cleaning

A vibrating screen cleaner is used for whole soybeans to remove foreign materials such as stones, splits, and so on. It has two slanting screen trays capable of vibrating. Normal size beans roll off the end of the first screen of small mesh to be fed onto the second screen, which passes only the normal size to a hopper. A few factories employ a spectrograph sorting machine which detects dark-colored soybeans or pebbles, which are removed and discarded automatically.

The screened soybeans are cleaned with a scrubber/grinder to polish and remove the dust from the hulls. The grinder also can be used as a dehulling machine. Another type of dehulling machine is a roller-type crusher, which can be used for partially dehydrated soybeans with moisture level less than 13%.

2. Washing and Soaking

The cleaned soybeans are washed with a stream of water in a washer with a screw conveyor and pumped up to a soaking tank. The soaked soybeans are transferred to a pressure cooker through a pipe by gravity or pump.

3. Cooking and Cooling

After draining, the beans are cooked with a soybean cooker. The most popular cooker is the batch-type rotary (see Fig. 3) or fixed-pressure cooker, which is capable of raising the internal pressure to 2 kg/cm^2 with steam supplied through pipes from the bottom and the upper side of the cooker (Fig. 4).

Cooking conditions, including temperature of water or steam and duration of cooking, vary to some extent depending on the type of miso. For example, soybeans are cooked under a pressure of 0.8 kg/cm^2 for 60–90 min for ordinary salty rice miso. For light-colored miso, the beans are first cooked in water at 100°C for 30–60 min, and then the water is drained. The precooked beans are steamed under a pressure of 0.75 kg/cm^2 for about 25 min. Finally, the pressure is reduced by opening the valves. Immediately after exhausting the steam is completed, the temperature of the materials is lowered rapidly by reducing the inner pressure as low as possible with a jet condenser. When the temperature comes down to approximately 50°C, the cooker is opened and the beans are cooled on a continuous cooler which consists of a stainless-steel mesh conveyor about 90 cm wide and 6 m long (see Fig. 5).

A continuous pressure cooker is a large cylindrical tube with two rotary valves: one located at the inlet and the other at the outlet to keep the inner pressure to a certain level above atmospheric pressure during cooking. The beans, loaded onto the stainless-steel mesh conveyor, are transferred at a slow speed to the outlet under steam pressure. The beans are cooked at 1.3–2.0 kg/cm^2 for 2–7 min. The continuous soybean cookers are employed by a few large plants.

4. Grinding (Mashing) and Extruding

The cooked beans are ground with a large metal meat chopper about 30 cm in diameter and 90 cm long. The machine extrudes the ground soybeans through holes 5 mm in diameter in the cutting plate.

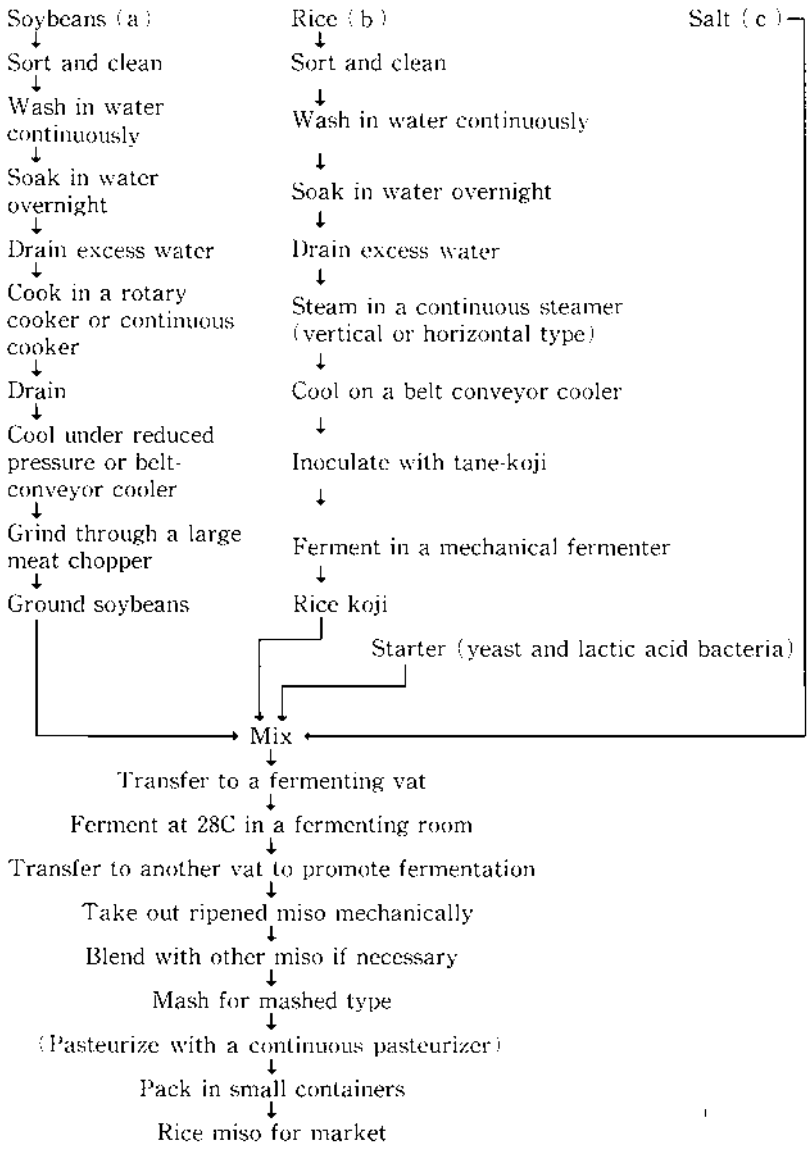

Figure 4 Flowchart of industrial/commercial production of rice miso today: (a) 10 tons; (b) 7 tons, (c) 4.2 tons. (Courtesy of the Central Miso Research Institute.)

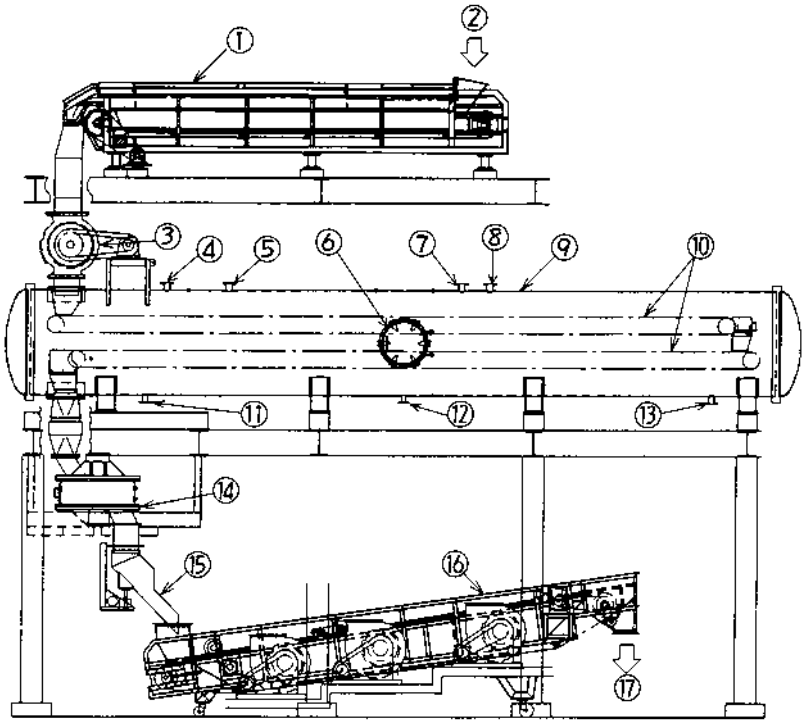

Figure 5 Continuous soybean cooker: (1) preheating screw, (2) raw material feeding hopper, (3) raw material feeding rotary valve (horizontal type), (4) equal-pressure piping, (5) steam inlet port, (6) sight (inspection) glass; (7) safety valve fitting nozzle, (8) steam feeding port, (9) steaming (cooking) equipment, (10) net conveyer, (11) drain nipple, (12) drain nipple, (13) Drain nipple, (14) cooked materials dicharging rotary valve (vertical type), (15) chute (16), cooling machine, and (17) cooled materials discharging chute. (Courtesy of Fujiwara Techno-art Co. Ltd.)

C. Koji Making

1. Screening (Sorting) and Cleaning

It is rare to start with brown rice in today's miso factories. Usually, milled and cleaned rice is supplied as it is for direct use. To minimize water pollution from the wash water, rice cleaning by brushing is carried out in some factories.

2. Washing and Soaking

Cleaned rice is washed in water with a bean washer equipped with a screw conveyor and soaked overnight, usually at about 150°C in a soaking vat.

3. Steaming

After draining for 1 h, rice is steamed in a specially designed cooker. Two continuous types are widely used. One is a horizontal belt conveyor about 90 cm wide and 7 m long made of a fine-mesh stainless-steel net which moves in a loop inside the machine (see Fig. 6). Moistened rice loaded on the conveyor belt is steamed through a steam conditioner injected into the spaces under the moving belt of the cooker. The temperature of the steam is adjustable. Rice is often steamed twice depending on its cooking quality. After the first steaming, a small amount of water is often sprayed to adjust the moisture to a favorable level for growing mold.

A vertical-type steamer is also employed at large factories. It is simply composed of a cylindrical steel body (900 mm in diameter) jacketed to heat with steam during steaming. Steam injectors are located at the bottom and upper sides. Moistened rice loaded through a stainless-steel pipe from the soaking tank upstairs is steamed in the cooker and unloaded from the bottom onto the cooling belt conveyor. The belt is composed of stainless-steel mesh and steamed rice is cooled by a stream of air blown so as to pass through the steamed rice layer. The rice is cooled to 35°C, inoculated with tane-koji, and transferred into a koji fermenter by belt or penumatic conveyors.

4. Koji Fermentation (Koji Making)

Although there are many types of koji fermenter, the following two types are most popular. One is an incubation chamber (about $9 \times 15 \times 2$ m). The wall of the chamber is well insulated and lined with stainless-steel plates. The floor is composed of stainless-steel plates with many perforations through which

Figure 6 Steel-belt-type continuous rice steaming equipment. (Courtesy of Fujiwara Techno-art Co. Ltd.)

air is supplied for the layer of koji. The inoculated rice is spread over the bed to ferment at about 30°C. The temperature, relative humidity, and oxygen content of the supplied air are automatically controlled with an air conditioner outside of the chamber. During fermentation, rice koji is turned and stirred twice with a turning machine that has many spikes mounted on a horizontally mounted rotating spindle moving on two rails fixed to both sides of the chamber.

In large factories, a rotary-disk-type automatic koji fermenter has been employed (see Figs. 7 and 8). The principle of fermenting the koji by supplying air is almost the same as in the incubating chamber method. The major difference is that the koji holding bed is replaced with a rotating cylindrical bed, and the movable turning and stirring machine is also replaced with a fixed turning machine.

5. Mixing Soybeans, Koji, Salt, and Inoculum

In small factories, these ingredients are mixed in a small cylindrical mixer with rotating spikes or in a rotating spindle tank capable of holding 2 tons. In large factories (see Fig. 9), automatic continuous mixing machines are employed for mixing the ingredients In this process, automatically weighed soybeans, koji, and salts (or a mixture of koji and salts) are continuously loaded by belt conveyors or pipes into a mixing machine with rotating spikes like a concrete mixer. The entire process, including weighing materials, determining the

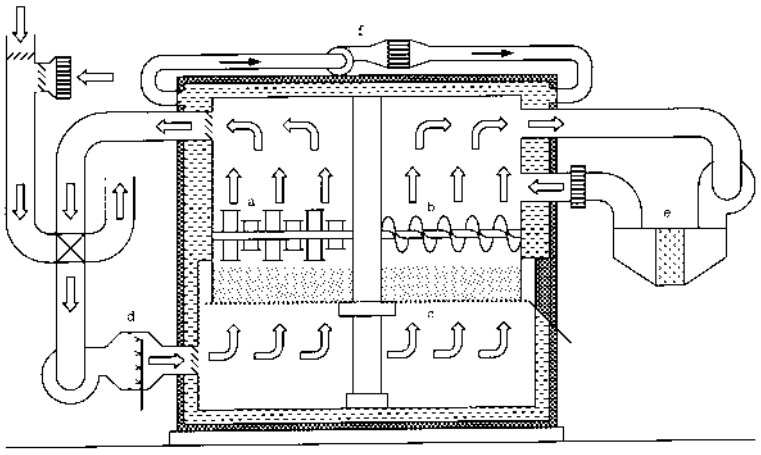

Figure 7 Rotary-disk-type automatic fermenter: (a) stirring equipment, (b) screw transfer, (c) rotary bed, (d) air conditioner for humidification, (e) air conditioner for dehumidification, and (f) dew protecter (by hot-air circulation). (Courtesy of Nagata Brewing Machinery Co. Ltd.)

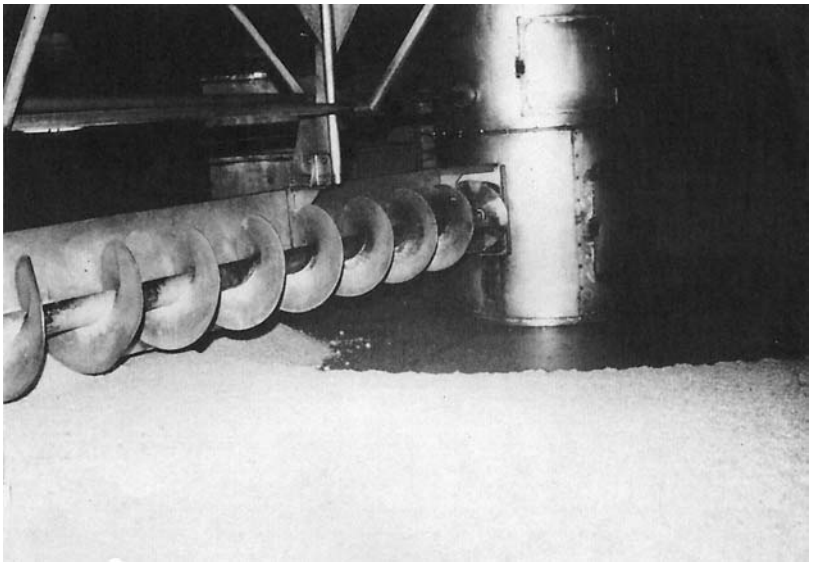

Figure 8 Rotary-disk-type solid-state automatic fermenter with a rotary bed and a transfer screw machine. (Courtesy of Fujiwara Techno-art Co. Ltd.)

duration of stirring with an inoculum, and determining salt content in the mixed materials, is computerized. When mixed homogeneously after a certain time, the materials are extruded through a grinder similar to a large meat chopper. The ground young miso is transferred by belt conveyor into vats to be fermented naturally at ambient temperature or to a fermenting chamber. Traditionally, wooden vats were widely employed and they are seen in small factories for natural fermentation. Recently, they have been replaced with stainless-steel or glass-lined resin vats or tanks with a capacity of 1–100 tons. Weights equivalent to 5–10% of the total miso are placed on top covered with a resin sheet. The ground young miso is packed into a fermenting vat on a platform truck or a fork-lift truck to be moved to a fermenting chamber.

6. Brine Fermentation

The young miso is usually fermented in a fermenting chamber, the temperature of which is controlled to 25–30 °C to accelerate the process (see Fig. 10). During fermentation, miso is transferred from the original vat to another to keep the fermentation homogeneously. For this purpose, one vat is turned over the other vat with a fork-lift truck or turning machine (see Figs. 11 and 12).

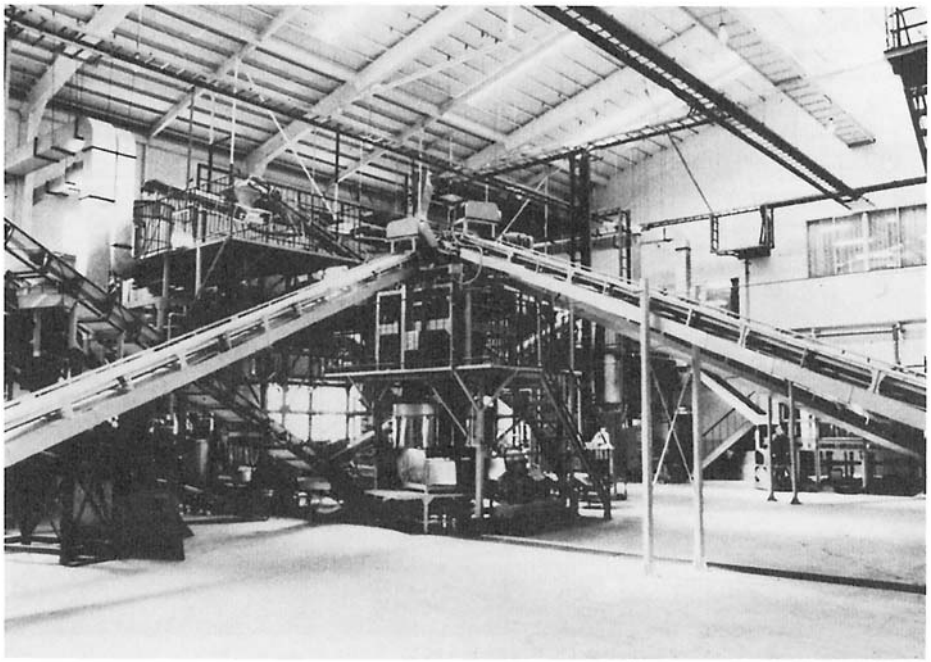

Figure 9 Mixing place of cooked soybeans, koji, and salt with the automatic mixer in the central part. (Courtesy of Miyasaka Jozo Co. Ltd.)

Fermented and ripened miso is then blended, if necessary, in a blending tank and ground and extruded through a grinder with a cutter plate having many perforations of 1 mm in diameter or less.

7. Pasteurization (Figure 13)

In order to prevent the refermentation of packaged miso resulting in swelling, packaged miso was often pasteurized by simply submerging it in hot water. A large pasteurizer of horizontally mounted metal tubes about 30 cm in diameter and 5 m in length is used in large factories (12,13). Hot water is circulated around many pipes in the jacket to heat the miso in the pipes. Miso loaded from the hopper of the heating part is moved in the pipes by pressure (7 kg/cm^2) to be heated up to 83°C and kept for several minutes in the central part of the machine to make sure that the heating is effective. Thereafter, pasteurized miso is pressed successively into the pipes in the cooling part to be cooled with circulating water.

Figure 10 Fermenting room. (From Ref. 10.)

8. Packaging

Miso is packed in resin bags or resin cups of 500 g or 1 kg for market. At large factories, automatic packaging machines are used.

V. CONTRAST BETWEEN INDIGENOUS AND MODERN PROCESSING

Generally, the difference between indigenous miso manufacturing and modern miso manufacturing is the scale of production. The annual production of the former is generally less than 200 tons and the latter is 1000 tons or more. There are, however, many exceptions. Some factories are highly modernized regardless of the small production, and others are deliberately retaining traditional processing. For example, manual koji-making methods are still employed at modern factories even though other processing steps have been remarkably modernized.

Figure 11 Fermenting room. Fermenting vats with capacity of 2 tons of miso are taken on racks in a fermenting room. (Courtesy of Sendai Miso Shoyu Co. Ltd.)

Figure 12 Turning a vat of miso (2 tons) transferred from the fermenting room with a fork-lift truck. (Coutesy of Sendai Miso Shoyu Co. Ltd.)

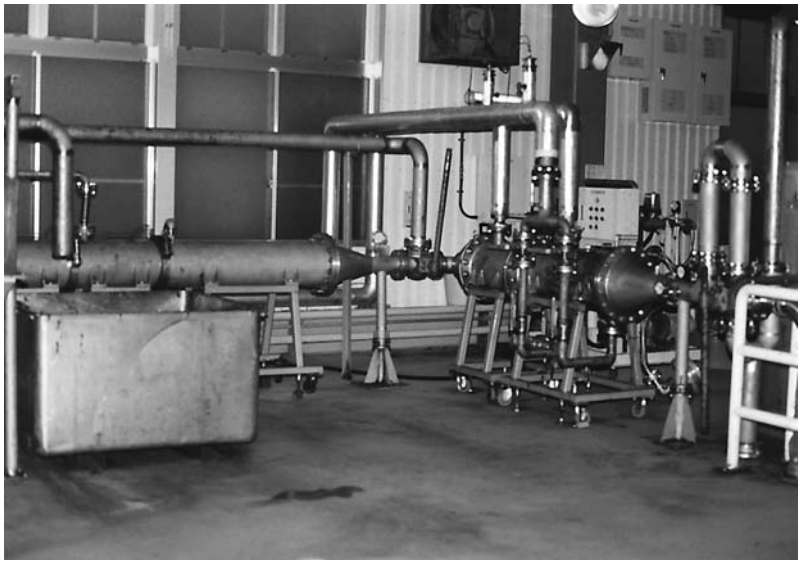

Figure 13 Pasteurization of miso with a continuous pasteurizer. (Courtesy of Sendai Miso Shoyu Co. Ltd.)

A. Ingredients

1. Modern Processing

Large-scale factories require a constant supply of large amounts of ingredients at a certain level of quality and at a reasonable cost throughout the year. For this reason, domestic ingredients are not always suitable and imported ingredients such as soybeans from China and barley from Canada and Australia are often used.

B. Sorting

Indigenous processing: Rice and soybeans are run through a winnowing machine to remove any chaff, straw, or other debris.

Modern processing: Soybeans are sorted and cleaned with a vibrating screen cleaner and a spectrograph sorting machine. For light-colored miso, soybeans are often dehulled with the cleaner. Milled and polished rice is used.

C. Washing and Soaking

Indigenous processing: Rice is washed in a washing keg with a stirring tool by hand, rinsed in baskets with water, and soaked in a soaking tub overnight.

The next morning, the soaked rice is drained by pulling a plug in the bottom of the tub. Soybeans are treated with the same tools and procedure as rice.

Modern processing: Rice and soybeans are washed in washers separately and transferred to the soaking tank mechanically.

D. Steaming and Cooling Rice

Indigenous processing: Soaked and drained rice is steamed in a wooden steamer ("koshiki"), placed on a cauldron, and cooled on cloth or canvas in a mixing box while being stirred and crumbled with spades.

Modern processing: Rice is steamed with a horizontal- or vertical-type continuous steamer and cooled continuously with a conveyor belt cooler as shown in Fig. 6.

E. Inoculation

Indigenous processing: When cooled to 35°C, tane-koji (0.1% of the raw rice) mixed with cooked rice in a koji tray is scattered over the cooled rice and mixed well using hands.

Modern processing: Crumbled rice cooled to 35°C with a cooling machine is inoculated and mixed thoroughly with tane-koji by means of an inoculating machine connected with the cooling machine.

F. Koji Making (Incubation)

Indigenous processing: Koji making is the most important and difficult process in miso making. Inoculated rice is transferred into a germinating crib in a koji chamber (see Fig. 14) which is warmed with charcoal or other fuel in winter. The next morning, when the temperature of the young koji is raised, it is turned over to crumble the lumps. After 2–3 h, about 1.5–3 kg of the young koji is transferred onto many koji trays to establish aerobic conditions for the growth of the mold. During fermentation, young miso koji is stirred twice by hand when the temperature rises to around 40°C. About 40 h after the inoculation, koji trays are taken out of the koji chamber.

Modern processing: Koji making is carried out in a koji fermenter operated almost automatically, as seen in Figs. 10 and 11.

G. Soybean Cooking

Indigenous processing: Soaked and drained soybeans are cooked in a steamer on a cauldron under atmospheric pressure for 5–6 h and often kept a few hours longer or overnight without strong heating. Cooked soybeans are

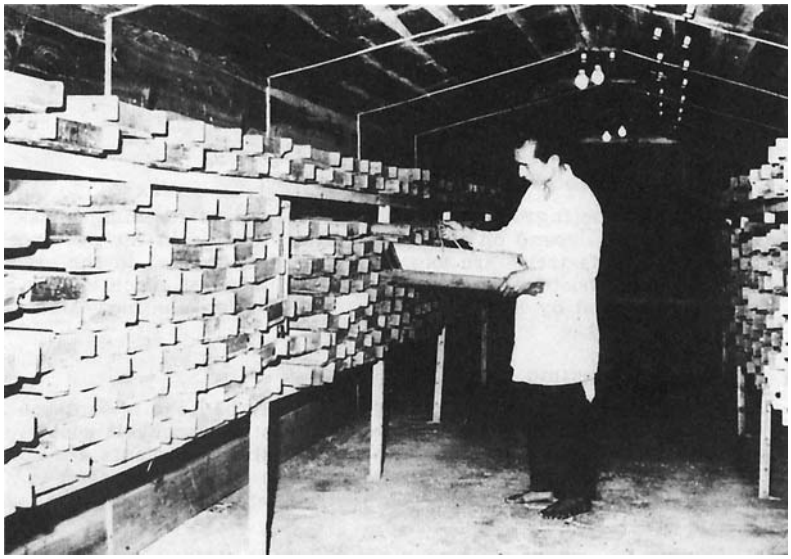

Figure 14 Koji chamber for indigenous miso manufacture. A craftsman is measuring the temperature of koji on a koji tray. (From Ref. 10.)

transferred into small kegs or molded into a sort of miso-dama and allowed to cool naturally, as seen in Figure 15.

Modern processing: Soaked soybeans are cooked in a pressure cooker for a shorter time. Cooked soybeans are cooled in the tightly closed cooker under reduced pressure and/or on a continuous belt cooler. Cooked soybeans are often ground and extruded through a machine while warm.

H. Mixing

Indigenous processing: Cooked soybeans, salted koji, seed miso, and a small amount of mixing water are mixed in a mixing tub and trodden underfoot with rice straw sandals. Mixed and partially mashed materials are carried to a wooden fermenting vat by craftsman.

Modern processing: Cooked and extruded soybeans, koji, salts (or salted koji), and mixing liquid containing a pure culture of lactic acid bacteria and/or yeast are mixed in a mechanized mixer. Mixed materials are transferred to fermenting vats or tanks by conveyor belt or packed into fermenting tanks on a fork-lift which conveys the tanks to a fermenting chamber.

Figure 15 Cooling of cooked soybeans molded into large miso-dama for cooling. (Courtesy of Central Miso Research Institute.)

I. Fermentation

Indigenous processing: Fermentation is carried out under natural conditions for 10 months or more. During fermentation, miso is turned and transferred to another vat with spades by craftsmen. All transference of miso is done by manpower with spades and kegs.

Modern processing: Fermentation is carried out in a fermentation tank placed in a chamber with a controlled temperature favorable to fermentation. Turning over or transference of the miso is performed mechanically.

J. Preparation for Marketing

Indigenous processing: Most miso is not ground before packing in kegs for marketing.

Modern processing: Miso is blended if necessary prior to grinding with an extruder and often pasteurized mechanically to prevent swelling (12). Finally, 300–1000 g of miso is packed in a small resin container. When pasteurization is not practiced, ethyl alcohol is mixed to a level of 2%, including alcohol produced by natural fermentation.

Over the past 40 years, modernization has been responsible for saving labor, shortening the fermenting period, leveling up productivity, establishing hygienic standards, and reducing production costs. However, there remains the criticism that modernization of the process could cause the loss of the characteristic flavor and aroma possessed by the traditional, indigenous miso.

VI. CRITICAL STEPS IN MANUFACTURE/FERMENTATION

Although remarkable improvements in the processing of miso have taken place since 1955, there are some critical steps. Soybean cooking is undertaken in steam and/or boiling water with a pressure cooker. For making light-colored miso, the beans are cooked in water using four times as much water as beans. This method, however, causes a loss of water-soluble matter of 20% or more of the solid in soybeans, resulting in water pollution. Because the BOD (biochemical oxygen demand) of the waste is 30,000 ppm or more, a large treating tank is required, as well as powerful air blowers and substantial electric power. For the improvement of this cooking method, high-temperature, short-term steaming with a continuous cooker with rotary valves has been introduced in large factories. However, this method does not always give satisfactory results. Koji making, even with a mechanized koji fermenter, takes about 40 h postinoculation. A computerized koji fermenter is now operating automatically. However, it is not successful in reducing the fermenting time. As a result of basic research, it is possible to reduce the fermenting time by employing partially germinated spores of tane-koji and by providing favorable conditions for the growth of the mold, including nutrients, temperature, and humidity.

Traditionally, the transference of miso was carried out with spades and kegs by craftsmen. In modernized factories, these have been replaced with conveyor belts and vats on lift trucks. However, these are not satisfactory from the viewpoint of food hygiene and labor efficiency. Transference by pipe and pump is expected to be applied. However, in this case, there is the problem of transferring the miso without it becoming sticky.

VII. MAJOR PROBLEMS IN THE INDUSTRIALIZATION

Production at miso factories ranges widely up to 40,000 tons a year. As seen in Table 2, the amount of miso production per worker has increased remarkably from 1965 to 1997 due to mechanized equipment. Furthermore, the productivity per worker in large factories is far higher than that in small factories. Modernization of the process improved not only the productivity but also the hygienic conditions, production costs, and quality control of the products to a

Table 2 Changes in the Productivity of Miso Factories Between 1961 and 1999 (Factories Grouped by Production Capacity)

		1965		1997	
Rank	Capacity (tons/year)	Tons[a]	Tons/man[b]	Tons[a]	Tons/man[b]
A	1–375	116,340	10.6	59,147	18.4
B	376–1125	102,231	28.4	46,846	44.3
C	1126–1875	55,042	28.4	38,258	61.2
D	1876–3750	95,592	39.9	43,800	56.5
E	> 3750	166,597	90.6	376,169	157.2

[a] Total production.
[b] Production per worker.
Source: Food Agency. Ministry of Agriculture, Forestry and Fishery, Japan.

certain level. Nevertheless, there is still the criticism that the flavor and aroma of the miso produced at the highly automated, computerized factories are rather flat and featureless compared to those of the well-fermented and ripened indigenous miso. This situation resembles the relationship between cheese made at modernized plants and farm-made cheese. The problem seems very difficult to solve because the roles of many varieties of micro-organisms during the fermentation of miso have not yet been determined thoroughly. As stated earlier, the indigenous process of koji preparation in small-scale factories is a manual production with koji trays in a koji chamber which provides suitable conditions for the aerobic growth of the mold. On the other hand, bulk production at a depth of 30 cm or more does not always seem to establish the best condition for the growth of the mold or for production of necessary enzymes and favorable substances for miso fermentation. Indigenous processing makes use of old wooden vats which provide the seed of characteristic micro-organisms for the unfermented miso and moderate change of temperature during fermentation.

Preservation of packaged miso is a new problem caused by the introduction of packaging the product in a small, sealed container. Miso is generally prone to oxidation by the air, resulting in browning and, ultimately, an off-flavor. This was not a problem when miso was dealt with as an indigenous product in a small keg or jar during distribution and storage. The oxidation is basically promoted by labile such as phenolic compounds, amino acids and reducing sugars, oxygen in the air, ambient temperature, cupric and ferric ions as contaminants, and the enzymes of the mold (*A. oryzae*). Viable micro-organisms such as yeast and lactic acid bacteria protect the miso from browning to some extent. So far, employment of plastic films as an oxygen barrier in packaging and the addition of an oxygen-absorbant

ferrous compound in the inner space is effective in preventing browning to some extent. Because this concerns the shelf life of the product, it is a very important problem.

Another problem arose after the introduction of small, sealed packages. Swelling of packaged miso is caused by refermentation by the remaining viable yeasts. To protect against swelling, chemical and/or physical treatments have been applied. Although the use of sorbic acid is permitted by food hygiene law, it is not welcomed by the consumer because it is a synthetic product. Instead, ethyl alcohol is widely used at a concentration of 2%, which includes the alcohol already present from the fermentation. Pasteurization is also employed for this purpose. Recently, there was criticism that heat treatment caused some degree of browning and spoiled the characteristics of raw miso, including the micro-organisms and their enzymes.

The waste from miso factories is usually rich in organic compounds, which increase the BOD value to 3000 ppm or more. It is generally treated biologically by employing activated sludge. The main process which produces the heavy load of BOD is the washing, soaking, and cooking of soybeans or rice. Specifically, the BOD of the waste from soybean cookers is 30,000 ppm or more, contributing the greatest amount of the waste. As a result of investigations into mechanical, chemical, and biochemical methods, biological treatment is generally employed. However, the amount of electric power for the continuous aeration amounts to half of the total factory energy consumption. This is an important economic problem. One solution is to find a new way to utilize the waste as a resource. An investigation was carried out to attempt to utilize the waste from the soybean cooker as an ingredient for the submerged culture of *A. oryzae* and then return the mycelium to miso to promote fermentation and improve the flavor of miso (13).

Miso is generally used as an ingredient of miso soup with other foods in Japan. To promote the consumption of miso, the development of new types of miso suitable for use in other ways in today's food culture is of primary importance for the miso industry. For example, miso in a dehydrated or liquid form convenient for use in the kitchen or at the table seems attractive because of the ease of handling. Low-salt, bright-color, or odorless miso would also have expanded uses.

VIII. OPTIMAL ENVIRONMENTAL CONDITIONS FOR MICROORGANISMS

A. Koji Making (14)

Aspergillus oryzae, the principal mold for miso-koji making, grows well around 30°C. Basic research has shown that the optimal conditions for the

germination of tane-koji are temperature at 36°C, relative humidity at 97% or more, 0.1% carbon dioxide, and 20% oxygen. The optimal conditions for mycelial growth are temperature at 35–37°C, relative humidity at 75% or more, < 1% carbon dioxide, and 20% oxygen. Practically, however, lower temperature (28–30°C) is used for germination of the koji mold and 30–35°C for cultivation in a koji chamber. The optimal moisture level of cooked rice is 36–38% and the optimal pH is 6.0–6.4. For the production of enzymes, the following temperatures are desirable: glucoamylase 30°C, (α)-amylase 35°C, protease 25–30°C, and carboxypeptidase 35°C. For the production of slightly acid and alkaline protease beneficial to miso fermentation, the pH should be around 6. The addition of sodium glutamate or sodium succinate is effective for maintaining the pH at 6–7 during fermentation, resulting in the production of higher protease activity.

B. Brine Fermentation of Miso (15)

The following conditions are optimal for the growth of *Zygosaccharomyces rouxii*: temperature 30°C, pH 3.5–5.0; for *Candida versatilis*, they are temperature 25–35°C, pH 4.0–4.5, and for *Pediococcus halophilus*, they are temperature 30°C and pH 7.0 (10).

IX. ESSENTIAL MICROORGANISMS FOR FERMENTATION

A. Mold

Aspergillus oryzae is an indispensable mold for making miso-koji (14). It is used as the inoculum for making tane-koji. Tane-koji is olive green in color and consists of the spores of the mold cultivated on the cooked brown rice with small amounts of hardwood ash. One kilogram of tane-koji is enough to inoculate 1000 kg of rice or barley. There are many varieties of commercial tane-koji, each having different capabilities for breaking down proteins, carbohydrates, and lipids in raw materials. It is very important to select the appropriate variety for the type of miso desired. For salty rice miso rich in protein, tane-koji of high proteolytic activity is suitable whereas for sweet rice miso rich in starch, that of high amylolytic activity is preferable.

B. Halophilic Yeast and Lactic Acid Bacteria (15)

Halophilic yeasts, *Z. rouxii* and *C. versatilis*, play a very important role in the fermentation of miso. The former is of primary importance for all varieties of miso except white miso, and the latter is useful for the production of a fully ripened aroma in salty red miso.

Culture media containing 10–12% raw soy sauce, 10% glucose, and 10% sodium chloride are used for the propagation of halophilic yeast. Incubation with aeration is carried out at 30°C until the count of viable yeast reaches the level of 10^8/mL.

As a lactic starter, 1 L of culture media containing 20% raw soy sauce, 2% glucose, and 5% sodium chloride is used. The pH value is maintained at 7 during cultivation. The microbial count reaches the level of 10^9/mL in about 60 h. One liter of culture is used for 1 ton of miso. These pure cultures are supplied by laboratories under the miso industrial cooperative or are commercially available from tane-koji makers.

C. Other Microorganisms

Streptococcus faecalis and *S. faecium* of the Enterococcus group are generally not essential micro-organisms for miso. However, they have behavior of interest. These lactic acid bacteria are often isolated from koji and young miso. The former grows during koji making as a contaminant and often controls the growth of unfavorable *Clostridium* species such as *C. butylicum* and *C. toanum*, which produce butyric acid causing off-flavors (16).

Bacillus subtilis, B. mesentericus, and B. pumilus were isolated from fermented miso (Mogi, private publication, 1976). *B. subtilis* seem to play some role in the ripening of miso, especially salty red miso (17).

X. POSSIBLE SPOILAGE MICROORGANISMS

A. Yeast

Film-forming yeasts such as *Hansenula* sp. and *Z. halomembranis* are detrimental when they grow on the surface of miso giving it a strong unpleasant odor. *Endomycopsis fibuligera* is a contaminant during koji making. It often reduces the activity of amylase and protease of the koji when it grows in large quantities (18,19).

B. Bacteria

Pediococcus acidilactici is one of the most detrimental contaminants during koji making. It is halophilic up to 15% sodium chloride and can survive at pH 4.7 in a medium consisting of miso extract. *T. halophilus* grows and produces lactic acid until the pH falls to 5.1.. Thereafter, *P. acidilactici* has a chance to grow, producing lactic acid and causing acidified miso when the salt level is low. Twelve percent salt in miso or the addition of *Z. rouxii* at a level of millions per gram is effective in controlling this lactic acid bacterium (20).

Bacillus subtilis and *B. pumilus* are also detrimental when they grow in koji. Large quantities of these bacteria result in a disturbance of the mold growth during koji making and fermentation (Mogi, private publication, 1976).

Clostridium perfringens, C. toanum, & C. fluvum are the most deleterious contaminants during koji making (16,20). Because they are salt-resistant up to a 10% salt concentration, they often grow rapidly in miso of a lower salt concentration, resulting in the explosive swelling of miso packages. *S. faecalis* acts to prevent their heavy contamination during koji making.

XI. CHEMICAL/BIOCHEMICAL CHANGES IN MISO DURING FERMENTATION

A. Changes Occurring in the Major Components

Proteins, lipids, and carbohydrates, the major components, are first hydrolyzed by the enzymes of koji to produce primarily amino acids, fatty acids, and sugars (21). Along with the degradation of proteins and polysaccharides, the texture rapidly becomes soft and "tamari" (liquid) is separated on the surface of miso in a tank. The color gradually changes from yellow to red-yellow, according to the Munsell color book, resulting in clear color. The saltiness, which is very strong during the first 3 weeks, becomes weaker and the sugar content ranges from 9.7% to 17.2%.

Soybean proteins are one of the most important components for the formation of flavors of the miso. During miso fermentation, soybean proteins insolubilized by denaturation with cooking are digested by koji enzyme into water-soluble nitrogen. Soybean proteins undergo the digestion within several days of the fermentation and the water-insoluble proteins of the miso are resistant to the further hydrolysis by the koji enzymes (22). Thus, solubilized protein is further digested by proteases to peptides and amino acids at an early stage of fermentation. The protein-solubility index [the ratio of water-soluble nitrogen to total nitrogen %] increases to approximately 60% and the protein-hydrolysis index [the ratio of formol-nitrogen to total nitrogen (%)] increases to 25% in a month at around 30°C after mixing.

During the hydrolysis, many varieties of peptides are produced and further degraded into smaller molecular peptides. Mochizuki et al. (23,24) sorted the peptides by the length of the molecules, as indicated by the APL (average peptide length) (Fig. 16). During fermentation, large APL number generally decreases, with the exception of APL 13–20, which are composed of glutamic acid, aspartic acid, and proline. The change of each amino acid during fermentation is shown in Fig. 17. Arginine is produced rapidly during the early stage of fermentation and decreases 1 month after mixing by the

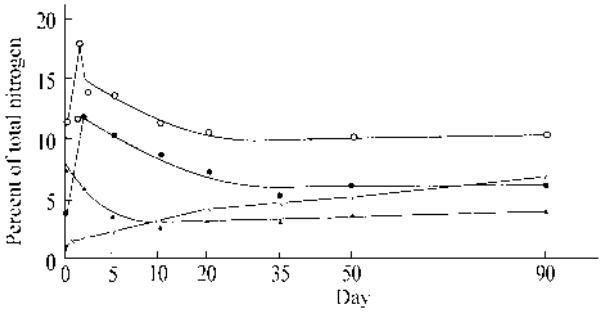

Figure 16 Change of polypeptides during fermentation of miso. (○) APL: 3–8, (●) APL: 3–4, (▲). APL 5, (×). APL: 13–20. (From Ref. 23.)

action of micro-organisms such as lactic acid bacteria (25). The liberation of glutamic acid, which has a meatlike flavor, and aspartic acid gradually progresses to only 10% of the level of total glutamic acid and aspartic acid. The total amount of glutamic acid in the miso fermented for 45 days is 2073 mg/100 g, which includes free-type (20.7%), glutamine (33.4%), and combined type (45.8%) (26,27). Glutamic acid is changed to pyroglutamic acid, which has no meatlike flavor, when fermented at 35°C or more. Because the

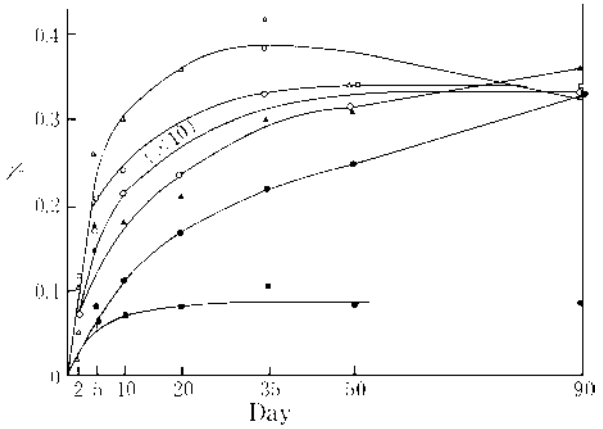

Figure 17 Change of free amino acids during fermentation of miso: (○) total free amino acids, (△) arginine, (□) lysine, (▲) glutamic acid, (●): aspartic acid, and (■) histidine. (From Ref. 27.)

liberation of glutamic acid is slow at 25°C, the formation of pyroglutamic acid is also slow (28). Some amino acids, including leucine, isoleucine, valine, and methionine, are decomposed to alcohols such as amyl alcohol, butyl alcohol, and methionol during fermentation by the yeasts. These alcohols or their ethyl esters give favorable aroma to miso (29).

Because lipid is the major component of soybeans, approximately 20%, its change during fermentation is important, affecting on the formation of flavor and aroma. The level of lipids in miso ranges from 3.0% to 10.5% depending on the amount of soybeans in the ingredients (24). According to Kiuchi et al. (30), the fatty acid composition of ordinary miso was similar to that of soybeans. During fermentation, lipids are hydrolyzed to free fatty acids and glycerol. The content of triglycerides, diglycerides, monoglycerides, and fatty acids in varieties of miso is shown in Table 3. It is noteworthy that the amount of diglyceride and monoglyceride are small in comparison with triglyceride and free fatty acids. Some of the fatty acids are further esterified with ethanol produced by yeasts to form such esters as palmitate, oleinate, and stearate. The average of the esters to fatty acid plus ester is 62.4% (31). These esters are important to the flavor and aroma. The addition of a small amount of lipase (0.3 units/g of *Candida* origin enzyme) in fermenting miso significantly increases the amount of fatty acids and their esters compared to ordinary miso (32).

Recently, it was revealed that the lipolytic enzyme produced by *Aspergillus* and found in miso consisted of three enzymes, L1, L2, and L3, which have different roles in hydrolyzing triglyceride, diglyceride and monoglyceride, respectively, at the first step of the fermentation, and they act as esterase when the amount of ethyl alcohol produced by yeasts increases to some level to form fatty acid ethyl esters (33).

Linoleic acid is often further decomposed by the yeasts (15).

The carbohydrate content of miso ranges from 14.9% to 37.9% (24). The polysaccharides in the substrates are hydrolyzed enzymatically to

Table 3 Glycerides and Fatty Acids of Miso

	TG	DG	MG	Fatty acid
Variety	(% of total lipid)			
Salty rice miso, light yellow	65.8	4.2	1.1	25.6
Salty rice miso, red	60.5	7.7	0.9	32.6
Barley miso	57.3	2.8	0.8	31.9
Soybean miso	43.6	2.0	3.1	47.3

Note: TG, triglyceride; DG, diglyceride; MG, monoglyceride.

oligosaccharides and sugars. The ratio of reducing sugar as glucose to total sugar reaches approximately 75%, the maximum level, in 20 days of fermentation. Thereafter, the level is reduced principally by the metabolism of micro-organisms resulting in the production of flavor and aroma. They are also reduced by an amino-carbonyl reaction causing browning. The predominant sugar in ripened miso is glucose (10–13%) followed by isomaltose, fructose, galactose, galacturonic acid, stachyose, mannose, melibiose, arabinose, and xylose (34).

B. Organic Acids and Flavor Compounds

The major organic acids are given in Table 4 (35). Furthermore, fumaric acid and (α)-ketoglutaric acids are produced during koji fermentation with volatile acids such as acetic and propionic acid (31). During fermentation of rice miso, lactic, acetic, pyroglutamic, succinic, and pyruvic acids increase, whereas citric and malic acids decrease along with the growth of micro-organisms. *P. halophilus* produces acetic, lactic, formic, and succinic acids from citric acid in shoyu mash (36).

During fermentation of miso-dama, the major acid is lactic acid followed by acetic, citric, pyroglutamic, malic, formic, glycolic, and oxalic acid in that order (37) (see Fig. 17).

As the result of fermentation, the titratable acidity increases accompanied by the lowering of the pH value. The change of the two parameters takes place along a certain line specific to the variety of miso. Therefore, these parameters can be used to indicate the approximate degree of ripening (see Fig. 18). In this connection, it is noteworthy that miso has a high buffer activity which maintains a constant pH value when miso is mixed with other foods. The activity differs from variety to variety. Soybean miso has the strongest activity, followed by salty rice miso, barley miso, and sweet rice miso. The activity is increased during fermentation along with the hydrolysis

Table 4 Organic Acids of Miso (mg/g)

Variety	Pyroglutamic acid	Lactic acid	Acetic acid	Formic acid	Malic acid	Citric acid	Succinic acid
Sweet rice miso, white	42	7	10	0	7	53	5
Salty rice miso, light yellow	353	100	79	2	13	360	61
Salty rice miso, red	330	31	40	5	19	257	15
Barley miso, red	329	21	40	5	20	113	48
Soybean miso	816	541	227	29	43	277	23

Source: Ref. 35.

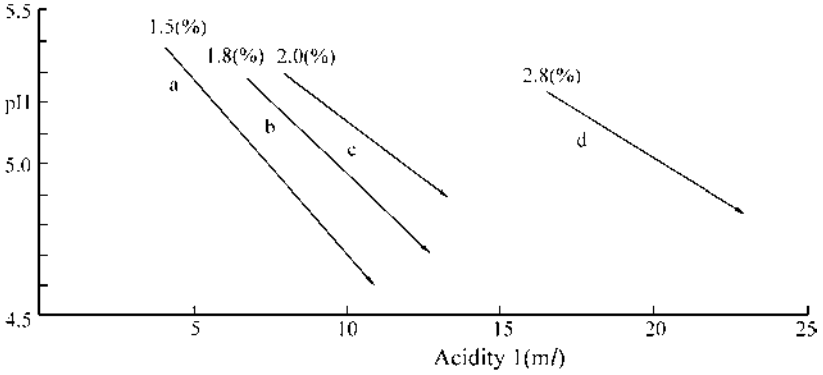

Figure 18 Change of pH and acidity of various misos during fermentation: (a) rice sweet miso and barley miso (T.N. 1.5%), (b) rice salty miso and barley salty miso (T.N. 1.8%) (c) rice salty miso (T.N. 2.0%), and (d) soybean miso (T.N. 2.8%). T.N. = total nitrogen. (From Ref. 38.)

of protein and its derivatives or formation of acid compound such as phosphorus compounds and organic compounds (38).

Ethanol and its esters are the most important aroma components in rice miso as well as barley miso. Isobutyl, butyl, and isoamyl alcohol produced by the yeast fermentation is a characteristic aroma of well-fermented miso.

Glycerol is produced by two ways—from the glycerides in the ingredients by lipase as stated earlier and from sugar by the fermentation of the halophilic yeast. Glycerol has a favorable effect on the flavor of miso. The amount of sugars and alcohols is shown in Table 5. Vanillic acid and ferulic acid are the major components of the characteristic aroma of barley miso (35). Ferulic acid liberated from lignin in the substrates is changed to 4-ethylguaiacol by *Torulopsis,* which gives a characteristic aroma of fully ripened miso (39).

Table 5 Amount of Carbohydrates and Alcohols in Good Quality Miso (%)

Variety	TS	DS	Ethanol	Glycerol
Salty rice miso, light yellow	16.5	13.5	0.48	0.82
Salty rice miso, red	12.8	10.0	0.19	0.95
Barley miso	22.3	16.9	0.06	1.17
Soybean miso	5.8	4.4	0.08	1.49

Note: TS, total sugar including starch and other polysaccharides; DS, direct reducing sugar.

C. Change of Enzymes and Nutritive Value During Fermentation

Enzymes found in the substrate are almost inactivated by cooking at 100°C. The major enzymes are produced and liberated by the koji, which produces 5–10% mycelium. The most important enzymes are the proteolytic and amylolytic enzymes, as well as lipolytic enzyme. In addition, many varieties of enzyme such as cellulase, hemicellulase, pectinase, phosphatase, phytase, esterase, and phospholipase seem to play important roles in the fermentation of miso, although they are not yet clearly understood. These enzymes liberated into miso from the mold mycelium of koji and of halophilic micro-organisms keep their activity for a certain period with gradual decrease or increase.

As stated earlier, because of enzymatic activity of koji, water-soluble components except sodium chloride, account for approximately 30% of salty rice miso. These water-soluble components include free amino acids, peptides, free sugar, and organic acids, which are highly digestible. Amino acid pattern of miso resembles that of soybeans (40). The pattern of fatty acids in lipid also resembles that of soybeans, as mentioned earlier. The peroxide value of fat and oil in fully ripened rice miso is comparatively low (41). The contents of sodium, potasium, and calcium are comparatively high in various types of miso. Because of the high calcium level,miso soup supplies about 25% of the calcium intake for the inhabitants of Tohoku province in Japan (42).

Thirty to fifty percent of thiamine in raw soybeans is lost during soaking and cooking (43). Although riboflavin and vitamin B_{12} are lost in the same way, they are reproduced in light-colored rice miso during fermentation to the level of 0.1mg and 0.1 (μg) g per 100 g respectively (24,44).

Phytic acid in soybeans and cereals is an antinutrient. It is easily binds to form stable complexes with nutritionally importamt minerals such as calcium, copper, iron, and zinc resulting in their insolubilization and reduced bioavailability (45). Phytic acid, however, is hydrolyzed by mold phytase producing mioinositol and phosphoric acid with no binding activity for minerals during fermentation (46). The ratio of inorganic P in total P of rice-koji miso, barley-koji, and soybean-koji miso are 88%, 94%, and 95% respectively (47).

D. Formation of Novel Flavorous Furanone

Recently, furanone compounds including 4-hydroxy-2(or 5)-ethyl-5(or 2) methyl-3(2*H*)-furanone (HEMF) and 4-hydroxy-2,5 dimethyl-3(2*H*)-furanone (HDMF) are seen as novel flavor compounds of miso (see Fig. 19). The former is especially interesting from the practical application in miso

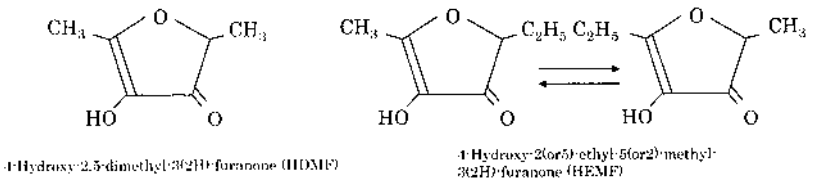

Figure 19 Molecular structures of 4-hydroxy-3(2*H*)-furanone (HDMF and HEMF). (From Ref. 48.)

production. HEMF was isolated and identified from shoyu (soy sauce) for the first time and investigated as a characteristic aroma of shoyu (49,50). It is worth noting that it has a very low sweet aroma threshold in water (0.02 mg/L) (51). It was also isolated later from rice-miso, employing a porous polymer, Tenax GC, for adsorption of the compound from miso suspension in water (52).

The sweet aroma of HEMF is important for the sensory evaluation of the aroma of rice miso. The addition of HEMF to the miso suspension of lower HEMF concentration yields a stronger misolike aroma. HEMF is considered to be produced by the halophilic yeast (*Z. rouxii*) added as the starter to young miso during brine fermentation, especially when the pH value fall below 5.6 (52–55).

The mechanism of the formation of the furanone in miso including HEMF and HDMF was intensively investigated by Hayashida et al. (48,56,57). According to the resultant hypothetical biosynthesis pathway of the furanone compounds, specific precursors are must be produced by halophilic yeast (Fig. 20). They are produced by a Maillard reaction among protein, carbohydrate, and their hydrolysates, which are rich in miso. For example, HDMF is produced from the precursor formed between hexose and amino acid, whereas HEMF is formed from the precursor between pentose and amino acid. On the other hand, Sasaki reported that HEMF was produced by yeast fermentation in a simple media based on shoyu-koji extract with added ribose 5-phosphate, the combination of xylulose 5-phosphate and ribose 5-phosphate, and also sedoheptulose 7-phophate and sugested that HEMF was formed by the yeast via the pentose–phosphate cycle (58,59).

For industrial production of miso, enhancement of the furanone compounds is affected by the yeast activation in miso and the amount of precursors originating in soybeans and rice or barley used as ingredients. For example, the contents of sugar and amino acid are varied by changing the ratio of soybean and rice in the substrate. They are also changed by the

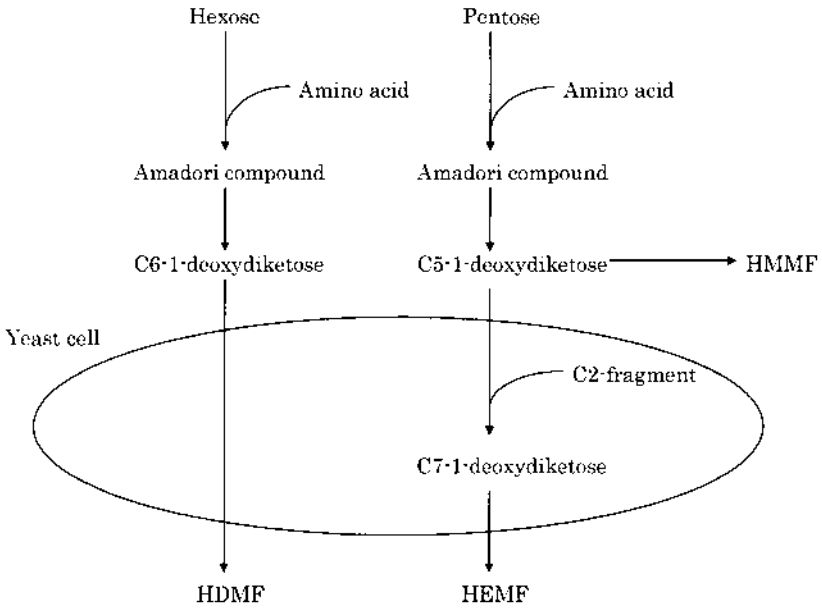

Figure 20 Hypothetical biosynthesis pathway of furanone compounds from hexose and pentose sugar by yeast. (From Ref. 48.)

enzymatic activity to produce precursor from the ingredients. Young miso, a mixture of cooked soybeans, rice or barley koji and salt is allowed to ripen, and after a certain period, it is mixed thoroughly to accelerate the fermentation. Although the optimum period differs from variety to variety of miso, a comparatively short term is necessary for the formation of the precursor for production of HEMF. Furanone compounds as HEMF and HDMF are revealed not only as important flavors and aromas of paratable miso and shoyu, but also as useful compounds for anti-oxidative and anti-tumor activity (60).

E. Isoflavones

Soybeans are a unique dietary source of isoflavones including daidzein, genistein, glycitein, and their glycosides. Miso has been indispensable for Japanese foods as nutritious multipurpose seasonings and has many physiological functions handed down from generation to generation which supported their daily life. Recently, the effects of miso were clarified by scientific studies.

According to Hasler (61a), soybeans have been in the spotlight during the 1990s. Not only is soybean a high-quality protein as reported by many articles, it is now thought to play preventive and therapeutic roles in cardiovascular disease, cancer, osteoporosis, and the alleviation of menopausal symptoms. The chemopreventive and chemotherapeutic effects of isoflavones in miso have led to "the change of physiological function of miso."

Regarding the change of isoflavones in misos, they are hydrolyzed by β-glucosidase to aglycones during fermentation. As seen in Table 6, the amount of isoflavones including daidzin and genistin and their aglycones in commercial matured miso varied from variety to variety, headed by soybean-koji miso, followed by barley-koji miso, and rice-koji miso. The ratio of aglycone to total isoflavone in matured miso is also headed by soybean-koji miso, barley-koji miso, and rice-koji miso in this order. Kudou et al. also examined the isoflavones in commercial miso. Daidzin and genistin in soybean (koji) miso were completely hydrolyzed into daidzein and genistein, whereas in sweet rice (koji) miso, they were not hydrolyzed. The isoflavones in the semisweet rice (koji) miso, salty rice (koji), and barley (koji) miso were partially hydrolyzed (62). According to Esaki et al., novel isoflavones, 8-hydroxydaidzein (8-OHD), 8-hydroxygenistein (8-OHG), and 6-hydroxy-

Table 6 Isoflavone Content of the Methanol Extracts from Commercial Mature Misos (μg/g dry weight)

Miso	Daidzin	Genistin	Daidzein	Genistein
RM1	171	286	422	571
RM2	177	286	404	614
RM3	606	1108	497	1170
RM4	686	1002	449	713
RM5	576	960	404	614
Average	413	728	531	736
BM1	204	299	287	457
BM2	171	236	296	428
BM3	167	232	1087	1299
BM4	130	76	808	1156
Average	168	210	619	835
SM1	171	261	1968	2683
SM2	167	168	2385	2997
SM3	130	421	1895	2882
Average	156	283	2065	2857

Note: RM = rice-koji miso, BM = barley-koji miso, SM = Soybean-koji miso, RM1, RM2 = Sweet rice-koji miso.
Source: Ref. 61b.

daidzein (6-OHD) were isolated from fermented soybean products with *Aspergillus* species and identified (63–66). These isoflavones showed significantly stronger antioxidative activities than those of daidzein and genistein. They have been also formed in miso-dama (cooked soybean balls described earlier) and exist in soybean-kojis for the preparation of soy sauce and tamari. These isoflavones were stable even to heating at 100°C for 30 min and stored at 30°C for 9 months. However, they were not detected in rice-koji miso nor barley-koji miso consisting of only cooked soybeans that are not fermented with *Aspergillus* starter.

The antioxidative activity of soybean miso which contain *o*-hydroxyisoflavones (ODI) showed a generally stronger value than those of other types of miso and there was a good correlation ($r = 0.821$) between ODI content and antioxidative activity.

According to Hirota et al., the 8-hydroxydaidzein and 8-hydroxygenistein were found and isolated from soybean miso as characteristic compounds. They had as high DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity as that of α-tocopherol. The antiproliferative activity of 8-hydroxygenistein toward the cancer cell lines showed the highest activity ($IC_{50} = 5.2$ μM) toward the human promyelocytic leukemia cell (HL-60), whereas 8-hydroxydaidzein and genistein demonstrated IC_{50} value of 26 μM and 33 μM, respectively (67–69b).

F. Saponin

Soybean seeds contain approximately 2% of glycosides composed of several kinds of saponin and isoflavonoid. Saponins consist of six group A saponins, four group B saponins, and two group E saponins which contain soyasapogenol A, B, and E as the aglycone, respectively. These saponins are isolated from the hypocotyl and cotyledone of soybean seed. In fact, A group of saponins of the most unpleasant taste (70) is found only in the hypocotyl and approximately 50% of the total saponin is included in the hypocotyl (71).

Generally, saponins have physiological activities which are often useful as components of herbal medicine, whereas undesirable taste such as bitterness and astringent have been detected. The undesirable taste, however, is an obvious disadvantage when employed as ingredients of food. Some devices to reduce the undesirable taste have been practiced empirically.

According to Kudou et al. soybean glycoside compositions of various misos and the effects of soybean saponin on the sensory evaluation were investigated in commercial misos collected throughout Japan (62). The soybean saponin A group having soya sapogenol A as an aglycone in miso is partially deacetylated or hydrolyzed at the sugar moiety during fermentation. The content of soybean saponin Bb, the principal saponin in the B group

saponin in rice miso, was in the range 16–173 mg/100 g and the mean was 57 mg/100 g on a dry matter basis. Among the misos tested, soybean miso was the highest, followed by barley miso, salty rice miso, semisweet rice miso, and sweet rice miso in that order.

Sensory evaluation of miso was carried out on 239 commercial miso samples by a well-trained panel for color, flavor, taste, texture, and overall quality (72).

In salty rice miso, the most popular variety ($n = 202$), the correlation coefficients between saponin Bb content and sensory scores for color and flavor were 0.150 ($p < 0.05$) and 0.205 ($p < 0.01$), respectively. This fact shows that the sensory evaluations for color and flavor show a tendency to be lower with increasing soybean saponin Bb content. The soybean saponin Bb content was less in miso made from dehulled soybeans than in miso made from whole soybeans, and the sensory evaluations on miso made from dehulled soybeans were higher than those of miso made from whole soybeans. As a practical method to make light-colored rice-koji miso, dehulled soybeans are employed or whole soybeans are dehulled by rubbing during cooking in small-scale facilities.

In fact, dehulling is effective in eliminating the hypocotyl, in which A group saponin of the most unpleasant taste is condensed.

XII. CHANGES OF PHYSIOLOGICAL FUNCTIONS DURING FERMENTATION

In a review on soy intake and cancer risk (73), 4 studies on laboratory animals and 17 epidemiological studies reported up to 1993 studied the effects of consumption of miso and miso soup on cancer development. In the four studies on laboratory animals cancer, including mammary, liver, and stomach, was controlled in three studies. In the 17 epidemiological studies on cancers, including breast, prostate, colon, rectal, stomach, esophagus, gallbladder, and pancreas, 9 studies revealed statistically significant control and 8 studies were statistically insignificant.

A. Epidemiological Study

According to Hirayama (74,75), daily intake of miso soup was found to significantly reduce standardized mortality rates for gastric cancer in an ongoing large-scale prospective study of 122,621 males and 142,857 females aged 40 and above in 29 health center districts from 1966 to 1978. Daily intake of miso soup significantly reduced the standardized mortality rate of gastric cancer both in males ($p < 0.001$) and females ($p < 0.01$). The mortality rates

for males with daily, occasional, and rare intakes and nonconsumers were 171.9, 210.2, 240.0, and 255.9 per 100,000 persons, respectively, and the corresponding values for females were 77.8, 85.3, 97.5, and 113.6, respectively. These relationships remained significant $p < 0.01$ when the subjects were classified according to socioeconomic status and smoking habits. Reduced mortality rates due to miso consumption were confirmed in case-control studies of both males and females in urban and also in rural areas (< 0.01).

B. Biological Study

As seen in the above epidemiological studies, daily intake of miso soup reduced mortality rates for gastric cancer, indicating that miso might have an inhibitory effect on gastric cancer; however, miso, the major ingredient of miso soup, was not yet cleared by biological experiments. In this connection, extensive research on chemoprevention of cancer by miso was started by Ito in 1989 (76). The effect of a miso diet fed to mice was investigated. Freeze-dried salty red rice miso, fully fermented, the most popular type, was selected as the ingredient of the miso diet. The mice used were of the C3H/HeN and B6C3F strains. The mice were exposed to either ^{252}Cf fission neutrons or diethyl nitrosamine (DEN) at 2 or 6 weeks of age as a hepatocarcinogenic insult. The mice were fed a diet of 10% miso daily up to 1 year and 3 months after carcinogen administration. In one study with the male C3H, development of spontaneous liver tumors were assessed. The incidence and multiplicity of liver tumor for miso (10%) diet and control were 32 : 89 (significantly different from control by $p < 0.05$) and 1.06 : 2.86, respectively. In a similar study, both sexes of B6C3F mice were first irradiated with 2 Gy of ^{252}Cf fission neutrons and subsequently given a 10% miso diet similar to the as C3H mice. In this study, control animals fed either the normal or 10% miso diet developed liver tumors at a frequency of 5%. Following irradiation, the incidence of liver tumors reached over 50% in the male and 32% in the female. The tumor incidence was decreased significantly by the miso diet (77). Miso contains various nutrients, including flavonoid; isoflavones such as biochanin A and genistein which are most effective for in vitro tumor inhibition (78) Biochanin A, resembling genistein, was used in the mouse hepatocarcinogenesis study, and it was significantly effective in reducing the multiplicity of liver tumors. However, 10% miso was much more effective than 20 ppm biochanin A (79).

C. Chemopreventive Potentials of a Combination of Miso and Tamoxifen

A menu shown in Table 7 was applied in a rat mammary tumorigenesis study. Mammary tumors were induced in female Sprague–Dawley (S-D) rats by

Table 7 Incidence and Multiples of Mammary Tumors Rats Treated with 10% Miso and/or 20 mg Tamoxifen

Group	*N*	Incidence of tumors (%)	Tumors/rat
Control	22	90	4.5 ± 1.7
10% Miso	22	77	2.4 ± 1.0[a]
Tamoxifen (TAM), 20 mg	22	68	1.4 ± 0.6[b]
10% Miso + TAM	20	10[c]	0.2 ± 0.3[c]

Note: All rats received 50 mg of a cholesterol pellet containing 2.5 mg of test ingredients and renewed every 4 weeks. The rats were observed for 18 weeks after MNU administration. *N* = number of rats.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.**
Source: Ref. 76.

injecting *N*-methyl-*N*'-nitrosourea (MNU) 40–50 mg/kg I.V. and the induced mammary tumors were scored for incidence and multiplicity. Ten percent miso, 2% and 10% soybean, and 10ppm and 50ppm of biochanin A were tested for efficacy. They were fed throughout the experimental period of 18 weeks. In the control group, the incidence of mammary tumors reached 90% and multiplicity was 4. By giving the above special diets, tumor incidence and multiplicity were significantly decreased; the lowest incidence was only 30% in the 10% miso or 50 ppm of the biochanin A diet groups. Tumor multiplicity also dropped to lower than 2 (80). In a subsequent study, similar means were applied in combination with tamoxifen. A single administration of 10% miso and 20 mg of tamoxifen was quite effective, as shown in Table 7.

Furthermore, combined treatment of miso and tamoxifen was almost completely inhibitory for the occurrence of mammary tumor. This experiment suggested that it may not only be applicable to primary chemoprevention of cancer but also for therapeutic treatment (81).

D. Difference of Physiological Function Between Young Miso and Fully Fermented Miso

As mentioned earlier, the constituents of the ingredients are changed during fermentatation, producing new compounds. Because the physiological function of miso seems to be changed during fermentation, it is important to clarify the difference between young miso and fully fermented miso. The radioprotective effect of miso on the survival time, crypt survival, and jejunum crypt dimension in male B6C3F1 mice was investigated. Freeze-

dried miso was mixed in the MF diet at 10% level and the chow was admimistered from 1 week before irradiation and thereafter. Animal survival in the long-term fermented (180 days) miso group was significantly prolonged after 8 Gy of ^{60}Co γ-ray irradiation (dose rate of 2 Gy/min) as compared to the short-term fermented (60 days) miso and MF (control diet) cases. At doses of 10 and 12 Gy irradiation the treatment with long-term fermented miso significantly increased crypt survival. Also, effects of irradiation on crypt dimension on the long-term fermented miso group were significantly increased as compared to those in the short-term and medium-term (90 days) fermented miso and diet groups. The above suggest that prolonged miso fermentation is very important for protection against radiation effects (82).

E. Inhibition of the Formation of Carcinogenic Heterocyclic Amines by Miso

According to Nguyen, heterocyclic amines (HCAs) are generated by heating foods at high temperature. The addition of miso ingredients such as boiled soybeans, rice-koji, or unfermented miso did not inhibit the mutagenicity of the HCA-generating model system containing creatinine, glucose, and glycine. However, the mutagenic activity of this model system or fried beef model was significantly decreased by the addition of soybean-koji miso or rice- koji miso of the sweet type. The decrease of MeIQx in the model system was also confirmed by high-performance liquid chromatography (HPLC). From the above results, it is concluded that miso can inhibit the formation of HCAs in a model system and that this inhibition is not caused by the components of raw material. Therefore, it is estimated that a component generated by the miso fermentation process has contributed to the inhibition of HCAs in model systems (83).

F. Removal of Allergens

One of the physiological functions of miso is the removal of harmful substances such as allergens deleterious to human health. The fate of the allergen in miso during fermentation was examined by ELISA (enzyme-linked immunosorbent assay) and immunoblotting with a monoclonal antibody or sera from soybean-sensitive patients. The allergen in three types of miso (rice-koji, barley koji, and soybean-koji miso) was clearly shown to be rapidly digested within 1 month as fermentation proceeded. These findings may provide useful information in the selection of miso as a safe and hypoallergenic food for soybean-allergic patients (84–87).

XIII. FORECAST OF THE ROLE OF MISO IN THE FUTURE

The consumption of miso is concentrated in east Asia at present. It is a key point for miso producers whether or not, in the future, miso becomes a food accepted throughout the world. According to Shurtleff and Aoyagi (6), the total U.S. miso consumption grew from 756 tons to 1970 to 1122 tons in 1975, and up to 2349 tons in 1982. By late 1982, Americans in the continental United States were consuming three times as much miso as they had in 1975 and the market was growing about 17% a year. Thereafter, the consumption was roughly estimated at 3270 tons of miso, including the domestic product and that imported from Canada and Japan in 2001, indicating a steady increase of 139% above the consumption in 1982. The miso was consumed by both Asian and non-Oriental Americans, both in growing numbers. Non-Oriental miso consumers came from several groups. The great majority came from the closely related natural food, macrobiotic, or vegetarian movements, but some came from the growing number of people interested in Japanese, Chinese, and other Asian cuisines. This indicates that miso could posssibly become acceptable to non-Orientals worldwide. For Western people who consume so mauch animal protein, including meat and meat products rich in saturated fatty acids and cholesterol, miso (which is usually eaten with vegetables or seaweed) would be a food recommented for its nutritional value. In short, the merits of miso can be summarized as follows:

1. Safety: Miso has been consumed traditionally for at least 1300 years without any ill effects. No micotoxin has been found in the koji or in the miso (5).
2. Nutrition and health: As stated earlier, miso is a highly nutritious and healthy food which may possibly prevent diseases, including cancer.
3. Acceptability: Because there are many varieties, including sweet, salty, meatlike, light yellow, dark red, and well-ripened miso, consumers can select their preference. Miso might be especially attractive to vegetarians because it has a meatlike flavor produced from genuine vegetable substrates.
4. Reasonable cost: Miso can be produced without expensive equipment and without significant loss of ingredients.
5. Long shelf life: Miso of the salty type can be stored at ambient temperature for at least 6 months. When the world population explosion takes place and the supply of food is extremely serious in the future, the production of foods, especially protein foods from agriculture and marine resources, will be of primary importance to cope with the situation. Although the production of new proteinous

foods might be undertaken for the present world production of soybeans, 100 million tons, containing approximately 40 million tons of protein, can provide 2.74 billion humans with 40 g of protein per person per day.

Miso is expectd to play an imortant role as one of the soybean foods.

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3

Industrialization of Sake Manufacture

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I. INTRODUCTION

A. Outline of Sake Brewing

Sake, a traditional alcoholic beverage, has been a favorite drink of the Japanese for more than a thousand years and is becoming popular in other parts of the world. The principal raw products used in sake brewing are rice and water.

The characteristic features in sake brewing are the use of "koji," a culture of *Aspergillus oryzae* grown on and within steamed rice grains, and parallel fermentation by sake yeast.

The technique for sake brewing probably originated in ancient China, but comparison of the production processes of sake and Chinese alcoholic beverages such as Shao-hsing rice wine show remarkable differences, especially with respect to the micro-organisms involved as will be described later. Koji, which is comparable to malt used in beer brewing, is used for liquefaction and saccharification of the starch contained in the rice grains.

Although fermentation takes place after filtration of the mash in beer brewing, in sake mash, which is called "moromi," glucose liberated from rice grain starch is fermented successively by yeast. "Parallel fermentation" refers to the combination of progressive saccharification of starch and slow alcohol fermentation at low temperature. This contributes to consid-

erable ethanol production, which can be as high as 20% (v/v) in moromi mash.

A flow diagram of the sake-brewing process is shown in Fig. 1. The first step in the procedure of sake brewing is the preparation of milled rice and its steaming. This is followed by the preparation of koji and then the preparation of moto mash, a starter for sake yeast prepared by mashing steamed rice, koji, and water. This is then inoculated with a pure yeast culture. To avoid the growth of harmful bacterial contaminants and to promote the growth of the inoculated yeast, about 0.5% lactic acid is added at an early stage in the preparation of the "moto" mash.

The next step involves the preparation of moromi mash, the main fermentation mash, by the addition of a large amount of steamed rice and koji to moto mash in a large fermentation vessel. The additions of steamed rice are divided successively into three batches over 4 days. The fermentation takes 15–25 days after the addition of the final batch. Contrary to the situation in beer brewing, the sake mash is very dense and mushy. The solids are considered beneficial in retaining large numbers of yeast cells in suspension during the fermentation, which is one of the reasons that sake contains as much as 20% (v/v) ethanol without resorting to distillation. The

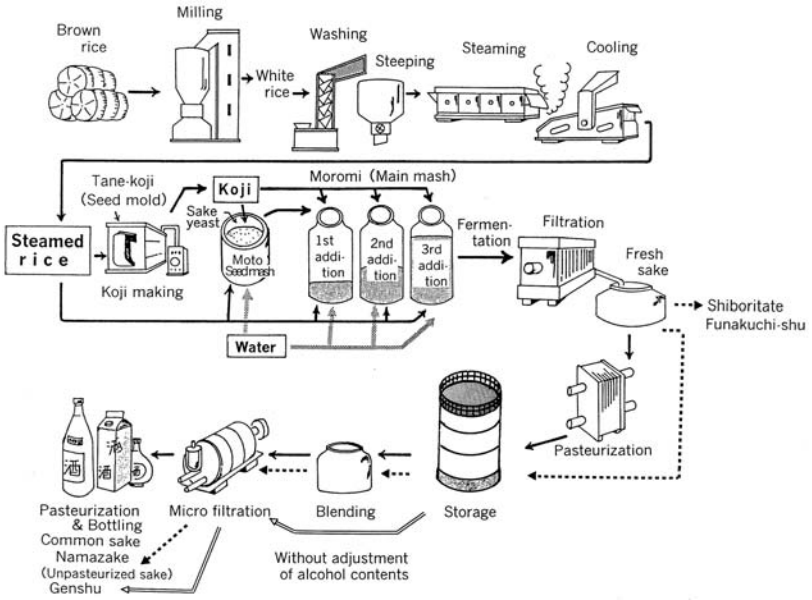


Figure 1 Flow diagram of the sake-brewing process.

final stage is the aging and bottling of the sake. Usually, after several months of aging, sake, matured and having developed a smooth taste and a golden pale yellow color, is finally blended, adjusted to the appropriate ethanol content (14–16% v/v) by adding water and, if necessary, treated with activated charcoal carbon followed by filtration, pasteurization, and bottling. Recently, there are some variations at this stage, resulting in the various types of sake: *Funakuchi-shu* or *Shiboritate* is just pressed fresh sake, sake-nouveau. *Nama-shu* (*Nama-zake*) is unpasteurized sake, or live sake. *Genshu* is undiluted sake having 18% to more than 20% alcohol content. *Koshu* is old sake, the sake aged for more than several years.

B. A Short History of Sake Brewing

According to ancient manuscripts of China, the ancient (2nd to 3rd century AD) people of Japan drank sake because it was their nature to appreciate alcoholic beverages. Even in mourning, callers for condolences gathered to perform a memorial service for the dead by singing, dancing, and drinking sake. Thus, from ancient times, the Japanese people already had sake for their own consumption, and they made it anytime and anywhere according to their need. In olden times, when they did not know the usefulness of mold for sake brewing, sake was supposedly brewed by chewing soaked or steamed rice so that salivary amylases instead of mold amylases saccharified rice starch. The technique of using mold for sake brewing presumably originated with the Chinese, but a comparison of processes for producing sake with those for producing Chinese alcoholic beverages shows remarkable differences, especially with respect to the micro-organisms concerned and the physical conditions of the raw materials.

Since prehistoric times, the Japanese have consumed rice as a staple food. The ancient people cooked and ate rice by roasting it, boiling it to gruel, or pounding it into cakes, and these rice foods easily became covered by molds if they were allowed to stand for a few days or more in the high humidity of the Japanese climate. Thus, it might also have been easy to select koji mold such as *A. oryzae* and to develop a unique koji-making method in which steamed rice grains were prepared in a loose heap. On the other hand, in producing Chinese alcoholic beverages, various molds such as species of *Rhizopus*, *Mucor*, and yeasts are grown spontaneously on uncooked wheat flour or other raw materials, most of which are prepared like a compact brick or dumpling and known as "Bing-qu." The techniques for making Bing-qu were introduced into Japan with other techniques for brewing of Chinese alcoholic beverages in the beginning of the 5th century AD and made a great contribution to improving the traditional Japanese skills in sake brewing which had already been established.

At the beginning of the 5th century AD, The Imperial Household and its government were the strong driving force in culture and industry, and they established many factories and employed many technicians and workers in various fields, all of which were monopolized, and sake brewing was not an exception. As a result, a group of sake brewers appeared who were sanctioned by the government, and sake brewing from the 5th to the 9th centuries AD can be referred to as "sake made by the Court "as opposed to" sake made by the general public."

After the 8th century, Buddhists and Shinto priests gradually began sake brewing, and at the end of the 12th century, they granted authorization for sake brewing to several specialists who were required either to offer sake or to pay tax in return for it. At the end of the 10th century, sake entered the commercial market. This meant that sake had to be subjected to long periods of storage as a commodity unlike the type brewed in olden times and introduced "sake made by professional brewers" in place of "sake made by the Court." By the middle of the 14th century, the technical know-how on sake brewing, which was, in principle, the same as today, had been established. At that time, sake was brewed through a process in which rice-koji and steamed rice and water were mashed successively in two steps. The application of spontaneous lactic acid fermentation effectively protected the mash from bacterial contamination and allowed dominant growth of sake yeast during moto preparation. Moreover, the activation of enzymes and promotion of microbial growth were carried out by stepwise elevation of the mash temperature. It is noteworthy that a document on sake brewing written in the second half of the 16th century described in detail a heating method to kill the micro-organisms that cause spoilage of sake. This is almost identical to the process invented in Europe by Louis Pasteur in 1865 for low-temperature pasteurization of wine and beer. This knowledge laid the foundation for the sake brewing of today.

From the 16th to the 19th centuries, under the feudal system, sake-brewing skill and methods which had been handed down from the former centuries spread, and this led to the establishment of the thousands of sake brewmaster groups. During this period, the leadership in sake brewing belonged to the brewers in Nada district and its surroundings. In addition to these brewers in the main producing districts, there emerged great numbers of small breweries run by landowners and rice traders.

At the end of the 17th century, the total number of breweries was reported to be more than 27,000. In the 19th century, with the collapse of the feudal system, there occurred a drastic change in the method of sake brewing under the influence of new brewing technology based on European science. Many attempts to improve sake brewing were made by direct application of European beer-brewing methods. These attempts ended in

failure, however, because many special techniques for sake brewing, such as the use of mold cultures, were very different from beer-brewing methods. This was a notable period, though, in that the old know-how regarding sake brewing was analyzed and given a theoretical basis through the influence of European science.

II. PRODUCTION AND CONSUMPTION

A. Production

Because sake is consumed within a year of production, its production, as with beer brewing, has generally kept pace with its consumption. The change in sake brewing and the numbers of sake breweries in Japan over a period of about a half-century are shown in Table 1.

During World War II, production decreased remarkably. Thereafter, production was restored and increased gradually to reach its maximum of 1,417,000 kL in 1974. Since then, the production has been decreasing gradually.

In general, from 1.8 to 1.9 kL of sake is brewed from a ton of brown rice. During World War II, a shortage of rice resulted in an increase in the

Table 1 Changes in the Amount of Sake Produced and the Number of Sake Breweries in Japan

Year	Sake production (× 1000 kL)	No. of breweries
1936	771	7499
1940	477	6870
1945	151	3160
1950	188	3692
1955	467	4021
1960	685	3980
1965	1089	3865
1970	1257	3533
1975	1350	3229
1980	1193	2947
1985	928	2586
1990	1060	2435
1995	980	2336
2000	720	2152

Source: Annual Report of the National Tax Agency, 2002 (www.nta.go.jp).

amount of sake made by adding ethanol, water, and sugars to fermented moromi mash. Thereafter, this method was improved and applied so widely that it plays an important role in providing sake with a mild and smooth taste, in addition to increasing the amount of sake produced. On the other hand, the sake production without alcohol addition (jummai-shu) is increasing recently, because breweries can use rice abundantly.

Table 2 shows changes in consumption of various alcoholic beverages in Japan since 1940. In 1938, only 739,000 kL of sake was consumed. Consumption has increased steadily (except during World War II) and reached a peak of 1,766,000 kL in 1973. Thereafter, consumption of sake has decreased, and in 2001, it was 974,651 kL, or 9.4 L per capita.

Although the number of sake breweries has decreased, there were about 2100 such breweries scattered throughout Japan in 2000. The most famous districts for sake production are in and around the cities of Kobe (Nada district) and Kyoto (Fushimi district). These two areas contribute about 47% of the sake produced in Japan. About 60.8% of the breweries produce less than 300 kL of sake per year. About 60 large breweries with modern industrialized factories, producing over 2000 kL of sake per year, ship nearly 70% of the total sake production.

Currently, sake is brewed also outside of Japan. There are seven breweries in the United States and China, respectively. There are three breweries in Korea and 2 breweries in Brazil. Thailand, Vietnam, and Australia also have 1 brewery each. Sake is being consumed by people other than the Japanese.

Table 2 Changes in Consumption of Various Alcoholic Beverages in Japan[a]

Year	Sake	Beer	Wines	Whisky	Shochu[b]	Total
1940	476	271	—	—	93	949
1945	173	98	—	—	29	340
1950	183	178	—	—	173	623
1955	507	406	—	—	272	1368
1960	751	932	—	—	260	2184
1965	1159	1986	40	66	212	3566
1970	1601	2982	33	137	213	5067
1975	1747	3908	51	257	202	6257
1980	1473	4532	66	371	254	6820
1985	1355	4860	88	299	625	7462
1990	1422	6586	147	253	591	9315
1995	1310	6979	170	181	666	9988
2000	977	5185	282	124	734	9520

[a] Consumption × 1000 kL.
[b] Japanese traditional spirits.
Source: Annual Report of the National Tax Agency, 2002 (www.nta.go.jp).

Because fermentation of sake mash proceeds in an open vessel usually below 15°C, sake is generally produced only in winter. Recent developments in production techniques in refrigeration and sanitation as well as in brewing have allowed sake production to take place year-round. Many large breweries have built modern factories equipped with air conditioning and various other facilities, thereby increasing the scale of production and decreasing labor costs. On the other hand, most of the small breweries have continued manufacturing sake in old-fashioned factories. It may be difficult in spite of their ardent desire for them to build modern factories due to financial circumstances unless they establish a joint concern or organize a cooperative association. Thus, the differences in profits between large and small breweries may increase unless the latter produce unique, refined, and, therefore, valuable sake by using their elaborate manual techniques. Economical and technical analysis of the sake-brewing industry, including combination and cooperation among breweries, is now under way.

B. How Sake Is Consumed in the Diet

Table 3 shows the consumption of sake. The taste of sake is soft, mild, and sweet, yet rich and stimulating. Sake is commonly drunk as an aperitif or

Table 3 Analysis of Average Sake

Item[a]		Alcohol (%,v/v)	Density[b]	Acidity[c] (mL)	Amino acidity[d] (mL)
Jummai-shu	Ave.	15.32	2.6	1.52	1.7
	SD[e]	0.73	5.1	0.38	0.39
Ginjo-shu	Ave.	15.72	3.6	1.32	1.32
	SD	0.77	2.2	0.24	0.33
Honjozo-shu	Ave.	15.45	3.3	1.29	1.57
	SD	0.61	3.1	0.19	0.35
Common sake	Ave.	15.32	1.9	1.19	1.34
	SD	0.51	3.1	0.19	0.34

[a] The sake brewing method and quality labeling standards of jummai-shu, honjozo-shu, and ginjo-shu are defined by the government ordinance (see text).
[b] Density: Expressed in sake meter value which is basically a Baume meter. Water is given a value of zero, which means the taste is neither particularly dry nor particularly sweet. A positive value indicates a drier sake (+10, extremely dry; +5, dry; +3, slightly dry). A negative value indicates that the same will be sweeter (−10, very sweet; −5, sweet; −3, slightly sweet).
[c] Acidity: Titration of milliliters of 0.1N sodium hydroxide solution taken to neutralize 10 mL sake.
[d] Amino acidity: Expressed in formol titration (mL).
[e] SD: standard deviation.
Source: Annual Report of the National Tax Agency, 2002 (www.nta.go.jp).

with foods. The flavor of sake complements traditional Japanese food such as sashimi, tempura, and tofu dishes and enhances the taste of these foods, especially raw or cooked fish. Sake is traditionally served warm. The proper temperature is from 35°C to 55°C, depending on one's taste. Sake can also be served cool, either refrigerated or poured over ice.

The sake-brewing method and quality labeling standards of junmai-shu, honjouzou-shu, and ginjo-shu are defined by government ordinance. They are summarized as follows: Junmai-shu is a sake brewed using rice of 70% or less of polishing ratio and does not utilize alcohol addition and it has good flavor and full-bodied taste with a rather stronger flavor and color. Honjouzou-shu is also brewed using rice equivalent quality to the former and is accepted with the addition of less than 10% of alcohol (the weight of 95 proof alcohol) to the weight of rice used. This sake is characterized by a lighter taste. Ginjo-shu is the most refined sake that has to brew using the rice-polishing ratio of less than 60% and slow fermentation at lower temperature that takes a longer fermentation period. This sake is characterized by a rich fruity flavor and a lighter good taste. When carrying out alcohol addition to sake, the same quantity as honjozo-shu should be a maximum. Ginjo-shu that does not utilize alcohol addition is called jummai ginjo-shu.

III. MANUFACTURE OF SAKE

A. Raw Materials

1. Water

Water, which accounts for about 80% (v/v) of sake, is considered one of the principal raw materials. It is used not only as an ingredient but also in other procedures such as washing and steeping of rice, bottle washing, and for boiling. In general, about 25 kL of water is necessary per ton of rice used for sake brewing.

The water for sake brewing needs to be colorless, tasteless, and odorless neutral or weakly alkaline, and to contain only trace amounts of iron (<0.02 ppm), ammonia, nitrite, and organic substances and no harmful micro-organisms (1). Several ions, especially potassium, contained in water in minor concentrations have been reported to affect fermentation and koji making (2–4). Iron is injurious to sake because it imparts an intense color and causes deterioration of flavor, as will be seen later. Appropriate treatments such as aeration, successive filtration, absorption and flocculation, absorption on activated charcoal carbon or ion-exchange resins, and flocculation with the reagent aluminum sulfate are generally employed to remove iron in brewing water (1).

2. Rice

Rice is a principal raw material of sake and the effect of its nature on the yield and quality of sake has been elucidated. Whereas the aroma of grapes largely affects the flavor of wine, rice has neither a characteristic flavor nor a profound effect on the flavor of sake, which is affected rather by the complicated procedure of sake brewing. Such properties as ease of milling and steaming, the high yield of sugars by enzymatic saccharification, and being well-covered with mold have been considered important in estimating the quality of rice.

Much work has been done on the structure and constituents of rice grain as they relate to saccharification, yeast or koji mold growth, fermentation, and formation of flavor compounds in the mash. "Japonica" short-grain varieties harvested in Japan have been found to be suitable for sake brewing. Long-grain varieties form less sugar in saccharification and contribute odor to the sake (5,6). Among many domestic Japonica varieties, some are considered especially suitable for sake brewing and are sold at a high price. "Yamadanishiki," the most famous variety suitable for sake brewing, as shown in Fig. 2A, has a radial arrangement of uniformly well-developed endosperm cells, in contrast to "nihonbare" (Fig. 2B), one of the small-grain varieties in Japan, in which the back side of the grain develops well and makes a tighter structure than the belly side (7,8). The latter contains more proteins and lipids than the former. Grains such as nihonbare are sometimes not steamed uniformly and thus produce less sugar and more amino acids in the mash fermentation (9). In the grains of yamadanishiki, the endosperm cells are arranged loosely, and at the center of the grain there is a cavity filled with starch granules in place of endosperm cells. This structure scatters light and forms a white core, "shimpaku," which is often seen at the center part of large grains. The rice grains with shimpaku absorb water quickly and are well steamed. Moreover, these grains are considered suitable as raw material for koji because the mycelia of *A. oryzae* can grow into the white core cavity as well as on the surface of the grains (10,11).

In mash fermentation, acid protease and carboxypeptidase originating in the koji decompose proteins to peptides and amino acids, some of which are assimilated by yeast and give sake a full, heavy taste. However, an excess of these compounds often gives sake a rough taste, a deep color, and accelerates deterioration of sake quality. Fatty acids, especially unsaturated ones such as linoleic acid and oleic acid and composed of acyl glycerides, largely decrease the formation of aroma eaters such as isoamylacetate by yeast during fermentation (12,13).

Brown rice contains about 7–9% protein and about 2–3% ethyl ether extractable lipid and about 0.7% fat-by-hydrolysis, incorporated in the α-

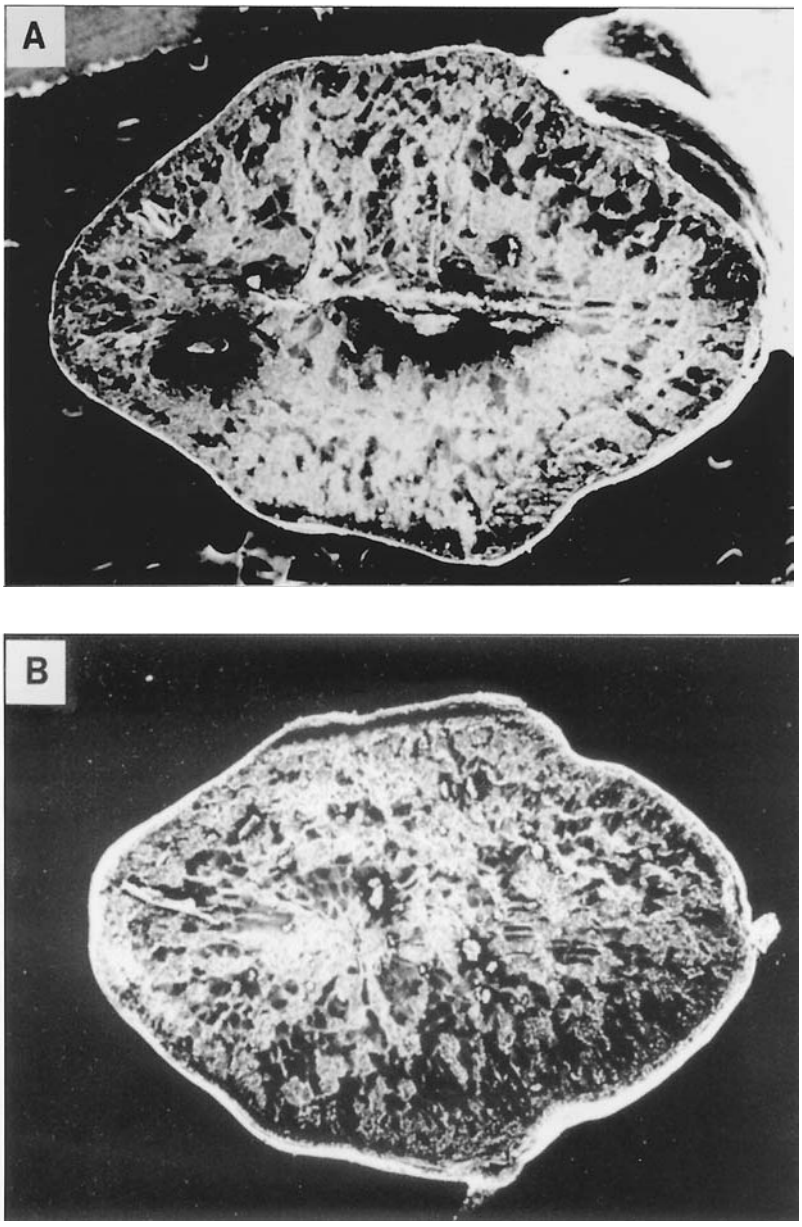


Figure 2 Scanning electron micrographs of the cross sections of (A) yamadanishiki rice grain and (B) nihonbare rice grain. (Reprinted with permission from Ref. 7 and Ref. 8).

helix structure of amylose. The radial distributions of these compounds and minerals in rice grains are shown in Fig. 3 (14).

Milling reduces the protein content of rice grain gradually. Rice overmilled to 75% of the polishing ratio usually contains 5–6% protein. The polishing ratio is termed as the ratio [159] of milled rice to the original brown rice. Because the ethyl ether extractable lipid exists mainly in the germ and aleurone layer, milling reduces the lipid remarkably to below 0.1% in overmilled white rice of 75% polishing ratio (4,15).

Recently, because consumers prefer sake with a clear, light taste and flavor, adjustments are being made in milling and other aspects of rice processing that will be described later. Usually, rice of 70–75% polishing ratio is used for sake brewing. The content of starch increases gradually with decreasing polishing ratio. For more refined sake (ginjo-shu), rice of over 50% polishing ratio is used and the sake produced has a fruity flavor and a very clear, light taste.

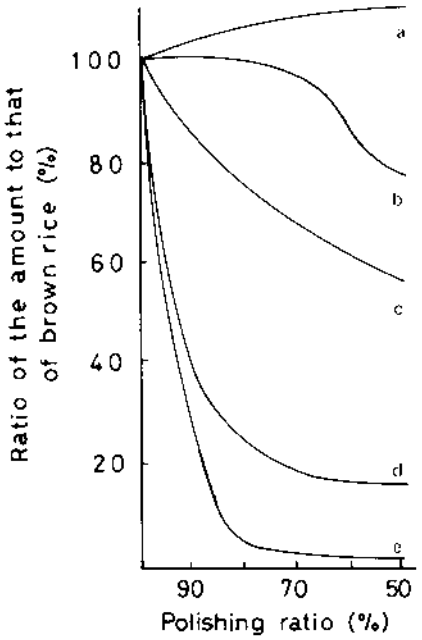


Figure 3 Changes in the content of various substances in rice grains with changes in milling ratio: (a) starch, (b) moisture, (c) proteins, (d) minerals, and (e) ether extractable lipids. (Reprinted with permission from Ref. 14).

B. Rice Processing

1. Milling

As mentioned previously, the main purpose of milling is to remove proteins, lipids, and inorganic substances such as potassium and phosphate, which are in excess in the germ and the surface layers of rice grain and are considered undesirable for sake brewing. As shown in Fig. 3, the content of ethyl ether extractable lipid and minerals decrease most rapidly, whereas protein content decreases gradually until the polishing ratio reaches 50%, after which it remains practically constant. The composition of the proteins and the kinds of fatty acids composed of acyl glycerides change with various polishing ratios; the percentage of glutelin and that of saturated fatty acid increase gradually with decreasing polishing ratios (4,15).

As shown in Fig. 4, by the end of the 19th century, milling was done with manual pound and millstones or a water mill. Currently, a type of abrasive roller mill is used (Fig. 5). Rice grains fed from the hopper are abraded in the milling chamber and fall to the bottom through the sieve, where rice bran and germ are removed. The conveyor basket carries rice grains from the bottom to the hopper. Thus, the operation continues until the grains are milled to the required ratio.

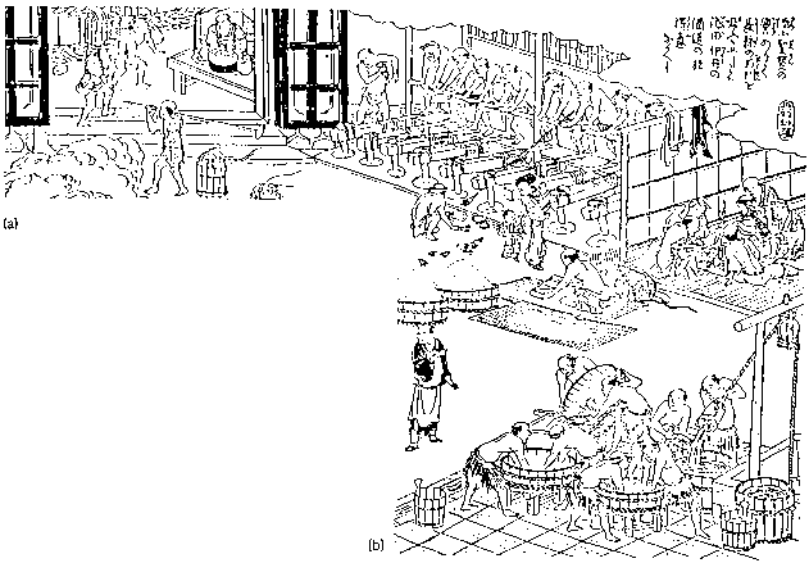


Figure 4 Rice processing: (a) milling with manual pounders and millstones; (b) washing. Scenes of sake making in 18th century. (Reprinted with permission Nihon Sankai Meisan Zu-e Vol. 1, 1798.)

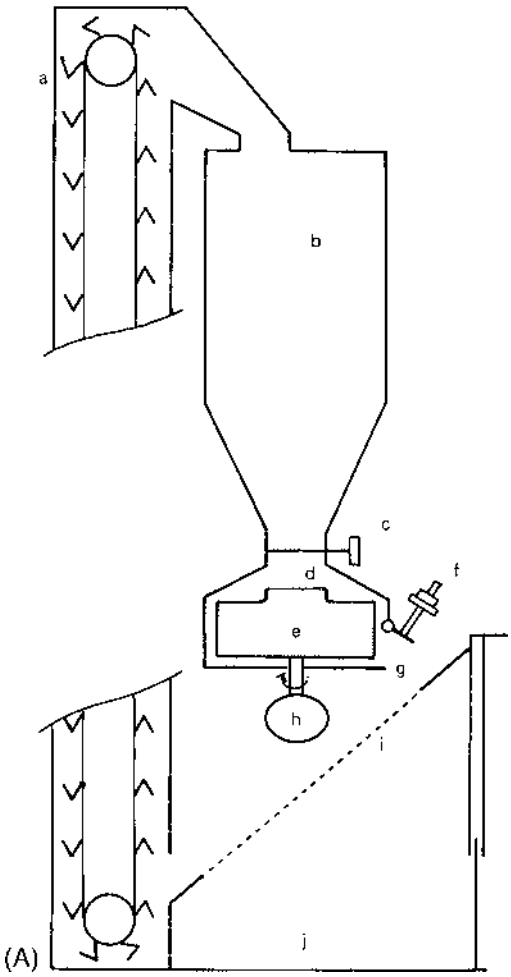


Figure 5 (A) Vertical-type of rice mill used in sake brewing: (a) basket conveyor, (b)rice hopper, (c) rice flow adjustment valve, (d) milling chamber, (e) roller, (f) resistance, (g) exit, (h) motor, (i) sieve, and (j) bran reservoir. (B) Abrasive roller mills in operation.

2. Washing and Steeping

Rice is washed and steeped in water prior to steaming. During washing, the grains are subjected to a kind of polishing caused mainly by the collision of rice grains in the water. This process removes some of the surface parts of grains, 1–3% of the total weight of the grains, which make up most of the suspended solids and account for most of the biochemical oxygen demand in

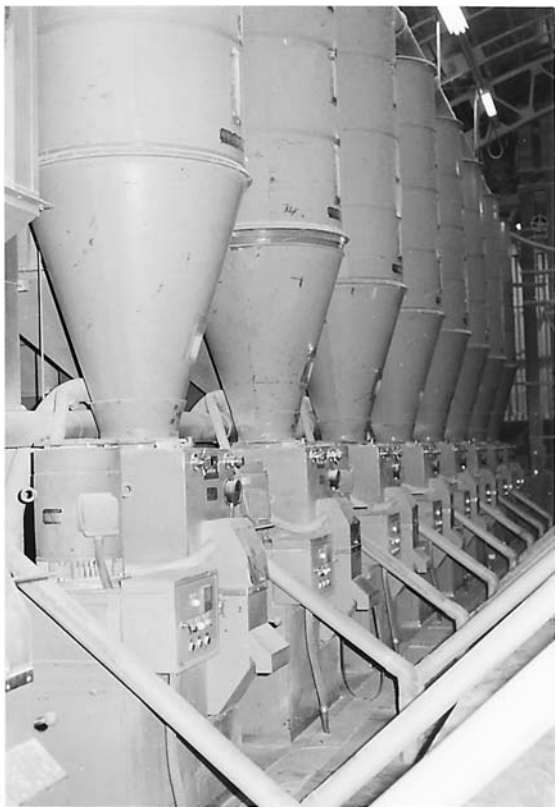


Figure 5 Continued.

wastewater discharged from sake breweries (16,17). During washing, the grains absorb water up to 9–17% of their weight. Washed rice grains are passed into a vat and immediately steeped in water. During the washing and steeping procedures, the grains absorb water to about 25–30% of their original weight. The moisture content of rice is much higher, the water absorption rate is lower, and the lower the moisture content, the more the water absorption rate increases (18). The absorbed water promotes penetration of heat into the grains during steaming and accelerates modification of the starch granules in the grains. Absorption of the appropriate amounts of water is a very important process in preparing properly steamed rice and controlling koji making and fermentation. After steeping, excess water is drained off from the grains for about 4–8 h before steaming.

3. Steaming

The rice is steamed for 40–60 min during which time the starch is changed to the α form and proteins are denatured to make them susceptible to enzyme action by koji. Steaming also sterilizes the grains. During steaming, the grains absorb water to 7–12% of the starting weight of the rice, thus resulting in a total water gain of about 35–40% from the beginning of the brewing process.

In olden times, and still in small breweries, steam is usually generated from boiling water in a large pot as shown in Fig. 6. A boiler is commonly used in many larger breweries for the steaming of rice. Steeped rice grains are heaped up in an insulated shallow tub with a special jet in the center of the base through which steam is blown. This is placed on top of a large kettle filled with water and steamed under atmospheric pressure. In modern breweries, two types of continuous steaming apparatus are used, namely a belt conveyor system (Fig. 7) and a cylinder system. The steamed rice is cooled to nearly 40°C for koji making and to about 10°C for preparing moto and moromi mash.

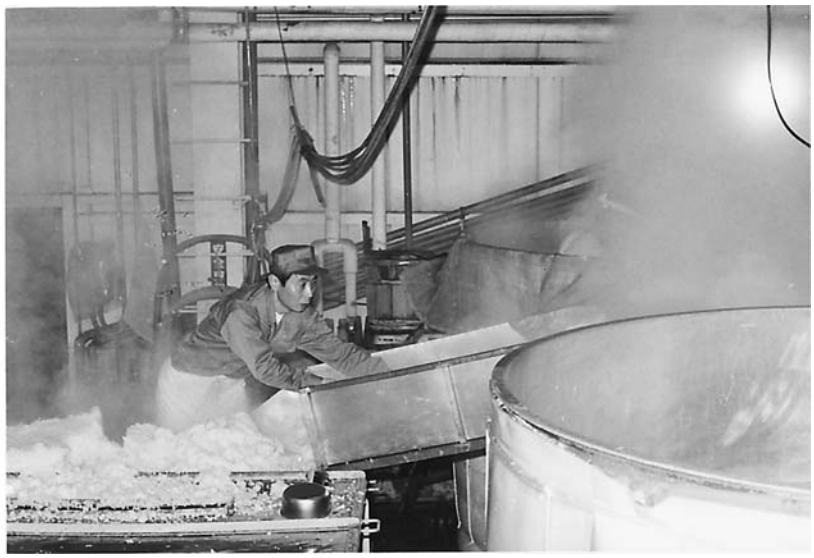
C. Preparation of Koji

Koji is a culture of koji mold, *A. oryzae*, grown on and within steamed rice grains, which accumulates various kinds of enzyme involved in sake brewing. For the preparation of koji, seed mold, which is called "tane-koji," is used. Tane-koji is a type of koji with abundant ascospores, especially prepared for commercial sake brewing.

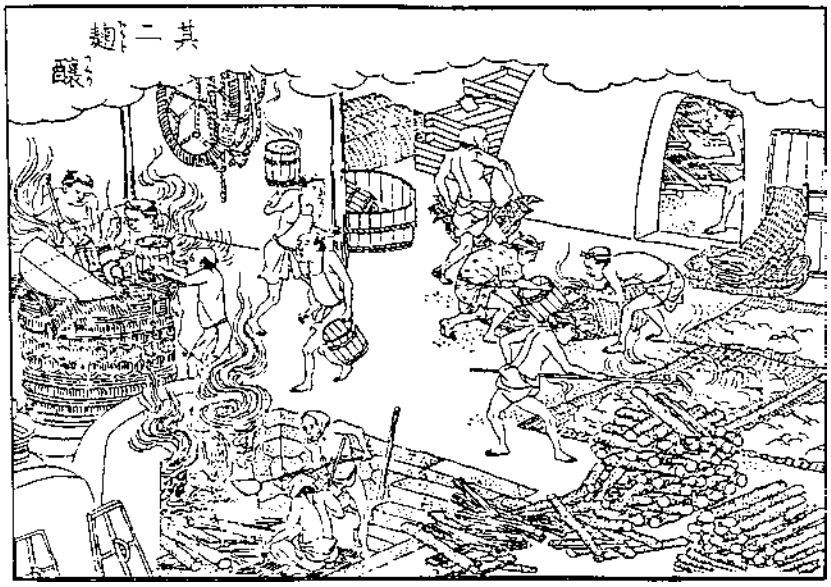
Aspergillus oryzae used for sake brewing and other Japanese fermentation foods, such as miso, is different from *A. flavus*. These two species are clearly distinguishable from each other on the basis of mycological characteristics of the authentic-type cultures of the two species and various industrial strains (19,20). It is worth noting that aflatoxin-producing strains have not been found among the Japanese industrial strains of koji mold (21–23).

Although more than 50 kinds of enzyme have been found in koji, the most important of these are α-amylase and glucoamylase and acid protease. Cultural conditions influence the formation of enzymes. In general, the higher the cultivation temperature (~40°C), the greater the activities of amylases; a lower temperature (~30°C) favors the development of protease activity appears in koji (24).

Some hydrolytic enzymes, important in sake making, especially glucoamylases, are produced in much higher amounts in solid-state culture than in submerged culture. The glucoamylase is encoded by the *GlaB* gene. The

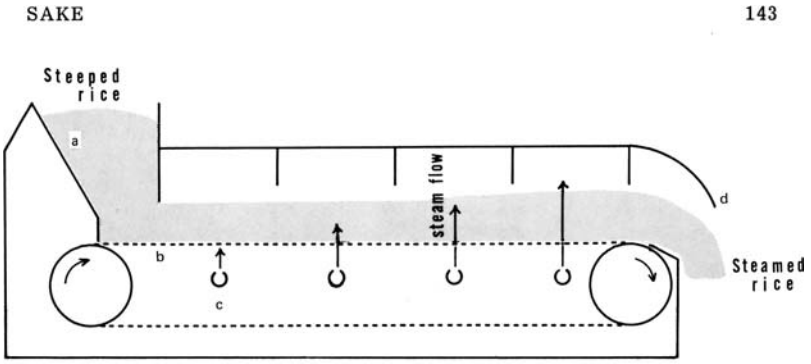


(a)

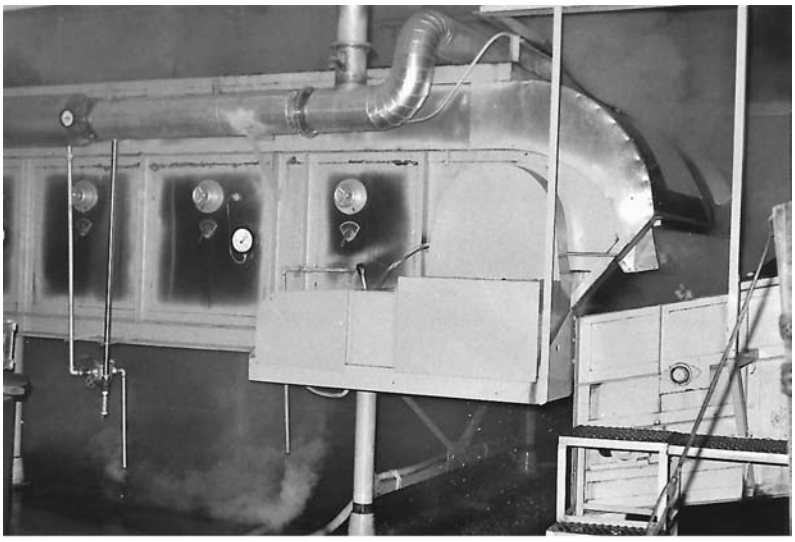


(b)

Figure 6 Steaming of rice and preparation of koji: (a) steaming of rice; (b) preparation of koji, taking the steamed rice into the koji cultivation room.



(A)



(B)

Figure 7 (A) Conveyor-belt-type apparatus for steaming rice: (a) entry port for steeped rice, (b) stainless-steel wire mesh belt conveyor, (c) steam nozzles, (d) exit port for steamed rice. (B) Conveyor belt system in operation.

conditions of low water activity, high temperature, and physical barriers to hyphal extension, as well as high contents of starch, are just traditionally developed koji preparation procedure, and the conditions enhance the *glaB* gene expression at the transcriptional level (25).

1. Process of Conventional or Traditional Koji Preparation

Taking the Steamed Rice into the Koji Cultivation Room. After the steamed rice has been cooled to 40°C, this moist rice is transferred into the koji cultivation room called "koji muro," a large incubation room where temperature and humidity are controlled at 26–28°C and at 80–90% relative humidity (RH), respectively. It is heaped on an insulated wooden bed called "toko" and covered with cloth for 2–3 h to achieve even temperature and moisture (Fig. 8).

Inoculation of Tane-Koji. The moist rice is spread over the bed and inoculated with ascospores of tane-koji by sprinkling with a sieve, followed by mixing and rubbing to attach the spores equally on the grains. Amounts of tane-koji inoculated are usually 60–100 g/100 kg of the original rice; thus, each grain will acquire approximately 2000 mold spores. The moist rice grains are again heaped up on the bed and covered with a few sheets of cloth. The finishing temperature of this process is 31–32°C.

Kirikaeshi: Breakup of the Grain Heap. Ten to twelve hours after the inoculation, the temperature of the grain mass rises slightly by respiratory heat of the growing molds. The moist rice grains lose their original smell and a few mycelial colonies of the mold begin to appear on the grain surface as white spots. To expel the heat and carbon dioxide generated inside the heap, the stiffened lump of grains is broken up. This process is called "kirikaeshi." Dispersed grains are again heaped on the bed and covered with cloths. The finishing temperature is 30–31°C.

Mori: Separating the Mass into Trays. Growth of the mold becomes vigorous and the temperature of the grain mass begins to rise about 24 h after inoculation. To control the temperature rise and lack of air, the large heaped mass of grain is separated into small trays. Usually, the grain is divided into 1.5-kg masses and heaped in a tray. Six or eight trays containing the grains are placed one on top of another. This process is called "mori." The temperature of the grain mass is about 33°C before and 31°C after the operation. As the temperature of the mass in the upper tray of a pile increases to become higher than that of the lower tray 3–4 h later, each tray is changed in its position in a pile to equalize the temperature. This operation is called "tsumikae." The temperature of the mass is 32–34°C at the time.

(a)

(b)

Figure 8 The inside of a koji cultivation room. (a) At the front, the moist rice is spread on the bed (took). After incubation with ascospores of tane-koji, the moist rice is heaped up on the took and covered with a few sheets of cloth. (b) Trays containing the grains are placed one on top of another in stacks of eight at the rear.

Nakashigoto: The First Mixing Operation. After 6–8 h of mori, the temperature of the mass rises to about 35–36°C. The mass in each tray is stirred and mixed to lower the temperature and expel carbon dioxide generated by mold respiration. After the mixing, the grains are again heaped in the tray. This operation is "nakashigoto" and the finishing temperature is about 33–35°C. After 3–4 h, the trays are also shifted in their position in the pile to adjust the temperature ("tsumikae").

Shimaishigoto: The Second Mixing Operation. As the growth of mold progresses, the temperature of the grain mass increases vigorously. About 6 h after the former mixing, it reaches 37–39°C and 80% of the grain surface is covered with the mycelia. At this point, the second mixing operation is performed to expel heat and carbon dioxide. The finishing temperature is 36–38°C.

Dekoji: Finishing of Koji Preparation. Several hours after shimaishigoto, the temperature of the mass reaches its highest point, 41–43°C. At this stage, trays are again shifted in their position in a pile to equalize temperature. Cultivation is further continued for 6–8 h from this stage. When a required quality of koji is obtained, the masses in each tray are gathered on a cloth and spread over the floor. After being stirred and mixed to expel excess moisture and lower the temperature to room temperature, the finished koji is taken out of the cultivation room.

Koji used for sake brewing is white because the growth stage of the mold is stopped before sporulation. Koji is relatively dry to the touch, consisting of individual kernels, not clumps, and each kernel is relatively hard. The mycelial growth is spread on the surface of the grain and also penetrates deeply into the kernel. Changes in temperature, composition, and development of enzyme activities in koji during cultivation are summarized in Fig. 9 (25).

2. Simplified and Industrial Preparation Methods

Big Tray Method. Because grain masses are separated into small trays of 1.5 kg each in the conventional method as described earlier, this is a troublesome operation. In the big tray method, large masses of about 15 kg are separated onto large trays. To control temperature rise and moisture content in the grain mass, the bottom of the big tray is made with wooden lattice or wire mesh; air comes through and expels heat, moisture, and carbon dioxide easily.

Koji-Making Machine. Some koji-making machines have been devised. In principle, however, they are almost the same, differing only in detailed parts such as temperature- and humidity-controlling systems or ven-

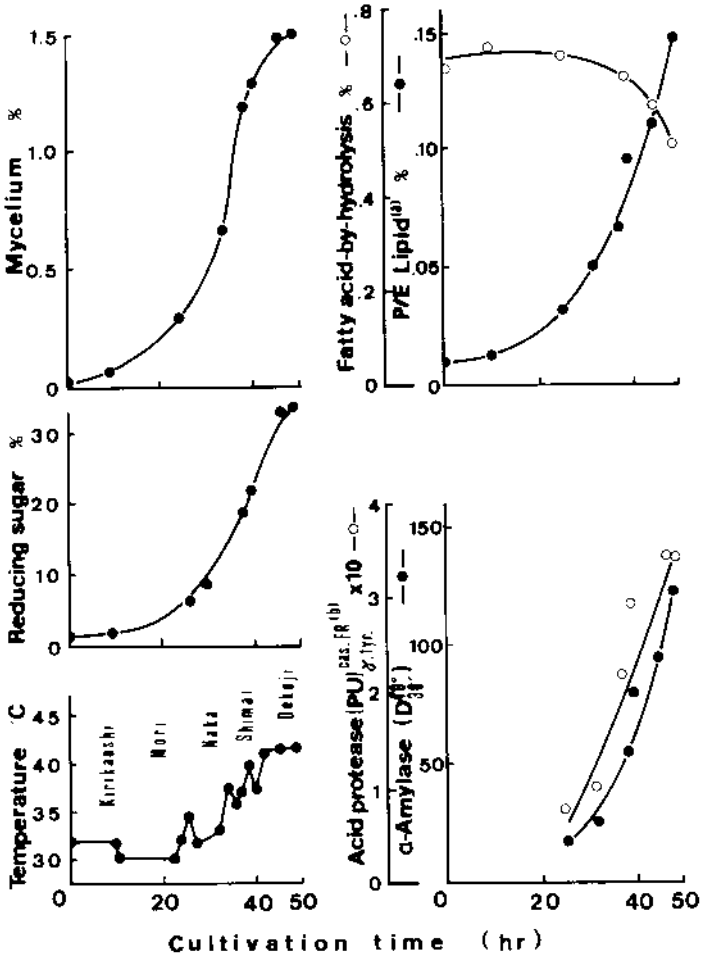


Figure 9 Changes of various components and progress of temperature during rice-koji making: (a) *n*-pentane/acetone (1:1) extractable lipid; (b) $[PU]^{cas.FR^{(b)}}_{\gamma.tyr.}$ denotes the unit of protease activity. One unit is the amount of protease that liberates 1 μg of tyrosine from 2% casein solution by reaction at pH 3.0 and 38°C for 1 min. Tyrosine is estimated by the Folin–Ciocalteu method. (Reprinted with permission from Ref. 26.)

tilation systems. Seed-inoculated moist rice is put into the machine, heaped in a fixed thickness, and its temperature and moisture content are controlled automatically by the flow of air. A koji-making machine is shown in Fig. 10.

D. Preparation of Moto, Seed Mash as Yeast Starter

Moto plays an important role as a starter of the yeast culture in carrying out fermentation of moromi or main mash. Moto is required to provide a pure and abundant yeast crop and to supply sufficient lactic acid to prevent multiplication of harmful wild micro-organisms during the preparation of moto and in the early stage of moromi fermentation. In the conventional procedure, lactic acid is produced in the mash by lactic acid bacteria, whereas in the modern industrial method, lactic acid is added to the mash at the beginning of moto preparation, so that little growth of lactic acid bacteria and other harmful micro-organisms derived from koji and the water supply takes place. The amount of rice used for moto preparation is usually 7% of the total rice used for a lot of sake mash.

1. Yamahai-moto: A Conventional Method

Preparation of "yamahai-moto" is a representative example of a traditional method in which lactic acid bacteria produce lactic acid. The principle of

Figure 10 Koji-making machine.

this method is still significant from the viewpoint of modern microbiology. The following is an example of the conventional method for moto preparation (27).

Seventy kilograms of steamed rice are mixed with 30 kg of koji and 100 L of water in a vessel at an initial temperature of 7–8°C. It is held for 3–4 days with intermittent stirring and agitation. During this period (called "utase"), the rice grains are partially digested and saccharified and the temperature falls gradually to 6–7°C. The mash is then warmed gradually at a rate of 0.5–1°C per day by inserting into the mash a wooden or metal cask filled with hot water or, as of late, an electric heater placed under the bottom of the vessel. Warming is continued for 10–15 days as the mash reaches a temperature of 14–15°C. During this period, successive changes in the microflora occur because groups of micro-organisms that require few nutrients are gradually replaced by others that have complicated requirements as the nutrients are dissolved from koji and steamed rice. As shown in Fig. 11 (28–32), in the early stages nitrate-reducing bacteria such as *Pseudomonas* and *Achromobacter* derived from koji and water appear, followed by lactic acid bacteria, including *Leuconostoc mesenteroides* var. *sake* and *L. sakei*. These bacteria multiply to reach a maximum count of about 10^5–10^8 per gram, but successively disappear before fermentation by sake yeast begins, owing to a high concentration of sugar and acidification resulting from their own metab-

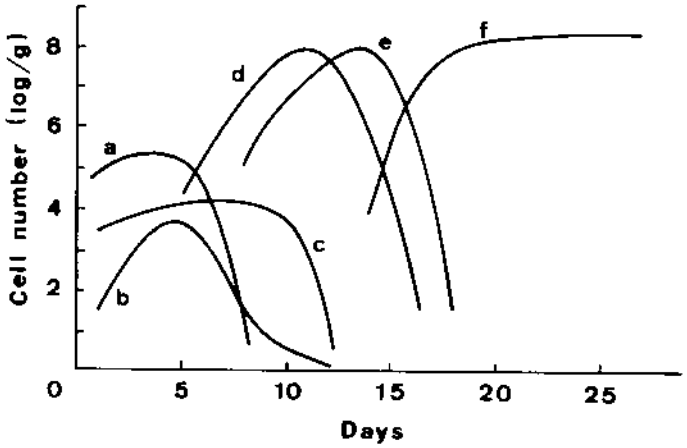


Figure 11 Changes in microflora during preparation of yamahai-moto, the naturally acidified seed mash: (a) nitrate-reducing bacteria; (b) film-forming yeasts; (c) wild yeasts; (d) lactic acid bacteria (cocci); (e) lactic acid bacteria (rods), and (f) sake yeasts. (Reprinted with permission from Ref. 32.)

olism. The wild yeasts, which are derived from koji and number about 10^4 cells per gram for the first 10 days, rapidly decrease to almost zero within another 2–3 days due to the toxic effect of nitrite, which can be produced at a rate of 5–7 mg/L by nitrate-reducing bacteria from nitrate contained in the water. The toxic effect of nitrite is multiplied by the existence of lactic acid. When wild yeasts have almost disappeared and the temperature of the mash reaches close to 15°C, nitrite has also disappeared from the mash. At this stage, only sake yeast (*Saccharomyces cerevisiae*) survives. Sake yeast can tolerate this severe environment containing 0.1–0.2% lactic acid with high sugar content. Today, purified culture of sake yeast is added to give a count of 10^5–10^6 cells per gram of mash. After 2–3 days, when the temperature has risen to 18–20°C, sake yeast reaches full growth (about 10^8 cells per gram) and fermentation begins. This stage is called "wakitsuki" (gushing out) and is maintained for 2–3 days, during which time the sugar content of the mash is reduced as a result of alcohol fermentation by yeast. A few days later, when the density of the filtrate is reduced to about 8–9 Baume, the mash is cooled to room temperature to prevent the yeast from dying or being weakened by a high concentration of alcohol and acid. Thus, by this procedure, a pure yeast population ideally suited for sake brewing is ultimately selected from a complex natural population of micro-organisms. After a resting period called "yasumi" (5–7 days), the moto is used for the fermentation of moromi mash (Fig. 12). An example of temperature progress and changes of composition during yamahai-moto preparation is shown in Fig. 13.

2. Sokujo-Moto: Rapidly Processed Moto, the Most Popular Industrial Method of Moto Preparation

"Sokujo-moto" is very popular nowadays in sake brewing. It is based on the principle that artificial addition of lactic acid is also effective in preventing contamination by micro-organisms. By this method, moto can be prepared in a relatively short time, 7–15 days, because the time required for lactic acid fermentation by naturally occurring lactic acid bacteria is saved and the solubilization and saccharification of the mash proceed more quickly with the high initial mashing temperature, 18–20°C. In this method, 600–700 mL of commercial lactic acid is added to 100 L of water at the beginning of moto preparation to give a pH value of 3.5–3.8.

Because the contamination of harmful bacteria is prevented by the early addition of lactic acid, wild sake yeast as well as the cultured sake yeast can develop during the moto preparation, and often the wild types of yeast finally predominate. This may be ascribed to the high mashing temperature and acidic conditions, which are close to optimum for the multiplication of both cultured and wild yeasts. In addition, as opposed to behavior in the

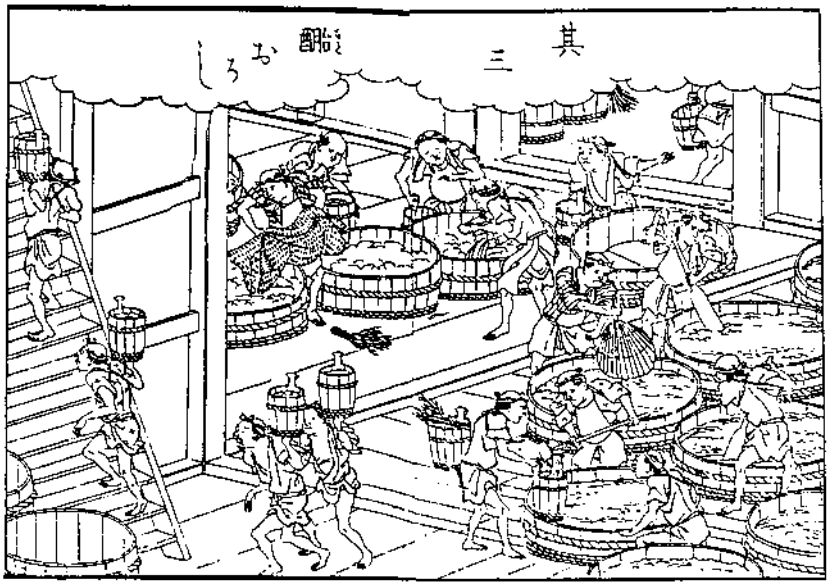


Figure 12 After the resting period (yasumi), the moto is used for the fermentation of moromi mash.

classic yamahai-moto preparation, no natural selection of wild yeasts by the toxic effect of nitrite occurs because nitrate-reducing bacteria do not appear in sokujo-moto owing to the presence of lactic acid. It is therefore very important to inoculate the cultured yeast at high levels at the start.

An example of sokujo-moto preparation is as follows: 60 kg of koji is mixed with 200 L of water treated with 140 mL of commercial lactic acid (75%), and a pure culture of sake yeast is added to give a count of 10^5–10^6 cells per gram. The temperature of the mixture will be about 12°C. One hundred forty kilograms of steamed rice is then added to the mixture, cooling it sufficiently to give a mash temperature of 18–20°C. It is kept for 1–2 days with intermittent stirring and agitation. The temperature will decrease to that of the surroundings, about 10°C. Warming of the mash is initiated after 2 or 3 days in the same way as yamahai-moto (i.e., by increasing the temperature at a rate of 1.0–1.5°C per day). This warming procedure contributes to the acceleration of the digestion of steamed rice and the growth of yeast, whereas in the yamahai-moto, it is very important in keeping the alteration of the microflora to a desired sequence. As the temperature rises to nearly 15°C, the inoculated yeast reaches full growth and fermentation begins. As contamination by harmful bacteria can be inhibited from the

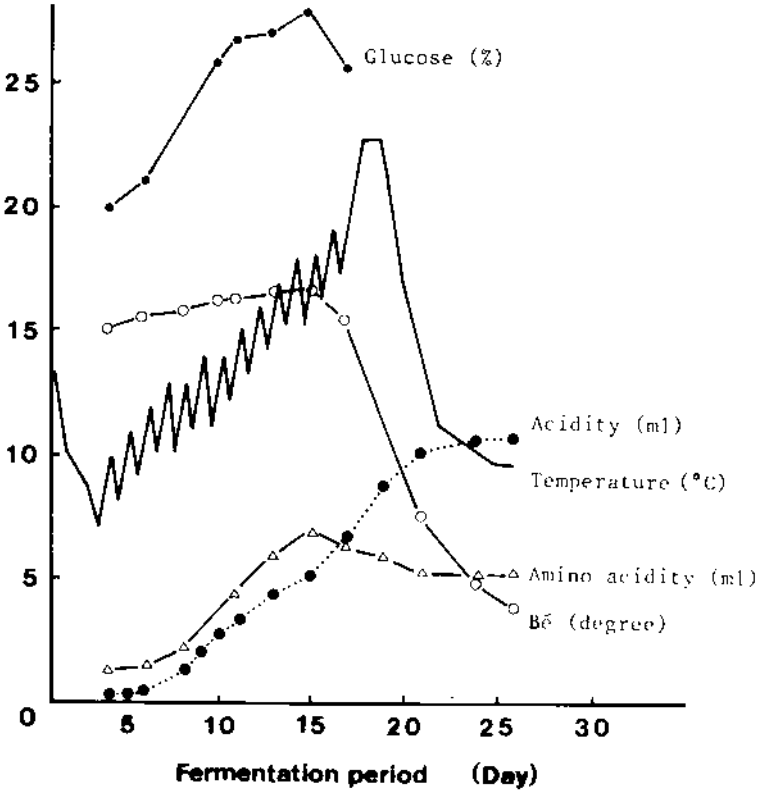


Figure 13 The progress of temperature, and changes in chemical compositions of yamahai-moto. The zigzag progress of temperature indicates the result of warming and the spontaneous cool-down and warming again on the next day.

beginning in sokujo-moto, temperature reduction of the mash at the early stages of cultivation is not always necessary. The cultivation period can be further shortened by starting the mashing at a temperature of 25°C and insulating the vessel so as not to cool the mash below 18–20°C. In a few days, adequate amounts of sugars and the nutrients are prepared and fermentation begins. Except for the several points mentioned earlier, the procedure is almost the same as that of yamahai-moto.

3. Ko-on-toka Moto: Hot Mashed Moto

This is a variety of sokujo-moto and it produces a sweetened mash prepared at a high temperature (55–60°C) in several hours, followed by inoculation

with a crop of pure cultured sake yeast. The following example is typical. To 220 L of water (out of a total 270 L) warmed to 60°C, 120 kg of steamed rice and 60 kg of koji are added in a vessel equipped with a water jacket and agitators. After incubation for about 6 h at 55–60°C, the mash is treated with 180 mL of lactic acid and rapidly cooled by adding ice blocks equivalent to the remaining water and by running water in the jacket. Composition of the mash is as follows: density, 16–17 Baume; reducing sugar, 24–25%; amino acid, 0.16–0.2%; total acids, 0.3–0.35%. When the temperature of the mash reaches about 25°C, pure cultured yeast is inoculated to give a count of 10^5–10^6 cells/g of mash.

The later procedures are almost the same as those for sokujo-moto. The advantage of this method is that excellent moto containing inoculated yeast of high purity is obtained more quickly in a week, because wild yeasts from koji are killed at a high temperature at an early stage and the broth is rich in nutrients for the growth of sake yeast during the hot-mashing process.

It is noteworthy that the amount of amino acids contained in yamahai-moto is two or three times higher than that of sokujo-moto (33,34). It depends on the properties of acid protease and carboxypeptidase which *A. oryzae* produced. The remarkable difference between yamahai-moto and sokujo-moto is the pH value in their initial stage, that is, in the early stages of yamahai-moto preparation, the pH value is almost neutral, but acid in sokujo-moto to which lactic acid is added. In yamahai-moto, the pH value from 6 to about 3.5 gradually decreases by spontaneous lactic acid fermentation. During this period, high amounts of protein solubilize from the protein body II (one of the storage proteins rich in glutelin contained in rice grain) with an action of acid protease under 20% or more of glucose coexistence and the solubilized proteins are further decomposed by carboxypeptidase and a substantial amount of amino acids accumulate in yamahai-moto. On the other hand, in sokujo-moto, the acid protease decomposes the protein body II rapidly at the optimal pH condition of the enzyme, and dipeptides and tripeptides accumulate. However, because the carboxypeptidase cannot decompose these peptides, amino acids do not accumulate and most peptides remain (35).

4. Sake Yeast

Sake yeast is classified taxonomically as a member of the *S. cerevisiae* group (36). However, sake yeast can be differentiated from other strains of *S. cerevisiae* by additional properties such as vitamin requirements (37), the osmophilic character for sugar and acid tolerance, adaptability to anaerobic conditions, and dominant growth in the fluid culture of koji mold. These

characteristics of sake yeast may be evaluated by a consideration of its dominance over the other micro-organisms, including wild yeasts, in the sake-brewing process carried out in the open and without sterile conditions. Recently, DNA polymorphism analysis has shown that the sake yeasts and shochu (a Japanese traditional spirit) yeast strains made up a different cluster from the wine, ale, whisky, and bakery yeast strains among the species of *S. cerevisiae* (38).

Generally, as shown in Fig. 14, a large amount of foam forms during moto and moromi mash fermentation. Foam formation depends on the nature of the yeast cell wall. The foaming ability depends on a GPI (glycosylphosphatidylinositol)- anchored protein having a molecular mass of 166,873 Da, which is a hydrophobic protein localized in the yeast cell wall. The protein is encoded in the the *AWA1* gene. It was confirmed that an *AWA1* disruptant shows a nonfoaming phenotype in moto and moromi (39).

Because the foam occupies approximately one-third of the capacity of the fermentation vessel, foamless fermentation would be of great value to the brewery, saving the space occupied by the foam and scaling up the amount of moromi produced. Foamless mutants, which have the same characters as the parent yeast except foam formation, can be obtained compar-

Figure 14 A large amount of foam is formed during moto and moromi mash fermentation.

atively easily by froth floatation (40,41). Many kinds of foamless mutants of sake yeast, *S. cerevisiae*, Kyokai Nos. 7, 9, 10, and 14–17, are now available. Presently, breeding of sake yeasts by hybridization is being carried out and one of these strains, Kyokai No. 13 is available for sake brewing (42).

A killer strain of yeast suitable for sake making was constructed by cytoducing the killer plasmid of double-strand (ds)-RNA into sake yeast Kyokai No.7 using a nuclear-fusion-defective killer strain (43). Such a killer strain, which produces a fine quality of sake, is useful in sake brewing, because it prevents not only the contamination by killer-sensitive wild yeasts but also prevents serious damage by the invasion of killer yeasts. The latter kills the culture yeast that has been seeded in the mash to cause abnormal fermentation (44).

The breeding of some antibiotic-resistant sake yeast mutants has been carried out (45) and some of them are now available. A cerulenin-resistant mutant shows from 4-fold to 10-fold more ethyl caproate productivity than its parent's strain (Kyokai No. 1601) and a 5,5,5-trifluoro-DL-leucine-resistant strain shows higher isoamyl acetate productivity. Of course, the "both antibiotics resistant" mutant (Kyokai No.1701) shows higher ethyl caproate and isoamyl acetate productivity (46).

The Brewing Society of Japan, Tokyo, distributes many kinds of these useful sake yeast as "Kyokai" sake yeast to breweries all over the country.

E. Preparation and Fermentation of Moromi, Main Mash

1. Characteristics of Moromi Mash

Moromi has an important role in the production of an excellent quality of sake. The qualities of the moto and koji have a large effect on the quality of moromi. All procedures hitherto described are preparations for desirable fermentation of the main mash, moromi.

Moromi is fermented in a large open vessel without special sterilization (Fig. 15). To achieve the desired fermentation and high alcohol contents (such as 20% v/v), some protocols are necessary.

Stepwise mashing, one of the characteristics of moromi, is carried out as follows: First, steamed rice, koji, and water are added to a prepared moto, with the amounts of the added materials being almost the same as that of moto. Thus, total acids and yeast populations in the moto are diluted by about one-half, but they still have an inhibitory effect against contamination by wild micro-organisms. The temperature of the first mashing is 12–14°C, and fermentation starts slowly on the second day. After a 1-day interval, when the population of sake yeast reaches about 10^8 cells/gr of mash (the same order as in the moto), a second addition of materials is made by

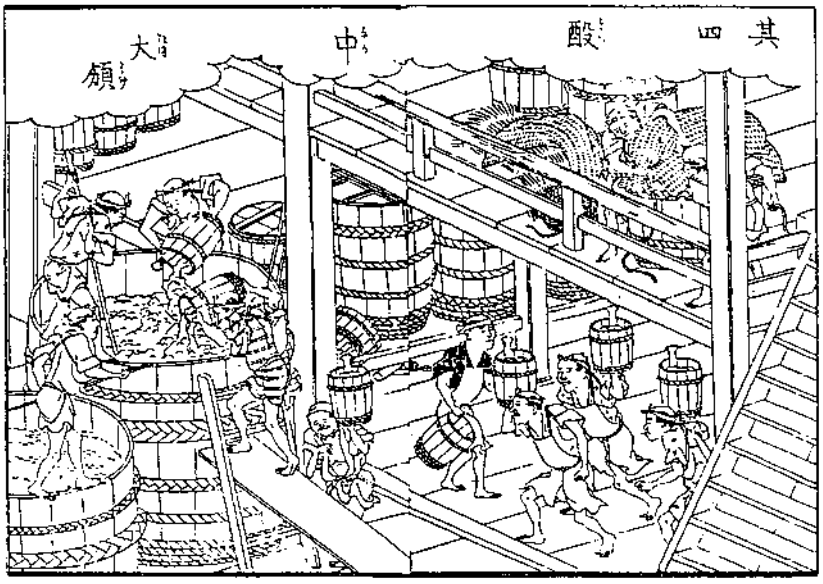


Figure 15 Preparation of moromi. The main mash is poured in a large open vessel made of wood.

adding an amount nearly twice as much as the first addition. Yeast population and total acids are again diluted to about half, now decreasing the inhibitory activity against contamination. Thus, the temperature at the second addition should be lowered to 9–10°C. The third addition of materials is made the next day, again doubling the amount. As total acids are diluted and lose most of their preventive ability against microbial contamination, the temperature is lowered further to 7–8°C. (In sake brewing, especially in the moromi stage, temperature is controlled precisely.)

Both saccharification of raw materials and alcohol fermentation occur simultaneously in moromi, whereas the processes are consecutive in moto. While sugars are released in small quantities from steamed rice and koji, they are fermented gradually by sake yeast until the alcohol content reaches nearly 20% (v/v). For as much as 20% alcohol, about 40% sugars are needed; if such a high amount of sugars appeared at once, the sake yeast could not ferment them.

Fermentation of the mash below 15–18°C is also characteristic of sake fermentation. As sake mash is fermented to prepare a balanced flavor and taste as well as to produce alcohol, the temperature of the mash has a great influence on the quality of the sake produced. The existence of suspended

solid materials such as steamed rice and koji with proteolipids from *A. oryzae* (47), contributes to the high alcohol production; yeast cells supported by these substances are always dispersed in the moromi.

2. Progress of Moromi Fermentation

Moromi mash is prepared by successive additions of materials in three steps, thereby increasing their amounts and lowering the temperature, as mentioned earlier. Table 4 gives an example of the proportion of raw materials used for a typical moromi mash.

After the third addition, a long, slow fermentation proceeds for about 15–25 days. Three to four days later, the density of the mash reaches a maximum, 7–8° Baume; the alcohol concentration is 3–4% (v/v). Foam resembling soapsuds gradually spreads over the surface and subsequently increases to form a thick layer. With fresh fruitlike aroma at this stage, the yeast cell count reaches a maximum of about 2.5×10^8 per gram. As the alcohol and acid concentrations increase, the foam becomes less dense and is easily dispersed. When the density of the mash decreases, the froth begins to recede, displaying numerous beautiful hemispheres, followed by various features, including a wrinkled scum, a smooth thick or thin covering, or no covering on the surface of the mash, according to the type of sake yeast and the physical and nutritional conditions in the mash.

The temperature of the mash reaches a maximum of 15–18°C by the day 6 to day 9, and this temperature is maintained for another 5–7 days, after which it decreases as fermentation subsides. On the 15th to 25th day, the alcohol concentration in the mash reaches 18–20% (v/v) and fermentation has almost ceased. Usually, at this stage, pure 30–40% alcohol is added

Table 4 An Example of a Formula of Raw Materials for Sake Brewing

Component	Total rice (kg)	Steamed rice (kg)	Rice koji (kg)	Water (L)
Moto mash[a]	240	160	80	260
First addition (hatsu zoe)	460	330	130	440
Second addition (naka zoe)	850	660	190	1000
Third addition (tome zoe)	1450	1150	300	2200
Alcohol[b]				850
Total	3000	2300	700	3900

[a] Sokujo (rapidly processed) moto and *S. cerevisiae* (Kyokai No. 7) was used.
[b] A 30% alcohol addition on the day 23 of the fermentation period. In the case of jummai-shu, the alcohol addition is omitted.

(not added for jummai-shu) to the mash to adjust the final concentration to 20–22% (Fig. 16). Quite often, in order to sweeten the mash, about 8% of the total amount of steamed rice is added during the final stage of the moromi process. By this means, a certain amount of glucose produced from starch by the saccharifying action of koji accumulates in the mash because of the weakened fermentative activity of the yeast in the presence of over 15% alcohol. Figure 17 (48) illustrates an example of the changes with time in various mash components and temperature during moromi fermentation.

3. Biochemical Changes During Fermentation

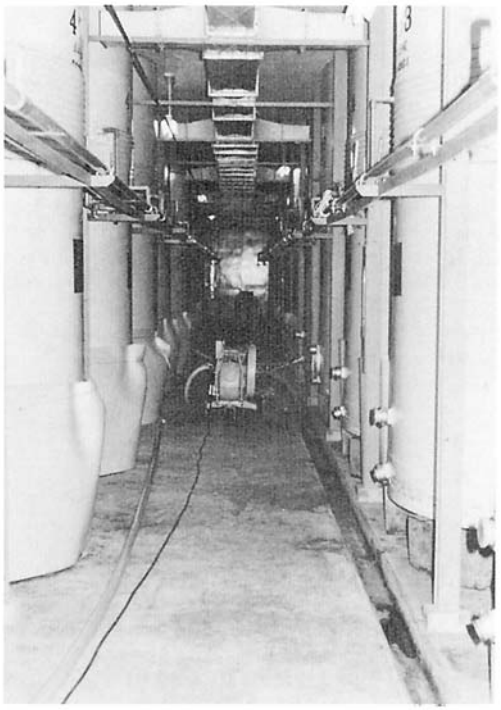
During fermentation, various biochemical changes occur in the moromi mash. First, starch changed to the α form by steaming is saccharified by koji amylases to glucose and other sugars such as maltose, isomaltose, and panose. Koji amylases are relatively stable and continue to act in the moromi throughout fermentation. Second, glucose is fermented by yeast to form ethanol and carbon dioxide. Lactic, succinic, and other organic acids are produced at the same time. In general, the first two acids, being nearly equal in concentration with malic acid, account for close to 90% of the total organic acids (49).

Protein in the rice grains is hydrolyzed by proteases and carboxypeptidase to peptides and amino acids, some of which are assimilated successively by sake yeast. Eighteen different amino acids have been identified in sake. These compounds may interact with various other substances to give smoothness and balance to the taste of sake.

Sake contains fairly large amounts of *n*-propanol, *i*-butanol, *i*-amyl alcohol, and their acetic acid esters, together with the ethyl esters of butyric and *n*-caproic (50,51). The higher alcohols are derived from glucose and partly from amino acids by sake yeast depending on the concentration of amino acids in the moromi mash, the fermentation temperature, and the yeast strain used. Because the Ehrlich pathway is important in the regulation of higher alcohol synthesis, the amounts of higher alcohols produced depend on the amounts and kinds of nitrogen sources (i.e., the concentration and composition of amino acids in the moromi mash) (52–54).

The synthesis of acetic acid esters in sake yeast cells occurs via alcoholysis of acetyl-CoA catalyzed with acetyl-CoA: alcohol acetyltransferase (AATase) with a molecular weight of 61 kDa and located in the inner cell membrane of sake yeast (55,56). The enzyme is encoded in the *ATF1* gene, whose expression is repressed by unsaturated fatty acid or oxygen (57). Two pathways are known for the formation of fatty acid ethyl esters, such as ethyl caproate, via esterase and via acyl-CoA: alcohol acyltransferase, and the locations of the two enzymes in the cell are different (58).

(a)



(b)

Figure 16 In modern sake factories, the moromi fermentation room has generally two stories. (a) The second story is the opening of the large vessel used for mashing. (b) Sake is pumped out from the tap of a large vessel after fermentation.

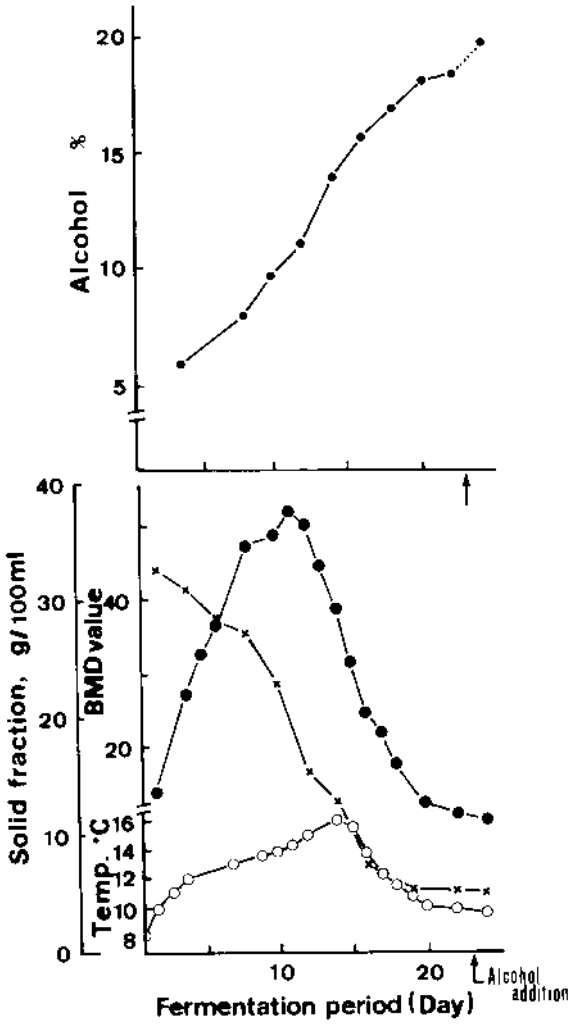


Figure 17 The progress of temperature, changes of BMD value, and solid fraction of the mash. BMD value = [density: (Baume)] × [fermentation period: (day)], ●, BMD value; ○, temperature; ×, solid fraction. (Reprinted with permission from Ref. 48.)

The most refined sake, "ginjo-shu," contains higher concentrations of esters such as ethyl acetate, ethyl caproate, ethyl capylate, ethyl caprate, and isoamyl acetate (59,60). Ginjo-shu is brewed by elaborate, skilled manual techniques of a veteran brewmaster using highly milled rice with a polishing ratio of 40–50% and containing little protein, at an extremely low temperature of 9–11°C and taking longer periods, of more than 25 days of slow fermentation. Thus, ginjo-shu contains the high concentrations of esters and rather low concentrations of nitrogenous compounds, organic acids, and sugars, all of which contribute to provide the special flavor and taste.

F. Filtration, Pasteurization, Storage, and Bottling

1. Filtration

After the addition of alcohol to the moromi, except for jummai-shu, the mash is divided into sake and solids by filtration in the conventional method. The mash is poured into hemp bags of about 5-L capacity (currently the bags are made of synthetic fiber), which are laid in a rectangular box. The pale yellow liquid, sake, is squeezed out under hydraulic pressure. In modern brewing factories, several automatic filter presses for filtration of moromi have been used (Figs. 18 and 19).

After complete filtration, the solids pressed in a sheet are stripped out of the bags. The cake, called "sakekasu," contains starch, protein, yeast cells, and various enzymes and is used for making foodstuffs such as soup and for preserving some kinds of vegetable and fish meat.

In general, about 3 kL of sake containing 20% ethanol and 250–300 kg of sakekasu are obtained from a ton of milled rice. The slightly turbid sake is clarified to separate lees by placing it undisturbed in a vessel for 1–2 weeks at a low temperature. During this process, the fresh sake matures gradually, probably because of continuing enzyme activity. Maintaining a low temperature for suitable periods is important in order to avoid overaging of the sake by autolysis of the yeast cells, which results in a deterioration in quality. After setting, the supernatant is filtered with activated charcoal carbon (if the color of sake is deep) cotton and membrane filters are also used for fine filtration. The clarified sake is blended in order to ensure uniform quality.

2. Pasteurization

After holding the clarified sake for another month, it is pasteurized to kill yeasts and harmful micro-organisms, especially hiochi-bacteria such as *Lactobacillus homohiochii* and *L. fructivorans*, if present, and to deactivate enzymes to adjust the rate of maturation. *L. homohiochii* and *L. fructivorans*

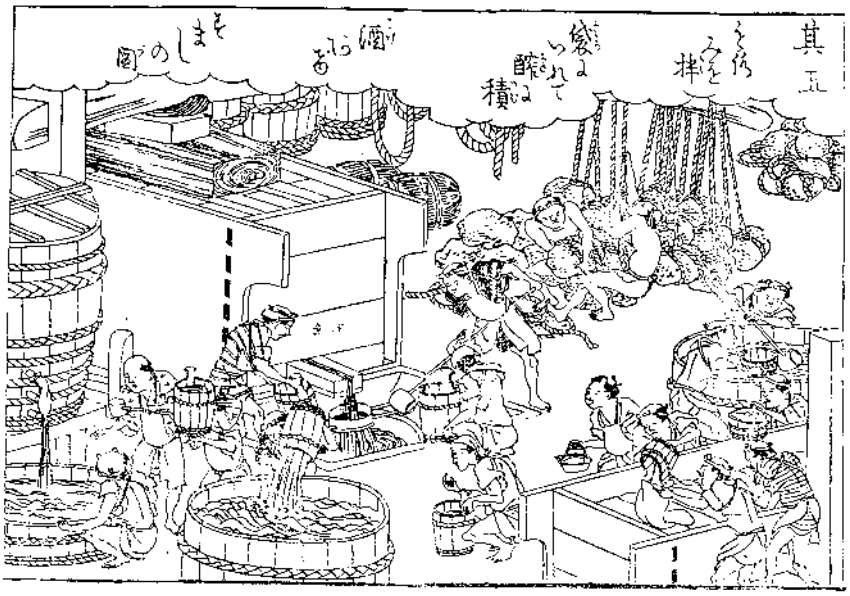


Figure 18 Filtration. Sake is squeezed out under the counterweight of stones instead of an hydraulic pressure.

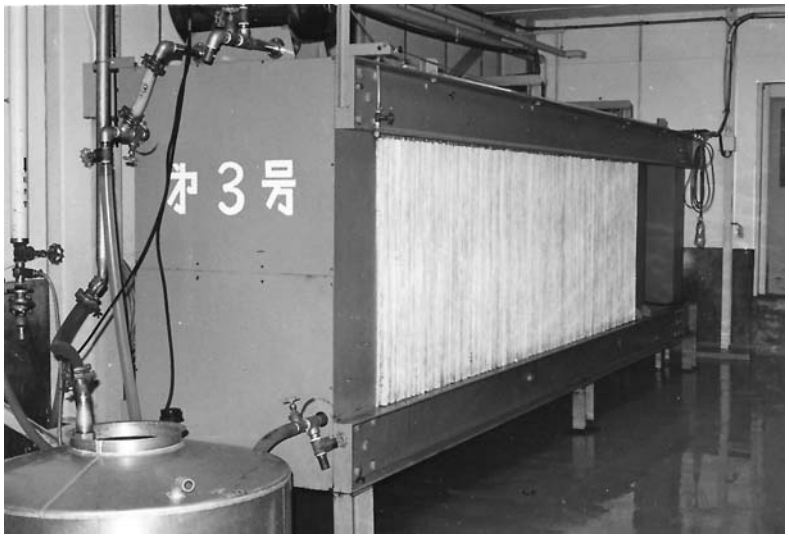


Figure 19 An automatic filter press for moromi filtration.

can grow in sake having 14–16% alcohol with a growth factor, mevalonic acid produced by *A. oryzae* (61). The sake is heated to 60–65°C by passing it through a tube-type heat exchanger for a short time. Recently, multitube or plate-type heat exchangers with a high efficiency of heat transfer have become available.

As mentioned previously, the history of pasteurization of sake began in the 16th century, before Pasteur's discoveries. Since the middle of this century, pasteurization has been used to sterilize sake produced during the warm seasons. Immediately after pasteurization, sake is transferred to sealed vessels for storage with or without the addition of activated charcoal carbon. Pasteurization and the high content of alcohol in sake prevent most microbial infections.

3. Storage (Aging) and Bottling

Storage. Sake matures gradually in storage. The maturation process is probably due to chemical oxidation and to physicochemical changes. The storage temperature should be carefully kept at 13–18°C for common sake (Fig. 20) and at 0–5°C for refined or fresh ones, with consideration given to the rate of maturation and the time of bottling.

Because sake may vary in flavor and taste in each batch during storage, blending is necessary to provide a uniform quality. The blended sake is diluted with water to the appropriate alcohol content, usually 14.0–16.5% (v/v) and is filtered through activated charcoal carbon to improve the flavor and taste and to adjust the color and clarity. In modern procedures, filtration is done through membranes or sheets having numerous pores of 0.45–1.0 μm in size, thus removing minute particles, including micro–organisms, if present. This procedure enables the sake producer to omit pasteurization in the bottling procedure and thus prevent deterioration of quality caused by heating sake (nama-zake).

Clarity is one of the most important properties of sake. The main turbidity-forming substances found in sake are enzyme proteins, especially saccharifying amylases from koji (62), which decrease in solubility upon heating and are suspended in sake. These substances react with bacterial and fungal proteinases to aggregate and settle out (63). The insoluble substances are also compounded by reacting with tannin from persimmon, followed by the addition of protein such as gelatin to complete the reaction (64–67).

Chemical Changes in Sake. During aging, some compounds such as 2-deoxyglucosone are found to increase in concentration in sake (68) and accelerate aminocarbonyl reaction to turn the color of the sake to brown. The product of the reaction, melanoidin, is the main coloring component of sake and contributes about 40–80% of the coloration (69). Ferrichrysin, one

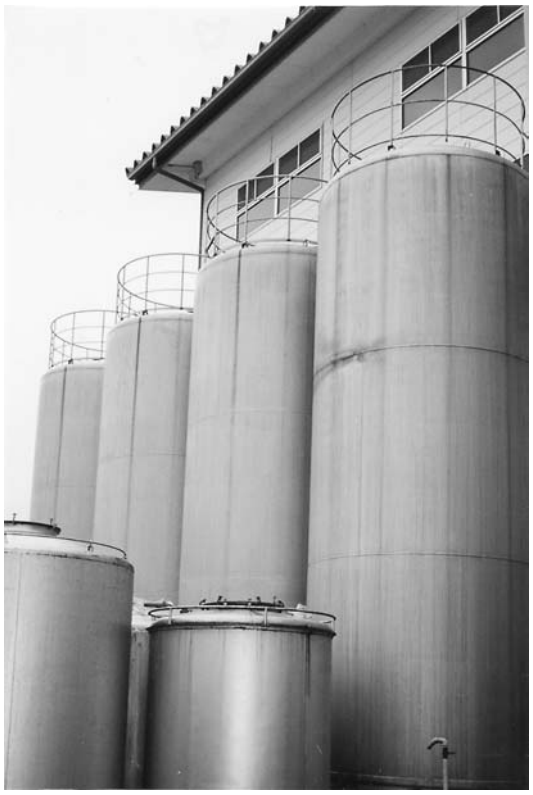


Figure 20 At some modern factories, the large-scale fermentation tanks and the storage tanks are installed outdoors.

of the ferrichromes, is the iron-containing coloring compound in sake (70) and turns the color of sake to impure brown. The colored sake is generally unmarketable. *A. oryzae* produces the colorless compound deferriferrichrysin, the iron-free form of ferrichrysin, during koji making. This compound develops a color by combining with ferric ions originating from rice grains, water, and various other contaminants in the sake-brewing process. Contrary to the ease with which melanoidin can be removed with activated charcoal carbon, it is difficult to remove ferrichrysin from sake. Breweries therefore take care to prevent contamination by ferric and also ferrous ions, which are easily oxidized to ferric ions in sake-brewing procedures.

Because sake is also very sensitive to exposure to sunlight (71), bottled sake increases its color intensity if it is exposed to sunlight during trans-

portation or in a window display and this is accompanied by a deterioration in quality. Brown or emerald green bottles, which are impervious to most wavelengths in sunlight, effectively prevent color development caused by exposure to this light.

G. Treatment of Wastewater

Water pollution is a serious problem that needs to be solved. Since 1972, sake breweries have been requested to decrease the suspended solids (SSs) content and biochemical oxygen demand (BOD) of the discharged wastewater and to adjust its pH value to that approved by Japanese governmental ordinance. About 25–30 kL of wastewater, containing from 500 to 100 ppm of SSs and BOD, respectively, is discharged per ton of rice used in sake brewing. In sake-brewing procedures, the bottle-washing process discharges about 40% of the total amount of wastewater, which contains few SSs and low BOD, but its high pH value should be adjusted by adding a solution of mineral acid.

The rice-washing procedure, followed by the steeping, accounts for 50–70% of the total BOD (72). Therefore, it is important to treat the wastewater discharged from rice-washing and steeping procedures in order to effectively lower the SSs and BOD of the total wastewater.

Two coagulation methods using respectively polyalminum chloride and ferric chloride, with high-molecular-weight coagulating reagents such as polyacrylamide, are effective and simple in lowering the SS content and BOD of the wastewater discharged from the rice-washing procedure (17).

Microbiological treatment using yeast, one of the strains of *Pichia anomala*, followed by activated sludge treatment can lower the BOD in discharged water to below 20 ppm (73). A unique bacterium *Rarobacter faecitabidus* inhabits the yeast-activated sludge tub. The peculiar bacterium digests living yeast cell wall causing the cells to burst and be killed (74).

IV. CONCLUSION

Advances in the understanding of the micro-organisms participating in sake brewing make it possible not only to confirm the fundamental soundness of many traditional techniques of sake brewing but also to improve the technology by eliminating certain unfounded or unnecessary procedures and by developing various new items of equipment such as those for continuous steaming, microfiltration, automatic koji preparation, and new computer control systems.

On-line monitoring of specific gravity, ethanol and sugar contents in sake mash (75) and automatic controls for fermentation temperature will be

introduced to sake-brewing processes. Liquefaction brewing is performed at some breweries as a new sake-brewing method (76). The advantages of this method are that (a) automatic control of moromi fermentation is realized because the rice and koji are liquefied and they have been homogenized, (b) the conversion rate of rice starch to alcohol is improved, and (c) the energy cost for materials processing is reduced.

These advances will bring about revolutionary changes in the traditional technology of sake brewing and will compel breweries to use large-scale processing. On the other hand, refined sake such as ginjo-shu, having an unique character, will continue to be made in small breweries by elaborate manual techniques using the traditional procedures. These refined sakes will continue to be highly appreciated by many connoisseurs, just as are the "chateaux wines" in France.

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4
Industrialization of Japanese Natto

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I. INTRODUCTION: DESCRIPTION AND HISTORY OF NATTO

A. Raw Materials

The main raw materials for natto production are soybeans, water, and natto starter. Additionally, salt and koji may be added to preserve the natto or to promote the degradation of soy proteins and carbohydrates.

B. Natto in East Asia

1. History of Natto

There are many kinds of natto in Southeast Asia; for example, douche in China, tua'nao in Thailand, pe-pok in Myanmar, kinema in Sikkim of India, Nepal, and Vietnam, chongkukjung in Korea, and so forth. The starters for these products are *Bacillus subtilis*.

Sasuke Nakao hypothesized that natto was first developed in the monsoon area of Southeast Asia where there are lucidophylluous forests (east asian evergeen forest) (1,2). He pointed out that Yunnan province in China should be the original center of natto. He assumed "the triangle of natto" hypothesis, because there are many kinds of natto in the triangle area connecting Yunnan province in China, Thailand, Myanmar, Bhutan, and Nepal to Indonesia and Japan. Although Indonesian tempeh is regarded as a kind of natto, the starter is a fungus, *Rhizopus oligosporus*, rather than a bacterium.

2. Natto in China (Douche)

There are three kinds of Chinese fermented soybean food: nonsalted douche, sweet douche, and heavily salted douche (3,4). The use of douche spread from China into Southeast Asia and east Asia (Fig. 1). In particular, the use of nonsalted douche spread widely in Southeast Asia. The starters of Chinese douche are usually bacteria belonging to *B. subtilis*, but fungi are used as starters for some Chinese nonsalted douche. Those products are

Figure 1 Douche sold in Yunnan province, China: douche mixed with red pepper and jinger (top); douche covered with pepper and ginger as insect powder (middle); douche shaped in thick disks (sold in Yunnan province, China) (bottom).

manufactured by fermenting steamed black beans or soybeans with *Mucor racemosus*, *Aspergillus oryzae*, *Rhizopus* sp., natto bacilli, and so forth and then brewing in brine water (5). The product is a fermented food holding the shape of whole beans during the fermentation, and after the fermentation salt, red pepper and/or ginger is often added to douche and the mixture is mashed for preservation (salted douche). Chinese douche has been classified as dried or wet by its moisture content. The former douche is produced by drying the latter. *A. oryzae* has strong protease and amylase activities. *Mucor* has a stronger cellulase than *A. oryzae* do, but the protease and saccharifying amylase activities of *Mucor* are weaker. The soybean coat is degradated by the cellulase of *Mucor*, and the coat of the douche becomes soft. The product, douche, holds its shape and has good flavor.

3. Natto in Japan

Itohiki-natto. There are two kinds of natto in Japan, namely nonsalted natto (called itohiki-natto) and salted natto called (daitokuji temple natto or hama-natto). Most Japanese natto is itohiki-natto, which is a sticky food (Fig. 2). In this chapter, we describe only the latter natto. According to legend, natto was accidentally produced in the East-Northern District by a military officer and used as a military food about 1000 years ago (6).

Yabe first isolated the natto bacterium in 1895 and analyzed the components of natto. Sawamura isolated and purified the natto bacterium in 1913 and named it *Bacillus natto* (7). In 1919, Professor Jun Hanzawa of Hokkaido Imperial University commercially marketed natto made with a purified starter of natto bacilli, and he also marketed the starter (8). Before his time, the packaging for natto had been made from rice straw, but he used packaging made from thin wooden shavings of Japanese cedar, which made industrial production of natto with the purified starter possible.

In 1949, Yamazaki and Miura developed a fermentation room for natto and created the basis for the natto industry (9). Natto had not been produced safely with rice straw packages by wild starters, but now natto production was controlled, and the industrial production of safe, high-quality natto could be conducted. Before World War II, natto was produced by farmers or manufactured in small factories and circulated in limited areas, but after the war, refrigerators, supermarkets, and automated, large-scale industrial manufacturing all became widespread. Large-scale production and marketing of natto, with product circulation all over Japan, began at that time (10) (Figs. 3 and 4).

Most modern equipment for natto factories is manufactured by Suzuyo Kogyo Co. (11,12). More than 90% of the instruments used in these factories are produced by this one company.

Figure 2 Sticky materials (called Ito in Japan) of natto.

Other Japanese Natto (13–16).

CRACKED NATTO. Parched soybeans are cracked, and they are used as a raw material for natto production. The soaking time is short (3–4 h) after since the endosperms are exposed. Cracked natto is popular among children and the elderly (17).

YUKIWARI-NATTO (ONE OF THE TRADE NAMES OF BARREL NATTO). Yukiwari-natto is a special product of Yamagata prefecture located in the northeastern part of Japan. Eighteen kilograms of cracked natto, 9 kg of koji, and an appropriate amount of salt are mixed and brewed in a large barrel in winter; the product was traditionally eaten during the season of inoculation of rice, when farmers are especially busy. Yukiwari-natto is a commodity of barrel natto, shipping to Tokyo, and so forth.

Figure 3 Natto packed in a polystyrol paper tray (PSP) package and its label covering the package.

Figure 4 Natto packed in a cup package and soy soup in a small plastic bag. Goods in a package of three are usually sold in Japan.

BARLEY NATTO. Barley is used as a raw material in addition to soybeans in this type of natto. Because natto bacilli grow abundantly on barley, barley natto is very viscous.

SALTED NATTO. It is said that salted natto was imported from China and is derived from the salted douche of China. Soy-koji (*A. sojae*) is grown on steamed soybeans and mame-koji (soybean koji) is produced; then it is brewed with salt and water.

DRIED NATTO. Dried natto is a food for preservation (Fig. 5). Natto sprinkled with salt is stored for about 100 days and then dried after the natto has lost viscosity.

SOBORO-NATTO. Soboro is a mixture of natto and pieces of white radish. Soboro-natto is also a food for preservation. After a white radish is cut into small pieces, the pieces are dried. After softening by soaking in soybean nijiru (juice drained from soybeans during cooking), the radish pieces are mixed with natto soaked in 4–5% brine water. This type of natto is a special product of Ibaraki prefecture of Kanto District located in the center of Japan.

How Natto Is Used (13).

MIXED WITH RICE. The ways in which natto is used as a food differ in various geographic areas. In Japan, natto is most commonly mixed with rice and eaten with chopsticks.

IN MISO SOUP. Natto can also be used as an ingredient in miso soup.

Figure 5 Dry itohiki-natto from Ibaragi prefecture, Japan.

NATTO-SUSHI. Natto can be used as an ingredient in sushi. This type of sushi is called natto-maki (maki means "rolled"); in this type of sushi, the natto is put on rice rolled with dried seaweed. Natto used for natto-maki is often mixed with Japanese pickles.

ACCOMPANIMENTS TO DRINKS. Natto is fried or is hardened tightly in a ball shape and baked. Natto prepared in this way is eaten as an ingredient with sake and tea.

MEAL OF RICE AND TEA MIXED WITH NATTO. Natto is sometimes added to rice, and tea is poured on the mixture.

NATTO PICKLES/SALAD. Natto, miso, salt, sugar, garlic, and other ingredients are mashed together and stored in chinaware for a few days to produce a pickled dish that is served as a salad.

4. Tua'nao

Tua'nao has different names in different areas (e.g., pe-pok in Myanmar and tua'si in Laos). Soybeans are cooked with an equal amount of water for 3–4 h, and the excess water is drained. The cooked soybeans are transferred to a bamboo basket, covered with banana leaves, and fermented for 3–4 h. The soybean loses its unique flavor, increases the viscosity, and develops ammonialike flavor, thereby yielding natto. Spices and other ingredients are added to the natto, which is mashed into a paste called "raw tua'nao" (18–20).

Before it is eaten, raw tua'nao is packed with banana leaves and steamed or baked for 30 min. A product with 50–55% moisture is obtained, which is called "cooked tua'nao." Its shelf life is only 2 days at room temperature. Raw tua'nao in a ball shape of 1.3 cm diameter is pushed into a tip and dried in the sun until the moisture falls to 10–15%; the resulting product is called "tua'nao tips." It can be stored for several months (Fig. 6).

Cooked soybeans are fermented and then mixed with salt and seasonings. The product, called pe-pok, is obtained by fermenting for 2–3 months.

Tua'nao is used as raw material for foods like fries (called as tua'nao sa koa), dressing (tua'nao sa koe) with a shallot, mint leaves, red pepper, sodium salt, sesame oil, and so forth, or a raw material of a seasoning, (nam-prik pong) (20,21).

The starter of tua'nao fermentation was isolated and identified as *B. subtilis* (22).

5. Kinema

According to Tamang and Sarkar (23), Tamang (24), and Nikkuni (25), kinema is an important food in the eastern Himalayan regions of Nepal, the Darjeeling hills and Sikkim in India, and the northeastern hills of India,

Figure 6 Semidried Tua'nao.

Bhutan, and Nepal. Kinema is a fermented whole-soybean food with a sticky texture, gray to tan in color, and flavorful. It is similar to natto. In the Sikkim Himalayas, small-sized (up to 6 mm) yellow soybeans are washed and soaked overnight and then cooked by boiling. After the soybeans are cracked, the grits are placed in a bamboo basket lined with fresh fern fronds and left to ferment naturally at ambient temperature (25–40°C) for 2–3 days in an earthen kitchen oven. In some villages, about 1% of fresh firewood ash is added to the cooked soybeans during production. The product is a white viscous mass with a typical kinema flavor and a slight ammonialike odor. The shelf life is 2–3 days during summer and a maximum of 1 week in winter without refrigeration. Sun-dried kinema can be stored for several months at room temperature.

Karki (26) reported that kinema is now limited to the eastern hills of Nepal and that the *B. subtilis* strain was found to be the dominant microflora of kinema fermentation. Nikkuni visited Hille, a town in the mountains located to the east of Nepal, which is an area where kinema is manufactured and consumed (25,27). According to his report, kinema is mainly made of black soybeans. Black soybeans are considered better than yellow soybeans. Soybeans are washed, soaked, and cooked. The cooked soybeans are cracked crudely with a wooden pestle while they are hot and then put in a bamboo basket in which banana leaves have been spread and wooden ashes have been

scattered (28). After the soybeans have been placed in the bamboo basket, the ashes are scattered over the soybeans. The bamboo basket is covered with banana leaves, placed in a jute bag, and left beside the fireplace in the kitchen during fermentation. No starters are added. The kinema is ready to eat within a few days. The flavor and savor of kinema are similar to those of natto, but kinema is not as sticky than natto. Kinema is eaten as an ingredient in a soup with green vegetables. It is also used as a flour to be sprinkled over main dishes.

Kinema is used as a curry ingredient because it emits a pleasant flavor that enhances curry. Onions, tomatoes, green chilis, turmeric, and other ingredients are included in kinema curry. According to Sarkar et al. (29), Kinema contains 7% salt, and it is an alkaline food, with a pH of 7.89. The representative isolate was *B. subtilis*, and, organoleptically, the monoculture fermentation of soybeans by *B. subtilis* produces the best quality kinema. Monoculture fermentations by other micro-organisms could not produce kinema, suggesting that *B. subtilis* is the sole fermentation organism for this product (30,31).

6. Chongkuk-jung

In Korea, chongkuk-jung is mixed and mashed with 7% salt, red pepper, and garlic and used as an ingredient of chige soup. Chige soup is cooked by adding seven parts of water to one part of chongkuk-jung and boiling. Most of the bacterial isolates from chongkuk-jung have been identified as *B. subtilis* (*natto*) (32,33).

7. Dawadawa

Professor S. A. Odunfa of Nigeria (34) brought the first information on dawadawa to Japan in 1985. Dawadawa is a nattolike food, and it is prepared as a traditional family art, practiced as a rural cottage industry. The preparation method is as follows: Dry locust beans are boiled in water in a covered earthenware or metallic pot for up to 24 h to soften the seed coats or testae. The boiled seeds are then put in a *mortar* and pressed by foot. Alternatively, it can be pounded with a pestle. Sand or other abrasive material such as wood ash is added to help in the removal of the testae; the cotyledons are then washed thoroughly, and the testae are removed using baskets or special sieves, such as earthenware perforated calabashes or pots. The process requires a small amount of water, and in some places, it is carried out near a stream. The washed bean cotyledons are boiled again for 1–2 h in a metallic pot. A softening agent locally called kaun in Yoruba or kanawa in Hause is added during the second boiling to aid in the softening

of the bean cotyledons if a softer variety of dawadawa is desired. Kaun contains sunflower seeds and native potash, a salt containing mostly potassium carbonate, and bicarbonate. After the second boiling, hot bean cotyledons are drained through raffia or baskets and spread on a clean calabash tray in layers about 4 cm deep. Two or three calabash trays containing cooked bean cotyledons are stuck together and wrapped in two layers of rucksack or cutting cloth to provide a warm, humid atmosphere. The hot cotyledons also provide moisture and increase the humidity for fermentation. The cotyledons are then left to ferment for 36 h or longer, depending on local practice. In southwestern Nigeria, the Yorubas allow 2 days for fermentation, whereas in Ghana, it may be up to 4 days. The temperature increases during the fermentation from the ambient temperature of 25–30°C to 45–50°C. Fermentation is terminated by removing the covers from the trays, thus exposing the beans to the air.

A kind of bacteria, predominantly *Bacillus*, grows in the beans during fermentation and produces a sticky, moisturized substance that covers and links the individual bean cotyledons. The fermented beans have a strongly proteolytic activity and slightly ammoniac smell. During the 3-d fermentation, the bean cotyledons change in color from light brown to dark brown and become softer. About 70 kg of fresh dawadawa is obtained from 100 kg of locust bean seeds.

To make soybean dawadawa, the soybeans are first fried to a tawny brown color and then ground to remove the seed coats or testae. The whole soybeans are cooked in water for 3 h. The cooked beans are drained using the calabash sieve and are spread in baskets lined with leaves. Some dawadawa from a previous batch is added in the basket to act as a source of starter bacteria. The mixed beans are covered with the same leaves used for lining the baskets. The covered baskets are placed in a warm place for 2 or 3 days for fermentation. The fermented beans are then sun-dried and pounded to a fine powder. The postfermentation treatment of dawadawa varies with local practice. The Yorubas of southwestern Nigeria add salt to the fermented beans. The salted beans are molded into bowls about 3 cm in diameter. Bowls of dawadawa are arranged in calabash trays for marketing. The calabash trays are covered with flat leaves of raffia to keep away the flies and other insects. Freshly prepared dawadawa must be sold within 3 d; otherwise, it becomes infested with maggots. The most important postfermentation treatment involves drying of the dawadawa. Sun-drying yields a stable dark brown to black product. Dry dawadawa can be stored in earthenware pots for up to 1 year.

The optimum temperature and time for dawadawa fermentation were found to be 35°C for 36 h and 40°C for 48 hs. The optimum relative humidity level for fermentation was 51% at 35°C and 71% at 40°C.

Dawadawa is a strong-smelling product and is normally used as a condiment to flavor soup or stew. Stews and soups are essential features of the diet of West Africans. Dawadawa may also be added to millet-based dumplings and porridges.

Kato visited Nigeria as a member of the agricultural biotechnology section of the Food and Agriculture Organization of the United Nations (FAO) in 1989 and investigated the actual production of dawadawa (35). He reported the following: After fermentation, the bean was mashed until the shape was completely lost; the cakelike product was then dried in the sun. The product was similar to dried miso and could be dissolved in hot water just like miso. It emitted the strong flavor of butyric acid. The soup made from dawadawa was cooked with corn, chili, and so forth and it was as essential to daily cooking in Nigeria as miso soup is in Japan. Kato mashed natto and cooked natto soup, and asked some Nigerian people to compare natto soup with dawadawa soup. The Nigerians liked the natto soup as much as they liked dawadawa soup and said that the products were similar. West Africa is the habitat of okra, and the people are so familiar with the viscosity of okra that they do not feel uneasy about the viscosity of natto soup. Soybeans were brought to Nigeria in 1906, and in the 1970s, a species appropriate to West African soil was selected for cultivation. As a result, soybean production has been increasing at a rapid rate in Nigeria over the past 10–15 years. The amounts of soybeans produced in Nigeria during 1986 and 1998 were 68,000,000 and 405,000,000 metric tons, respectively. No information is available on the proportion of Nigerian soybean production utilized in the making of dawadawa.

8. Making of Natto in the Home

Various kinds of natto are manufactured by farmers and small factories. In Japan, natto was formerly made at home in many districts, but now commercial natto is extensively utilized.

II. PRODUCTION AND CONSUMPTION

The amount of soybeans consumed for natto in Japan was 130,000 metric tons. Because about 2 g of natto can be produced from 1 g of soybeans, this means that about 260,000 metric tons of natto were produced, corresponding to 5.20 billion 50-g packages. The Japanese market share of natto was 160.5 billion yen in 1999 (36).

Approximately 500 companies produce natto in Japan, but the largest 10 companies dominate the market, accounting for 85% of total production.

III. THE FERMENTATION PROCESS

Before fermentation, soybeans, one of the raw materials, must be cleaned to maintain quality and remove contaminating materials. After harvest, soybeans are purified at local dealers' factories and then stored in a low-temperature warehouse at about 15°C, with the humidity kept at about 60%. Soybeans to be used in 4–7 days are shipped from the warehouse to a silo of a natto factory. In the raw material treatment factory, contaminants are removed and soybeans are separated according to size. Soybeans are stored in the charge tank and prepared for production.

On the first day, the natto manufacturing process begins with the removal of the soybeans from the charge tank and measurement of their weight. The next procedures are washing, to remove foreign materials that may have stuck to the surface of the soybeans, and soaking at 15°C for 18–20 h.

On the second day, the steaming process is performed. Soybeans are steamed at 2 kg pressure (at 132°C) for 30–40 min. The pressure is decreased to normal, and the soybeans are inoculated with natto bacilli while they are still hot. A total of 30–50 g of inoculated soybeans are placed in a package and covered with a polyethylene film, small packs of soy sauce and mustard are placed on the film, and, finally, the package is covered and sealed. Then, 35–42 packs are put in a layer on a container, and 12–15 containers are piled onto a cart and taken to the fermentation room.

The fermentation process follows the bacterial growth curve, passing from the lag, log, and stationary phases to the death phase. The fermentation pattern is controlled by a computer to create optimal conditions depending on the properties of the raw materials, the kinds of package used, and other factors. The approximate fermentation time is 20–22 h.

The main purpose of the first part of the fermentation process is to grow natto bacilli. During the lag phase, the room temperature and humidity are kept at 37–40°C and 85–90%, respectively. During the log phase, oxygen is provided by an air supplier and a ventilator. During the stationary phase, the temperature of the soybeans is kept at 52°C for 4 h; this temperature is optimal for the synthesis of viscous materials. The humidity is lowered and the temperature is decreased to 20°C by compulsory cooling to remove moisture from the natto and to inhibit the deamination caused by the growth of natto bacilli.

On the third day, natto is removed from the fermentation room and aged outdoors. Then, natto is carried to the first refrigerator and cooled to 0–5°C.

On the fourth day, the packaging process is performed. Natto is taken out of the refrigerator and two, three, or four packages are put together and packed using a film label instrument. Cups are packed using shrinkwrap, stamped with a date, packed in corrugated cardboard, and then stored in a

product refrigerator kept at 0°C. Natto is usually shipped within 1 day, but even if it is stored, aging continues and good flavor develops.

IV. MATERIALS USED FOR TRADITIONAL AND MODERN NATTO PRODUCTION

As discussed earlier, the raw materials for natto are soybeans, water, and natto bacilli.

Soybeans were distributed from continental China to Japan, and the species of soybean used are now more than 400. Soybeans for natto have been cultivated in many places, and large, medium, and small soybeans have been used. Currently, however, small soybeans are usually used.

Water that has met the Japan Industrial Standards is usable for natto production. Both municipal water and underground water have been used for producing natto without significant problems.

Traditionally, natto was produced by packing cooked soybeans in a straw package; the straw served as a source of wild natto bacilli for the fermentation. Natto production has been improved by using purified organisms and a hygienic package since 1919, and in the 1940s, the scientific method became popular. Today, marketed natto bacilli are produced and supplied by three starter makers. Since the 1980s, studies on the improvement of natto bacilli have been reported. Some of the recently developed strains have been put into use to produce natto with special characteristics, such as stronger or weaker viscosity, mild flavor, or special food functionalities (37).

V. MODERN MANUFACTURING METHODS

The factories of the 10 largest natto manufacturers are scattered all over Japan. A factory can treat 15 tons of soybeans in a day and produce 550,000 packs, each containing 50 g of natto, manufactured in a day.

The natto industry started later than other fermentation industries. However, after 1960, the industry developed hygienic, power-saving production systems, solved problems pertaining to preservation techniques, and produced appropriate machines, electronic instruments, and refrigerators, thus leading to modern industrial natto production.

Figures 7–9 explain the methods of natto production, showing the flowsheet, the flowchart, and diagrams of each process.

Raw soybeans appropriate for natto production are used. Today, 96.2% of the soybeans used in natto production in Japan are imported, primarily from the United States, Canada, and China; only 3.8% are domestically produced. Even foreign soybeans are bred and developed on

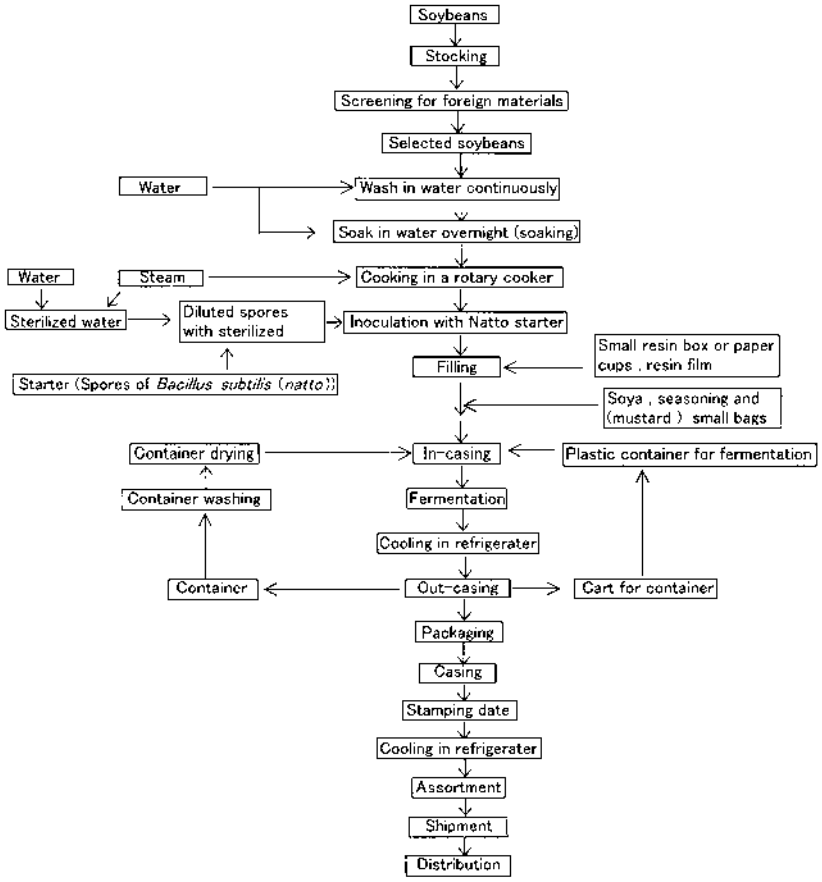

Figure 7 Flowsheet of industrial/commercial natto production today. (Courtesy of Suzuyo Kogyo Co., Ltd.)

the basis of the original Japanese species for natto. To allow uniform production, soybeans are separated by size and graded as follows (38):

Large: at least 7.9 mm in diameter
Medium: diameter 7.3 mm or greater but less than 7.9 mm
Small: diameter 5.5 mm or greater but less than 7.3 mm
Extrasmall: diameter 4.9 mm or greater but less than 5.5 mm

After selection, the soybeans are stored in a low-temperature warehouse at 15°C and 60% moisture to avoid the quality deterioration that could result from a rise in temperature.

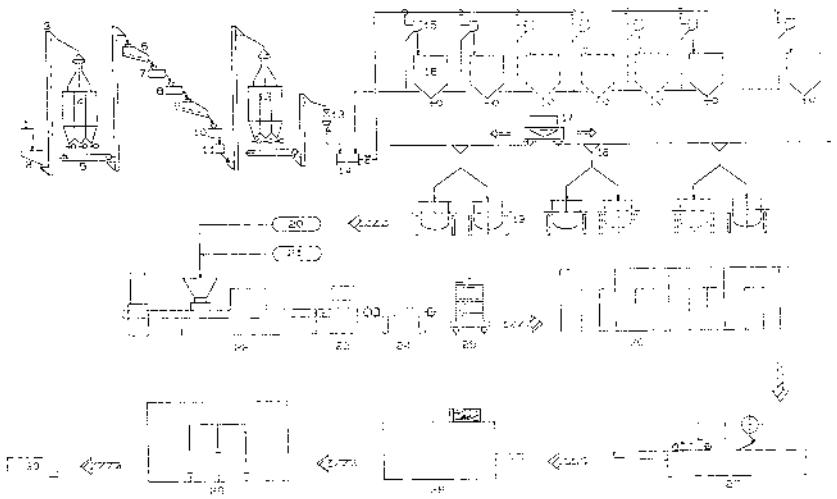

Figure 8 Flowchart of industrial/commercial natto production today (example 1). (1) Soybean throwing, (2) hopper, (3) upconveyer, (4) storage tanks (silo), (5) Flat conveyer, (6) screening machine, (7) stone remover, (8) scrubber, (9) screening by size of beans, (10) spectrograph sorting machine, (11) metal remover, (12) charge tanks, (13) measuring scale, (14) soybeans washer, (15) separator (soybeans from water), (16) soak tank, (17) soaked beans carrier, (18) soaked beans shooter, (19) rotary cooker, (20) cooked beans, (21) natto starter [*B. subtilis* (*natto*)], (22) filling machine, (23) weight checker, (24) flat conveyer, (25) containers/cart, (26) automatic control fermenter, (27) wrapping machine, (28) corrugated cardboard caser, (29) refrigerator, (30) shipment (Courtesy of Suzuyo Kogyo Co., Ltd.)

A. Storage of Raw Soybeans and Purification Process

Domestic and foreign soybeans are purified at producing districts and harbors and then stored in low-temperature warehouses. Within 4–7 days, the soybeans are usually shipped to the factories and stored in silos.

Foreign materials, such as metals and stones, are removed from the soybeans and then soybeans are separated by size at each natto factory. Raw soybeans are stored in a charge tank after they have been treated and checked with various purifying instruments, including a vibrating-screen cleaner, a stone remover, a scrubber, a screening machine, a spectrograph sorting machine, and a metal inspector (Fig. 10 and 11).

B. Processes of Washing and Soaking

Soybeans that have been purified and preserved in a charge tank are then measured, washed with water, and transferred to a soak tank. A screw-type

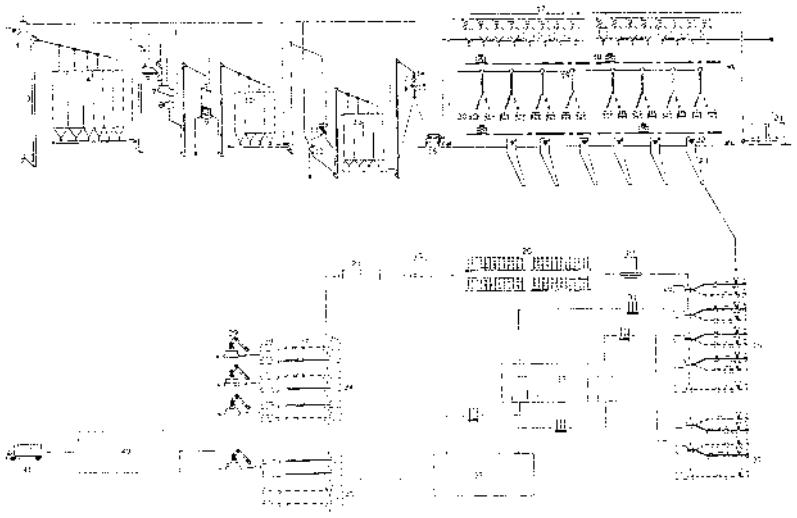

Figure 9 Flowchart of industrial/commercial natto production today (example 2). (1) Dust collector, (2) hopper, (3) upconveyer, (4) storage tanks (silo), (5) screening machine, (6) screening blaster, (7) stone remover, (8) scrubber, (9) belt screener, (10) buffer tanks, (11) first spectrograph sorting machine, (12) second spectrograph sorting machine, (13) charge tanks, (14) metal checker, (15) measuring scale, (16) soybean washer, (17) closed-type soak tanks, (18) soaked beans carrier, (19) soaked beans shooter, (20) rotary cooker, (21) CIP (ceaning-in-place) unit for soak tank cleaning, (22) inoculater, (23) inoculated cooked beans shooter, (24) container washer, (25) stacker, (26) container stock yard, (27) traverser, (28) automatic in-caser carrier, (29) box filling line, (30) cup filling line, (31) automatic case carrier, (32) automatic control fermenter, (33) first refrigerator, (34) box wrapping line, (35) cup wrapping line, (36) automatic uncaser, (37) wrapping machine, (38) corrugated cardboard caser, (39) palletizing robot, (40) second refrigerator, (41) shipment. (Courtesy of Suzuyo Kogyo Co., Ltd.)

soybean washer is used to remove adhesive substances by eluting them into the water (Fig. 12); heavy foreign substances such as stones and metals fall to the bottom of the tank, and light foreign substances rise to the top and overflow. Soybeans are washed by physically rubbing them and by rinsing to remove foreign substances and micro-organisms. Then, they are sent by a water pump to a soak tank (Fig. 13) and transferred into the soak tank by a water separator. The soak tank is usually of the batch type, and most soak tanks hold about 1–2 tons.

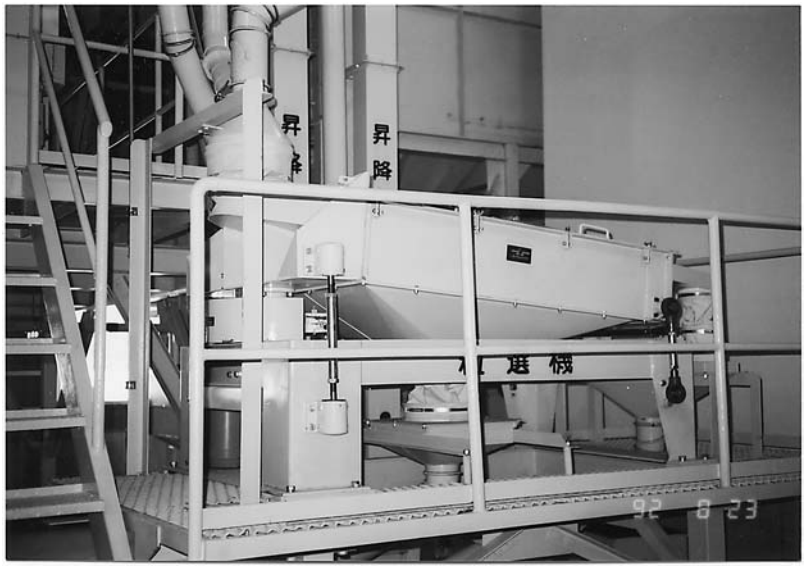

Figure 10 Upconveyer and screening machine. (Courtesy of Suzuyo Kogyo Co. Ltd.)

Soaking is performed at a low temperature to inhibit the growth of micro-organisms. Water for soaking is cooled by a chiller to 10°C, and the soaking process takes about 18 h. The soybeans take up water during soaking; their weight after soaking is about 2.2–2.3 times their weight before soaking. After the soybeans are removed, the soak tank is washed by the CIP (cleaning-in-place) system, by which micro-organisms, components eluted from the soybeans, and other materials are removed.

C. Steaming and Inoculation Processes

The soaked soybeans are steamed under 2 kg pressure by a rotary cooker (Fig. 14) to inhibit the growth of natto bacilli and soften the soybeans. The steaming process requires about 1.5–2 h. Modern equipment, including automatic controls and self-control systems, have improved the uniformity and conditions of the steaming process.

The steamed soybeans are transferred onto a cart, which is connected to a filler and equipped with an inoculation spray on the upper part. Natto bacilli are uniformly inoculated in a mist onto the soybeans.

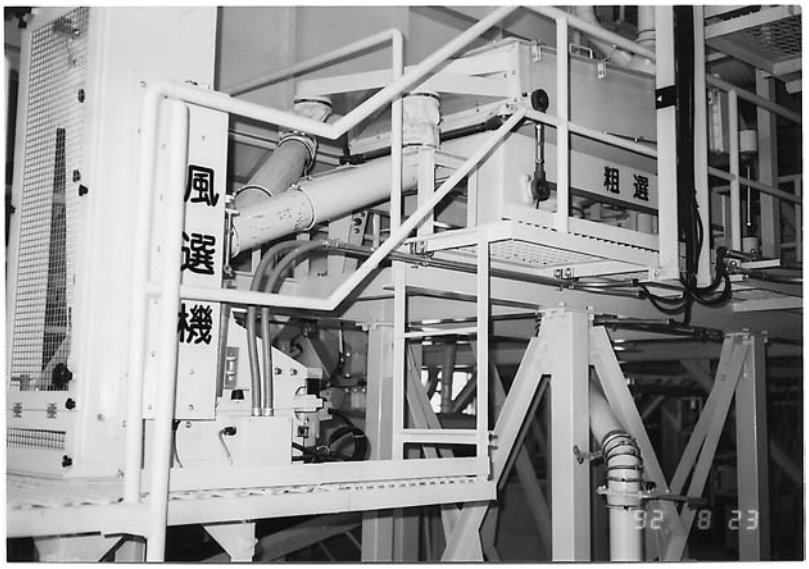

Figure 11 Screening machine and screening blaster. (Courtesy of Suzuyo Kogyo Co. Ltd.)

Figure 12 Soybean washer. (Courtesy of Suzuyo Kogyo Co. Ltd.)

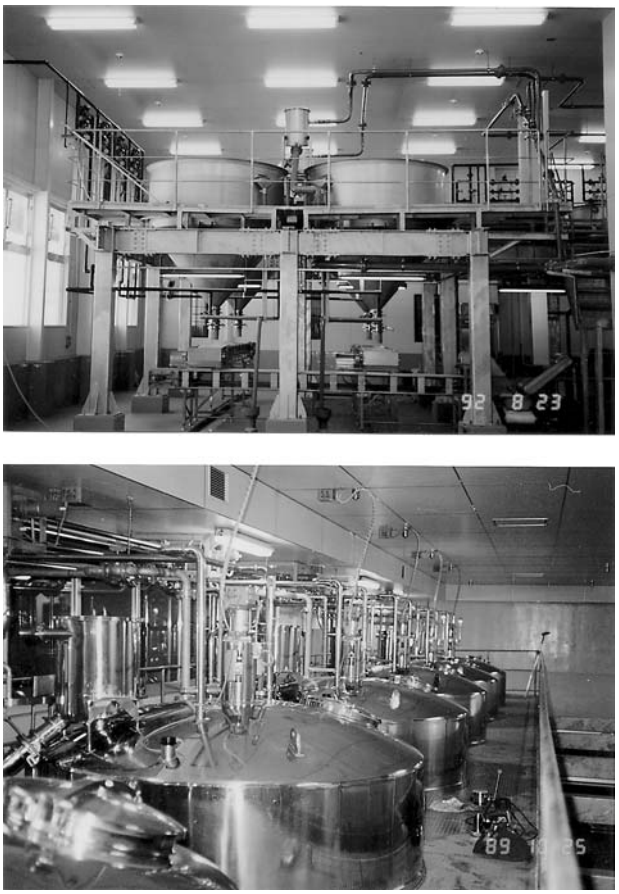

Figure 13 Soak tanks and water pipe line: (upper) open-type soak tanks and (lower) closed-type soak tanks. (Courtesy of Suzuyo Kogyo Co. Ltd.)

D. Natto-Filling Process

The automatic filler is equipped with a vibration feeder. Using the filler, at a speed of 120 packages/min, 30–50 g of steamed soybeans are filled into square PSP (polystyrol paper tray) packages, each 100 mm × 100 mm × 25 mm, or into paper cups 75 mm in diameter. An exclusive high-speed filler can fill 180 packages/min (Fig. 15).

Formerly, the Natto-filling process required many hands, but, today, it is highly automated. The automatic filling system carries out all of the steps

Figure 14 Rotary cooker. (Courtesy of Suzuyo Kogyo Co. Ltd.)

involved in quantitative filling of soybeans into packages, covering with film, depositing of bags of soy sauce and mustard, and sealing of the lids of packages. In the case of cups, the top seal is covered after filling. The packages pass a weight checker and metal detector, and substandard packages are removed. A constant volume of filled packages is packed by an autocaser (Figs. 16 and 17) at a constant speed into a container, which is supplied from a container yard (Fig. 18). The containers are transferred to the fermentation room (Fig. 19) on a cart or by a small remote-control car (Fig. 20).

Figure 15 The interior of a filling room and an automatic filling machine. (Courtesy of Suzuyo Kogyo Co. Ltd.)

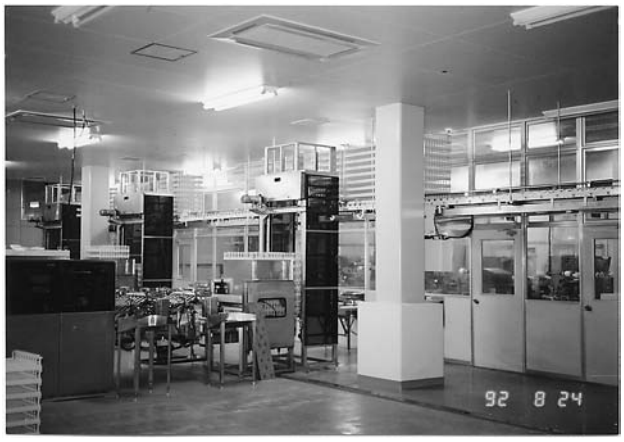

Figure 16 Filling room and automatic in-caser. (Courtesy of Suzuyo Kogyo Co. Ltd.)

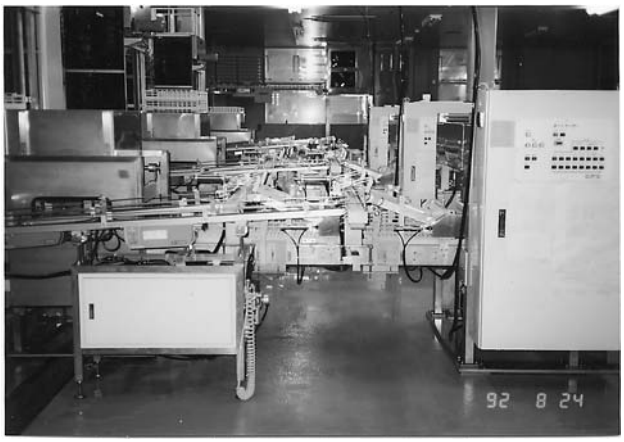

Figure 17 Automatic in-caser (slide system). (Courtesy of Suzuyo Kogyo Co. Ltd.)

E. Fermentation Process

This process is the most important one in modern natto manufacturing. Inoculated and filled steamed soybeans are fermented in their packages, which are sold as commercial products. The fermentation is a short-term process, and the quality of the product is determined in only 16–18 h.

In the first part of the fermentation process, the top priority is to encourage sufficient growth of the natto bacilli; in the latter part of the

Figure 18 Container stock yard. (Courtesy of Suzuyo Kogyo Co. Ltd.)

Figure 19 Automatic control fermenter. (Courtesy of Suzuyo Kogyo Co. Ltd.)

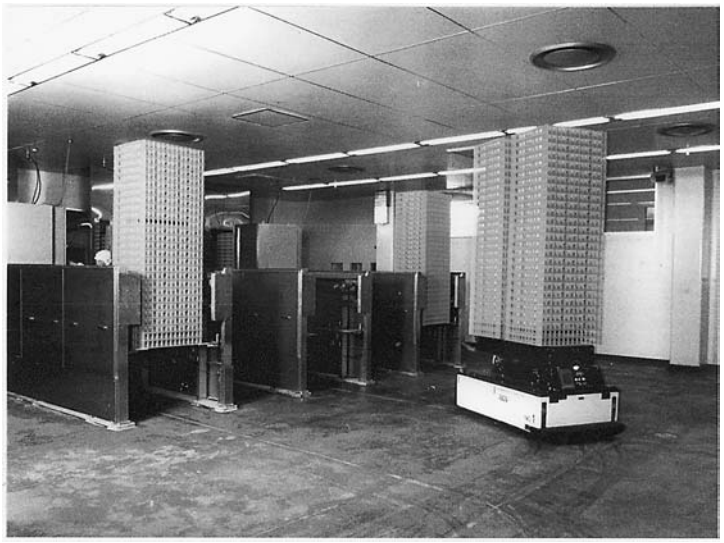

Figure 20 Automatic case carrier. (Courtesy of Suzuyo Kogyo Co. Ltd.)

process, promoting sufficient enzyme activity is most important. The equipment in a modern fermentation room allows the temperature, moisture, air supply, and ventilation to be adjusted in order to optimize bacterial growth and enzyme activity.

F. Refrigeration and Aging Processes

Refrigeration performs important functions in the first cooling to adjust the patterns of lag, log, and stationary phases, and in the second cooling to inhibit the growth of natto bacilli and the production of ammonia and to promote aging and stabilize the quality of the product (Fig. 21).

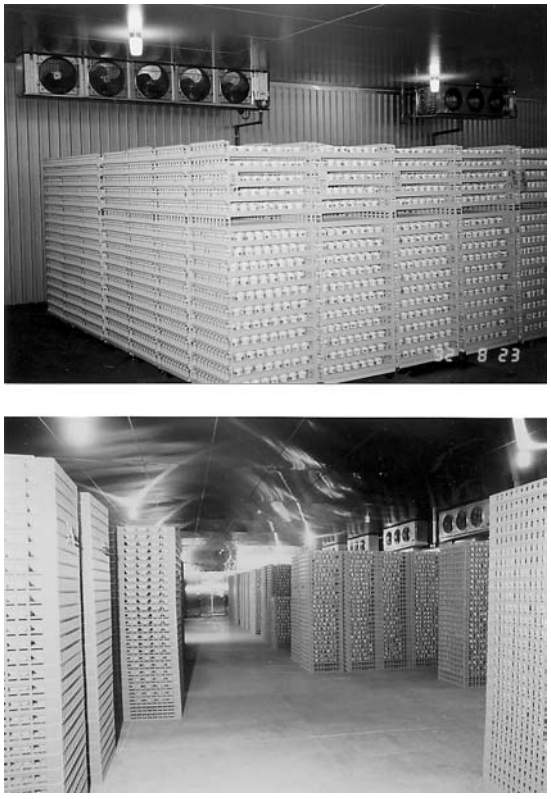

Figure 21 First refrigerator. (Courtesy of Suzuyo Kogyo Co. Ltd.)

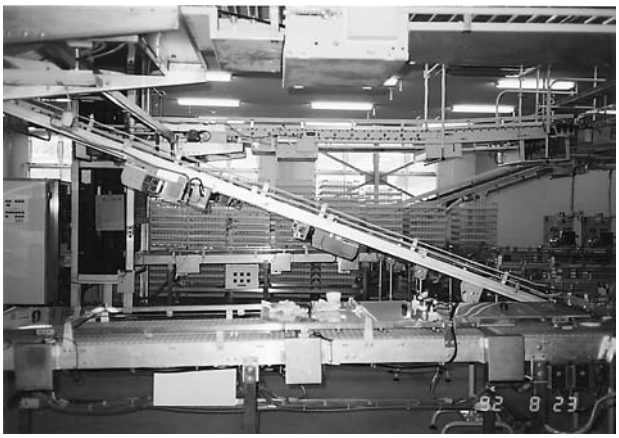

Figure 22 Container conveyer. (Courtesy of Suzuyo Kogyo Co. Ltd.)

G. Second Packaging and Shipping Processes

The product, which has been cooled at 0–5°C and has undergone aging in the refrigerator, is taken from a container (Fig. 22), packed for a second time, and prepared for shipment.

The package form is two to three layers of PSP packages or shrink packages of three cups (Fig. 23). Packed products are filled into a corru-

Figure 23 Packaging room and cup wrapping machine. (Courtesy of Suzuyo Kogyo Co. Ltd.)

Figure 24 View of modern natto plant. (Courtesy of Suzuyo Kogyo Co. Ltd.)

gated cardboard box and stored at 2°C in the second refrigerator. After the product has cooled sufficiently, it is shipped to supermarkets and other retail outlets in a refrigerated truck (Figs. 24 and 25).

VI. CHANGE FROM TRADITIONAL PROCESS TO MODERN MANUFACTURING PROCESS

Natto was once a homemade seasonal product, produced only in the autumn and winter when the weather was cold. However, it was rapidly transformed into an industrial product, available year-round, during the 20th century.

The first technical revolution that contributed to the later success of the natto industry began with the study of microbiology at the dawning of

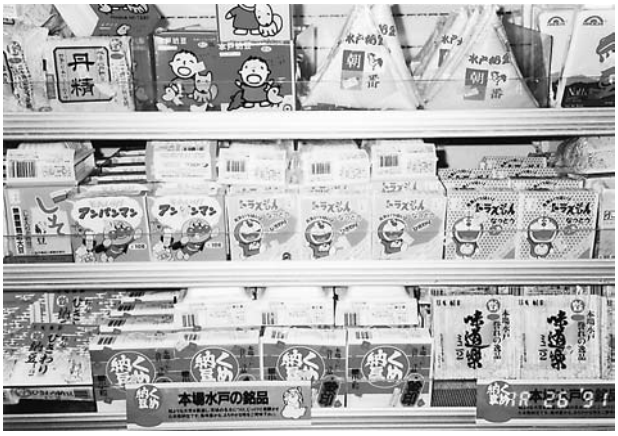

Figure 25 Various natto packages in a supermarket in Los Angeles, CA.

science in the 1890s. Studies begun at that time led to the development of a scientific method of manufacturing Natto in a hygienic package by Professor Hanzawa of Hokkaido Imperial University in the 1920s. Thus, the commercially stable production of natto became possible.

A second technical revolution took place in the years after World War II. During this period, the rise of supermarkets, the mechanization of food processing, and the invention of new packaging techniques and materials all contributed to major changes in the Japanese food industry. All of these developments contributed to the success of commercial natto production. However, the single most important advance that allowed large-scale, year-round production and sale of natto was the development and widespread use of refrigerators. Modern commercial refrigerators, which can maintain closely controlled low temperatures, are essential for the production, shipment, and storage of uniform, high-quality natto. Moreover, the availability of refrigerators in the home is essential to the success of natto as a modern food product. Natto is highly perishable; if kept at room temperature, it spoils within a day. No one could make a profit by selling natto on a large scale until people had a way to keep the product cold in their homes. Home refrigerators first became common in Japan after World War II. By 1962, approximately 30% of Japanese homes had refrigerators; today, essentially all Japanese homes have them. The availability of refrigeration transformed natto from a seasonal product to a year-round food. Natto became one of the fermented foods that can be appreciated wherever and whenever we want. Large natto factories were constructed in the 1980s and the natto industry became a prosperous one.

The major differences between traditional and modern natto production is summarized as in the following subsections

A. Cultivation and Storage of Raw Soybeans

1. Traditional Process

Soybeans were cultivated at home and stored at home in many places in Japan.

2. Modern Process

Soybeans are cultivated both domestically and in foreign countries, and they are stored in low-temperature refrigerators.

B. Selection Process

1. Traditional Process

Selection is made at home, using a straw sieve, a Chinese sieve, and a Japanese sieve.

2. Modern Process

Selection is made by a machine in the natto factory. The process is crude selection, removal of stone, grinding, separation by color, and removal of metals.

C. Washing and Soaking Process

1. Traditional Process

After washing by hand, soybeans are soaked.

2. Modern Process

After washing, soybeans are soaked at a controlled low temperature.

D. Steaming and Inoculation Process

1. Traditional Process

Originally, the soybeans were cooked in an iron pot. Later, the pot might have been equipped with a steamer. Rice straw is served as a source of natto bacilli.

2. Modern Process

A high-pressure rotary cooker is heated by steam from a boiler. After steaming, a diluted suspension of natto bacilli is sprayed on the soybeans.

E. Filling Process

1. Traditional Process

A package was made from dried and purified straws. Steamed soybeans were packed in straw packages soaked in water, and the water was removed. A piece of straw was inserted in the steamed soybeans so that natto bacilli would grow.

2. Modern Process

From about 1920, a hygienic package and a box made from a thin plate of Japanese cedar were used, and air was circulated in the fermentation room. From the 1960s, PSP packages and paper cups began to be used.

An automatic filler equipped with a vibrator has been developed. The filling process, which had been performed by hand, became automatic. Modern equipment can perform the whole filling process. The machinery used includes a package supplier, an automatic filling instrument, an automatic film covering instrument, a depositor of soy sauce and mustard, an automatic sealer, a weight-measuring detector, a detector of metal admixture, and an autocaser that loads packages into a container.

F. Fermentation Process

1. Traditional Process

Natto production requires places where cooked soybeans can be kept warm and natto bacilli living on the straw package can be allowed to grow. It also required a heat source to maintain the desired temperature during the lag phase of bacterial growth.

To produce natto, people made use of warm places such as the inside of a pot where water was warmed to body temperature, the inside of a barrel which was warmed after boiling water was stored, the inside of soil where straws were burned, the inside of a charcoal kiln after finishing burning charcoal, the inside of a hearth, a fixed body warmer with waste heat, and so forth. It took a few days to produce natto in these conditions.

After fermentation, natto was cooled in air and stored at as cool a temperature as possible.

2. Modern Process

Nowadays, a fermentation room is generally made of FRP (fiber-reinforced plastic). In the room, an air conditioner in the center of the ceiling blows air to every side, and heating and cooling equipment are used to produce the desired temperature changes. A humidity controller, an air conditioner, an exhaust pipe, room thermometers, and equipment to measure the temperature and moisture of the soybeans are all used. The whole process of fermentation is controlled step by step by a computerized automatic control panel.

Rolling and inverting the fan, the air conditioner keeps the temperature and humidity in the room constant in a turbulent flow.

The packages are packed in a container and loaded on a cart; they are laid out in lines with space between them in the fermentation room. During the fermentation, the lag, log, and stationary phases take 8, 4, and 4 h, respectively. After that, aging and cooling continue for 6–8 h; the fermentation is completed and the finished products are ready for refrigeration and shipment the next morning.

G. First Refrigeration Process

1. Traditional Process

Refrigeration was not available. Therefore, after fermentation, the product cooled to room temperature. Unless the ambient temperature was cold, the natto could not be stored for long without spoiling.

2. Modern Process

Natto is cooled to a temperature below 5°C in the refrigerator to prevent deamination that might otherwise result from the rise of temperature during the packing process.

H. Packaging Process

1. Traditional Process

Natto was sold without an outside package.

2. Modern Process

Two, three, or four packages are packed with a film label. Cups are packed with shrink packaging. Packages are dated to show the limits of salability/consumability. The packages are packed in corrugated cardboard.

I. Second Refrigeration Process

1. Traditional Process

Refrigeration was not available. The product was left on a shelf in rack, and cooled by the wind.

2. Modern Process

The product is kept refrigerated so that its temperature will not rise after shipping (9,39–41).

VII. CRITICAL STEPS IN THE MANUFACTURE AND FERMENTATION OF NATTO

There are three unsolved problems in the modern industrial production of natto. The first problem involves the steaming of the raw materials. As described earlier, soybeans for natto are currently steamed by a batch process. Unfortunately, the current technique causes lot-to-lot differences in product quality and it results in the elution of a high concentration of fluid (called nijiru in Japanese), which removes substantial amounts of nutritionally valuable components from the soybeans and also contributes to the load of wastewater from natto factories. A continuous steaming process would be preferable because it would lead to a more uniform product and could also reduce the production of nijiru. However, attempts at the development of a continuous steaming process have failed because the equipment caused excessive damage to the soybeans, thus lowering the quality of the finished product.

The second problem involves the filling process. Steamed soybeans are inoculated with natto bacilli, which are added in the hopper of a filler. At first, the soybeans are not viscous and are easy to handle, but they become very viscous and difficult to handle as time passes.

Currently, filling is performed by a vibration feeder after inoculation. The setting of the amount of soybeans for a package is now performed by measuring the volume on the basis of the standardized weight. It is difficult to do this properly with viscous materials. Thus, the filling process leads to much loss of soybeans. Measurement by a computer has been tried but in vain because the instrument was too large and too costly and problems occurred during washing.

The third problem involves the fermentation room. The goal of the fermentation process is to produce a uniform, high-quality final product. There is little problem with uniformity in the small-scale production of natto.

However, in large-scale production, which is currently conducted as a batch process in a large fermentation room, differences in temperature in different parts of the fermentation room lead to variations in product quality. A turbulent flow type of air conditioner, an automatic air supplier, and a ventilator help to control the temperature and airflow in the fermentation room. However, much heat is emitted during the log phase of fermentation, and the temperature rises and continues to be high in the space between containers placed in the upper part of the fermentation room. Air circulation cannot prevent the temperature difference between the upper and lower parts of the fermentation room, and it does not have a good effect on the growth of natto bacilli and the emission of heat. Efforts are being made to solve this problem through the development of a horizontal-flow type of air circulation in the fermentation room (42) and the development of a continuous fermentation process (43–45).

VIII. IMPORTANT PROBLEMS IN THE INDUSTRIALIZATION OF NATTO

First, the industrialization of natto product cannot be explained without mentioning the influence of the Japanese supermarket system. In the 1970s, natto began to gain recognition as one of Japanese healthy food products. It was almost the same time that the American supermarket system, which carries almost everything from garden tools to foods, was introduced to Japan from the United States. The attraction of this newly introduced marketing system which sold goods much less expensive than others was unbelievable and attracted enormous numbers of people from entire village or towns. Natto was one of the best selling items of the food product corner. Supermarkets needed large amounts of natto from producers to meet the demand of buyers.

This new structural reform of the marketing system forced changes in the new production system in order to supply large quantities of natto at the right times and at the right price. Necessity is the mother of invention. They needed to change to a high rate of production.

This influenced the retail sale of natto so that competition started among natto manufacturers. Some manufacturers increased the amount of natto that they produced and the size of their factories. Hygienic and quality management were improved, and a reduction of manpower had been promoted. Natto is a unique solid food, which is packed and fermented in a small unit of 30–50 g. In spite of mass production, uniform production is required.

For expansion and industrialization of natto production, unique techniques of the natto industry have been developed. Additional new techniques will be needed in the future.

A. Soaking Process

Soaking is usually done as a batch process. As production amounts have become larger, manufacturers have turned to the use of large soak tanks. There are two problems with the process. One problem is that soil micro-organisms grow as the water temperature increases. The growth of the micro-organisms causes the loss of soybean components during soaking, and metabolites produced by the soil micro-organisms inhibit the growth of natto bacilli.

Another problem is that the absorption ratio of water and the degree of swelling of the soybeans differ between the lower and upper parts of the soak tank. The soybeans soaked in the lower part are pressed and steamed; they actually become soft, although they are thought to become hard.

The first problem has been solved by supplying cold water to adjust the water temperature to 10°C during soaking and by washing the inside of the soaking tank. The other problem has not been considered seriously, but the author thinks it will be solved in the future by changing to a continuous rather than batch soaking process.

B. Steaming Process

Steaming is usually performed by a high-pressure batch process, but the loss of desirable nutritional components is so great that it is useful to recover eluted nijiru. It is expensive to dry, preserve, and reutilize these components. However, if the nijiru is simply disposed of, the cost of waste treatment is also very high. Filling by a rotary cooker takes time, so that the properties of steamed soybeans change during filling, and the soybeans become viscous. Unfortunately, as described earlier, attempts to solve this problem by adopting a continuous cooker have not been successful because of damage to the soybeans. The development of new steaming methods to produce less nijiru and without any injured soybeans is required.

C. Filling Process

After inoculating natto bacilli on steamed soybeans and filling a volume of sufficient for a meal into a package, Natto is produced through fermentation. Just after steaming, soybeans are not viscous, but some time later, when the temperature of the soybeans decreases, the moisture on the surface of the soybeans is absorbed and viscosity increases.

Filling is currently performed by measuring a volume corresponding to the weight to pack in a package. The soybeans are measured by a vibrator, opening or shutting an upper plate and a lower plate. There is a measuring error and the amount of soybeans per package must be increased

by about 10% to compensate. A weight-measuring method has been studied as a possible way to correct a trace weight error, but it proved too inefficient in practice.

The package is covered with a film after filling; small bags of soy sauce and mustard are placed on top; and the upper lid of the package is sealed. Packages are then piled in a plastic container with holes for good ventilation and piled on a cart and moved to the fermentation room. This used to be laborious work, requiring many laborers to pile packages in containers. However, an automatic encaser can now perform the work at the same speed as that of a filler.

D. Fermentation Process

The fermentation room consists of an adiabatic structure. An air conditioner that increases and decreases the room temperature is set in the center of the ceiling. The conditions inside the room are monitored by thermometers that measure the temperatures of both the room and the soybeans and by a hygrometer. The whole fermentation process is computer controlled.

The fermentation process finishes in 22–24 h. During this time, the temperature and moisture must be appropriately controlled at each step so that natto bacilli grow well and the fermentation proceeds well. The process ends by cooling the natto for aging.

The problem with this process is as follows. All of the push carts cannot deliver containers into the fermentation room at the same time and, thus, there are differences in the lengths of time that various packages stay in the fermentation room. Also, heat produced by fermentation collects in the upper part of the containers on each push cart, causing a temperature difference between the upper and the lower parts; this leads to differences in the quality of the natto produced.

To solve the problem, it may be necessary to change the air conditioner from a turbulent-flow type to a horizontal-flow type, although the latter type has some undesirable properties for the fermentation. A continuous fermentation room might be needed to eliminate the problems that result from differences in fermentation time experienced by different packages.

IX. OPTIMUM CONDITIONS FOR FERMENTATION

Fermentation is the most important process in the manufacture of natto. Figure 26 shows that the management of temperature and humidity during the fermentation process is a pattern of graded control, which modifies the fermentation process to obtain ideal conditions (46). The pattern changes for

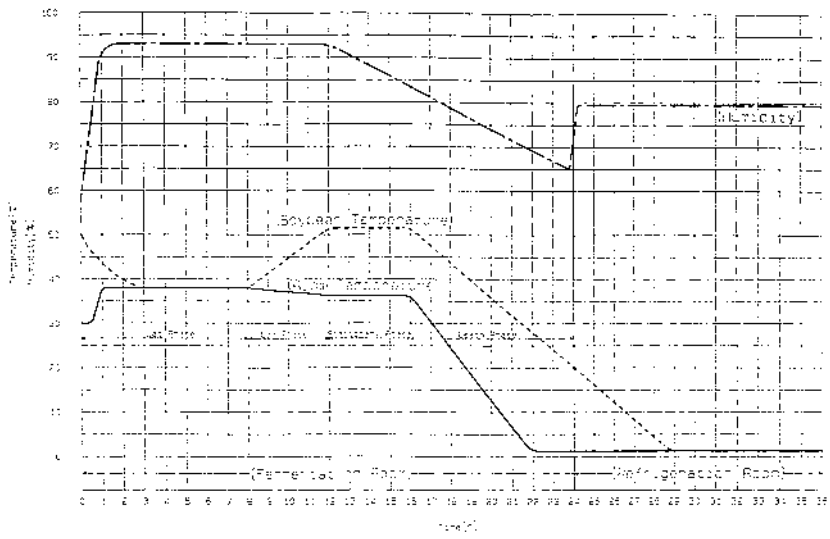

Figure 26 The management of temperature and humidity during the fermentation process.

different kinds of raw materials and package materials, so the best pattern must be set for each product.

A. Initial Stage of Fermentation (Lag Phase)

The purpose of initial stage of the fermentation is to make natto bacilli grow sufficiently on the surface of the steamed soybeans. The temperature is set at 37–40°C, which is the optimum growth temperature for the micro-organisms. It is important to preserve water on the surface of the soybeans so that the natto bacilli can absorb nutritional components from the soybeans. Therefore, the humidity is kept higher than 80%.

B. Middle Stage of Fermentation (Log to Stationary Phases)

During the log phase, the natto bacilli emit a great deal of heat produced by the fermentation process. The temperature of the soybeans begins to increase after about 8 h, and the temperature finally rises to higher than 50°C at about 12 h. Much oxygen is consumed during this time. Sufficient oxygen must be supplied in the fermentation room, and metabolic gases and fermentation heat must be exchanged gradually with outside air by an air

conditioner and a ventilator, which also help to remove moisture from the surface of the soybeans.

In the case of whole soybeans, the synthesis of viscous materials by natto bacilli is encouraged by keeping the soybeans constantly at 50–52 °C for 4–5 h. When packages are tightly shut, the temperature of the soybeans rises to high. The room temperature must be lowered to maintain the optimum temperature for the growth of natto bacilli. On the other hand, when packages are loosely closed, they radiate too much heat and the room temperature does not increase to the optimum. Therefore, the room temperature must be raised to increase the temperature inside the packages.

C. Latter Stage of Fermentation (Death Phase)

After the stationary phase, some vegetative cells of natto bacilli produce spores and reach the death phase. Natto bacilli undergo autolysis gradually. Cell walls and membranes dissolve, scattering cell substances such as proteins, endoenzymes, vitamins, and volatile components on the surface of the soybeans. Those materials penetrate into the tissues of soybean cells, promoting fermentation. The lysis of aggregated cells and colonies can be observed with the naked eye at this point, and the cells of natto bacilli gradually die. Soy proteins are degraded to amino acids or peptides by proteases and peptidases—exoenzymes produced by natto bacilli.

Eleven percent of glutamic acid has been liberated at 16 h after the beginning of fermentation, and the ratio of free amino acids has reached 11% (47). In parallel with those changes, organic acids are synthesized by the action of endoenzymes, and umami taste increases greatly.

X. MICROBIOLOGY AND BIOTECHNOLOGY

A. Determinative or Systematic Characteristics of Natto Bacilli

The natto bacterium was named *Bacilllus natto* by Sawamura (7). However, *Bergey's Manual of Determinative Bacteriology*, 6th ed. (48,49) stated that the organism should actually be classified as *B. subtilis*. According to the manual, *B. subtilis* is a Gram-positive, endospore-forming rod of less than 1 μm in diameter. The systematic characteristics of natto bacilli correspond very well to those of *B. subtilis* as described in the seventh and later editions of Bergey's manual (50–53).

Aoki et al. (54) showed that transformation and transduction occurred between seven strains of *B. natto* and 23 strains of *B. subtilis* from the culture collections of the Institute of Applied Microbiology of Tokyo University.

Takahashi et al. (55) reported that *B. natto* IAM8-1259 is highly homologous to *B. subtilis* Marburg, indicating that the two species are in fact the same.

B. Difference Between Natto Bacilli and Other *B. Subtilis* as Natto Starters

The natto bacterium is not acknowledged internationally as an independent species. However, even if *B. subtilis*, *B. cereus*, *B. megaterium*, *B. mycoides*, and so forth grow on cooked soybeans, they cannot produce a satisfactory natto product (56). In Japan, natto bacilli are described as *B. subtilis* (*natto*), to distinguish them from strains of *B. subtilis* that are not suitable for natto production.

Bacillus subtilis (*natto*) grows on cooked soybeans very abundantly. These bacteria also grow on various beans other than soybeans, on other plant foods, such as cereals, and on animal foods such as meat, fish, shellfish, and dairy products. *B. subtilis* (*natto*) utilizes glucose, sucrose, fructose, and so forth as carbon sources. Sucrose is necessary not only for growth but also for the production of viscous materials (57).

Soybeans contain about 20% carbohydrates, and, other than sucrose, polysaccharides such as arabinogalactan are degraded and utilized by the natto bacilli (58). *B. subtilis* (*natto*) requires biotin; vegetative cells cannot grow on media without biotin and their spores do not germinate (59). Media containing B vitamins are appropriate for growth and spore germination. Soybeans contain these vitamins.

In order to produce good quality Natto, it is necessary to provide good conditions for the germination of spores and the growth of natto bacilli. The optimum temperature for germination is about 40°C. The optimum temperature for growth is between 39°C and 43°C. Growth will stop at 55°C, and lysis of the vegetative cells will occur (60,61).

Natto bacilli grow at about neutral pH. Germination and growth are inhibited at pH 4.5 or lower. Oxygen is required for the growth of the organisms. Measurements with an oxygen electrode have shown that the natto bacilli can grow at concentrations of more than 3% oxygen (60).

With repetitive subcultures of natto bacilli, they sometimes lose the production of sticky materials. They recover the production by changing from one medium to another one (62,63).

C. Enzymes and Other Materials Produced by Natto Bacilli

The viscous materials produced by natto bacilli consist of polyglutamic acid and levan (fructan). Fujii (57,64,65) reported that the viscous materials

produced in a culture medium with glutamic acid were composed of polyglutamic acid, 40% of which was D-glutamic acid, and that the viscous materials produced in a culture medium with sucrose were a mixture of polyglutamic acid and levan. Saito et al. purified a viscous material (which they called natto mucin) and found that the viscous material was composed of 58% γ-polyglutamic acid and 40% polysaccharide; the ratio of L- to D-glutamic acid was determined to be 58 : 42, and the weight and *z*-average molecular weight of γ-polyglutamic acid were 2.08×10^5 and 2.22×10^5, respectively. Natto is known to become much less sticky when mixed with soy sauce, which has a pH of about 4.5 and contains about 15% salt. Furthermore, Iso et al. (66) studied the viscometric behavior of natto viscous materials and reported that a 2% solution of a natto viscous material behaved as a thixotropic flow at pH 5.7, although the flow of the same concentration was apparently Newtonian at pH 4.3. These researchers found that the viscous material molecule, which is mainly constituted from γ-polyglutamic acid, is randomly coiled at pH 5.7 and a rodlike molecule at pH 4.3 (66,67).

The protease of natto bacilli is called subtilisin and is an endoprotease (68). The optimum pH for the enzyme is 7.0 when casein is used as a substrate, and the activity at pH 10 is similar to that at pH 7. Nattokinase and elastase are also proteases of natto bacilli and will be described later.

According to Noda et al. (69), natto bacilli produce γ-transpeptidase (γ-GTP), which makes bridges between polyglutamic acid molecules and plays an important role in the formation of viscous materials.

Hara et al. (70) reported that natto bacilli had plasmids, on which genes for γ-GTP exist. They measured the gene size of the plasmids of natto bacilli, which they isolated from various natto samples from Southeast Asia. They also made a dendrogram of the plasmids of natto bacilli from the base sequence and classified the plasmids into three groups, namely Nepali, Chinese, and Japanese types. According to their hypothesis, plasmid pNKH isolated from the Nepali strain of natto bacilli separated 160 million years ago; Chinese and Japanese types separated 130 million years ago; and both types separated into a Japanese natto type and a Chinese douche type 70 million years ago. The plasmid pLS11 of *B. subtilis* separated from the Thai type 50 million years ago.

However, Nagai et al. recently reported, from genetic engineering studies, that the gene for poly-γ-glutamic acid (PGA) production is on the chromosome, not on the plasmids (71). Nagai and Itoh (72) recently found a generalized transducing phage from a laboratory stock of natto phages and applied for the analysis of the production of PGA using transposon insertional mutation technique acid (72,73). As the result, it was found that

their IS discovered in *B. subtilis* was related with the loss of ability to produce PGA during storage (74).

Ogawa et al. (75) extracted and purified γ-GTP from the culture medium of natto bacilli and investigated the enzymatic characteristics. γ-GTP was made from two subunits with molecular weights of 45,000 and 22,000, respectively. The low molecular-weight subunit had the same N-terminal amino acid as that of the subunit isolated from *Escherichia coli*. The enzyme uses glutamine as a glutamyl donor and an acceptor of γ-polyglutamic acid, and dipeptide glutamyl is a better substrate than free glutamic acid.

Katsumata et al. (76) determined the base sequence of γ-GTP and obtained a Japanese patent. According to it, the gene codes γ-GTP, which is made up of two subunits of molecular weight 38,000–44,000 and 20,000–25,000, respectively.

XI. OTHER ASPECTS OF THE MICROBIOLOGY OF NATTO

A. Phages of Natto Bacilli

Fujii found in 1967 that unusual fermentation of natto is caused by phages (77). Phages were found in natto that was insufficiently viscous or not viscous at all. Many phages of natto bacilli have been detected in natto factories, soils of rice fields, drain systems of paddies, and so forth.

Phages isolated from natto grew only in natto bacilli, but some phages can grow even in strains of *B. subtilis* not suitable for natto production. Phages were divided into four types from the species of the hosts and into three types serologically. Fujii et al. (78) investigated the effect of phages by mixing natto bacilli with their phages, inoculating on steamed soybeans, and culturing at 37°C. A high concentration of phages inhibited the development of viscosity from the beginning of the growth of the natto bacilli, and a low concentration of phages caused a gradual loss of viscosity from the fermented soybeans. The inhibition of viscosity is caused by degradation of polyglutamic acid (PGA); the molecular weight, optimum temperature, and optimum pH of the enzyme responsible for the degradation were 20,000, 45°C, and 7.0, respectively. PGA was degraded to small substances such as tetrapeptides, tripeptides, and dipeptides.

The relationship between PGA production and phage infection has been studied by Hara et al. (79). The phages are highly specific in that they do not grow in ordinary strains of *B. subtilis* but do grow in natto bacilli. A cured strain of natto bacilli lost phages as a result of acridine orange treatment and became phage-resistant. However, the transformant, transferred

from *B. subtilis* Marburg, which was resistant to phages of natto bacilli, could produce viscous materials and became sensitive to phages.

B. Plasmids of Natto Bacilli

Tanaka et al. (80,81) found 14 plasmids from 37 strains of *B. subtilis* (including 10 strains of natto bacilli) and classified them into 7 groups by molecular weight and the patterns of degradation by several limiting enzymes.

They found eight strains that had both small plasmids of 3.6–4.0 MDa and large plasmids of 31–35 MDa, a strain that had only small plasmids, and a strain that had only large plasmids. All plasmids were cryptic, with ambiguous physiological characteristics.

Hara et al. (79) found that *B. subtilis* (*natto*) Asahikawa had a plasmid of 5.7 kb. A PGA nonproducing strain (stg-), which was obtained by curing with acridine orange, lost not only plasmid pUH1 but also the γ-GTP activity. These researchers also isolated 8 plasmids from marketed natto and 13 plasmids from 10 natto bacilli. All of the plasmids except for one plasmid were the same kind: a functional plasmid, pUH1, which controlled the biosynthesis of PGA. The plasmid with a code for the PGA gene of 5.7 kb should require biotin. This plasmid is widely distributed in nature.

XII. CHEMICAL AND BIOCHEMICAL CHANGES DURING FERMENTATION

A. Soybean Characteristics on Steaming

According to the *Standard Tables of Food Composition in Japan*, 5th ed. (82) a Japanese soybean contains 35.3% protein, 19.0% lipid, 28.2% carbohydrate, 5.0% ash, etc., whereas American and Chinese soybeans contain 33.2% and 32.8% proteins and 21.7% and 19.5% lipids, respectively. Japanese soybeans contain a little more protein and a little less carbohydrate than the others do. In addition to these components, soybeans contain tocopherol, trypsin inhibitor, hemaglutinin, and other constituents.

Because soybeans are very hard, they are first made softer by soaking and removed of excess water in the processing of natto. During the subsequent heat treatment process, sterilization and simultaneous denaturation and inactivation of poisonous substances such as trypsin inhibitor and hemaglutinin take place.

Natto is firmer than steamed soybeans. The increase in hardness is dependent on soybean species, having no relationship to the size of the soybeans. Soybeans with a high ratio of hardness between steamed soybean and

natto are rejected, and soybeans in which hardness does not increase during processing are accepted.

Taira et al. (83–88) found that there is a positive relationship between the hardness of steamed soybeans and ammonia nitrogen. They deduced that in hard, steamed soybeans, proteins would be degraded only near the hulls of the soybeans and the odor of ammonia would be emitted easily, but in soft soybeans, the supply of sugars and the appropriate degradation of proteins during the fermentation leads to high-quality Natto.

B. Relationship Between the Components of Natto and Those of Soybeans

Sunada (89) described soybeans with low fat content and high carbohydrate content as appropriate for natto. He compared the constituents of 11 types of soybean produced in Japan, China, Canada, and the United States with the natto produced from those soybeans. There were relationships between the fat content and the appearance of soybean (−0.6689) and between fat content and the viscosity of natto (0.7542).

C. Changes in Soybean Constituents During Fermentation

1. Carbohydrates

Although the sugar content of a soybean is only 4–6%, it is important as a key constituent for umami. During soaking and steaming, the oligosaccharides of soybeans are partially lost in soaking water or cooking drain water and a part of them are decomposed. During fermentation, sucrose decreased to about one-seventh, and both raffinose and stachyose decreased to about one-third of the starting levels within 10 h after starting fermentation (90).

Taira et al. reported that soybeans with a high free-sugar content, especially with high raffinosse and stachyose content, are appropriate for natto (87,88,91). They deduced that sucrose is easily degraded by natto bacilli during fermentation, and there is a possibility that fermentation would stop too soon if the sucrose content is high, but raffinose and stachyose are difficult to degrade by the organisms and the fermentation would proceed adequately if they are present.

2. Fatty acids

Kiuchi et al. reported that fatty acid analyses showed that the fatty acid composition of natto was the same as that of soybeans. This indicates that natto bacilli do not have lipase or do not secrete it (92,93).

3. Organic Acids and Other Volatile Components

Natto emits unique flavor. Gas chromatographic analyses of natto flavors have been carried out, and many organic acids have been detected. In the odor concentration of the cooked soybeans, hexanal, (*E*)-2-hexanal and hexanol contributing to the green and grassy odor of soybeans disappeared or decreased while the cooling was in progress. 2-Pentylfuran and 1-octen-3-ol contributing to the beany odor remained even if the soybeans were cooked for 8 h and were fermented into natto. In the odor concentrate of natto, pyrazines and sulfur-containing compounds were important contributors to the characteristic odor of natto (94).

Obata and Matano identified acetic, propionic, caproic, and phenylacetic acids from the extract of natto (95,96). Kikuchi et al. detected acetic, formic, propionic, iso-butyric, iso-valeric, 2-methyl butyric acids, and diacetyl as the volatile metabolic product of natto (97).

Kanno et al. identified acetoin-2,3-butane-diol and acetic, propionic, isovaleric, 2-methyl butyric, and 3-methyl butyric acids (98,99). These constituents necessarily constituted part of the flavor of natto, but they did not account for the complete flavor, which means that the flavor presumably consisted of many volatile components. It is still uncertain how many volatile components contribute to the flavor of natto.

The flavor components of chongkukjung and natto were analyzed by gas chromatography–mass spectrography and identified 112 volatile constituents. Among them, ethanol showed large peaks and the other main peaks were those of acetoin and 3-methyl butyric acid. Ammonia, 2,5-dimethyl pyridine, and 2-methyl butyric acid were identified from the peaks of both chongkukjung and Natto (100,101).

Katagiri (102) reported that esters of long-chain fatty acids were not identified and the degradation of glycerides was hardly detected. The volatile acids identified were acetic, propionic, and isobutyric acids.

Kikuchi et al. (103,104) compared the effects of Japanese and Canadian cracked soybeans on the syntheses of ammoniac nitrogen, volatile acids, acetoin, 2,3-butane-diol, diacetyl, and so forth and they also evaluated the changes in these components during the fermentation. The fermentation was abundant with cracked soybeans, which have a large surface area. Positive relationships were found between acetic, propionic, isobutyric, and isovaleric acids and ammoniac nitrogen. On the other hand, the relationships between ammoniac nitrogen and 2,3-butane-diol and acetoin were very negative.

The ammonia odor of natto gradually becomes strong after production and the quantity of natto deteriorates. Therefore, the shelf life of natto was determined to be 7 days. During preservation, many small yellowish white, hard crystals (usually less than 1 mm in diameter) occur on the surface of natto, which are called "tyrosine crystals." The components of the crystal are

tyrosine and/or struvite (ammonium magnesium sulfate) (105). Muramatsu et al. showed that the syntheses of ammonia and magnesium causes the crystal (85,86,106).

4. Size of Soybeans

It is believed that Japanese soybeans are best for natto processing. Breeding to obtain soybeans of small size, of good color, and that produce the strong flavor of natto has been performed, and various processing aptitude tests of soybeans for natto have been carried out repeatedly in many districts (17,107,108).

Tochigi prefecture in Kanto District produces large amounts of natto, and a constant supply of good soybeans to meet the demand of natto production and improvements in the quality of soybeans are always required. Takahashi et al. investigated the quality of soybeans cultivated and harvested at 11 areas in Tochigi prefecture, using foreign or imported soybeans as a control (109). The species of Japanese soybeans were *Kosuzu*, *Natto-shoryu*, and *Natto-gokushoryu* and the control foreign or imported soybeans were Jizuka of Ibaragi prefecture and those imported from China, the United States, and Canada. The soybeans produced in Tochigi prefecture eluted less solid materials during soaking and steaming than *Jizuka* and the soybeans imported from the Unites States and Canada and were softened by brief steaming. The quality of natto produced with soybeans of Tochigi prefecture was superior to the others (103,104).

5. Steaming Condition

Matsumoto et al. investigated the relationship between steaming conditions and the quality of natto (110). Setting a steaming pressure of 1.5 kg/cm and 1–60 min of pressure holding time, soybeans were steamed and natto was produced. Nitrogen solubility ratio, nitrogen-degradation ratio, and ammonia nitrogen were estimated. Steaming for 30–40 min was found to be optimal for the production of appropriately aged natto; steaming for longer than 40 min yielded excessively aged natto.

XIII. STARTER CULTURES

A. Marketed Starters

When natto was homemade, previously produced natto was stored and used as a starter by mixing it with cooked soybeans. However, marketed starters used by natto makers now consist of only spores of natto bacilli. Natto may be produced with the vegetative cells of *B. subtilis* (*natto*), but the numbers of

viable vegetative cells change readily during the transfer and preservation of the cells. Therefore, it is difficult to produce a product of consistent quality from a starter containing vegetative cells. Spores of natto bacilli must be prepared completely, as even a piece of vegetative cells that do not leave, or spores may germinate to vegetative cells before utilization. Because spores do not germinate for lack of any nutritional substances in the suspension and in case that the portions of cell components like proteins, carbohydrates, and so forth are left in the suspension, they are degraded to smaller molecules with bacterial enzymes to become nutritional substances and, as a result, bacterial germination will occur.

Three marketed starters, miyagino, naruse, and takahashi starter, are sold by the three starter makers, respectively. The methods of starter production are considered trade secrets. Therefore, it is difficult for scientists, institutes, companies, or universities that study the development of new starters to put their research findings into practice. Furthermore, it is impossible to preserve natto bacilli by subcultures because the production of viscous materials by natto bacilli decreases during repeated subculturing. There may be some chance to regain viscous material production by changing the media of the subcultures, but it is difficult to recover it completely.

Commercial starters are marketed in the form of a spore suspension in distilled water or as lyophilized spores. Marketed starters contain (1–4) $\times$ 10^8/g. Natto makers purchase the starters and dilute them with sterilized distilled water or tap water, spraying 10^3 g of starter on the steamed soybeans while the temperature of soybeans is higher than 85°C or sprinkling the spore suspension on the soybeans with a watering pot. The reason that inoculation is carried out while the soybeans are hot is that the natto bacilli are resistant to heat, but many other bacteria that might contaminate the natto are not. Natto bacilli grow filamentarily on the surface of soybean at the initial stage of the culture, and after 6 h of culture, cells form septa and produce materials like slime, and then cells form short rods (111).

B. Development of New Starters

Many natto starters and natto products have been developed (37,73,112,113). Nagai et al. developed a starter producing both high umami and highly viscous materials (114). Koguchi et al. developed starters with high umami, producing natto with a weak ammonia flavor and white appearance, respectively (115–118).

It is said that 2- and 3-methyl butyric acids play important roles in the formation of natto flavor. People who do not like natto dislike the flavor and/or viscous materials of natto. Tamura et al. developed a starter with a mild flavor, using a temperature-sensitive mutant (119). The scientist group

of Mitsukan Co. obtained a 3-methyl butyric acid-less recombinant by a genetic engineering technique to make the natto flavor mild (120,121).

In Kansai District, natto is not as popular in the western districts as in Kanto and Tohoku districts, the eastern and northeastern districts, and there are many people who do not like natto. Hoshino et al., whose research institute is in Kansai District, one of the western districts, has developed a mutant that scarcely produces viscous materials (112).

In Japan, many people believe that natto is a healthful food. There are many descriptions in *Natto History* published in 1975 (122) indicating that natto has beneficial effects on digestion and/or controlling intestinal conditions or that natto contains potentially beneficial enzymes, including protease, amylase, cellulase, saccharase (invertase), and urease. It has also been reported that natto contains vitamins B_1, B_2, and B_{12} and nicotinic acid (niacin), that dipyridine carboxylic acid in natto has a preservation effect and removes radioisotopes, or that natto has an anticancer effect, and many other benefits.

Some studies that analyze the functionality of natto have been performed. Tamura et al. (1991) obtained a mutant producing natto with low vitamin K content. Hasegawa et al. obtained a mutant with vitamin B12 productivity by the cell fusion method (123).

Sumi et al. studied the effect of natto on thrombosis. They discovered an enzyme of natto that had fibrinolytic activity (124,125). They named it nattokinase and purified the enzyme. In an article by Sumi et al., it was reported that the research group of Yamada Foods Co., which is one of the representative natto companies in Japan, developed a highly nattokinase-producing strain by the cell fusion method (113). Yang et al. reported that fibrinolytic activity was enhanced in plasma by oral administration of chongkuk-jung and they purified a fibrinolytic enzyme from chongkuk-jung (126,127).

Bacillus sp. are used as probiotics for humans and animals. According to Hosoi et al., when mice were intubated with intact and autoclaved *B. subtilis* (*natto*) spores, only intact spores changed the fecal microflora, and the patterns of the changes differed depending on the diets. (128,129).

Terada et al. (130) reported that ingestion of natto affected the composition and metabolic activity of the human fecal microflora. Ingestion of natto increased the number of *B. subtilis* (*natto*) and *Bifidobacterium* spp.

Hosoi et al. examined the growth of three strains of lactobacilli cocultured aerobically with *B. subtilis*. The addition of *B. subtilis* (*natto*) to the culture medium increased the number of viable cells of all lactobacilli tested. Both catalase and *B. subtilis* (*natto*) enhanced the growth of *L. reuteri*, one of the intestinal microflora, whereas *B. subtilis* (*natto*), but not catalase, enhanced the growth of *L. acidophilus*.

As described earlier, Muramatsu et al. developed a highly elastase-producing strain and purified the enzyme (131–133). They also developed natto with high elastase activity (53,134).

To breed a high-vitamin-B_{12} producible natto strain, Hasegawa et al. utilized protoplast fusion between the two strain of natto bacilli (135). They isolated three fusants which were capable of producing stringiness and vitamin B_{12} on SG (sucrose–glucose) medium and observed that they produced the strong stringiness on a steamed soybean in a manner similar to *Bacillus subtilis* (*natto*).

The story of natto is an excellent example of the way in which modern techniques can improve an indigenous fermented food. Some people seem to think that modern technology inevitably ruins traditional, old-fashioned products, but that is not true for natto. Modern technology has transformed natto from a locally distributed seasonal product of uncertain quality and safety to a consistent, nationally distributed, high-quality product that can be safely enjoyed year-round.

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5

Tapai Processing in Malaysia: A Technology in Transition

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I. DESCRIPTION OF FOOD

Tapai is a popular Malaysian delicacy, normally consumed as a dessert. There are two main types of tapai, namely tapai pulut, which is made by fermenting glutinous rice (*Oryza sativa glutinosa*), and tapai ubi, which is made from tapioca or cassava tubers (*Manihot esculenta* var. *Cranz*). In the Malay communities in peninsular Malaysia, it is served at functions such as festivals and weddings.

Tapai tastes sweet yet slightly alcoholic, with a pleasant, fragrant aroma. The glutinous rice or cassava is soft and juicy, and there is also some liquid produced as a result of the fermentation. Both types are produced for the market on a cottage industry scale only and in the homes for family consumption.

Tapai is a very perishable product because the fermentation continues even after the optimum stage of fermentation has been reached. Thus, it has to be consumed immediately, but if it is chilled, it may be kept for about 2 weeks. Overfermentation results in a sour, alcoholic product not acceptable to the consumers. In the eastern state of Peninsular Malaysia, "tapai ubi" is more popular and some households use tapai to prepare sweet delicacies. "Tapai pulut" can also be made into other delicacies.

Tapai and products similar to tapai are found in many countries in Asia, although the names vary. All of these products are made using starters, and, again, the names vary from country to country although the product is

Table 1 Tapai and Tapai-like Products from Different Countries

Country	Name	Description
1. Tapai-like products		
Malaysia	Tapai pulut	Fermented glutinous rice
Indonesia	Tape ketan	
China	Lao-chao	
Malaysia	Tapai ubi	Fermented cassava
Indonesia	Tape ketella	
Java	Tape telo	
Sudan	Peujeum	
2. Starter		
Malaysia	Ragi	Starter for tapai
Indonesia	Ragi	
Philippines	Bubod	Starter for tapai-like products
China	Chiu-yeu	
Malaysia (Chinese)	Chiu-piah	
3. Alcoholic beverages		
East Malaysia	Tapai	Alcoholic liquid from fermentation of rice
Philippines	Binuburan	
Philippines	Tapuy	
Indonesia	Brem	
Malaysia	Samsu	Distilled product
Japan	Sake	

Source: Compiled from Refs. 1–4, 18.

basically the same. Table 1 summarizes tapai-like products and similar products in other countries.

II. EARLIEST KNOWN REFERENCE

According to Cronk et al. (5). Went and Geerligs (6) were reported to be the first to isolate and identify the microorganisms believed to be essential for the fermentation of tapai in 1895. A mixture of *Candida oryzae* and *Endomycopsis burtonii* produced a good tapai with 2.7% ethanol (v/v) and 17% reducing sugars.

Van Veen (7) also reported that work on fermented glutinous rice, cassava, and corn (''tape'') was done in the early 1900s in Java. A publication in Dutch by Heyne (8) gave a summary of the early research.

III. SUBSTRATES

The same substrates used in ancient times are still used today. However, depending on locality, the rice or cassava type may be more popular. Tapai can also be made from black glutinous rice (pulut hitam), but its production is very much less than for the other two types. Production of tapai from other raw materials is rarely done in Malaysia.

IV. MICROFLORA IN TAPAI

Research has been carried out by workers in many institutes to study the microflora of ragi, tapai ubi, and tapai pulut. The microorganisms isolated from traditional ragi samples from different localities in Malaysia (9) compared favorably with the microflora of ragi reported by other workers elsewhere (2,10–12). Table 2 lists the organisms that have been reported to be present. It can be seen that although a wide range of organisms have been found in ragi, only a few genera are present in tapai ubi and tapai pulut, indicating that most of the other organisms are contaminants.

V. STARTERS

The starters used for the production of tapai are known as "ragi." There are two main types in Malaysia. One is "ragi tapai," which is used for the production of tapai and probably originated from Indonesia. The other is "ragi samsu," which originated in China and is used for the production of rice wine.

Both types of ragi are dry, white, hemispherical balls, about 5–6 cm in diameter. Ragi tapai is about 0.5 cm thick, whereas Ragi samsu is at least 3 cm thick. The production of ragi is still done on a cottage industry scale in Malaysia, and the techniques of production vary from locality to locality.

No special equipment is required for the production of ragi, but the actual proportions of ingredients used are kept as trade secrets. Clean rice flour is mixed with water to make a thick paste. This is shaped by hand or by simple molds into hemispherical balls. Some ragi from a previous batch is ground into powder and sprinkled as inoculum over the balls. They are then placed on bamboo trays and covered with muslin cloth. The organisms will grow while the rice balls dessicate slowly at room temperature for 2–5 days.

Some manufacturers in Penang, in northern Malaysia, use a method adapted from Thailand. The rice flour is first mixed with "spices" which are

Table 2 Microflora of Ragi, Tapai Ubi, and Tapai Pulut

Product	Genera	Species	Ref.
Ragi	*Candida*	*C. guilliermondii*	12, 13
		C. humicola	12, 13
		C. lactosa	11
		C. melinii	11
		C. solani	11, 13
		C. intermedia	13
		C. pelliculosa	13
		C. parasilosis	11, 13
		C. mycoderma	13
		C. japonica	13
		Candida sp.	14
	Endomycopsis (*Saccharomycopsis*)	*E. chodati*	2
		E. fibuliger	2
		Endomycopsis sp.	14
	Hansenula	*H. anomala*	2, 11
		H. malanga	11
		H. subpelliculosa	11
		Hansenula sp.	14
	Saccharomyces	*S. cerevisiae*	12
		Saccharomyces sp.	14
	Torulopsis	*T. indica*	2
		Torulopsis sp.	14
	Rhodotorula	*Rhodotorula* sp.	14
	Mucor	*M. circinelloides*	12, 13
		M. dubius	2
		M. javanicus	2, 13
		M. rouxiii	2, 13
	Rhizopus	*R. arrhizus*	11
		R. oligosporus	11
		R. oryzae	2, 13
		R. stolonifer	2
	Chlamydomucor (*Amylomyces*)	*C. oryzae*	2
		Chlamydomucor sp.	13
		A. rouxii	2, 11
	Aspergillus	*A. flavus*	11
		A. niger	2
		A. oryzae	11
		Aspergillus sp.	14
	Penicillium	*Penicillium* sp.	14
	Fusarium	*Fusarium* sp.	12, 13

Table 2 Continued

Product	Genera	Species	Ref.
Tapai ubi	*Candida*	*C. guilliermondii*	12
		C. intermedia	12
		C. japonica	12
		C. mycoderma	12
		C. parapsitosis	12
		C. pelliculosa	12
		C. solanii	12
	Saccharomycopsis	*S. fibuliger*	1
	Chlamydomucor	*C. oryzae*	1
	Mucor	*M. circinelloides*	12
		M. javanicus	12
Tapai pulut	*Chlamydomucor* (*Amylomyces*)	*Chlamydomucor* sp.	3
		C. oryzae/*A. rouxii*	1, 15
	Saccharomycopsis (*Endomycopsis*)	*S. fibuliger*	15
		Saccharomycopsis sp.	1, 3
	Hansenula	*H. anomala*	15
		Hansenula sp.	3
	Rhizopus	*R. oryzae*	1
		R. chinensis	1

Source: Compiled from Refs. 1–3 and 11–15.

assumed to carry the desirable microorganisms. It is then mixed with sugarcane juice/extract instead of water and made into balls as above. The makers feel that by adding sugarcane juice/extract, the "ragi" will make a sweet tapai. If the ragi is to be used for making rice wine, then water is added in place of sugarcane juice/extract.

The ragi is harvested after 2–5 days, depending on the temperature and humidity. The final product is a dry cake that can be stored for long periods of time in dry, airtight containers. No control of any environmental factors is performed. Any microorganisms that settle on the ragi will grow. The environment surrounding the preparation area is usually saturated with normal ragi flora. However, ragi made during the rainy seasons are found to contain a lot more *Mucor* sp. and *Rhizopus* sp. due to the higher relative humidity and the longer period required for drying.

While the paste is still wet, the microorganisms grow and multiply, so that by the time the ball is dried out, the total viable count of the yeasts and fungi has increased significantly. Because the traditional process is nonsterile,

a good ragi will depend on the established load present in the environment and the containers and the inhibition of growth of other undesirable organisms. Microbiological analysis of ragi from different origin showed fungal counts of 8×10^7 to 3×10^8/g, yeast counts of 3×10^6 to 3×10^7/g, and bacterial counts of less than 10^5/g (19). The microorganisms isolated from ragi samples from different localities compared favorably with the microflora of ragi reported by other workers (2, 10–12). It is interesting to note that the ragi always contain an amylolytic genus, either *Amylomyces* or *Aspergillus*. As this is not a controlled fermentation, a wide variety of bacteria, yeast, and fungi have been isolated from the ragi.

Research showed that the organisms necessary to produce a good tapai pulut consist of a mixture of *Amylomyces rouxii* (*Chlamydomucor oryzae*), *Endomycopsis* (*Saccharomycopsis*) *fibuliger*, and *Hansenula anomala*. For a good tapai ubi, the essential organisms are *A. rouxii* and *E. fibuliger* (3, 15). These organisms were selected from ragi obtained from several areas in Malaysia. The identity of *E. fibuliger* and *H. anomala* were confirmed by Miller (personal communication, 1983) of the Department of Food Science, University of California at Davis.

The individual pure cultures were tested for their ability to produce tapai. It was necessary to have an amylolytic strain present to break down the starch to fermentable sugars. These were then converted to alcohol, aromatic esters, aldehydes, and acids if the fermentation was allowed to continue to completion, as in the tapai of East Malaysia. As a result of the fermentation process, the sticky glutinous rice or cassava is converted to a soft, juicy substrate with a sweet alcoholic flavor.

Tapai fermentation is not an anaerobic process and this partly accounts for the low alcohol content. Furthermore, the fermentation is normally arrested before all of the sugars are fermented, as the desired end product should not be alcoholic if it is to be consumed by those opposed to alcohol consumption for religious or other reasons.

VI. MICROORGANISMS THAT CAUSE SPOILAGE

The main microorganisms that cause spoilage are the fungi of the *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. Some bacterial spoilage also occurs, primarily due to acid-producing organisms such as acetic acid bacteria.

These spoilage organisms usually are present in traditional ragi because the preparation of ragi is exposed to contamination. Contamination can also occur through unhygienic preparation or premises. *Neurospora* sp. is known to cause spoilage and imparts an orange color to the product.

VII. TRADITIONAL METHODS OF PREPARATION

In the traditional method for preparing tapai pulut, the glutinous rice is washed, then soaked overnight and cooked (see Fig. 1). When cool, it is mixed with powdered, commercial ragi, wrapped in banana leaves or placed in containers, and allowed to ferment at room temperature (28–30 °C) for 1–3 days. Production of tapai ubi follows a similar method. The young tubers are peeled, cut, washed, and cooked. When cooled, the surface is sprinkled with powdered ragi. They are then placed in banana leaves or containers and allowed to ferment as for tapai pulut.

The taste of the resultant tapai is very much dependent on the inoculum used (i.e., the ragi). Ragi from different sources will produce tapai with

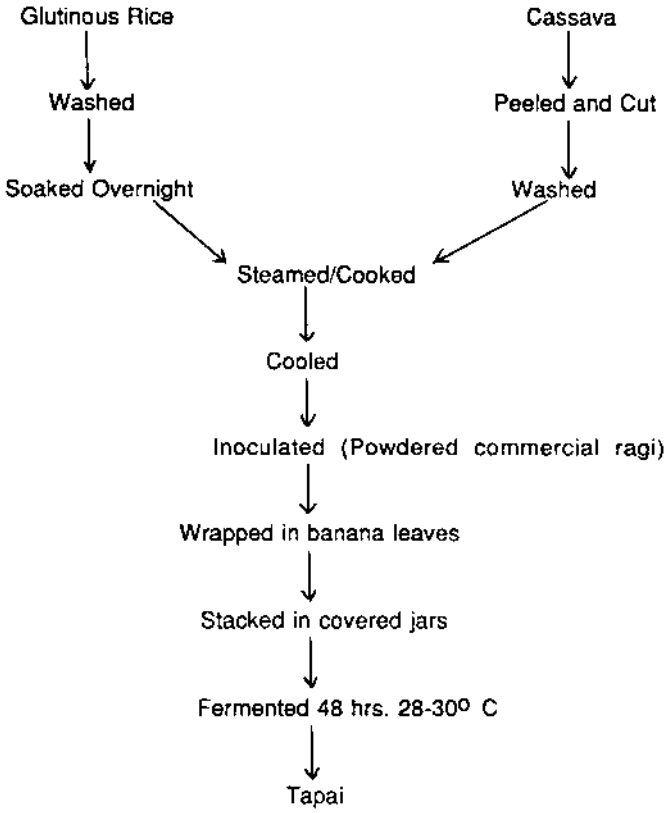

Figure 1 Tapai production (traditional).

varying degrees of sweetness and alcoholic flavor. Analysis of the ragi samples has shown variation in their microflora.

VIII. PREPARATION METHOD BY SMALL INDUSTRY

The method adopted for the small-scale industry or village industry in Malaysia does not differ much from the traditional preparation. As shown in Figs. 2 and 3, the emphasis is on the use of selected pure cultures of the desired organisms. Currently, the inoculum is produced by the Malaysian Agricultural Research and Development Institute (MARDI) and sold to processors at a nominal price.

Using MARDI's ragi, the processor needs to add only 0.2% inoculum based on the dry weight of rice. However, the processor may wish to scale up the inoculum to avoid frequent requests to MARDI as well as to cut costs.

Scaling up inoculum consists of inoculating every 100 g of sterile ground rice that has been moistened with 30 ml of sterile water with 1 g of inoculum and incubating it at room temperature for 4 days. This moistened inoculum can be used over a period of 2 weeks if kept uncontaminated under refrigerated storage.

The selection of raw materials is important for tapai, be it from glutinous rice or cassava. The glutinous rice should not be contaminated with other rice because the chemical composition is different and will affect the

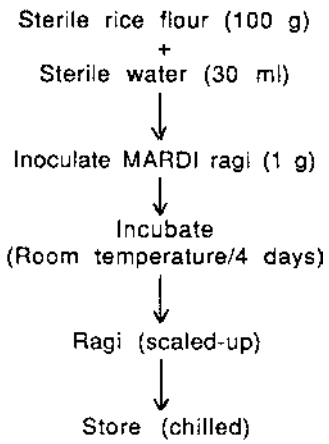

Figure 2 Scaling up of inoculum production.

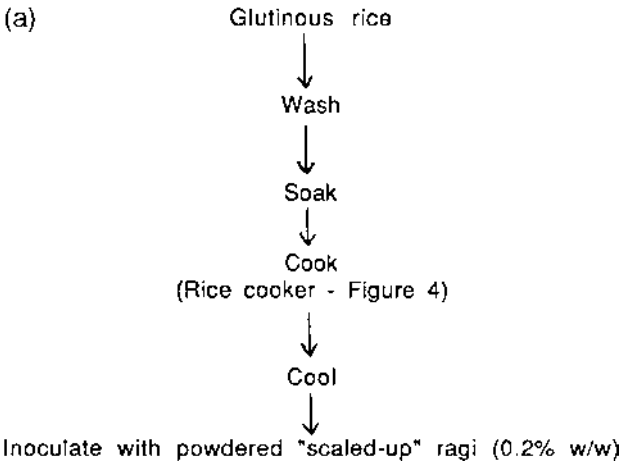

Figure 3 (a) Tapai pulut production; (b) tapai ubi production.

final product, especially in terms of the degree of sweetness and the amount of liquid in the tapai.

For cassava, the age of the tuber will determine the acceptability of the product because old tubers will be too fibrous. The optimum age for tubers is 10 months after planting and the maximum age should not exceed 12 months. It is also important that the tubers be freshly harvested, as stored tubers will not give the desired characteristic qualities.

At this stage of development, there is little mechanization of tapai production. Perhaps mechanical steamers as reported by Nunokawa (16) for steaming rice for sake production can be adopted for tapai pulut once the industry reaches that scale of operation.

IX. CONTRAST BETWEEN INDIGENOUS AND MODERN PROCESSING

Tapai is processed, usually by housewives, in the homes using very traditional preparation techniques. Occasionally, men may help in the preparation when it is meant for sale as a small-scale business.

An important step in the preparation of the cassava tubers is the scraping of the tubers to create tiny grooves to improve the attachment of organisms. Otherwise, the smooth surface of the tubers will not allow

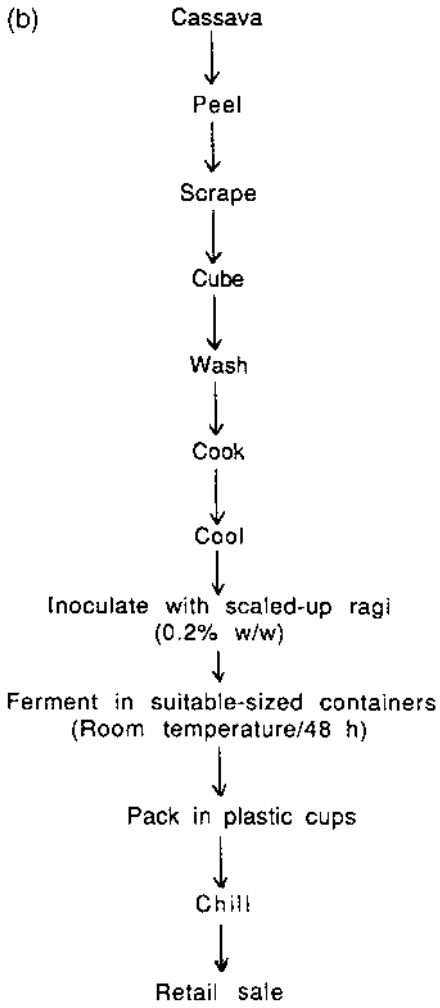

Figure 3 Continued.

sufficient hold, for the organisms and fermentation may be slow and incomplete.

The soaking time for rice prior to cooking can affect the quality of the product because insufficient soaking can cause the center of the grains to remain hard. However, excessive soaking can cause breakage and affect the appearance of the final product. The right amount of water needs to be used in

cooking because too much water will reduce the sweetness of the product and affect its texture.

The packaging used in traditional fermentation is banana leaves or any kitchen container if for home consumption, whereas the modern method takes advantage of the ever-popular and easily available plastic cups (Fig. 4).

The starter used in traditional methods is ragi, a dried cake form of uncontrolled organisms in a rice medium produced by village elders also using traditional methods. The starter for modern processing is powdered inoculum consisting of a single organism or a combination of three organisms produced by microbiological techniques. Currently, this pure inoculum is supplied by MARDI and can be stepped up by the processor to reduce cost.

Traditionally, the rice is steamed, although currently the automatic rice cooker is sometimes used (Fig. 5). For larger volumes, cookers can be fabricated locally (16). The traditional method of making tapai is shrouded with superstition and old wives tales which were used as an excuse for failure.

Due to poor presentation and short shelf life, traditional tapai is either sold in the marketplace or from house to house. The practice varies according to location. Because tapai is packed in leaves, much of the liquid, which is sweet and alcoholic, is left in the fermenting jar. In some places, especially those with door-to-door sales, the liquid is placed in a bottle and poured onto

Figure 4 Packaging in plastic cups.

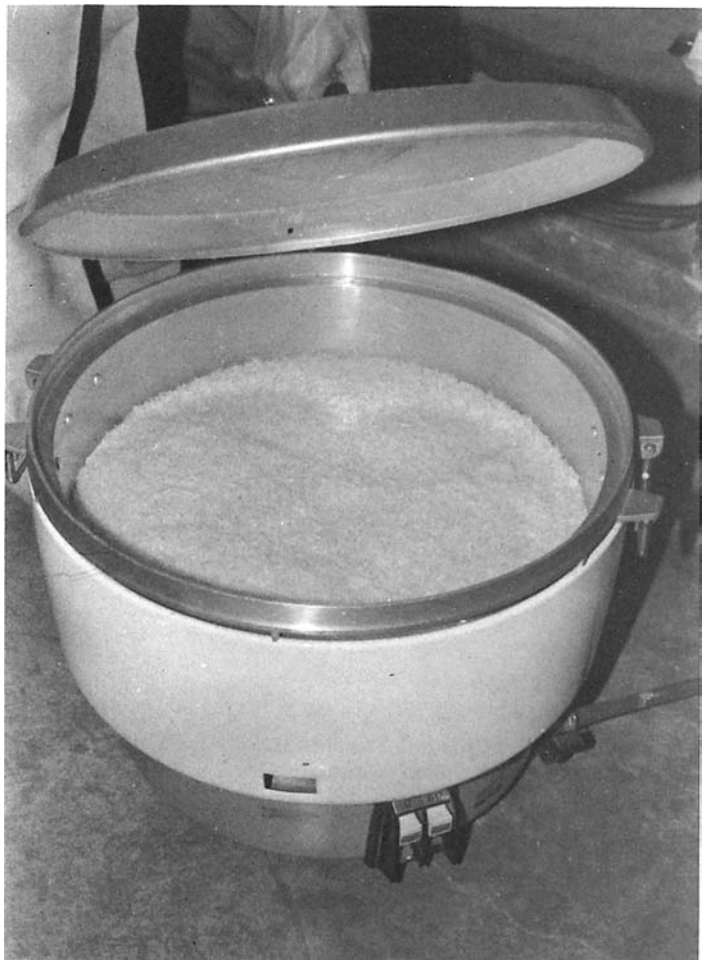

Figure 5 Automatic rice cooker.

the tapai at the point of sale. In other places, the liquid is not sold. The liquid can also be used in making other fermented products such as "apam" (a traditional cake).

With the introduction of the new packaging and the availability of refrigeration, the product can be kept longer. The packaging gives a new image to tapai. It marked a major change in the packaging and retail presentation of traditional foods.

Traditional tapai has a short shelf life, as it turns sour due to overfermentation if kept more than 2 days after completion of fermentation. Modern requirements of long-distance distribution, the consumers' expectation of convenience in food purchase and storage, the popularity of supermarkets, and the availability of chilled storage make the plastic cup an ideal container.

The cups (Fig. 4) with fitted lids (approximately 250 mL capacity) provide the following features: easy handling during distribution and storage prolonging shelf life, a microaerophilic environment for desired fermentation, convenience to consumers, attractive design to improve eye appeal, and printed labels on the package for the benefit of the consumers.

X. CRITICAL STEPS IN PROCESSING TAPAI

Tapai is a safe product. All of the necessary precautions must be taken to ensure a good product. The product is alcoholic (1.58% ethanol) with a high sugar content (32.06% sugar) (4). There is no known case of food poisoning caused by tapai. Spoilage of the product or quality deterioration, such as appearance, odor, or taste, is easily detected and is a reliable preventive measure against consumption. No significant biological, physical, or chemical hazards exist in the processing of tapai (see Tables 3–5). Ethanol production as a quality factor can be minimized by using properly made inoculum and controlling the fermentation time and temperature, and the quality of the product is ensured by following the correct processing and fermentation steps (in Table 6) (Figs. 6–8).

XI. MAJOR PROBLEMS IN INDUSTRIALIZATION

No heat processing is applied to the final product and, as such, the product has a relatively short shelf life. Kept at room temperature, traditional tapai lasts for 2 or 3 days. After this period, it becomes overfermented and sour. The tapai packed in plastic cups can be kept for about 2 weeks in the refrigerator. In the frozen form, it can be kept for several months, but the texture is adversely affected. The main problem in industrialization, then, is the shelf life of tapai. The product has to be kept refrigerated and this means additional cost. Furthermore, if there is a breakdown of refrigeration or electrical supply, the product becomes spoiled and has to be discarded. Distribution is also a problem, as it has to be done in insulated containers or refrigerated trucks, which, again, means additional cost.

Table 3 Hazard Analysis Worksheet for Raw Materials for Tapai

	Potential hazard (State whether B, C, or P)	Rational for inclusion or exclusion as a hazard	Is this significant hazard? (Yes/No)	What preventive measure or control can be applied to prevent this significant hazard?	Is this a sensitive raw material? (Yes/No)
Raw material					
Glutinous rice	B—toxic fungi	No known case	No		NO
	C—pesticide or fumigant residues	Not a problem in glutinous rice	No		
	P—stone, foreign matter	May be present in rice	Yes	All foreign matter removed at subsequent steps	
Water	B—none	Water used is potable water from municipality supply	No		
	C—none		No		
	P—none		No		
Packaging material					
Plastic cups and lids	B—none	Use of food-grade plastic containers, thus not a problem	No		
	C—none		No		
	P—none		No		

Ragi inoculum	B—toxic fungi or pathogens C—none P—none	Unlikely, as it is prepared under controlled conditions	No		
Cassava	B—none C—cyanide compound P—stone	No known case of biological hazards; cyanide compound, if present, will leach out during cooking and stones may be present	No	All foreign matter removed at subsequent steps	NO
Banana leaves (in place of plastic cup)	B—insects and pathogens C—none P—none	May be present	No	Leaves thoroughly cleaned and wiped dry before use	NO

B = biological, C = chemical, P = physical.

Table 4 Hazard Analysis Worksheet for Tapai Pulut

Processing steps	Potential hazard (State whether B, C, or P)	Rational for inclusion or exclusion as a hazard	Is this a significant hazard? (Yes / No)	What preventive measure or control can be applied to prevent this significant hazard?	Is this step a critical control point? (Yes / No)
1. Receiving of dry glutinous rice	B, C—none P—stone, foreign matter (FM)	Small stones and other FM may be present	Yes	Select good supply and hand-pick FM from rice; remove any remaining FM at step 7	NO
2. Washing	B, C, P—none				
3. Soaking	B, C, P—none				
4. Cooking	B—microbial spores may survive C, P—none	Pathogens not known to survive fermentation	No	Cooking is done to the required time	NO
5. Cooling	B—microorganism C, P—chemical and physical contaminants	Recontamination may occur	No	Ensure clean environment and no recontamination is possible	NO
6. Inoculating with ragi	B—pathogens C, P—none	Unlikely, as it is prepared under control condition	No	Ensure good ragi is used	NO
7. Mixing	B, C, P—none				
8. Filing into plastic cups	B, C—none P—FM	Unlikely to occur, as earlier steps would have eliminated FM	No	Last step to remove FM	NO
9. Weighing	B—microorganism C, P—chemical and physical contaminants	Recontamination may occur, but very short exposure time	No	Observe basic hygiene	NO
10. Fermenting	B, C, P—none				
11. Storing chilled	B, C, P—none				

B = biological, C = chemical, P = physical.

Table 5 Hazard Analysis Worksheet for Cassava Tapai

Processing steps[a]	Potential hazard (State whether B, C, or P)	Rational for inclusion or exclusion as a hazard	Is this a significant hazard? (Yes / No)	What preventive measure or control can be applied to prevent this significant hazard?	Is this step a critical control point? (Yes / No)
1. Receiving of fresh cassava tubers	B, C—none P—stone, dirt, foreign matter	Tubers may contain dirt and other FM	Yes	All foreign matter removed at subsequent steps	NO
2. Washing	B, C, P—none				
3. Scrape and cut into small pieces	B, P—none C—cyanide compound	Cyanide compound will leach out from tubers	No	Remove all leachate from tubers	NO

[a] Subsequent steps 4–10 are the same as in Table 4.
B = biological, C = chemical, P = physical.

Table 6 Quality Parameters in Processing of Tapai Pulut and Cassava Tapai

Processing steps	Potential quality problem	What preventive or control measure can be applied to prevent this problem?	Is this step critical in manufacturing/ fermentation? (Yes / No)
1. Receiving of raw materials: 1.1. Glutinous rice 1.2. Cassava 1.3. Ragi 1.4. Plastic cups	Poor quality products	Selection of raw materials which meet specifications	YES
2. Washing of rice/preparation of cassava	Contamination with foreign matter	Sorting and proper washing, and for the cassava, scraping of the tuber surface	YES
3. Soaking of rice	Gritty product	Sufficient soaking	YES
4. Cooking	Unacceptable texture and appearance	Cooking at the right temperature and time	YES
5. Cooling	Insufficient cooling may inactivate the inoculum, whereas long cooling may result in contamination from the environment	Cooling to room temperature and minimizing cooling period	YES
6. Inoculating	Uneven or no fermentation	Use of a good, suitable inoculum at the correct level	YES
7. Mixing	Uneven fermentation, mushy product	Thorough mixing of inoculum and substrate; however, rough mixing will result in a mushy product	YES
8. Filling	None	None	NO
9. Weighing	None	None	NO
10. Fermenting	Under fermentation or overfermentation	Controlled time and temperature	YES
11. Chilled storage	Spoilage	Products left at room temperature will continue to ferment, resulting in overfermentation and spoilage; chilled storage will prolong the shelf life	YES

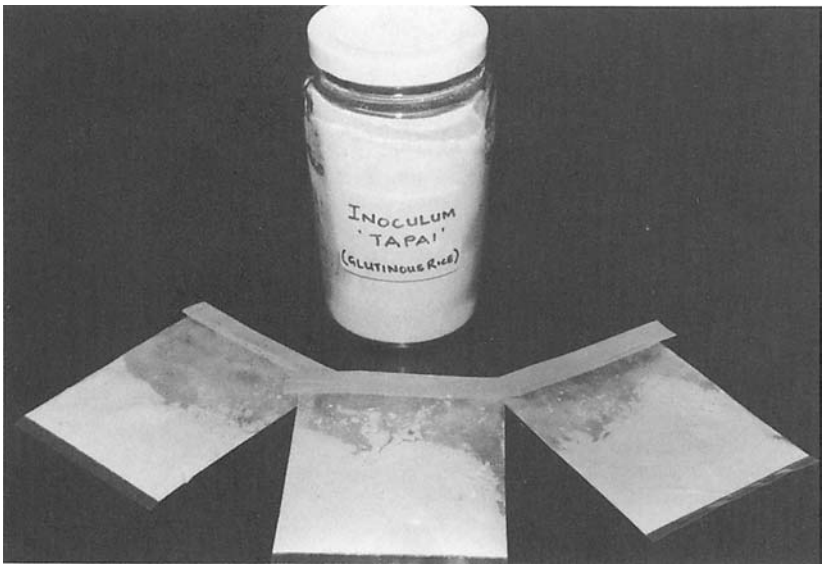

Figure 6 Tapai inoculum in powder form.

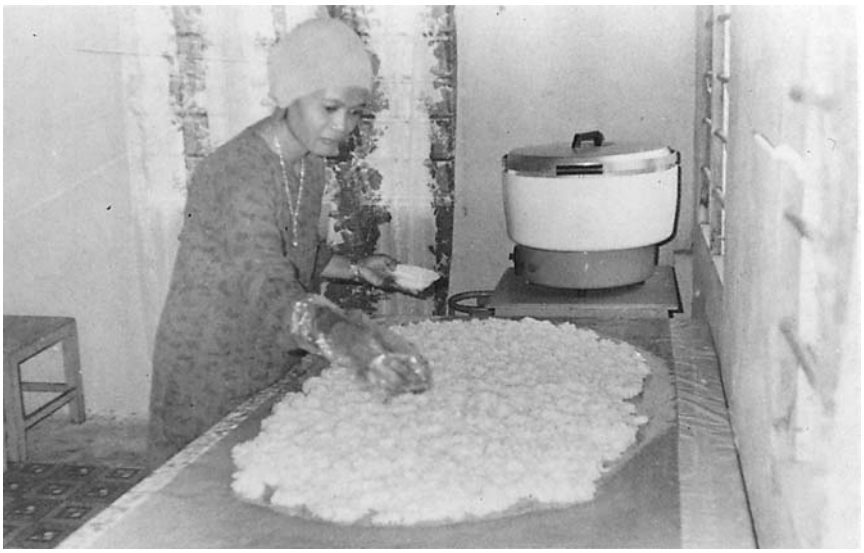

Figure 7 Processor inoculating the cooked glutinous rice.

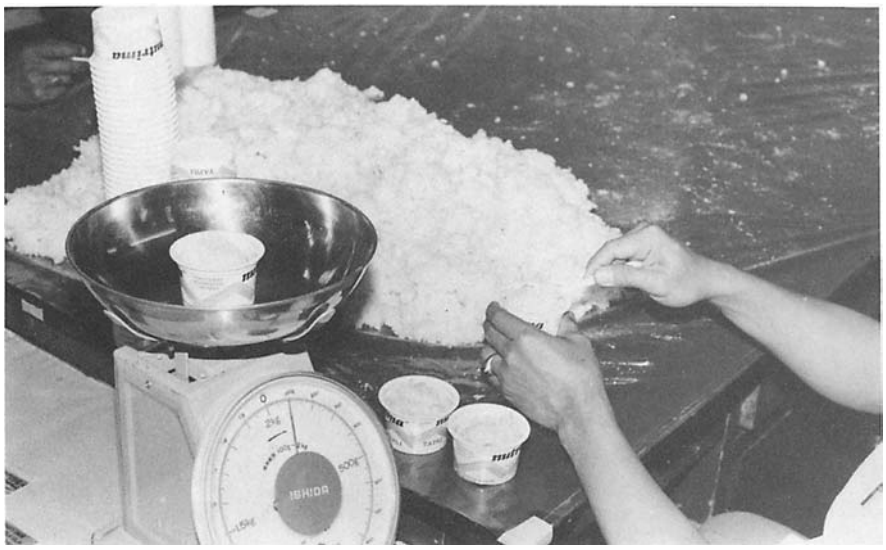

Figure 8 Packing in cups.

Good hygiene and sanitation must also be observed during processing because the product is susceptible to spoilage by contaminating microorganisms, especially fungi.

Currently, the process is not mechanized; thus, it tends to be rather labor intensive. Because of the nature of the product, rough handling has to be avoided to prevent it from becoming mushy. The scale of production is therefore limited.

XII. OPTIMUM FERMENTATION CONDITIONS

Tapai fermentation is best carried out under microaerophilic conditions. Under anaerobic conditions, the fungi are unable to grow and will not hydrolyze the starch. However, under aerobic conditions, there is good growth of both the fungi and yeast, but flavor development is affected because alcohol formation is decreased under these conditions. In this respect, the plastic cups with covers introduced by MARDI suit the conditions perfectly, as the covers are tight but not absolutely airtight. This closed fermentation system also helps to prevent contamination by other organisms.

Temperature also affects the rate of fermentation. Although a lower temperature of 25°C results in a product with a higher alcohol content after

144 h (5), this is not desirable in Malaysia, where the popular product is one with a low alcohol content.

Furthermore, a shorter fermentation period is preferred, and the MARDI product is usually fermented for 45 h at room temperature (26–30°C).

Trials carried out in the laboratory showed that acceptable tapai was produced after 45 h if tapai was incubated at 30–37°C. The product at 37°C was sweeter, however. If the rice was incubated at 25°C, a longer fermentation period of 3 days was required. After 45 h, the rice was slightly soft, but was tasteless and had no tapai aroma. Thus, the optimum temperature range for incubation is 28–37°C (17,19,20).

XIII. CHEMICAL/BIOCHEMICAL CHANGES

Evaluation of tapai from glutinous rice (pulut) prepared under aseptic conditions in the laboratory using single and mixed cultures, as well as commercial samples (5), showed the results presented in Table 7.

Ethanol was the only alcohol tested for in the tapai samples. The organisms used for producing tapai in MARDI were selected for low alcohol production, in response to consumer demands; thus, there is a significant difference between the alcohol levels in the MARDI samples and the commercial samples.

Table 7 Chemical Analysis of Tapai Pulut

Sample	Organisms in starter	% Ethanol (mean ± SE)	% Total sugar (mean ± SE)	% Red sugar (mean ± SE)
A	*A. rouxii*	0.92 ± 0.05	32.55 ± 0.63	32.50 ± 0.65
B	*A. rouxii* + *S. fibuliger*	1.03 ± 0.08	31.77 ± 0.30	31.75 ± 0.29
C	*A. rouxii* + *S. fibuliger* + *H. anomala*	1.10 ± 0.04	32.93 ± 0.38	32.01 ± 0.35
D[a]	*A. rouxii* + *S. fibuliger* + *H. anomala*	1.58 ± 0.07	32.06 ± 0.38	32.01 ± 0.35
E[b]		3.48 ± 0.38	26.33 ± 1.23	26.18 ± 1.25
Range		2.2–4.9	21.5–31.1	21.5–32.1

[a] MARDI's commercial tapai.
[b] Commercial samples.

The results show that the alcohol and sugar content of samples A, B, C, and D are quite similar. The fungus *A. rouxii* can hydrolyze the rice starch to sugars. Practically all of the sugars present were reducing sugars. *Amylomyces rouxii* is also an alcohol producer, producing 0.92% ethanol after 45 h of fermentation. Use of mixed cultures of *A. rouxii* and *S. fibuliger* produced a tapai with a slightly higher alcohol content but a lower reducing sugar content. Both samples tasted sweet and mildly alcoholic but did not have a strong tapai aroma. On the other hand, the tapai produced using a mixture of three organisms had a typical tapai flavor. Thus, it can be seen that *H. anomala* is essential for flavor production.

Changes in the alcohol content of tapai during storage at room temperature were also monitored. Results showed that the alcohol content of commercial tapai increased from 3.0% to 5.2% after 2 days, whereas that for MARDI's tapai increased from 1.6% to 2.7%, still substantially lower than the commercial sample. However, if the tapai is kept at a low temperature (7°C), fermentation continues but at a slower rate. The total sugar content remained stable for up to 2 weeks and the alcohol content showed only a slight decrease after 7 days. However, sensory evaluation showed that the product was acceptable for up to 2 weeks.

The results obtained are similar to those obtained by Wang and Hesseltine (1) and Cronk et al. (5) who reported that *A. rouxii* was able to hydrolyze starch as well as produce alcohol. The yeasts were found to be more involved with the flavor development in tapai. Cronk et al. (5) reported that *A. rouxii* in combination with *E. burbonii* was able to reduce the total solids of tape ketan by 50% in 192 h at 30°C and at the same time raise the protein content of the rice substrate to 16.5% on a dry basis. They also reported on the production of fusel oils, such as isobutanol, active amyl, and isoamyl alcohol in tapai by *A. rouxii* in combination with various *Endomycosis*, *Candida*, and *Hansenula* species.

Cronk et al. (5) found that rice lipids were hydrolyzed during tape ketan fermentation. The main fatty acids detected were oleic, palmitic, linoleic, stearic, and myristic. The amount of linoleic acid also increased as a result of fermentation. In MARDI's tapai, the main organic acids detected were acetic, lactic, citric, and succinic. The product had a titratable acidity of 0.2% (as acetic acid) and a pH of 4.2.

XIV. CHANGES IN NUTRITIVE VALUE

According to Cronk et al. (5), the protein content of rice is doubled to about 16% after fermentation as a result of losses of total solids and synthesis of protein by the microorganisms. In tapai from cassava, the protein content is

increased from 1% or 2% to 4%. Changes in the amino acid composition of rice after fermentation with *A. rouxii* alone and in combination with *E. burtonii* were also reported by Cronk et al. (5). In general, there is some decrease in the amount of amino acids after fermentation, especially glutamic acid, which falls by about 17.5%. However, lysine increases by 11% and tyrosine by 9.8%. There is significant increase in the thiamine content, which increases from 0.04 mg/100 g to 0.10 and 0.12 mg/100 g respectively when *A. rouxii* alone or in combination with *E. burtonii* is used.

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6

Industrialization of Africa's Indigenous Beer Brewing

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I. DESCRIPTIVE PROFILE OF SORGHUM, MILLET, AND MAIZE BEERS

A. Product Characteristics

Indigenous African beers are soured, fermented drinks made with malted sorghum, millet, or maize. Identified by the grains used in their production, these beers are often called "sorghum," "millet," "maize," or even "corn beers." The same beers have also been known, at various times, as "kaffir beer," "bantu beer," and "opaque beer." Collectively, this chapter will refer to them as "SMM" (sorghum, millet, and maize) beer, "indigenous" beer, or simply as "beer."

Those acquainted with the pleasures of imbibing Africa's indigenous beers realize how much they differ from the "clear beer" commonly consumed in the United States, Europe, and elsewhere. Indigenous African beers are opaque and generally a pinkish brown in color due to the large quantity of solid particles and yeast suspended in solution (1). Much thicker than clear

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beer, indigenous beers resemble a thin gruel. They have a distinctly sour, yogurtlike taste, which makes them refreshing even when drunk warm—as they always are. Because sorghum, millet, and maize beers must be sold in an actively fermenting state, they spoil rapidly, within 1–5 days. Although it may vary between 1% and 8%, the alcohol content of these drinks normally lies between 2% and 3% (1–10). In fact, the government of South Africa legally restricts the alcohol content of factory-brewed sorghum beer to a maximum of 3% when leaving the factory (11).

Because of the low alcohol content and the large quantity of solids suspended in the beer, many consumers and market observers consider indigenous beer as much a food as a beverage (12–14). Numerous consumer tests have shown that buyers continue to purchase sorghum beer even when the alcohol content is reduced as low as 0.7%, so long as the distinctive sour taste and the beer body are not diminished (1,14). Moreover, many industry specialists consider a soured, nonalcoholic maize porridge, mageu, to be a primary market competitor to indigenous beers (11). In the lexicon of the Pedi of South Africa, protocol requires that host and invitees begin their sorghum beer drinking by each asking, "Mother may we eat?" (15). Similarly, when asked why they like sorghum beer, consumers in Botswana repeatedly respond, "It fills my stomach" or "It keeps me strong" (16, and survey data).

Africa's brewers confect a wide variety of indigenous beers using two principal inputs: malt and a starchy adjunct. For malting, both home and factory maltsters prefer to use sorghum or millet, although home brewers in the humid regions of west and central Africa sometimes malt maize instead. As their second input, the starchy adjunct, factory brewers almost invariably use maize grits, whereas home brewers employ a wider variety of adjuncts. Although their starch sources commonly include unmalted sorghum, millet, or maize, home brewers in some regions also use malted grains. In some cases, they even brew with cassava as their starchy adjunct.

Not surprisingly, the many possible combinations of malt and starch result in a wide assortment of indigenous beer recipes across the African continent. Tables 1 and 2 display the malt and starch combinations used by brewers in different countries as well as the different local names by which the beers are known.

The SMM beers remain the drink of preference in arid, temperate, and highland areas of sub-Saharan Africa, whereas palm wine reigns supreme in the humid, coastal regions (23,39,40) (Tables 1 and 2). Thus, regions of heavy indigenous beer consumption include a broad swath across the savannas of western Africa (including the Sahel plus the northern regions of Ivory Coast, Ghana, Togo, Benin, and Nigeria), virtually all of eastern and southern Africa, and the arid and highland areas of central Africa.

Table 1 Characteristics of Home-Brewed Indigenous Beers in Sub-Saharan Africa

Location	Local name of beer	Type of malt	Source of starch
Western Africa			
Benin	Chapalo	Millet	Millet
	—[a]	Sorghum	Sorghum
	—	Maize	Maize
Burkina Faso	Dolo	Sorghum or millet	Sorghum or millet malt
Ghana	Pito	Sorghum or millet	Sorghum or millet
Ivory Coast	Dolo	Sorghum or millet	Sorghum or millet
Mali	Dolo	Sorghum or millet	Sorghum or millet
Niger	Dolo	Sorghum or millet	Sorghum or millet
Nigeria	Pito	Sorghum or millet	Sorghum or millet
Togo	Dam, dolo	Sorghum	Sorghum malt
Central Africa			
Angola	Walwa	Maize	Maize
Cameroon	Amgba	Sorghum	Sorghum
	—	Sorghum or millet	Sorghum or millet
Central African Republic	—	Sorghum or maize	Sorghum or maize
Chad	—	Millet	Millet
Congo	Gwalo	—	—
Rwanda	Ikigage	Sorghum	Sorghum
Zaire	—	Sorghum, maize, or millet	Sorghum, maize, cassava, or millet
Eastern Africa			
Ethiopia	Bouza	Sorghum	Sorghum
Kenya	Busaa, pombe	Sorghum or millet	Sorghum, millet, or maize
Sudan	Merissa, marisa	Sorghum	Sorghum
Tanzania	Pombe, Bwalwa	Sorghum or millet	Sorghum or millet

Table 1 Continued

Location	Local name of beer	Type of malt	Source of starch
Uganda	Busaa	Millet	Sorghum
	Omwenge	Sorghum or millet	Banana
	Omuganda		
	Malwa	Millet	—
Southern Africa			
Botswana	Bojalwa ja setswana	Sorghum or millet	Sorghum, millet, or maize
Lesotho	Yalwa, joula	—	—
Malawi	—	Maize	Maize
South Africa			
Ndebele, Zulu	Utshwala, tshwala	Sorghum	Sorghum or maize
Pedi	Bjalwa ja mabelethoro	Sorghum or millet	Sorghum or millet malt
Shagane	Mqomboti	Sorghum	Sorghum or millet
South Sotho	Joula, joala, jalwa	Sorghum	Sorghum or maize
Swazi Shosa	Utshwala	Sorghum	Sorghum or maize
Tswana	Bogule	Sorghum or millet	Sorghum or maize
Venda	Mela	Sorghum or millet	Sorghum or maize
Zambia	7-Day beer	Sorghum or millet	Sorghum or millet
	Katata	Millet	Maize
Zimbabwe	Zezuru	Sorghum or millet	Maize

[a] Indicates no information available.

Source: Benin: Refs. 17 and 18; Burkina Faso, Ivory Coast, Mali, and Niger: Ref. 19; Ghana: Refs. 20 and 21; Nigeria: Ref. 21; Togo: Refs. 19 and 22; Angola and Central African Republic: Refs. 23 and 24; Cameroon: Ref. 22; Chad: Ref. 25; Congo: Ref. 24; Rwanda: Ref. 26, and Csete, personal communication, 1987; Zaire: Ref. 23; Ethopia, Kenya, Sudan, Tanzania, and Uganda: Refs. 21, 22, 24, and 27–30; Botswana: Ref. 31; Lesotho: Refs. 24 and 32; Malawi: Ref. 23; South Africa: Refs. 15, 32, and 33; Zambia: Ref. 21; Zimbabwe: Ref. 34.

Table 2 Characteristics of Factory-Brewed Indigenous Beers in Sub-Saharan Africa

Location	Brand name	Type of malt	Source of starch
Western Africa			
None	n.a.[a]	n.a.	n.a.
Central Africa			
None	n.a.	n.a.	n.a.
Eastern Africa			
Kenya	Chibuku	—[b]	—
Tanzania	Chibuku	Sorghum	(Maize)
Uganda	(Chibuku)[c]	Millet	—
Southern Africa			
Botswana	Chibuku	Sorghum	Maize
Malawi	Chibuku	(Sorghum)	(Maize)
South Africa	Tiokwe, ijula, zebra, and many others	Sorghum	Maize
Swaziland	Imvelo	(Sorghum)	(Maize)
Zambia	Chibuku	(Sorghum)	Maize
Zimbabwe	Chibuku Ngwebu	Sorghum	Maize

[a] not applicable.
[b] indicates no information obtainable.
[c] parentheses indicate an informed speculation.
Source: Ghana and Kenya: Ref. 35; Tanzania: Refs. 21 and 31; Uganda: Ref. 21; Botswana: Ref. 31; Malawi: Ref. 36; South Africa and Swaziland: Ref. 37; Zambia: Ref. 38; Zimbabwe: Ref. 31.

B. History of Production

Indigenous beers have held a prominent place in the African diet for many centuries at least—probably for millennia. Egyptian hieroglyphics depict women brewing beer, probably from barley, as early as 3500 BC (41). Although durable records of brewing procedures are less readily available south of the Sahara, the very earliest chronicles of outside visitors remark on the prevalence of sorghum and millet beer in the region. In southern Africa, Portuguese explorers have reported on the prevalence of sorghum and millet beer production since their earliest voyages in 1505. Likewise, Heinrich Barth and Mungo Park, two of the earliest explorers of western Africa, commented on the existence of indigenous beers in the areas they visited in the 1800s [5,12]. Thus, we have written testament to the prevalence of sorghum and

millet beers dating back about five centuries and every reason to believe they were common well before then. Novellie, one of the foremost students of sorghum beer production, asserts that the brewing of indigenous beers dates from "prehistoric times" (1).

From the earliest times, indigenous beers have formed an important part of African social fabric (1,5,13,19,42–45). In early times, women brewed indigenous beers for their own families' consumption, for use at ceremonies and social occasions, and for exchange in cooperative work parties. From the Sahel to eastern, central, and southern Africa, beer occupied a central place in the celebration of harvest and at weddings, funerals, and social gatherings of all kinds (3,8,19,21,44–52). It also served as a primary motivation in mobilizing the village labor for cooperative work necessary for preparing fields, harvesting, or building houses (3,8,12,19,21,24,34,44,53) Figures 1–7 depict the use of sorghum beer in religious and initiation ceremonies.

Beer consumption in these early days took place in a variety of locations and normally outside of meal times (3,5,15,34,52,54). In the case of cooperative work parties, workers drank at the work site as they worked. During

Figure 1 Beer and snuff are being sacrificed to the ancestors at home altar, Molepo–northern Sotho (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

Figure 2 Before an initiation ceremony, Venda girls bring beer to the house of the chief. The beer is then tested ceremonially as an indication that it is drinkable (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

ceremonies, revelers imbibed at the village center or in the compound of the host. When entertaining friends at home, hosts normally served beer separately from meals.

As African economies became monetized, so too did the distribution of home-brewed beer. Some women came to specialize in the production of beer which they sold for cash. In central, eastern, and southern Africa, the earliest reports of cash home brewing date its emergence at between 1880 and 1920 (3,40,47,48,55–57). However, in western Africa, cash home brewing probably began much earlier, possibly as early as the 1300s when cowries became common currency in the region (19,46,52,58,59). Today, available evidence from across the continent indicates that over 90% of both rural and urban home brew is produced for cash sale (19,31,60). In southern Africa, today's large cash home brewers are known as "shebeen queens." In western Africa, they are known as "dolotieres." Everywhere, they are known to the multitude of discriminating customers.

Factory brewing first began at the instigation of municipal governments in South Africa and Zimbabwe. The municipalities of Durban, South Africa and Buluwayo, Zimbabwe made initial attempts at factor brewing in 1908 and

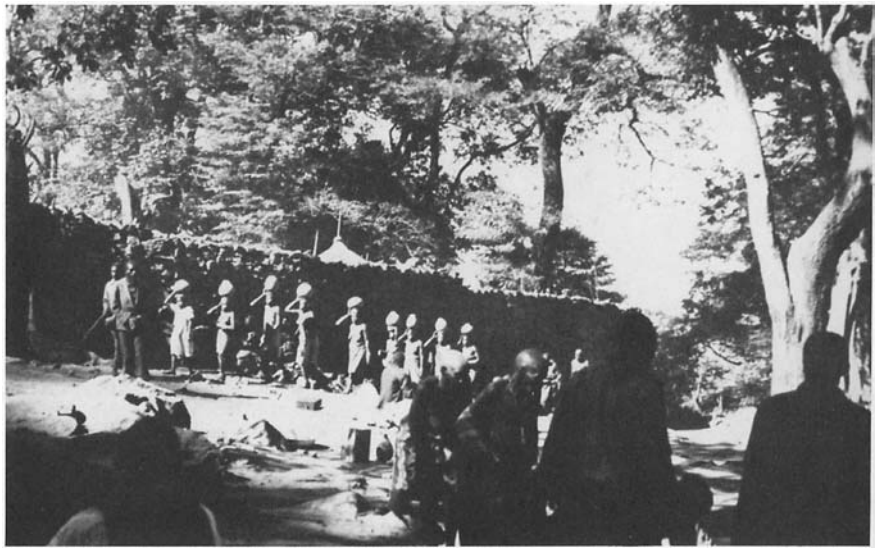

Figure 3 Delivery procession approaches chief's compound (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

1910 (45,56). However, technical difficulties plagued factory brewers in their initial efforts at scaling up home-brewing recipes to factory levels. Consequently, factory brewing languished; it was not until four decades after the pioneering efforts that factory brewing began its rapid rise to prominence (1,11). In 1937, the government of South Africa forced recalcitrant municipalities into action by enacting legislation requiring the municipalities to assure the supply of sorghum beer within their jurisdictions (61). In the face of continued technical difficulties, the municipalities, in 1954, commissioned the South African Council for Scientific and Industrial Research (CSIR) to undertake applied research on behalf of the municipal factory brewers of sorghum beer (1,62). The CSIR began what was to become an extremely successful research effort, leading to the establishment of a permanent Sorghum Beer Unit within the CSIR and the resolution of several key problems which made it possible to scale up production to an industrial level. From the mid-1950s onward, factory brewing of indigenous beers has mushroomed, beginning in South Africa, Zambia, and Zimbabwe.*

* See Refs. 1, 31, 63, and 64 for greater detail on the historical evolution of factory and cash home brewing of indigenous beer.

Figure 4 Beer arrives at the chief's compound (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

C. Consumption Patterns

The emergence of cash home brewing and factory brewing have altered some features of indigenous beer consumption, although not others. Today, as earlier, those who partake frequently consume their beer away from the household, although today they frequently drink in specialized home-brewer retailing outlets—"shebeens," "dolo bars," and "beer clubs"—or in licensed beer halls rather than in the compounds of friends. Still, on some occasions, customers purchase sorghum beer from either home or factory brewers and carry it home to drink with friends. Although precise data are not available, it appears that the bulk of SMM beer today is consumed away from the home—in home and factory brew retailing establishments (16,65).

One major change accompanying the parallel rise of cash home brewing and factory brewing has been the noticeable increase of drinking among young adults whose access to SMM beer was previously limited by social pressures and protocols. However, they now return from mines and cities with cash in hand and cannot be denied access to as much indigenous beer as they can afford (3,45,66–70).

Figure 5 Beer is displayed in preparation for the ceremony (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

Figure 6 Final preparations (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

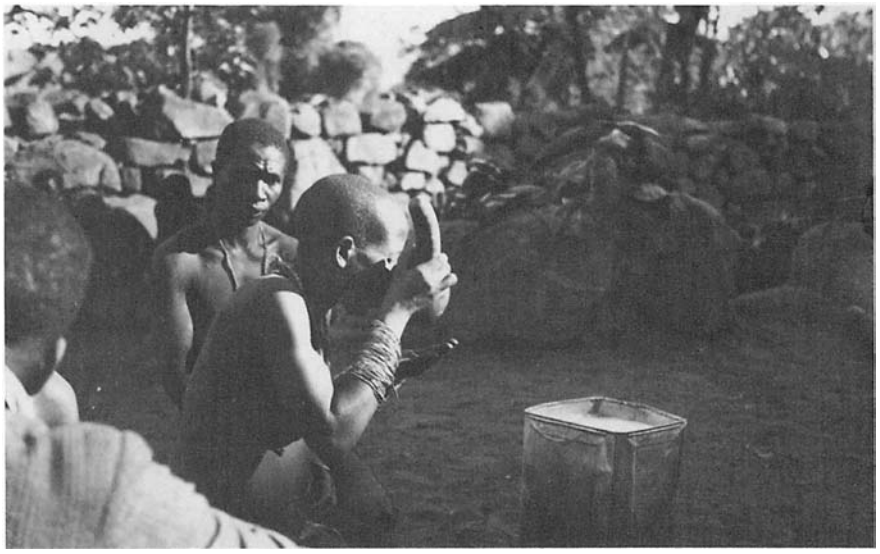

Figure 7 Ceremonial testing of the beer (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

Today, as earlier, indigenous beers occupy an important place in the diets of Africans living outside of the humid subtropical regions of the continent. Observers in many parts of the continent have commented on the large portion of cereal harvest consumed in the form of indigenous beer. Reports from the 1930s provide estimates that range from 12.5% of the grain harvested in Malawi to 33% in the Logone Plains of Chad (12,71). Today, in what are still predominantly grain-based diets, the share of grain consumed in the form of SMM beer stands at about 19% in Botswana, 13% in Burkina Faso (rising to 50% in the capital city of Ouagadougou), 6% in Kenya, and 9% in South Africa (16,21,60,72,73).

In terms of calories, estimates of the contribution of indigenous beer vary dramatically. Quin and Bryant report that in the late 1800s, in the months following harvest, Zulu men in South Africa virtually lived on sorghum beer (5,15). Maquet (54) has made similar claims about the Tutsi in Rwanda and Burundi. Likewise, Platt (12) indicated that in certain seasons, some of the Malawian men he studied in the 1930s derived 35% of their calories from indigenous beer. Quin's dietary data imply that, in the late 1950s, Pedi men in South Africa gained 13% of their annual caloric intake from sorghum beer (15).

Although contemporary nutritional data are subject to wide margins of error, judicious use of available sources can indicate orders of magnitude of the energy contribution of SMM beer. The Food and Agriculture Organization's (FAO) food balance sheets for 1975–1977 indicate that sorghum, millet, and maize beers contribute between 1% and 5% of total national caloric intake in the countries of Burkina, Gambia, Guinea-Bissau, Togo, Cameroon, Sudan, Burundi, Rwanda, Kenya, Tanzania, Botswana, Lesotho, Swaziland, Malawi, and Zambia, and they indicate that Ugandans derive 7.2% of their calories from sorghum, millet, and maize beer. However, at least some of these figures may represent serious underestimates. For the countries of Burkina, Botswana, and Kenya, it is possible to compare the FAO estimates with those of specialized brewing studies (19,21,31,60,74). In each case, the FAO estimates underestimate SMM beer consumption by a factor of about 3. Given that underestimate and given that children drink very little of the indigenous beer—other than the very nutritious dregs—it is possible that adults in the above-enumerated countries receive on the order of 6–12% of their calories in the form of sorghum, millet, and maize beer. Males, especially in certain seasons, may depend to an even greater extent on caloric intake in the form of indigenous beer.

In addition to their caloric contribution, indigenous beers are widely viewed as providing an important nutritional supplement to African diets because of the vitamins and proteins the beer contains (3,13,14). Recent laboratory tests indicate that prior claims must be tempered slightly, as not all the nutrients available in the beer can be assimilated by humans (64,75). Nonetheless, indigenous beers do appear to provide significant nutritional supplements in the form of calories, protein, and B vitamins, all of which are frequently deficient in grain-based African diets (3). Van Heerden (76), for example, has established that 1 L of sorghum beer provides more calories, protein, iron, and B vitamins than an "average" meal consumed by black South Africans.

Supplying the huge volumes of indigenous beers demanded by consumers is big business. Even in South Africa, Zimbabwe, and Zambia, where factory brewing has developed most fully, home brewers appear to supply at least half of the indigenous beer market, holding roughly a 50–66% market-share (11,16). Outside of those three countries, home brewers still provision the overwhelming majority of the market. This makes cash home brewing one of the largest cottage industries and, consequently, one of the major nonfarm employers in many African countries. Data from Botswana, Burkina Faso, Kenya, Uganda, and Zambia indicate that production and distribution of home-brewed indigenous beers provides employment—although not necessarily full-time employment—for 7–20% of the labor force in regions where it is prevalent (28–31,60,77–82).

Two factors fuel demand for indigenous beers. First, centuries of habit have attuned African palates to the distinctive sour taste of SMM beer. Second, SMM beers are affordable to a wide range of consumers, selling at a fraction of the price of other beverages. For example, in Botswana during the early 1980s, unpackaged sorghum beer retailed for 10–12 cents per liter, packaged sorghum beer sold for 17 cents, and clear beer cost 1.35 per liter, 8–10 times as much as sorghum beer (31). In Burkina, the price spread is smaller although still considerable. There, in 1980, sorghum and millet beer retailed for about 10 U.S. cents per liter, whereas clear beer sold for four times as much (19).

Today, as in olden times, indigenous beers are probably, by volume, the most widely consumed alcoholic beverage on the continent (71). Even in the Southern African Customs Union countries (Botswana, Lesotho, Swaziland, and South Africa), where aggregate economic statistics are dominated by the extremely wealthy and westernized economy of South Africa, sorghum beer accounted for 74% of the volume of all alcoholic beverages sold in 1977 (11). In Botswana alone, that figure rises to 77%, whereas in Burkina, it stands at 75% (16,19). This results in adult per capita consumption of 347 L/year in South Africa, about 285 L/year in Botswana, and between 230 and 470 L/year in Burkina (16,19,75). By way of comparison, the West Germans, the largest group of clear beer drinkers in the world, consume about 300 L of clear beer per adult, whereas U.S. adults drink about 160 L (calculated based on Ref. 83).

II. EVOLUTION OF THE BREWING PROCESS

A. An Overview of Brewing Procedures

Sorghum and millet constituted the key substrates used by African brewers in the earliest centuries of indigenous beer production. Indigenous to Africa—or at least introduced millennia ago—they were the only grains available for brewing until the introduction of maize by the Portuguese in about the 16th century (3,5,71). As maize cultivation spread, some brewers did incorporate maize into their brewing recipes. Because it does not malt as well as sorghum or millet (84), the use of malted maize is exceptional but not unknown among home brewers in humid regions where sorghum and millet are not readily available (23). Home brewers today probably malt sorghum more frequently than millet, given the sparse and often Islamic population in the arid millet-producing regions and given that African sorghum production is approximately 10% greater than that of millet (72).

For factory brewing as well, sorghum malt predominates. However, in some varieties of millet malt as well as sorghum, the much smaller size of the

millet grains presents complications for the mechanization of large-scale malting operations (22,85). Thus, whereas some maltsters in Zimbabwe were reportedly malting millet in the mid-1950s (6), today they undoubtedly follow the standard practice of their counterparts in South Africa and malt primarily sorghum.

Although brewers do not commonly malt maize, it has become popular as the starchy adjunct used in brewing. Today, virtually all factory brewers and a large segment of home brewers—especially the shebeen queens, the regular cash brewers in southern Africa—prefer maize as their starchy adjunct. Nonetheless, many home brewers still use sorghum and millet where they are more readily available, especially in sahelian western Africa.

Over the years, brewers and researchers have clearly identified the following key stages in the production of indigenous beers: malting, souring, boiling, mashing, straining, and alcoholic fermentation. Malting of any grain simply involves germinating the grain for several days, then drying and milling it. Germination produces amylolytic enzymes, not present in the original grain, which subsequently break down grain starches to sugars. The sugars are fermented to lactic acid by lactobacilli, and during alcoholic fermentation, yeasts convert them to alcohol, producing beer.

Unlike the brewing of clear beer, production of sorghum, millet, and maize beers involves two fermentations; the first for lactic acid and the second for alcohol. The lactic acid fermentation, or souring, confers on indigenous beers their characteristic sour taste and also controls many features of the subsequent mashing and alcoholic fermentation.

While alcoholic fermentation continues, brewers strain SMM beers through a coarse filter, leaving many solids as well as the yeasts still suspended in solution. Brewers sell the beer while it is still fermenting, while frothy and effervescent. Because the beer is not pasteurized, micro-organisms resistant to low pH continue to grow after straining, leading to a very short shelf life of 1–5 days. In flowsheet form, Figs. 8–10 embellish this basic sequence, detailing the many specific actions required at each stage in the home- and factory-brewing processes.

Several cautions are necessary before proceeding with a more detailed description of home- and factory-brewing procedures. First, our knowledge of ancient brewing procedures is imperfect because of an absence of written records about brewing in centuries past. Hence, knowledge of olden brewing must be obtained by combining observations from a few early reports describing practices between about 1900 and 1930 (3,5), with inferences based on the spectrum of technology observed in modern times, especially in remote rural areas.

Second, brewing procedures followed by home brewers today vary across the continent, even from brewer to brewer in the same village; and,

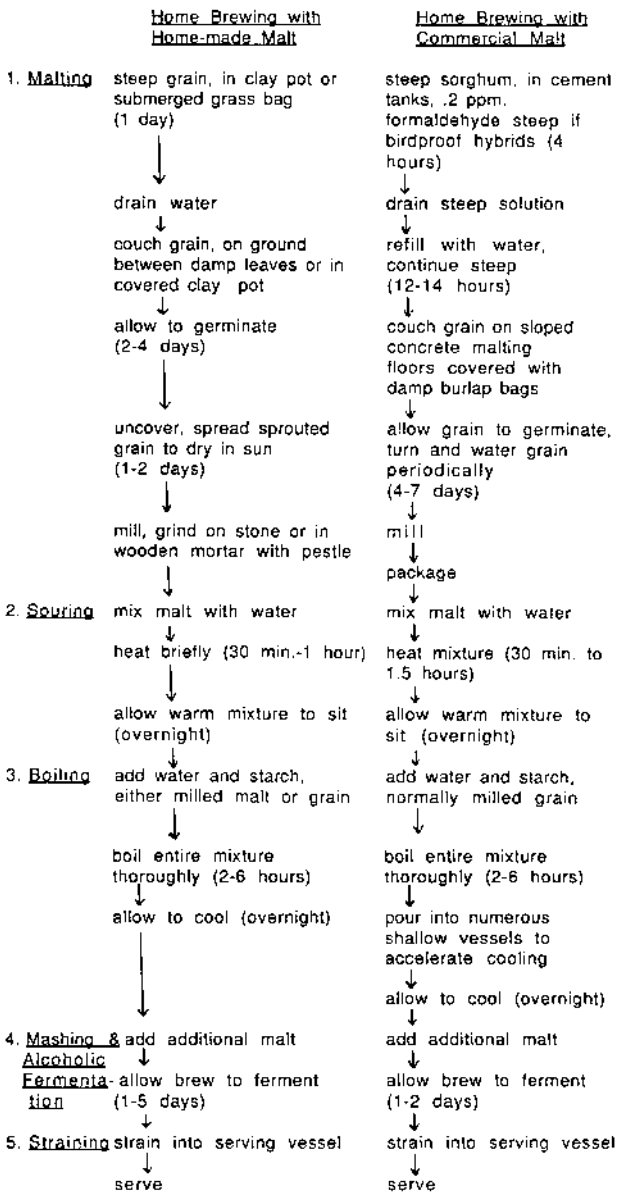

Figure 8 Flowsheet of home brewing procedures. (Adapted from Ref. 16.)

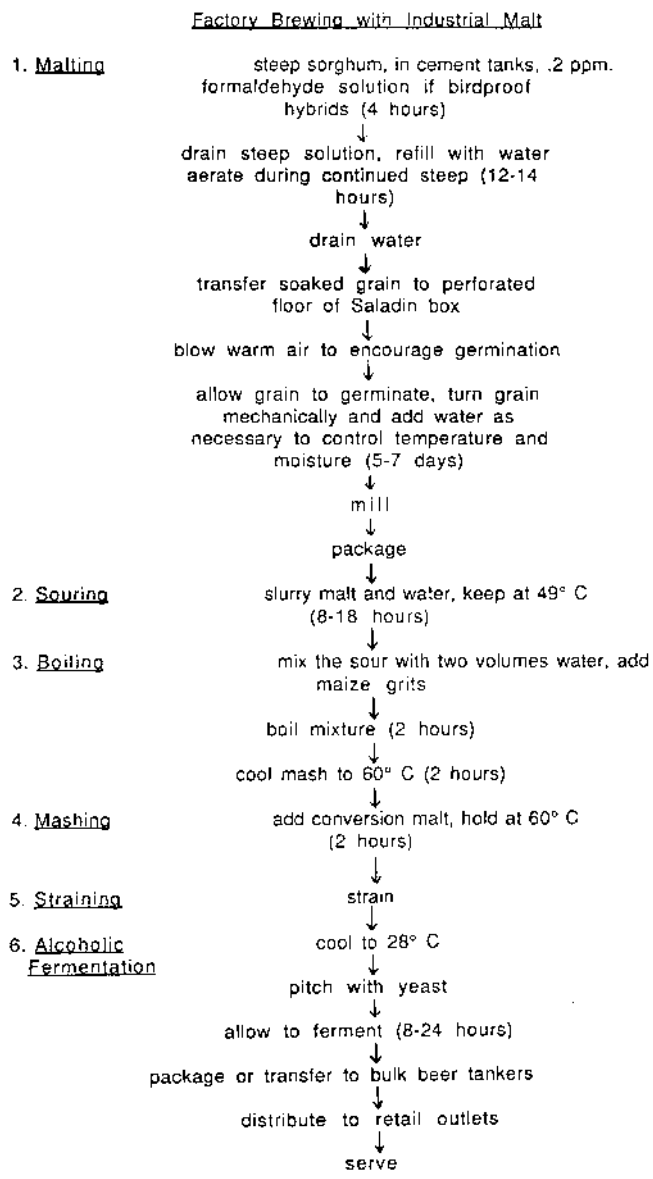

Figure 9 Flowsheet of South African factory brewing of reef-type sorghum beer. (Adapted from Refs. 1 and 64.)

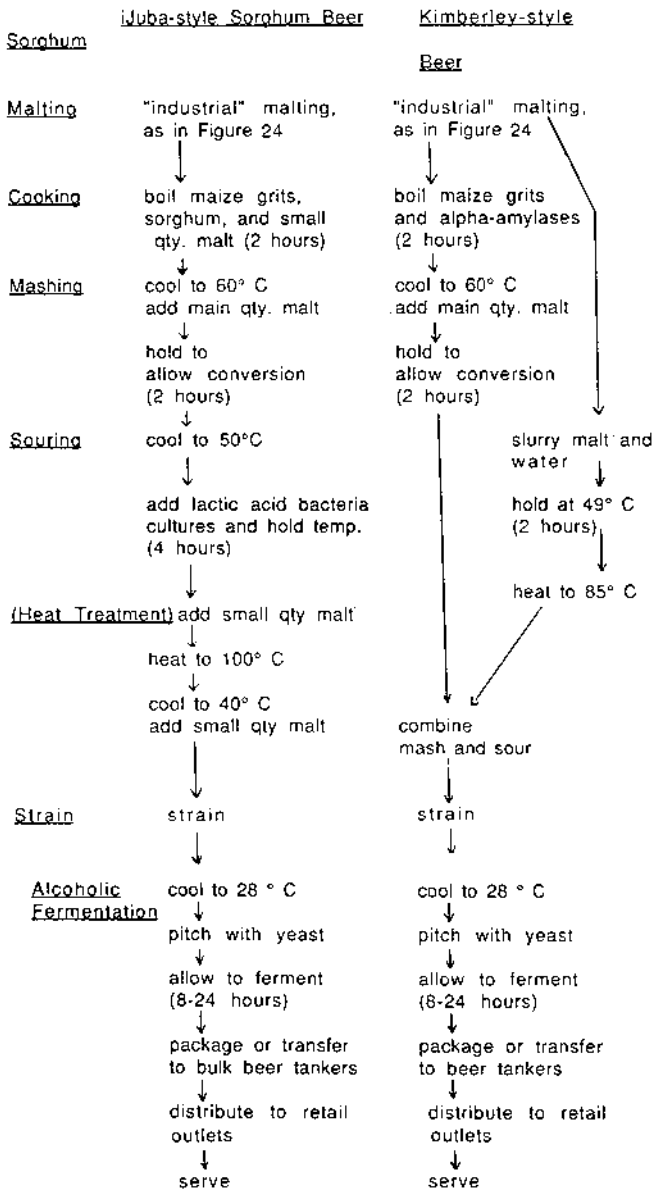

Figure 10 Flowsheet of factory brewing of ijuba- and Kimberley-style sorghum beer. (Adapted from Refs. 1 and 64.)

of course, each brewer jealously guards the secrets of minor variations and additions they are convinced confer a special aura on their brews. In view of this variation, the following description of home brewing is necessarily synthetic. Even so, it closely represents careful descriptions of home-brewing procedures coming from as far afield as South Africa, Burkina Faso, Botswana, and Togo. A feel for the variety in home brewing—as well as occasional glimmers of trade secrets—can be found in Refs. 1, 3, 5, 13, 15, 16, 19, 21, 22, 32, 34, 64, and 86–88.

In spite of the diversity among home brewers, it is clear from interviews with hundreds of home brewers and careful study of the recipes described by observers across the continent that virtually all home brewers adopt procedures which achieve the six essential brewing steps described in Fig. 8: malting, souring, cooking, mashing, straining, and alcoholic fermentation. Although a striking majority of brewing recipes can be interpreted as following this sequence, it must be recognized that the home brewers' inability to rigidly maintain brews at optimal temperatures for each step leads to a spilling over of processes across steps. Following a more detailed review of brewing microbiology and biochemistry, it will become clear that, to some extent, several of the six stages in fact overlap, both in factory and in home brewing.

B. Brewing in Ancient Times*

In centuries past, women who brewed indigenous beers invariably began by malting their own grain, either sorghum or millet. After harvesting and threshing the grain, they stored it for future use (Figs. 11–13). Brewers began malting by soaking the grain for about 1 day, either in large, water-filled clay vessels or in woven grass baskets placed in nearby streams. After draining the steep water, they allowed the damp grain to germinate by placing it in grass baskets, back into their clay steeping vessels, or on the ground between grass mats or moistened leaves. Brewers allowed the grain to germinate 2–4 days, longer in the winter than in the summer. Then, after the roots and shoots were about 3–5 cm long, they spread the sprouted sorghum or millet out on the ground to dry (Fig. 14). When it was dry, the women ground the malt by stamping it with a mortar and pestle or by hand grinding on stone (Fig. 15).

To begin the brewing proper, brewers would mix a portion of their malt with hot water. Some slurried the malt and water and then cooked the mixture for about half an hour, while others first heated the water which they then added to the malt. In either case, they covered the warm mixture and allowed

* This section is based on Refs. 1, 3, 5, 13, 15, 16, 19, 21, 22, 32, 34, 64, and 86–88, as well as interviews with about 100 home brewers in Botswana.

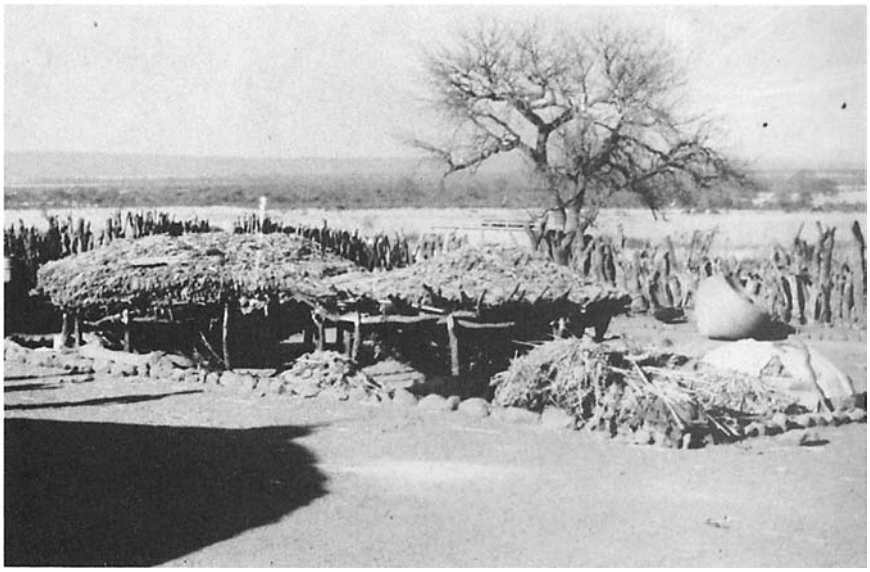

Figure 11 Platforms used for drying sorghum, Ngwato-Tswana (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

Figure 12 Threshing of sorghum by Pedi women (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

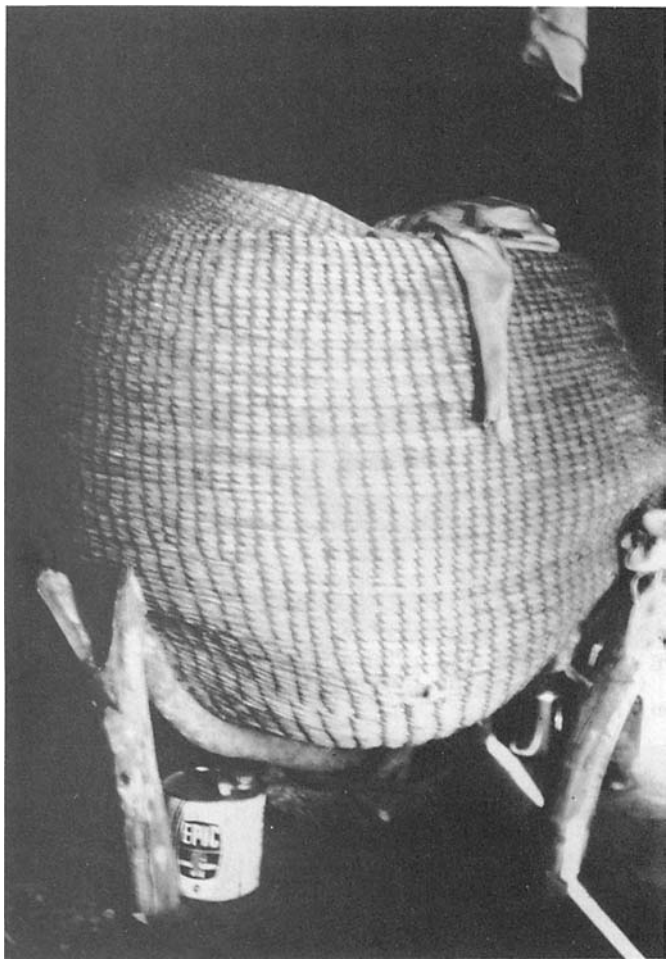

Figure 13 Grain container in hut, used for storage of sorghum (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

it to stand overnight to sour. On the following day, the women diluted the soured wort with additional water and added either more malt or ground unmalted sorghum or millet grain. They boiled the entire mixture thoroughly, probably between 2 and 6 h, and then allowed the mash to cool overnight. The brew thickened noticeably as it cooled. On the following day, after the brew had cooled, brewers added a second measure of malt. If tasting revealed that the brew was not progressing satisfactorily, some would cook the mixture a

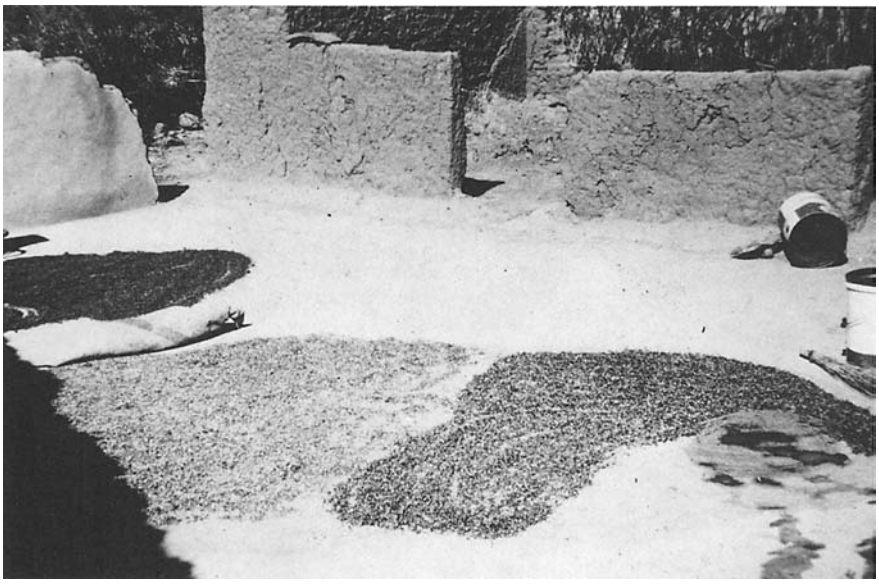

Figure 14 Drying of sorghum malt after germination, Hurutshe (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

second time, cool it, and add yet more malt. In the cooled mixture, the bubbling from carbon dioxide generated by the yeast action created an effervescence as alcoholic fermentation proceeded. Brewers allowed their brew to ferment for several days. When they and their experienced colleagues agreed that it was ready, they strained the brew through woven grass strainers, cones of plaited straw, or by placing vegetable fibers across the bottom of a broken clay pot (Figs. 16 and 17). They strained the beer into a clay serving vessel from which it was dished out in gourds and served with great ceremony to friends and visitors.

C. Modern Home Brewing*

Today's home brewers operate with recipes and procedures passed from generation to generation and refined based on centuries of experience and

* This section draws on Refs. 1, 13, 15, 16, 19, 21, 22, 32, 34, 64, and 86 as well as interviews with about 100 of Botswana's home brewers, the observations of factory brewers in Botswana, members of the Sorghum Beer Unit in Pretoria, South Africa, and Staff of the Brigades' Food Laboratory in Serowe, Botswana.

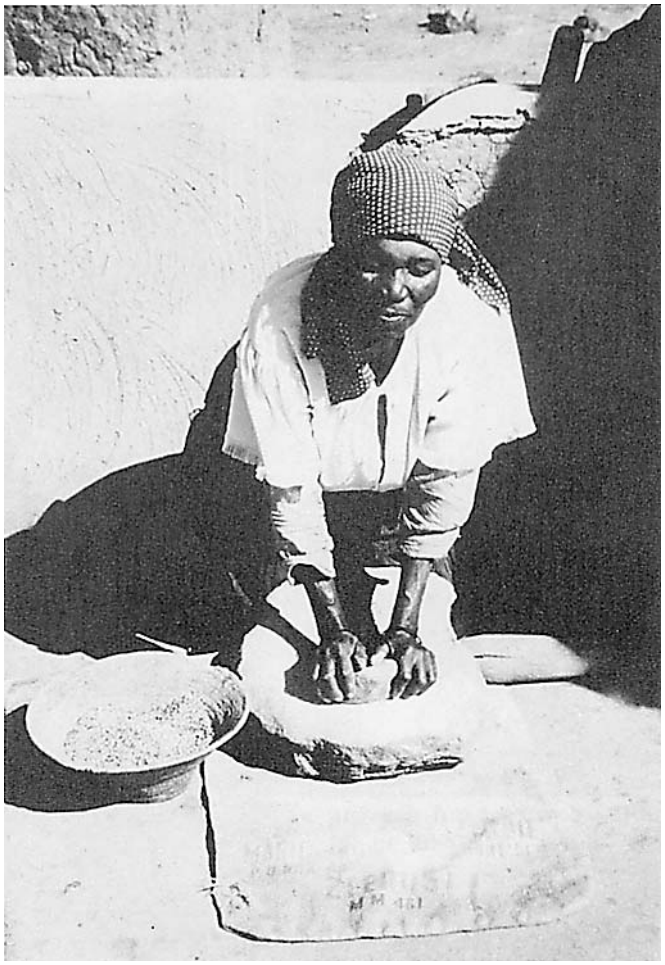

Figure 15 Sorghum is ground on the grinding stone, Hurutshe (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

experimentation. They follow essentially the same procedures as brewers of old, although they brew larger batches, with greater regularity and with more standardized inputs and procedures.

They brew larger batches for several reasons. Whereas in olden times all women brewed beer, the bulk of today's brew is produced by specialized professionals. To supply the market with a smaller number of specialized brewers, each needs to brew larger batches than their ancestors. Certainly, the availability of larger brewing vessels has facilitated this scaling up of home-

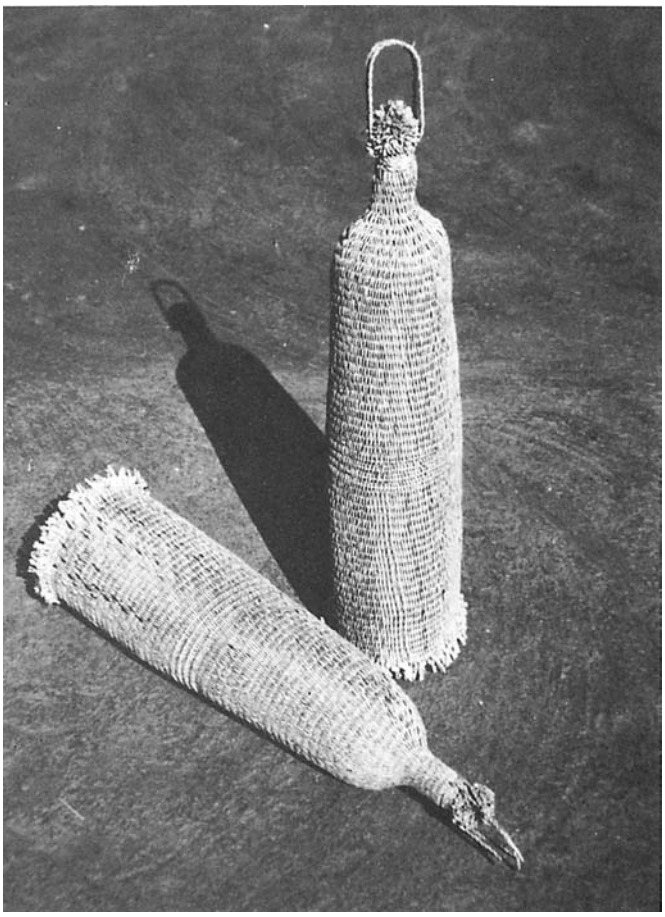

Figure 16 Beer strainers, northern Ndebele (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

brewing procedures. In olden times, brews ranged in size from 20 to 100 L, probably averaging around 40, whereas today they fall between 100 and 200 L, with 200 being the common standard (1,3,16).

Several economic changes explain why today's home producers brew with more regularity than their predecessors. Input marketing networks have developed steadily over time, making grain and malt more readily available year-round than previously. In reality, cash employment has grown dramatically, translating into a steady year-round demand and combining with input availability to dampen what were formerly very pronounced seasonal fluctuations in brewing activity.

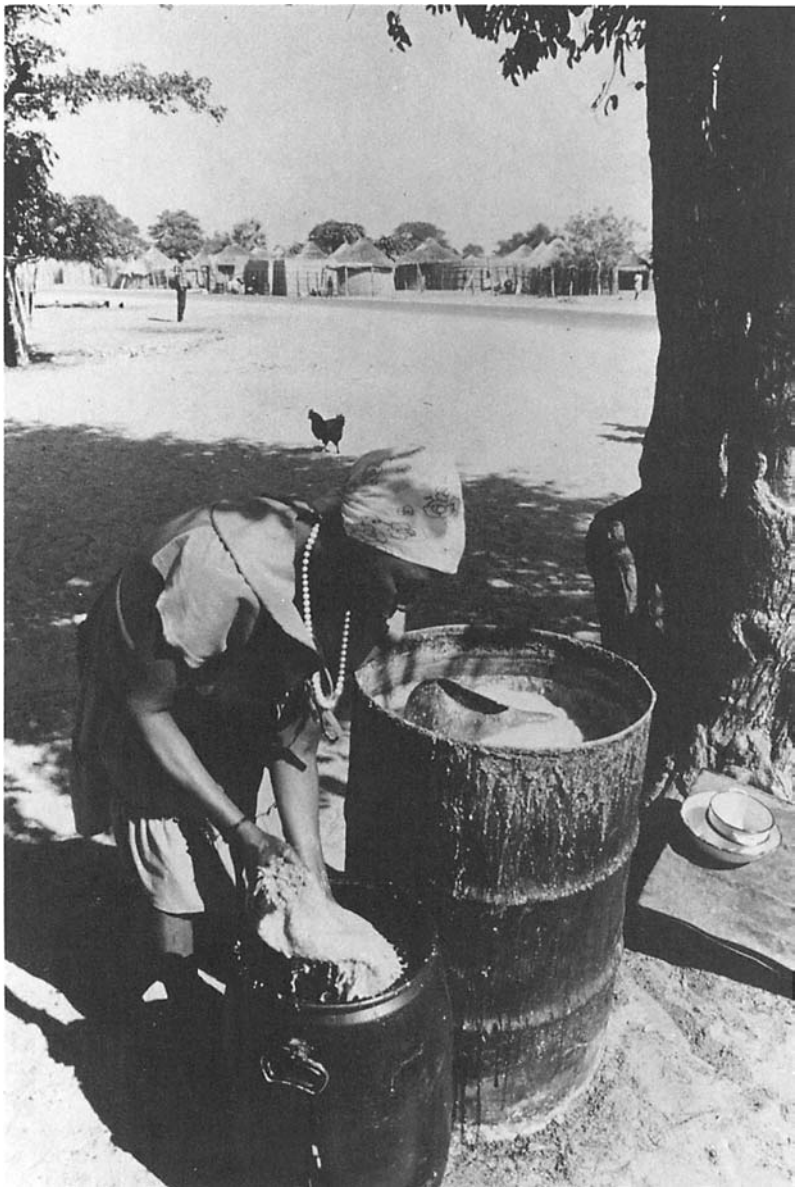

Figure 17 Straining the beer, Tswana (Maun, Botswana).

The final embellishment instituted by today's home brewers is their use of more standardized procedures than their predecessors. This regimentation has been made possible by the increased regularity of brewing, along with the availability of standardized input measures, brewing vessels, and inputs, especially malt.

1. Malting

The majority of today's home brewers still begin beer production by malting their own grain. In western, central, and eastern Africa, all use home-made malt. Most make their own, although some—primarily urban home brewers—procure home-made malt manufactured by home brewers in grain-exporting regions (19,26,89,90).

However, in southern Africa, home brewers now have the option of purchasing factory-made malt from any one of about 40 commercial maltsters. Originally referred to as "trade malt," the product of factory floor malting is now referred to as "commercial malt" (1,64). Factory malting grew up in southern Africa around the 1940s to supply malt for the budding factory-brewing industry and to supply urban home brewers producing for their own consumption. Although early factory brewers malted their own grain, they rapidly devolved this function to specialized factory maltsters (1). The differences in equipment and facility requirements as well as the temporal discontinuity between malting and brewing allowed this specialization. As research, especially in the latter 1950s, clarified the biochemistry of sorghum beer production and identified optimal brewing procedures, factory brewers scaled up production and demanded malt of increasing quality and consistency. To supply this rapidly growing factory-brewing market, some maltsters introduced more sophisticated equipment and methods, specializing in the production of what was originally called "municipal malt" but is now known as "industrial malt" (1,64). Others retained older vintage equipment and procedures and found that they could service a growing market niche among the urban home brewers who did not have the space to produce malt in towns.

Today, urban home brewers in countries such as South Africa, Botswana, and Zimbabwe use primarily factory-produced commercial malt, whereas many home brewers, especially those in rural areas, continue to malt their own grain. In the predominantly rural country of Botswana, the only country in southern Africa for which data are available, 30% of all home brew is produced with commercial malt, whereas in urban areas that figure rises to 100% (16). Yet outside of southern Africa, home brewers continue to use exclusively homemade malt.

Home Malting. Home maltsters follow the same procedures as their forebears with only very minor modifications (Figs. 8 and 11–15). In addition

to steeping their sorghum or millet in water-filled clay pots, today's brewers also use wooden tubs, plastic cans, steel drums, or burlap bags immersed in nearby streams. In addition to old-time sprouting equipment—grass baskets, clay vessels, grass mats, or moistened leaves—brewers today frequently germinate their grain under dampened burlap bags. Instead of hand grinding, they commonly mill their malted grain in diesel- or hand-operated mechanical mills. Yet, aside from these minor equipment substitutions, home-malting procedures remain essentially unchanged from ancient practice, with sun-drying and milling as the final steps.

"*Commercial*" *Malting*. Although commercial maltsters use slightly more modern equipment and facilities than do home brewers, they also malt outdoors following procedures strikingly similar to those of home brewers. Commercial maltsters begin by cleaning their grain, then steeping it in large water-filled steel or concrete tanks for an average of 16–18 h (6,84,91). Many add a small dose of formaldehyde to the steep to complexate the polyphenols and tannins in the bird-proof sorghum, producing a concentration of 0.02–0.1 parts per million. After about 4 h in the formaldehyde steep, they drain and replace the steep with water for the remaining 12–14 h.

South Africa's Sorghum Beer Unit developed this formaldehyde treatment in the mid-1970s in response to several problems caused by the introduction of bird-proof varieties of hybrid sorghum from the United States. The high polyphenol content of the bird-proofs posed serious problems for the brewers, rendering malt amylases insoluble and impairing lactic acid fermentation (1,92). Because the formaldehyde treatment was found not only to address these problems but also to soften proteins and improve the shelf life of the beer (92), some maltsters now use the treatment routinely, even for non-bird-proof sorghum. The process is now protected by a South African patent (92).

After the steep, commercial maltsters drain away the water and spread their soaked grain out on slightly sloped concrete malting floors, normally outdoors, although sometimes under corrugated tin roofs on poles. They, like home maltsters, couch the grain in beds varying in thickness from 13 to 90 cm and cover the beds with damp burlap bags (6,91). The thickness of the bed varies according to the time of year. Thinner layers are appropriate in the summers, when high temperatures accelerate malting, whereas cool weather necessitates thicker beds to facilitate the buildup of sufficient heat to promote adequat germination of the grain (1,84,91). Because the warmer bottom levels germinate more quickly than the upper layers, trade maltsters periodically turn the grain gently with a shovel to allow all segments of the grain couch to germinate. However, even with careful turning, uneven germination is a problem leading to malt of uneven diastatic power; at temperatures outside the optimal 22–25°C temperature range, germination and concomitant α-

and β-amylase production is impaired. Commercial maltsters germinate their grain for 4–7 days, sprinkling several times over that period to keep it moist while sprouting (1,6,91).

Like home maltsters, they dry the sprouted grain by spreading it in the sun to dry. They then mill and package the commercial malt for distribution. Packaging ranges from bulk 60-kg bags to 10-, 5-, and even 1-kg packages, premeasured sizes convenient for many home brewers.

2. Modern Brewing

Today's home brewers follow essentially the same procedures as their ancestors (Figs. 8, 17, and 18). The mix their malt—either commercial malt or homemade—with water, heat it for about 30–60 min, and allow the mixture to cool overnight to sour. The next day, they combine the soured wort with additional water and grain starch and boil the entire mixture for about 2 h but

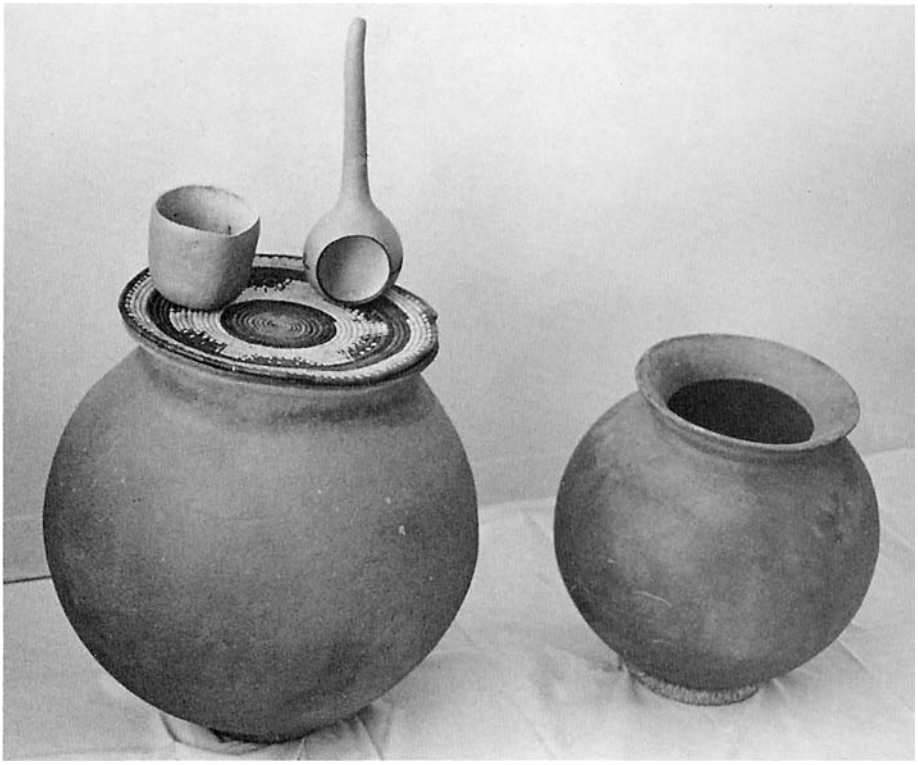

Figure 18 Clay pots, covers, and serving vessels used by home brewers in Burkina.

Figure 19 Different types of clay pots used for brewing, storage, and serving of beer (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

sometimes as much as 7 h. In contrast with old-fashioned brewers, many today add maize meal rather than malted or unmalted sorghum or millet as a starchy adjunct. Although some home brewers continue to cook in clay pots (Figs. 18 and 19), many now operate with three-legged iron pots or steel drums (Figs. 20 and 21). In what may be a modern innovation induced by larger brew sizes, many of today's home brewers encourage cooling by pouring the boiled brew into a series of shallow plastic, metal, or wooden containers so that the heat will dissipate more rapidly.*

* Early factory brewers followed exactly this procedure, cooling their mash in shallow wooden troughs. However, they converted quickly to cooling towers and internal water coils because, given their large brew sizes, cooling by dissipation could take as much as 2 days. See Refs. 4 and 6.

Figure 20 Stirring home-brewed sorghum beer, Tswana (Serule, Botswana).

On the following day (the third), they reassemble the thickened mixture into one vat—wooden, metal, or plastic—to which they add additional malt. They allow the mixture to thin and ferment for 1–3 days, then strain and serve the beer. Although old-fashioned grass and straw strainers are still in use (Figs. 16 and 17), many of today's home brewers instead use metal screen mesh custom-fitted by local metal workers onto frames that fit across the serving vessels. In some countries, such as Burkina, Rwanda, and Tanzania, the brew is transferred in bulk to special market places for retailing. However, elsewhere, such as throughout Botswana, South Africa, and Zimbabwe, it is most frequently sold in the same compound where it is brewed (Figs. 1–7 and 22–25). During a weekend or at the end of the month, after payday, brewers of good reputation may sell their product within a day or even an afternoon. However, during a slow period or with a brewer of lesser standing, it may take several days to liquidate an entire brew.

D. Factory Brewing

1. "Industrial" Malting

The most sophisticated arm of today's sorghum malting industry services factory brewers exclusively. Maltsters in this category produce the highest

Figure 21 Brewing the beer, Kono–northern Sotho (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

available grade of sorghum malt, now referred to as "industrial malt" but originally called "municipal malt" because of the municipal government monopoly on factory brewing in South Africa (1,64).

Unlike home and commercial malting, industrial malting is a highly mechanized process carried out indoors. As Fig. 11 indicates, industrial maltsters begin by steeping their sorghum in water for 16–18 h in steel or concrete tanks, aerating during the steep (1,84). When bird-proof varieties of hybrid sorghum are malted, industrial maltsters, like the commercial maltsters, steep the grain in a 0.02–0.1-ppm formaldehyde solution for the first 4 h (92). After draining off the steep water, they transfer the soaked sorghum to specially prepared malting boxes, commonly onto the perforated floor of a Saladin box. There, they spread grain out and turn it periodically with specially designed mechanical arms to achieve even germination. They carefully monitor temperature, airflow, and humidity during the 5–7-day germination period. To encourage germination initially, many blow warm air up into the grain through the perforated malting floor. Because germination generates substantial heat, cool air is normally blown in during later stages of malting (1,6,84).

Figure 22 Retailing outlet in front of brewer's compound, Tswana (Maun, Botswana).

Industrial maltsters dry their germinated grain by blowing warm air through the perforated malting floors. They do not kiln-dry the malt, as high temperatures would destroy the enzymes produced during germination. After drying the malt, producers mill and package it for distribution. Industrial malt must meet carefully specified tender specifications. Normally, factory brewers require malt achieving a diastatic power of 28–35 sorghum diastatic units (SDUs) (1,6,84).

2. Brewing*

Reef-Type Factory-Brewed Sorghum Beer. In regions where sorghum beer is produced on an industrial scale—in eastern and southern Africa—factory brewers do not practice identical brewing procedures. Even so, the most common remains the reef-type beer developed in the gold-mining region of South Africa, a region referred to as the "reef." Reef-type beer accounts for

* The standard, reef-type South African factory brewing procedures described in this section are summarized based on Refs. 1, 6, 64, and 84.

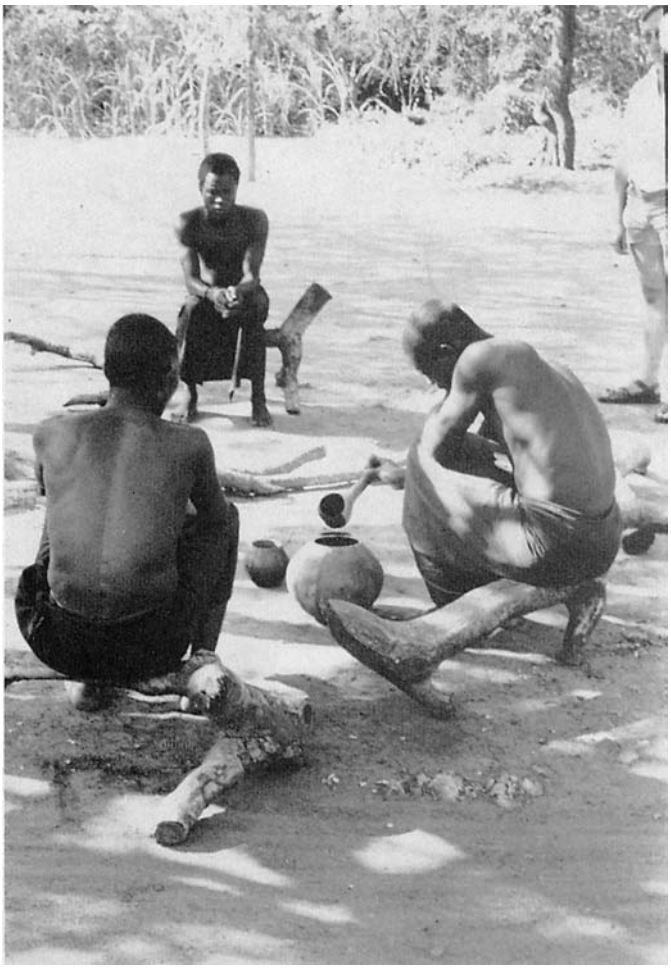

Figure 23 Tsonga men drinking beer (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

60% of South African factory production of sorghum beer (64). Given that a close variant is produced in several other major brewing countries (Zambia, Zimbabwe, Botswana, and probably others), reef-type beer and its near-relatives may account for as much as 75% of factory-brewed sorghum beer continentwide. Figures 26–43 offer views inside typical breweries.

Production of reef-type sorghum beer begins by fermenting the lactic acid, most commonly by producing a soured mixture of sorghum malt and

Figure 24 Retailing in a home brewer's "shebeen," Tswana (Maun, Botswana).

water. As the flowsheet in Fig. 11 indicates, brewers slurry a mixture of sorghum malt and water, then heat it to about 49°C for 8–18 h until the pH reaches 3–3.4. Most of South Africa's factory brewers do not inoculate the sour with pure cultures of lactobacilli (1), although they do inoculate with starter from a previous successful sour (Figs. 27 and 28). After fermenting for lactic acid, brewers dilute their sour with two volumes of water, then add brewers grits (refined maize meal) as a starchy adjunct.

Outside of South Africa, many factory brewers of reef-type sorghum beer induce lactic fermentation differently. Instead of slurrying sorghum malt and water to induce spontaneous fermentation, they add a highly concentrated, industrially produced inoculum of lactic acid bacteria directly to the maize grits prior to cooking (16). This apparently results in souring of the slurry during the time required to heat the mixture in preparation for cooking. Factory brewers in Botswana, and likely in corporate affiliates in Zimbabwe and Zambia, use this method, procuring their inoculant from outside suppliers and thereby avoiding the need for in-house production of starter culture portrayed in Fig. 27.

In both South Africa and elsewhere, factory brewers of reef-type sorghum beer next boil the entire soured mixture to solubilize the grain starches. Brewers control the cooking process by regulating temperature and

Figure 25 Pedi women drinking beer (South Africa). (Courtesy of the National Cultural History and Open Air Museum, Pretoria.)

time. Some use steam injection for in-line cooking for about 2 h, although most prefer shorter cooking times under pressure (Fig. 29). One brewery, the Kwaggaskop Brewery in Pretoria, has recently begun extrusion cooking of the maize meal (Fig. 30). The extruded product requires no further cooking and is added directly to the substrate before mashing.

After solubilizing the grain starches, brewers cool the boiled wort by circulating cool water through an internal coil to bring the temperature down as rapidly as possible to 60°C. The mash becomes quite viscous at this point because of the gelatinized starches.

When the mash reaches 60°C, brewers add additional "conversion" malt. To accelerate conversion of starch to sugar, some also add a small amount of conversion malt at about 80°C as the mash is cooling. The malt

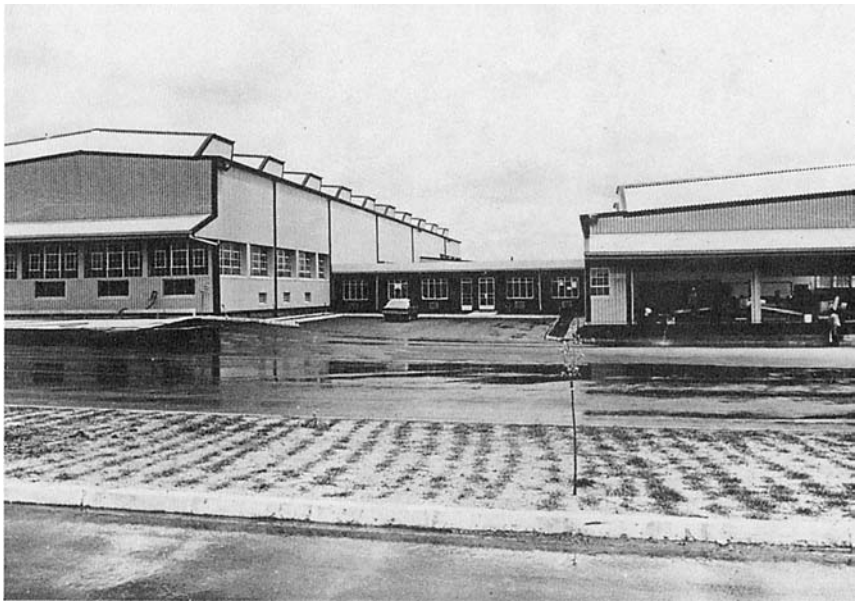

Figure 26 General view of a medium-sized (800,000 hL) sorghum beer brewery (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

enzymes thin the brew as they convert the gelatinized starches to sugars and dextrins. In this procedure, pH rather than temperature regulates the rate of sugar formation. The low pH of about 3.8 limits the enzymatic starch conversion, preventing the production of too thin a beer. Factory brewers outside of South Africa supplement their conversion malt with commercially cultured enzymes, most likely α-amylases, to facilitate thinning and diminish malt requirements.

After allowing starch conversion to proceed for about 2 h, brewers strain their mash at 60°C by centrifugation and/or filtration. In the early days of the industry and in small factories today, factory brewers use small capacity strainers such as Almaks. Today, large factories first centrifuge the mash in solid bowl centrifuges (Fig. 31), then remove remaining solids by straining through a vibrating screen. After straining, the mash is referred to as "wort."

Brewers cool the wort to bring the temperature of the brew down to 28°C as quickly as possible. They transfer the wort to stainless-steel vessels of 15,000–27,000 L capacity (Figs. 32 and 33) and then add pure cultures of yeast to initiate alcoholic fermentation. Brewers allow the mixture to ferment for 8–

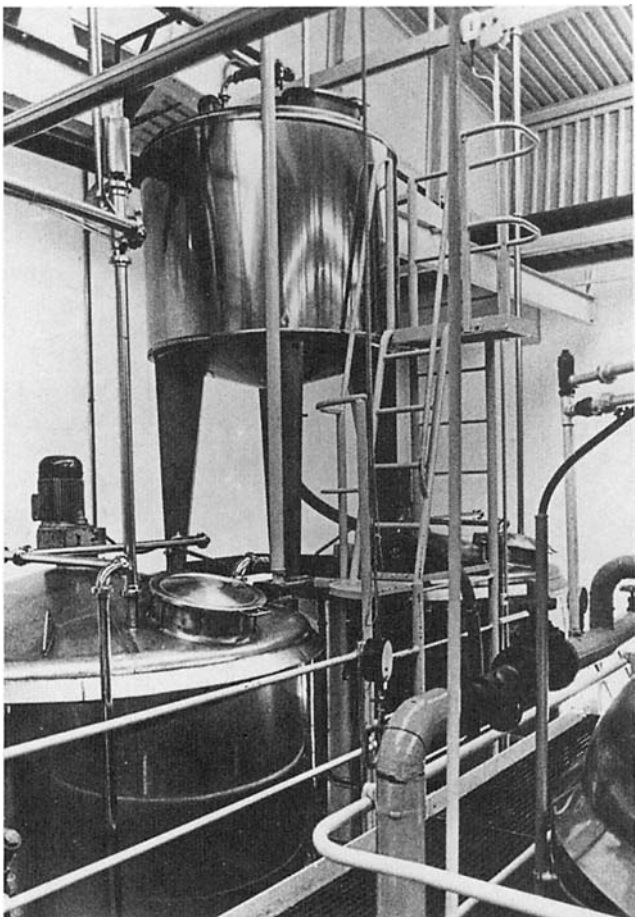

Figure 27 A "sour start" vessel (center) arranged to provide an inoculum to the two souring vessels (bottom) for lactic acid fermentation (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

24 h, depending on the demand profile of the moment, and then transfer the brew to bulk containers or package it for distribution. They transport bulk beer by road tankers (Fig. 34) to beer halls which can serve up to 5000 customers from stainless-steel holding vessels. Packaged beer is filled into containers of different sizes, ranging from the most popular 1-L nonreturnable wax paper carton to 2-, 20-, and 50-L polyethylene containers (Figs. 35–41).

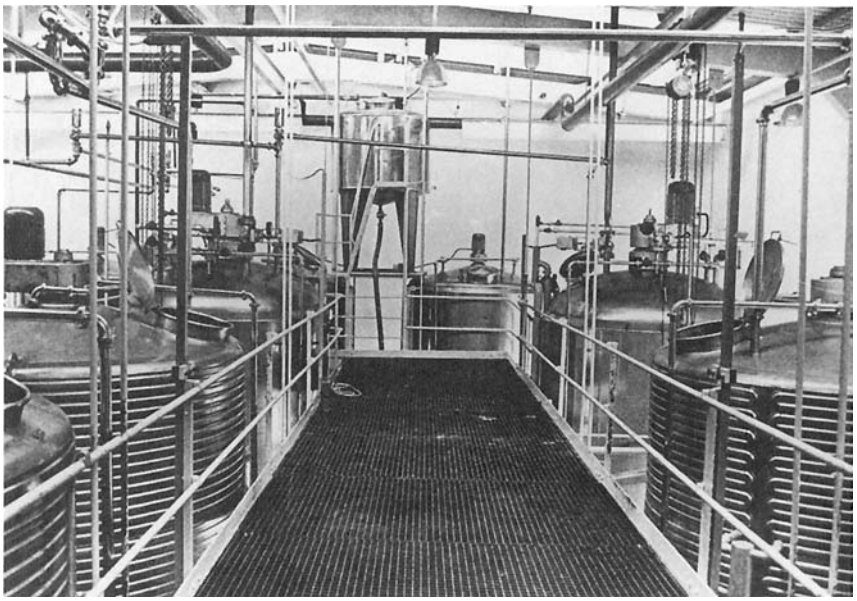

Figure 28 General layout of a brewing hall showing a sour start vessel (top center) providing inoculum to a sour vessel (bottom right off-center), two coil-heated pressure cookers (left and right of center), three jacketed conversion vessels (extreme left and right), and associated pipelines (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

Because the beer is actively fermenting when sold, packaged beer must be distributed in vented containers.

The share of factory brew distributed in packaged form has risen steadily over time. In South Africa, factory brewers packaged only 20% of their brew in the mid-1960s; however, in the mid-1980s that figure had risen to 70%, with 60% of total production sold in 1- and 2-L nonreturnable cartons (see Table 3 and Ref. 1). Similarly in Botswana, in the early 1980s, factory brewers distributed 80% of their production in 1-L nonreturnable wax paper cartons such as those depicted in Fig. 35 (16).

Other Factory Brewing Procedures. Two other, less prominent factory-brewing procedures are described in the flowsheets in Fig. 16. One, iJuba brand sorghum beer, accounts for 30% of factory-brewed sorghum beer in South Africa. Its prominence outside of South Africa is not known with precision but is likely negligible, as it aims at producing a slightly thinner product than normal to meet the taste preferences of the Zulu people. The

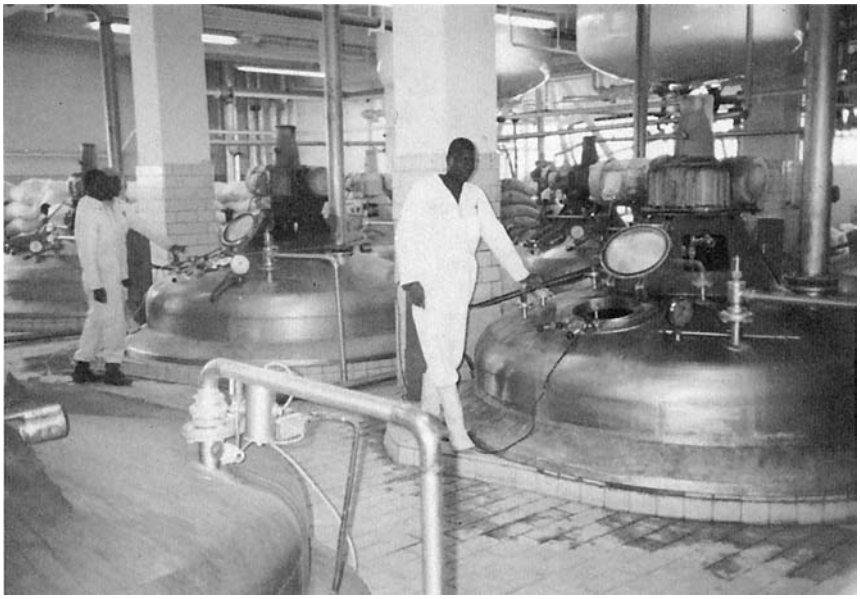

Figure 29 Pressure cookers for cooking grain adjunct (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

principal distinction between iJuba and reef-type sorghum beer is that iJuba producers delay the souring until after cooking and starch conversion. Thus, conversion takes place at natural pH, where enzymes act more quickly than at low pH, resulting in a thinner brew than reef-type beer in accordance with Zulu taste preferences. The iJuba brewers sour after conversion by adding pure lactobacilli cultures. They also heat-treat the mash after souring, then cool to 28°C before adding yeast, packaging, and distributing the beer.

Kimberley-type sorghum beer is the second of the alternative factory brewing technologies described in Fig. 12. It accounts for only about 10% of South Africa's factory sorghum beer production and is apparently limited outside that country as well. Like iJuba production, Kimberley beer involves cooking maize adjunct and adding conversion malt at natural pH. While conversion proceeds, brewers of Kimberley beer slurry sorghum malt and water to produce their sour alongside, combining the two mixtures after conversion. They strain, add yeast, allow the wort to ferment, then package and distribute it. Both Kimberley and iJuba beer production will be discussed further when reviewing the biochemistry and microbiology of brewing.

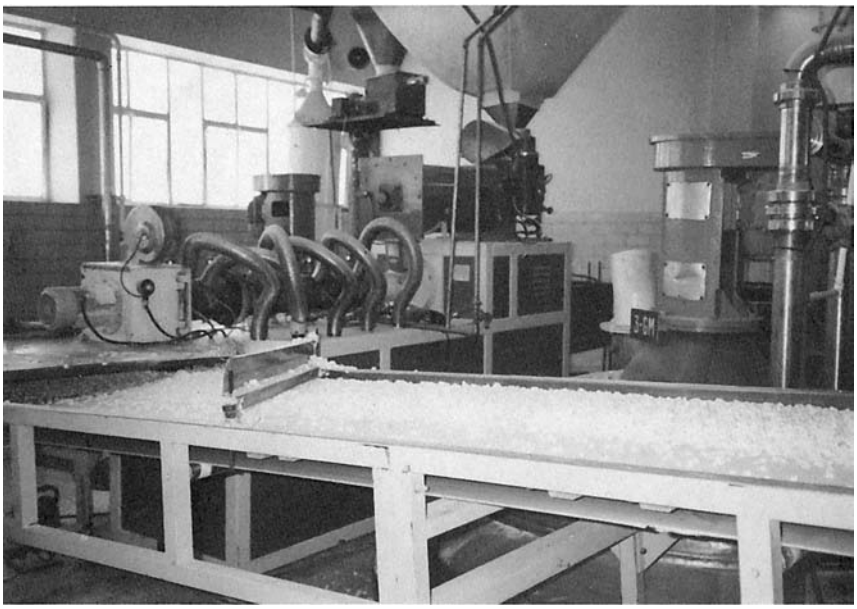

Figure 30 Extruder process for preparing precooked maize meal. This product is used as adjunct and is added to the substrate without any further cooking procedure (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

More recent developments include the prospective use of a widely cultivated cocoyam variety, *Xanthosoma sagittifollium*, in Nigeria. It was assessed for its suitability for lager beer production, using a malted white variety of *Sorghum vulgare* for saccharification. Results showed the cocoyam to be superior to barley and sorghum as a substrate because of its potentially higher carbohydrate content (71–78%) compared to barley (65%) and sorghum (70–73%). Kiln-dried *X. sagittifollium* gave a dark lager beer with good aroma and excellent flavor when compared to commercial lager beer (93).

Another development comprises the brewing of a high-gravity Zimbabwean traditional opaque beer (94). The three types produced, differed according to the varieties and ratios of raw materials used in the initial stage of beer preparation, with sorghum meal and sorghum malt in the ratio 2:1 in the first type. The second type was prepared with sorghum meal and finger millet malt in the ratio 2:1, whereas the third type contained sorghum meal, maize meal, and finger millet malt in the ratio 1:2:2. The time required to attain the desired sourness and final products varied according to the raw materials used

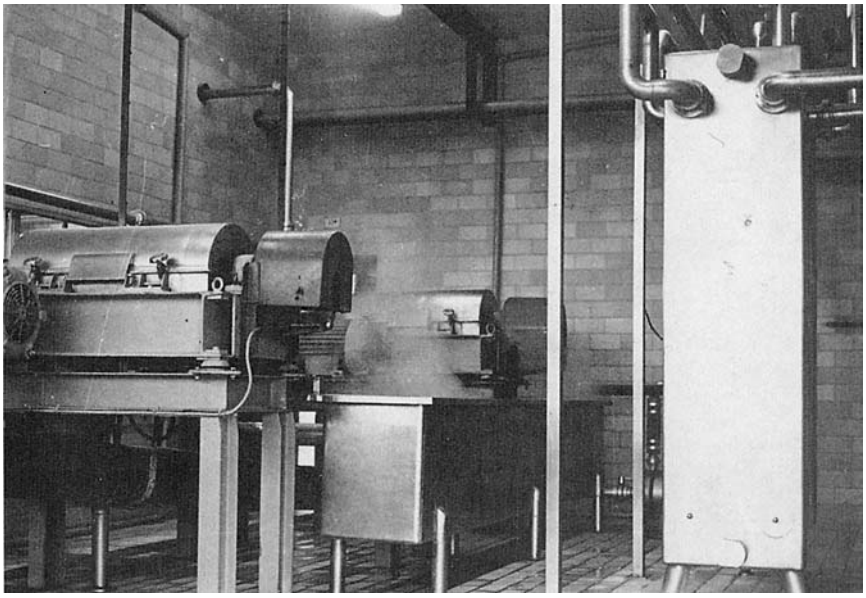

Figure 31 Two centrifugal decanters discharging sorghum beer wort to a collecting vessel (center) before pumping through the wort heat exchanger (right). Husks are conveyed away by the screw conveyor under the decanters (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

for beer preparation. Higher ethanol levels were obtained for the high gravity beers when compared with the normal beers, with maximum ethanol levels ranging up to 4.74% (v/v) for beer 3. Beers 2 and 3 had the highest overall acceptability.

E. Contrasts Among Brewing Processes

1. Contrasts Between Home and Factory Brewing

The brewing technologies observed today—home brewing with homemade malt, home brewing with commercial malt, and factory brewing with industrial malt—represent three stages in an evolutionary spectrum. Ancient brewing procedures, closely akin to today's home brewing with homemade malt, have given way to modern home brewing. Often employing commercial malt, today's modern home brewing is a more standardized, larger-scale version of procedures that remain otherwise strikingly similar to ancient

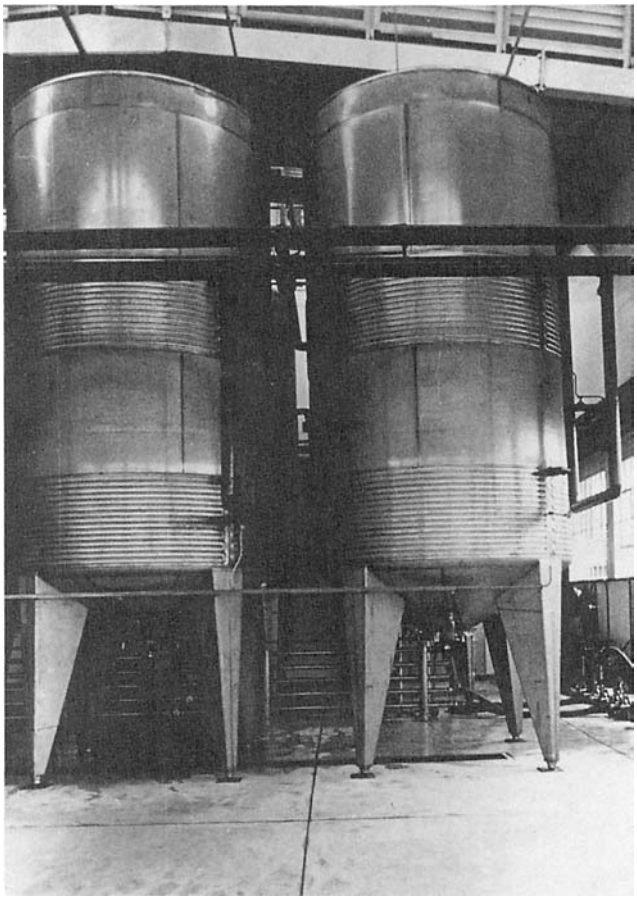

Figure 32 Jacket-cooled fermentation vessels with capacity of 27,000 L (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

practice. Although the move to factory brewing began as nothing more than a side-by-side replication of cash home brewing procedures (45,56), technical research and experimentation have enabled rapid transformation and scaling up of factory production over the past 30 years.

Today, many similarities remain between home brewing and the factory production that grew up by standardizing home-brewer procedures (6,32). Ultimately, all scales of brewing are alike in that all achieve the same combination of biochemical and microbiological processes: synthesis of

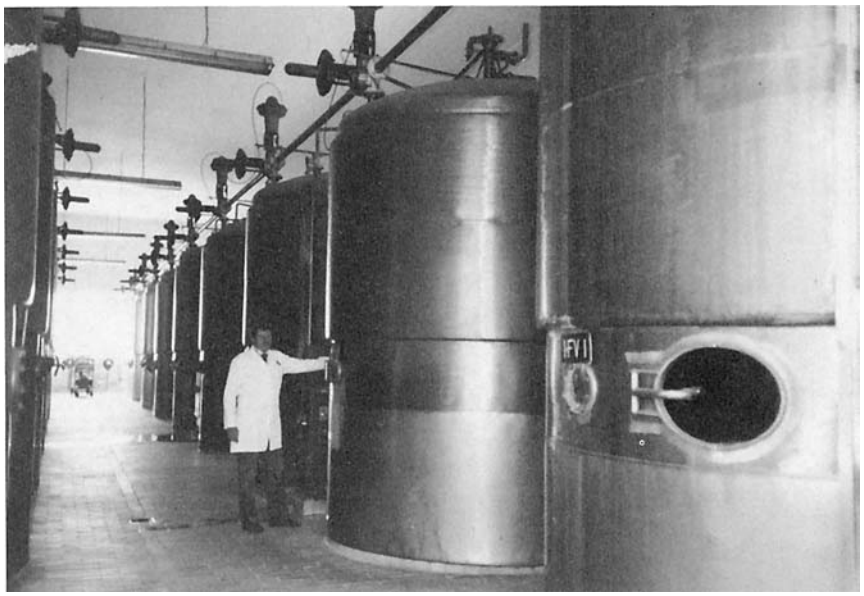

Figure 33 Fermentation vessels (reactors) used for the initiation of alcoholic fermentation (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

enzymes during malting, formation of lactic acid, gelatinization of starches, thinning and conversion of the starches to sugar, alcoholic fermentation, straining, and, ultimately, spoilage.

Yet, several important differences have emerged. The first is one of scale. Whereas the move from ancient to modern cash home brewing represented about a quadrupling in the scale of production, the move to factory brewing represents a further 100-fold increase. Instead of 200-L cash home-brewer batches, factory brewers have standardized on 15,000–27,000-L brews (16,32,64) (Figs. 18–21, 28, 29, 32, and 33). This dramatic jump in scale has been made possible by the development of carefully specified input quality standards and the use of specialized equipment that enables monitoring and control of each brewing step as well as rapid heating and cooling to optimal temperatures for each process. Whereas home brewers employ little more than wooden paddles and 200-L drums, factory brewers operate with 15,000–27,000-L stainless-steel brewing vessels, steam jets for rapidly heating brews, mechanical agitators for mixing the mashes, pumps for transferring brews

Figure 34 Bulk beer tanker at filling bay (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

from vat to vat, and internal cooling coils for rapidly bringing temperatures down to ideal levels for souring, mashing, and alcoholic fermentation (Figs. 16–21 and 27–33).

A second difference lies in the factory brewers' use of more carefully standardized inputs at several stages in the brewing process. They specify in tender contracts that industrial malt must measure between 28 and 35 SDUs with a moisture content of less than 10%, whereas home brewers operate with no such guarantee. All maltsters—home, commercial, and industrial producers—recognize that genetics plays a key role in malting quality, and all begin with good malting varieties of grain. Under ideal conditions, the home and commercial maltsters can produce high-quality malt, controlling temperature, humidity, and airflow by watering the grain during germination, turning it regularly, and adjusting the thickness of the couch to maintain temperature control. However, without sophisticated heat and humidity control equipment, they simply cannot match the consistency or average quality of industrial malt. Commercial maltsters, however, seem able to come closer than home maltsters because of access to scientific publications and

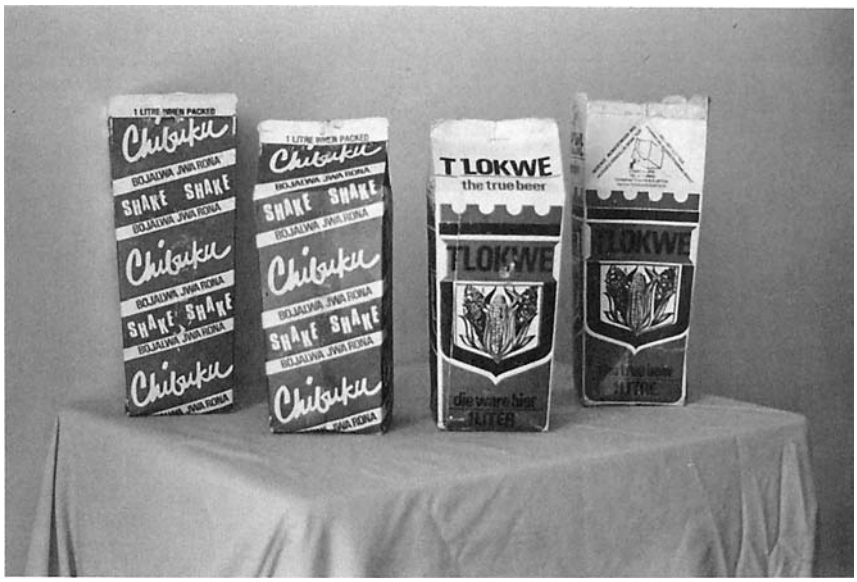

Figure 35 Common 1-L nonreturnable vented wax paper cartons (Botswana and South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

simple laboratory tests that allow them to more closely maintain ideal germination conditions. Commercial maltsters are also aware of the advantages of a low-concentration formaldehyde steep which many practice, thus improving the quality of their malt. Table 4 gives an indication of the differences in diastatic power among homemade, commercial, and industrial malts. It also demonstrates the variations in malt quality among different maltsters and even from batch to batch for a given producer.

In addition to higher-quality malt, factory brewers pitch brews with pure yeast cultures rather than depending, as home brewers do, on wild yeasts present in the malt (6,27,32,84). Many factory brewers, particularly outside of South Africa, also add pure cultures of lactobacilli and commercially cultured enzymes, whereas home brewers again depend on cultures and enzymes present in their malt (16,27,96).

The combination of standardized inputs and temperature control leads to a third—and striking—difference in the quantity of grain and malt required by different categories of brewers. As Table 5 shows, home brewers using commercial malt are able, on average, to use both less grain and less malt than women brewing with homemade malt. Although some of this difference may

Figure 36 Returnable 2-L beer containers used by different companies (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

stem from the greater regularity of the predominantly urban brewers who use commercial malt, a portion at least must stem from the higher diastatic power of commercial malt available in Botswana. The diastatic power of industrial malt generates even more striking economies in malt-to-adjunct ratios, and factory brewers partially replacing malt with commercial enzymes and pure cultures of lactobacilli achieve still greater economy in sorghum malt use, as shown in columns 3 and 4.

Finally, home brewers are unable to separate the individual fermentations as rigidly as factory brewers do because home brewers operate with less carefully standardized inputs—especially malt and yeast—and because they do not have the equipment necessary to rapidly raise and lower temperatures to optimal levels for each biochemical process. Although home brewer procedures—especially among the larger professionals—approximate the factory optima, home producers are unable to achieve the desired temperatures as rapidly or maintain temperatures as steadily at optimum levels. Consequently, in home brewing diastatic activities are not well controlled. This leads to varying concentrations of fermentable sugars and, hence, uneven rates of

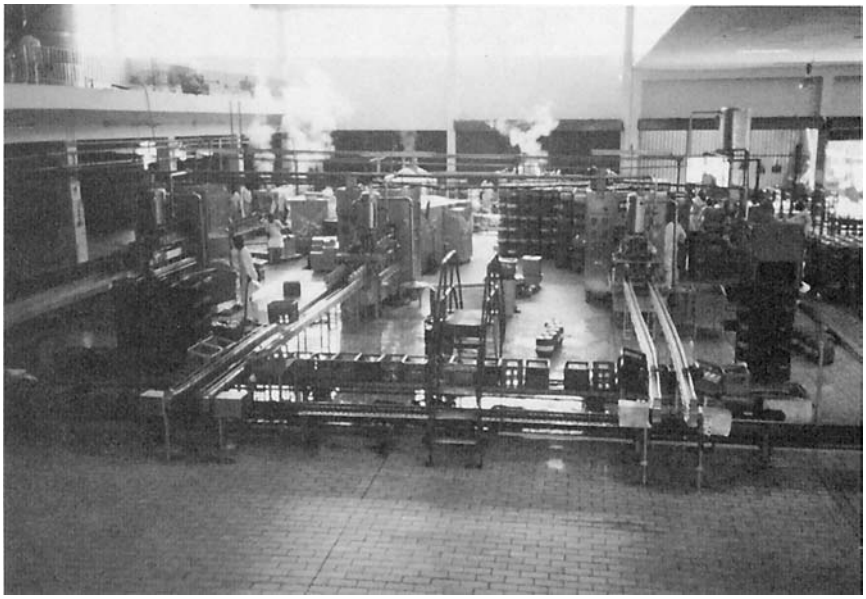

Figure 37 General view of the filling and packaging area (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

both lactic and alcoholic fermentation. Of course, even in factory brewing, there is some overlap in the sequence of mashings and fermentations, but in home brewing, the sequence is less rigidly separated. In addition, home brews are clearly more vulnerable to spoilage because they pass through suboptimal temperature ranges for longer periods than do the factory brewers.

2. Contrasts Among Factory Brewing Procedures

Some differences observed today stem from differences in consumer taste. The iJuba consumers prefer a thinner beer than others, allowing brewers to cook the maize grits and initiate starch conversion at normal pH, at which malt enzymes operate at a faster rate than under the low pH prevailing in reef-type beer production. To give the brew its characteristic sour taste, brewers of iJuba beer sour later, after conversion.

Other differences in factory-brewing procedures arise because brewers experiment with the substitution of inputs in efforts to reduce cost and improve brew consistency. Kimberley beer, for example, was developed in the mid-1970s in an effort to minimize brew inconsistencies associated with

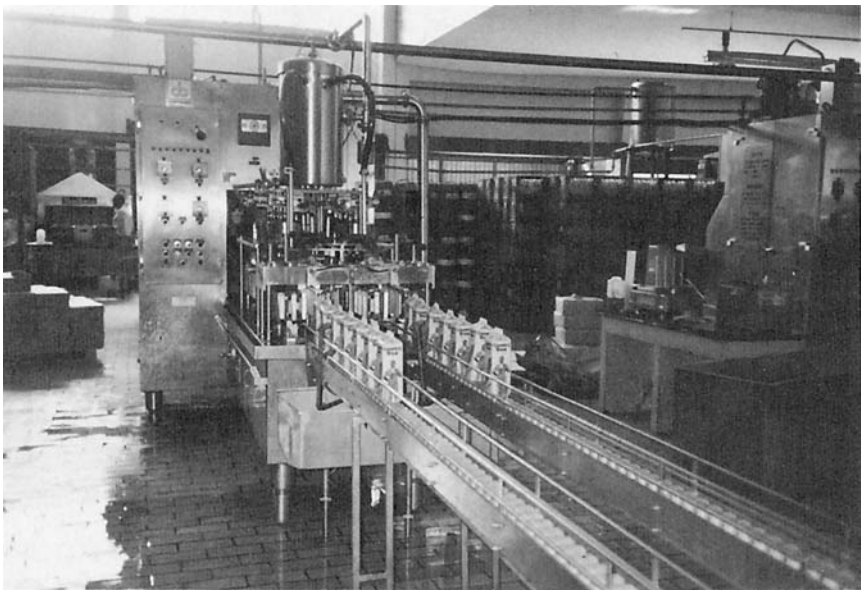

Figure 38 Filling line for 1-L beer cartons (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

low-quality malt produced following the import of U.S. sorghum hybrids to South Africa at that time. Brewers of reef-type beer in Botswana and Port Elizabeth as well as producers of iJuba beer substitute commercially cultured lactic acid for brewery-produced starter mixtures to reduce expenditure on malt and improve consistency by procuring pure lactobacilli strains. For similar reasons, brewers of Kimberley beer, along with factory brewers of reef-type beer in Botswana, Zimbabwe, and Zambia, use commercial α-amylases to partially substitute for sorghum malt. Over time, factory brewers everywhere have gradually reduced the sorghum-to-maize ratio of their brews in an effort to reduce use of the more expensive sorghum grain and malt (1,98) (see Table 12).

In their efforts to increase shelf life, factory brewers have experimented with a variety of short or low-temperature heat treatments aimed at destroying spoilage organisms. Brewers of iJuba and reef-type beer outside of South Africa have adopted heat treatments of the converted mash prior to straining, whereas producers of Kimberley beer heat treat their sour only (see Fig. 10). Efforts at actual pasteurization will be discussed in the section on alternative technologies (Sec. III.C).

Figure 39 One-liter wax paper carton being filled with sorghum beer (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

F. Critical Steps in Manufacturing and Fermentation

Temperature and pH control are important at all steps in the brewing process. Temperature determines which cultures of bacteria will grow most rapidly at each stage of the brewing process. It controls the rate of enzyme action during hydrolysis as well as the rate of alcoholic fermentation. Acidity affects virtually all biochemical processes in brewing, influencing the rate of sugar formation and bacterial growth.

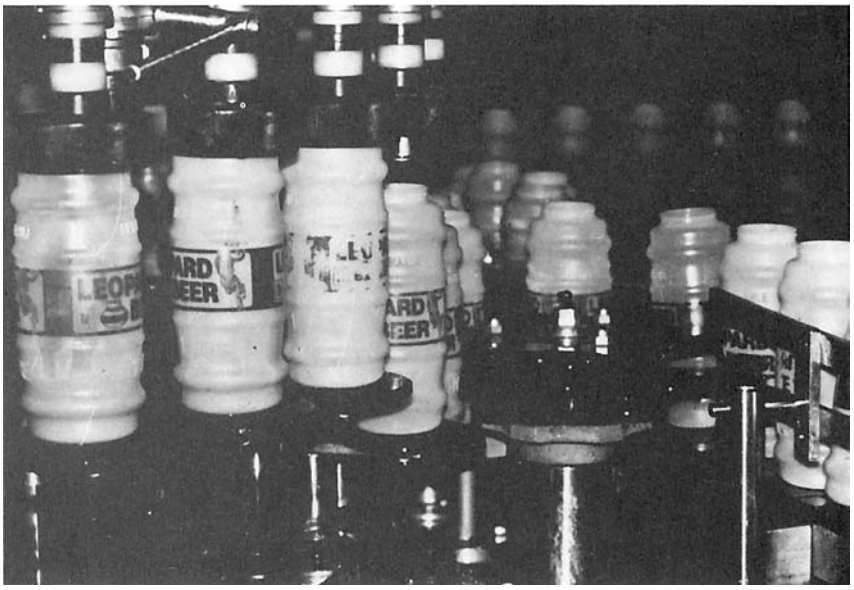

Figure 40 One-liter high-density polyethylene containers being filled on a low-vacuum filling machine (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

In addition to temperature and pH, two individual stages of the brewing process are particularly important to successful indigenous beer production. First, malting is crucial to both consistency and economy in brewing. The diastatic power of conversion malt governs the speed of starch hydrolysis and, hence, the body and alcoholic content of the beer. It also determines the malt-to-adjunct ratio required in brewing. Because malt is much more costly than unmalted adjunct, this ratio is critical to economy in brewing. Consequently, the availability of consistently high-quality malt has been central to factory brewers' ability to standardize procedures on scales as large as they do.

Souring is the second key stage in brewing. It produces not only the distinctive taste of the beer but also controls the entire brewing process. The acidity softens proteins, making them more digestible and accelerating gelatinization of starch granules they encase (84). The low pH of about 4.0 also prevents the growth of pathogenic bacteria, limits the rate of saccharification thus assuring a low-alcohol beverage, and controls the thinning and, thus, the body of the beer. It is no wonder that most observers of South African factory brewing emphasize how souring "controls" the rest of the brewing process (1,27,84,99).

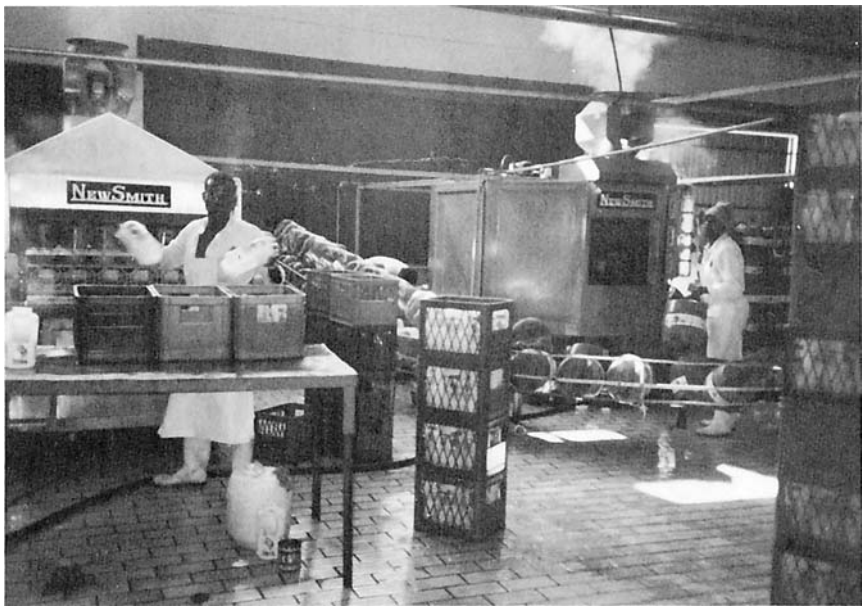

Figure 41 Washing of returnable 2- and 20-L plastic beer containers (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

As factory brewers and researchers continue to experiment with alternative brewing procedures, such as those embodied in the iJuba and Kimberley processes, it may become possible to control body, alcohol content, and taste by other means, through careful monitoring of temperature and the use of commercially cultured amylolytic enzymes and lactobacilli. However, today, among the home brewers who produce probably 80% of SMM beers in sub-Saharan Africa and reef-type factory brewer who account for roughly a further 15%, souring remains crucial.*

* Home brewers supply 100% of the SMM beer market in western Africa, over 75% in eastern and central Africa, and over half in southern Africa where factory brewing began and today remains most heavily developed. Figures available for countries in southern Africa suggest that home brewers supply 77% of the sorghum beer produced in Botswana and between 50% and 66% in South Africa. See Refs. 11, 16, and 31 for details.

Figure 42 Well-equipped laboratory at sorghum beer factory for routine microbiological, chemical, and sensorial analyses of the product and raw materials (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

G. Major Problems in Industrialization

In their efforts to scale up sorghum beer production to factory levels, factory brewers have confronted two major problems: malt quality and rapid spoilage of their final product. Availability of suitable quality malt has twice surfaced as a key problem, constraining the factory brewing industry in both the mid-1950s and again in the mid-1970s (62). Poor keeping quality has plagued factory brewers consistently.

1. Malting

Malting was historically the first stumbling block to successful scaling up of sorghum beer production to factory levels. For this reason, the first applied research on sorghum beer focused on malting. In 1954, at the urging of South African municipal brewers, that country's government research umbrella, the Council for Scientific and Industrial Research (CSIR), initiated research on

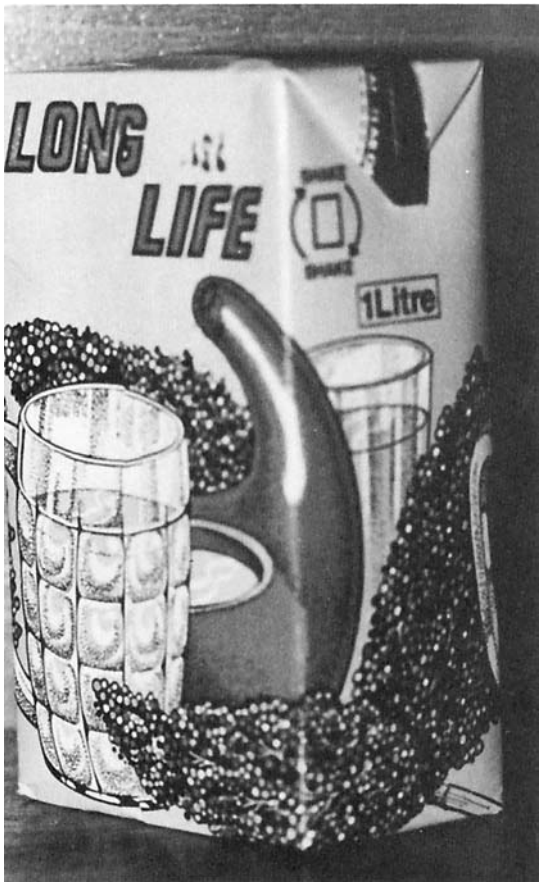

Figure 43 Aseptically packaged 1-L carton of pasteurized, degassed sorghum beer, with a 3-month shelf life (South Africa). (Courtesy of the Sorghum Beer Unit of the Council for Scientific and Industrial Research, Pretoria.)

sorghum malting (62). After 2 years of investigation, researchers succeeded in identifying key features of both the biochemistry and production of sorghum malt and established a standard method (method 235) for measuring malt quality (62). They assisted both factory brewers and maltsters with application of the tests and production techniques that would ensure the even malt quality so essential to factory brewers. This initial malting research was crucial to the development of factory brewing, enabling a virtual explosion in the volume of factory brewing from the mid-1950s onward (1,6,11).

In the course of this work, it became clear that these efforts were bearing fruit and that understanding sorghum malting was inextricably linked to

Table 3 Share of Packaged Sorghum Beer Sold in Different Containers, South Africa First Quarter 1986 (%)

Type of packaging	Metropolitan boards	Nonmetropolitan boards	Total
Bulk beer	8.5	20.8	29.3
16-, 20-, and 25-L containers	7.1	1.2	8.3
1-Liter nonreturnable	37.2	17.5	54.7
2-Liter nonreturnable	2.9	1.7	4.6
1-Liter returnable	2.0	1.1	3.1
Total	57.7	42.3	100.0

Source: Sorghum Beer Unit of the CSIR, Pretoria.

understanding the biochemistry of brewing. So in 1956, the CSIR established a permanent Sorghum Beer Unit that has continued since that day to undertake technical research in support of South Africa's municipal sorghum beer brewers. Since 1962, that work has been supported by a research levy charged on every liter of factory brewed sorghum beer sold (1). Imposed at a rate of 0.75% on the retail value of sorghum beer, the levy, in 1986, generated revenues of about R1.6 million (800,000) per year.

A second malting crisis arose in the mid-1970s, when growers introduced U.S. hybrid sorghum to South Africa to the chagrin of maltsters, who quickly discovered that the hybrids exhibited several undesirable malting properties. The high polyphenol content of the hybrids not only inhibited growth of lactobacilli necessary for souring but also rendered amylolytic malt enzymes insoluble and hence of no value for brewing (85,92,100). Technical research, again by the Sorghum Beer Unit, led to the development of a formaldehyde steep which successfully overcame this difficulty. Patented in 1976, this procedure not only neutralizes the polyphenols' inhibition of souring and starch hydrolysis but was also found to improve the keeping quality of the beer and to soften proteins (92).

What may be a third-generation malting problem has risen as a result of efforts to improve the shelf life of sorghum beer through pasteurization. A recent study by Mitchell (101) showed that malt may become infected with thermophilic bacteria, possibly *Bacillus coagulans*, which because of their heat resistance may survive pasteurization.

2. Spoilage

The short shelf life of sorghum beer has been a consistent problem, one far less tractable than those related to malting. The 1–5-day shelf life of sorghum beer, combined with its low value for weight, makes distribution a very difficult problem. Because demand jumps dramatically on weekends and at

Table 4 Variation in Malt Quality

Type of malt	Sample	Moisture content (%)	Sorghum diastatic units
Homemade malt, Botswana 1982			
Brewer 1	1	7.2	22.8
2	1	7.0	24.4
3	1	7.9	17.8
4	1	8.5	24.6
5	1	8.3	23.8
6	1	8.9	23.9
7	1	8.5	24.6
8	1	6.9	25.6
9	1	7.4	24.8
10	1	9.6	24.1
11	1	7.8	24.1
"Commercial" malt, Botswana 1982			
Maltster 1	1	10.3	28.9
"Industrial" malt, South Africa 1970			
Maltster A	A1	10.5	43.0
	A2	10.0	43.5
	A3	7.9	20.8
Maltster B	B1	6.9	41.7
	B2	9.9	48.9
	B3	8.7	47.6
Maltster C	C1	11.4	28.3
	C2	10.4	33.6
Maltster D	D1	9.4	38.2
	D2	8.2	46.1
	D3	9.3	53.0
Maltster E	E1	11.3	41.5
	E2	10.2	34.6
	E3	11.4	34.9
Brewery X	1	10.8	34.4
Brewery Z	1	16.2	42.5

Source: Botswana home-brewer and commercial malt analysis courtesy of Foods (Botswana) Pt. Ltd.; South African industrial malt analysis by de Schaepdrijver and Van Bellinghem 95.

Table 5 Comparative Malt-to-Grain Adjunct Ratios, South Africa[a] and Botswana,[b] 1982 (kg/100 L beer)

	Home brewing		Factory brewing	
Input	With homemade malt[b]	With commercial malt[b]	Industrial malt only[a]	Industrial malt, commercial α-amylases, and lactobacilli cultures[b]
Sorghum malt	18	8.8	4.5	3
Grain adjunct	7	6.9	11.5	12
Total	25	15.7	16.0	15
Malt/ adjunct	2.6	1.3	0.39	0.25

[a] Ref. 97
[b] Ref. 64.

month ends and because factory brewers have been unable to store the beer, their production schedule is tied to this widely fluctuating demand profile. Although the fluctuations are predictable, physical limitations on the speed of distribution require breweries to operate at different rates of throughput over the course of the month and week.

Indigenous beers spoil rapidly because they are actively fermenting when sold, with organisms in addition to yeasts flourishing in the rich medium. Although pathogenic bacteria are inhibited at the low pH of the final beer, several other micro-organisms remain active. The metabolic activity of mesophilic lactic acid bacteria are primarily responsible for spoilage. These, along with other undesirable bacteria, produce acetic acid, volatile off-flavors, fruity odors, and pellicles which render the taste, odor, and texture of the beer unacceptable to consumers (27,88,102).

Although pasteurization would improve the keeping quality by eliminating the major spoilage microbes, until recently it has not been possible without altering the character of the beer unacceptably—destroying yeasts and producing an uncharacteristically flat beer as well as thickening it dramatically by further gelatinization of starches and destruction of thinning enzymes (32,64). Moreover, slime-producing acetic acid bacteria have been reported to cause ropiness in packaged, pasteurized sorghum beer (103).

Increasing the keeping quality of sorghum beer has been at the forefront of research efforts in recent decades. Recently, some promising techniques have emerged, and they will be discussed under alternative technologies described in Section III.

The feeding of sorghum beer residues has been associated with hazardous effects [e.g. spinal cord degeneration in adult dairy cows (104)]. The epidemiology, clinical signs, and pathology suggested that the disease was associated with the fungus *A. clavatus*, which probably resulted from mould growth after collecting the industrial sorghum beer residues.

In contrast with industrial beers, South African traditionally brewed beers still seem to be contaminated with varying levels and types of mycotoxins. Especially maize and barley, frequently used for producing locally brewed beer, are frequently contaminated by mycotoxin-producing moulds. A study was undertaken by Odhav and Naicker (105) to investigate whether these toxins are present in raw grains and the traditional beers consumed by the local African population. The observations showed that the raw ingredients (sorghum, sorghum malt grain, and maize grits), and also commercially produced traditional beers (Utshwala and Utshwala special) and home-brewed beers (Umqombotha, Isiqatha, Imfulamfula) were contaminated by bacteria and fungi. Grain samples were found to be infected by *A. flavus*, *A. alliaceus*, *A. clavatus*, *Penicillium* spp., *Rhizopus* spp., and *Mucor* spp. Sorghum malt grain samples contained zearalenone. Although no mycotoxin-producing fungi could be detected in the fermented beers, two of six commercial beer samples contained aflatoxins (200 and 400 μg/L) and 45% (13 of 29) of the home-brewed beers contained zearalenone within the range 2.6–426 μg/L and/or ochratoxin A from 3 to 2340 μg/L.

III. BIOCHEMISTRY AND MICROBIOLOGY OF BREWING AND MALTING

A. Reef-Type Factory-Brewed Sorghum Beer

1. Malting

As sorghum germinates, it produces diastatic and proteolytic enzymes necessary to break down grain substrates into sugars and nitrogen compounds which support yeast growth and alcoholic fermentation. The diastatic enzymes in sorghum malt include α- and β-amylases, the major sources of diastatic activity, as well as limit dextrinase and maltase (α-glucosidase). In contrast to malted barley, the α-amylases predominate in sorghum malt, accounting for about 75% of amylolytic activity (106,107). The α-amylases are all formed during germination, whereas the β-amylases increase from levels present in the original grain. Maltase, also present in the sorghum grain, does not increase during malting (100,108). The diastatic activity developed during malting depends primarily on the cultivar selected for germination but also on the malting conditions, especially temperature, moisture level, and duration (109).

Protein breakdown also occurs during malting as grain structure changes to produce roots and shoots. The proteolytic activity in sorghum increases about 10-fold during germination. Because the formation of new plant protein is not completed, malting produces a substantial increase in free α-aminonitrogen (FAN) over levels found in the original grain (64).

Sorghum and millet malts and grain house a wide variety of micro-organisms which are responsible for both lactic acid and alcoholic fermentations as well as spoilage. The micro-organisms include yeasts, many species of bacteria, molds, and bacterial endospores (1,27). As Table 6 indicates, sorghum malt may contain microbial populations far in excess of 100 million/g, dominated by wild yeasts, aerobic endospores, and lactic acid bacteria (102). Many species of yeasts are present, but most commonly *Saccharomyces cerevisiae*, *Candida kursei*, and *Kloeckera apiculata* (27). Bacteria include lactobacilli, pediococci, streptococci, and other genera (27,102). Lactobacilli may be homofermentative or heterofermentative. The homofermentative lactobacilli produce lactic acid as a major end product from glucose, whereas the heterofermentative lactobacilli produce acetic acid or ethanol and CO_2 in addition to lactic acid.

2. Souring

Souring, or lactic acid fermentation, is the first of two fermentations involved in the production of SMM beers. Spontaneous souring occurs when lactic acid bacteria, present in the malt, convert the fermentable sugars to lactic acid. The temperature at which fermentation takes place determines which microbial groups will eventually dominate in the sour. In the lower-temperature range, from room temperature to 37°C, mesophilic lactobacilli, pediococci, streptococci, and even yeast may develop (27). The low temperature

Table 6 Effect of Irradiation on Microorganisms Present on Sorghum Malt (Surviving Organisms per Gram of Sorghum Malt)

Irradiation dose (kGy)	Total	Yeasts	Molds	LAB[a]	Endospores	
					Anaerobic	Aerobic
0	9×10^8	6×10^8	4×10^3	1×10^6	3×10^5	4×10^8
2.5	2×10^7	1×10^7	$<10^2$	2×10^3	3×10^5	2×10^7
5.0	7×10^6	7×10^6	$<10^2$	8×10^2	2×10^3	1×10^5
7.5	4×10^5	4×10^5	$<10^2$	5×10^2	$<10^2$	1×10^4
10	2×10^5	2×10^5	<10	<10	<10	20
12.5	7×10^4	2×10^4	<10	<10	<10	<10

[a] Lactic acid bacteria.
Source: Ref. 102 and unpublished work.

also favors the growth of heterofermentative lactic acid bacteria, *Betabacterium* spp., and *Leuconostoc* spp. which, in addition to lactic acid, produce ethanol and acetic acid, leading to early spoilage and off-flavors that render the product unsalable.

Higher temperatures, in the range 48–50°C, are optimal, as they inhibit the growth of these undesired microbes and instead favor the development of more desirable homofermentative thermophilic lactic acid fermentations. This, of course, explains why factory brewers carefully maintain temperature between 48°C and 50°C during souring. *Lactobacillus delbrueckii* appears to be the dominant thermophilic bacterium active in sorghum beer production.* At the end of a successful souring, the pH of the wort lies between 3.0 and 3.5 (1,32).

Although the lactobacilli can initially feed off the small amount of sugars present in the malt, their continued growth requires amylolysis of the malting starches to produce enough additional sugars to sustain the microbial growth. Thus, although often overlooked, successful souring depends on a simultaneous mashing of malt starches by the amylases present in the malt. Although 50°C is not optimal for sugar formation, sufficient sugars are produced at that temperature to sustain the lactic acid fermentation (99).

Research has shown that the thermophilic lactobacilli desired by brewers are not present on all malts (64). In the absence of *L. delbrueckii*, growth of the less acid-resistant *Bacillus coagulans* and *Pediococcus* spp. is promoted, resulting in the production of off-flavors. Factory brewers have, therefore, generally adopted the practice of using a small volume of a "successful" souring as inoculum. In this manner, they obtain a predictable rate and "purity" of acid production.

Unfortunately, the use of successful sourings for inoculation does not always provide satisfactory results, especially in the modern, sophisticated sorghum beer brewery. Even in quick souring malts, several undesired bacteria may still be present: The thermophilic lactobacilli may also constitute a mixed population with variable acid-producing capabilities. To address this problem, brewers and researchers have attempted to introduce pure strain starter cultures of *L. delbrueckii* in the industry. These efforts have not been successful, perhaps because of high cost and the overgrowth by other thermophilic lactobacilli present in the malt. A second attempted remedy, the use of deep-frozen, concentrated cultures of *L. delbrueckii*, has proven technically feasible, although brewers are not likely to adopt this on a wide scale. High cost and logistics make this procedure unattractive, as it requires a

* Although the name *Lactobacillus leichmanni* is often used in the literature, Weiss et al. (110) have shown this species to be a subjective synonym for *L. delbrueckii*.

sophisticated supply network and liquid-nitrogen storage facilities at the brewery (111).

One South African brewery has successfully introduced inoculation with a single strain starter culture of *L. leichmanni*. To do so, they cultivate cultures in the laboratory starting from a stab inoculation in MRS-agar in malt substrate (111). The intermediate culture may be stored at 5°C for several days until required for inoculation.

3. Boiling

By diluting their sour with about two volumes of water and then adding cereal adjunct, factory brewers raise the pH of their brew to about 3.7–4, a level which prevails throughout the subsequent mashing (32). Maize grits account for the bulk of the additional starch, although brewers may also include degermed maize, degermed sorghum grits, and sorghum meal as well. Boiling the entire mixture for 2 h gelatinizes the grain starches from both the malt and the starchy adjunct. The heat combines with the high acidity to soften the imprisoning protein matrix, allowing the starch granules to break free. They burst open, producing a thick tangle of starch polymers: amylose and amylopectin. Amylose usually constitutes about one-fourth of the total mass with amylopectin accounting for the remaining three-fourths (84,99).

Boiling also decontaminates the brew, destroying all micro-organisms in the vegetative form. Because these include the lactobacilli, boiling arrests lactic acid formation. The extreme heat also destroys the malt enzymes, thereby stopping the thinning action of the malt enzymes on their own substrate. Boiling, of course, also affects the flavor of the product by giving it a cooked taste.

4. Mashing for Alcoholic Fermentation

After cooling the boiled mixture to 60°C and adding additional malt, conditions are ideal for conversion of starch to sugars (1). By adding the conversion malt, brewers introduce soluble sugars, free aminonitrogen, protein, proteinases, starch, and diastatic enzymes to the mash. The amylolytic enzymes present in the malt—α- and β-amylases, maltase, and dextrinase—break down the mass of intertwined starch polymers, thinning the mash and producing sugar which can subsequently be converted to alcohol. The α-amylases generate the most effective thinning action, cutting the linear amylose chains in the middle and chopping the branched amylopectin polymers at the nodes (99). The β-amylases split off pairs of glucose units from the nonreducing end of the starch molecule, producing maltose, which the maltase subsequently breaks down into glucose. The dextrinase produces linear dextrins during mashing. Although some saccharification during this stage is due to the action of molds present in the malt, research has shown that

molds alone are insufficient to convert the starches (1,13,27). Rather, the major conversion is effected by the malt enzymes.

Because the acidity of the mash slows the action of the diastatic enzymes—especially the α-amylases—the pH of 3.7–4 slows the thinning of the brew, giving it its characteristic body, and also slowing sugar and consequently alcohol production. Insufficient acidity at this stage results in both too much thinning and too much alcohol production (84,112). Because the α-amylases are more heat resistant than the β-amylases, some early thinning of the mash can be achieved by adding a small dose of malt at about 80°C while the boiled mixture is still cooling (1). Although this is not the optimal temperature for adding conversion malt, a small early dose can facilitate handling of the viscous wort as it cools. Laboratory tests have shown that 60°C is optimal for sugar production (1).

Concurrent with the mashing and alcoholic fermentation, a secondary souring takes place as lactobacilli present in the conversion malt feed off the rich mash sugars to produce additional lactic acid. However, this secondary souring takes place too late to control the mashing process (32,84).

The typical sorghum beer mash contains about 10% protein. During mashing, proteolytic enzymes from the malt hydrolyze part of the proteins, producing amino acids and small peptides, jointly referred to as free α-aminonitrogen or FAN (64). Although the sorghum proteins are mainly insoluble, proteases such as proteinases and especially carboxypeptidases hydrolyze polypeptides and peptides to produce FAN. (The FAN production at this stage, however, is less than that produced during malting.) These enzymes show optimal activity at low pH values and are also active at the high mashing temperatures (64). During this action, a fraction of especially the insoluble reserve proteins, prolamin and glutelin, is hydrolized. These proteins constitute about 80% of the total nitrogen content after mashing. The major amino acids at this stage are aspartic and glutamic acids, each of which contributes 15–20% of the total FAN, and proline, which contributes 10%. These amino acids are valuable yeast nutrients and therefore necessary for alcoholic fermentation.

5. Straining

Factory brewers strain their mash, subsequently referred to as "wort," in final preparation of the substrate for alcoholic fermentation. They strain at 60°C rather than at 30°C in order to take advantage of the protective action of the temperature in preventing possible contamination of the brew by microorganisms present on the strainers (84). Straining removes up to 30% of total solids. Because the beer solids are enriched in protein, this results in a loss of protein to consumers of the beer and makes the residue a valuable feedstuff (64).

6. Alcoholic Fermentation

Brewers pitch their wort with active dry brewers' yeast, a top-fermenting strain of *S. cerevisiae*, to initiate alcoholic fermentation. Optimum conditions for alcoholic fermentation include a pH range of 4.0–4.5 along with temperatures between 20°C and 35°C (113).

During alcoholic fermentation, the secondary souring continues. However, the prevailing temperature, between 28°C and 30°C, favors the growth of mesophilic lactobacilli and other spoilage organisms as described below.

7. Packaging and Distribution

After fermentation has proceeded for about 24 h, factory brewers package and distribute their beer. Because most do not pasteurize their beer, alcoholic fermentation proceeds during packing and distribution. When customers purchase the beer, 1–5 days after fermentation begins, they consume a product which is still actively fermenting. The final product has a pH of about 3.5 and an alcohol content of about 3%, the legal maximum for beer leaving the factory in South Africa (1).

8. Spoilage

Indigenous beer spoils in 1–5 days, mainly because of the microbial contamination introduced into the wort by the conversion malt. After the addition of the conversion malt, no further boiling or heat treatment is applied, whereas conditions during mashing and especially alcoholic fermentation favor the growth of the contaminants. The low temperatures prevailing during alcoholic fermentation and distribution favor the growth of mesophilic lactic acid bacteria, which are the main spoilage organisms. Pathogenic and most anaerobic endospore-forming bacteria are sensitive to the low pH and are either inhibited or killed by the high acidity of the beer. Mesophilic, homofermentative lactic acid bacteria such as *Pediococcus* and *Streptobacterium* are responsible for "oversouring" as well as the production of volatile off-flavors in the beer. The heterofermentative lactic acid bacteria, *Leuconostoc* and *Betabacterium*, produce acetic acid or ethanol and CO_2 in addition to lactic acid (Table 7), contributing to unacceptability of the product.

No comprehensive study on the oversouring of beer has thus far been undertaken. However, preliminary investigations of 159 *Lactobacillus* isolates typically associated with sorghum beer spoilage have shown *L. plantarum*, a mesophilic lactobacillus, to be the dominant species accounting for 42% of the total isolates (102). Another 28% of the isolates were also grouped as mesophilic, homofermentative lactobacilli; thus, this group accounted for 70% of the spoilage organisms studied. Only 3% of the strains were identified as thermophilic lactobacilli, which most probably do not play

Table 7 Spoilage Association and Patterns of Sorghum Beer

Micro-organism	Chemical produced	Odors	Other effects
Acetobacter	Acetic acid	Vinegar	Pellicles
Pediococcus	Lactic acid		Ropiness
Lactobacillus (homoferm)	Lactic acid		Ropiness
Lactobacillus (heteroferm) and *Leuconostoc*	Lactic acid and acetic acid		Ropiness, turbidity
	Diacetyl	Butter/honey	
	2,3-butanediol	Sweet	
Zymomonas	Ethanol, CO_2, acetaldehyde	Rotten apples	
	H_2S	Rotten eggs	
Obesumbacterium	Dimethylsulfide	Parsnip, cooked cabbage moldy	
Yeasts			
Candida		Fruity	Pellicle
Pichia			Pellicle
Rhodotorula			Red coloration
Hansenula			Pellicle
Saccharomyces (wild strains)	Diacetyl	Phenolic, butter/honey	Superatenuation

Source: Compiled from Ref. 114.

any significant role in mesophilic beer spoilage. The heterofermentative lactobacilli represented 27% of the isolates, with *L. fermentum* the most prominent representative.

Using conventional and Petrifilm plating, Pattison et al. (115) determined the typical microbial populations associated with commercially produced South African sorghum beer, involving 46 commercially produced sorghum beer samples from retail outlets in the Johannesburg area. Yeast counts of 7.84 log CFU/mL were detected with conventional methods as compared to 7.85 log CFU/mL using Petrifilm (CFU-colony-forming unit). Lactic acid bacteria counts were 6.44 and 5.31 log CFU/mL, respectively, aerobic plate counts 5.96 and 5.34 log CFU/mL. Characterization of 419 predominant bacterial isolates yielded 88.0% lactic acid bacteria, 8.4% *Bacillus* species, 2.9% *Micrococcus* species, and 0.7% Gram-negative bacteria.

Under aerobic conditions, acetic acid bacteria oxidize the ethanol in the beer to produce acetic acid. *Zymomonas lindneri* ferments sugars producing,

in addition to ethanol and CO_2, off-flavors such as acetaldehyde and H_2S. In addition, wild yeasts, those other than *S. cerevisiae*, may cause fruity odors and pellicle formation (Table 7).

Although pasteurization would improve the keeping quality of the beer by destroying spoilage microbes, no such process is applied on a large scale for conventional sorghum beer. Early attempts at pasteurization failed because they led to an unacceptable increase in beer viscosity—through further gelatinization of starch and elimination of amylolytic enzymes—and also eliminated its characteristic effervescence by killing the active yeasts (32,64). Details are discussed in Section III.C.

Radurization of the conversion malt may also delay spoilage by reducing the population of microbial contaminants present on the malt. Radurization at 10 kGy has proved effective in reducing the total microbial population of sorghum malt by about 99.5%. The wild yeasts showed the highest resistance, whereas the lactic bacteria, fungi, and anaerobic endospores were eliminated to below the level of detection, extending shelf life by 2–3 days. As the results in Table 8 show, a maximum shelf life of 5 days was achieved with untreated malt as compared to 7 days with radiated malt.

B. Home Brewing and iJuba and Kimberley Factory Brews

The same enzyme formation, lactic acid fermentation, starch hydrolysis, amylolytic breakdown, alcoholic fermentation, and protein modification

Table 8 Extension of Sorghum Beer Shelf Life by the Use of γ-Irradiated (10 kGy) Malt

	Radiated malt		Untreated malt	
Sample	Acidity (°B)[a]	Shelf life (days)	Acidity (°B)	Shelf life (days)
1	3.62	7	5.01	5
2	3.93	7	4.95	5
3	4.35	6	6.44	4
4	4.95	5	5.82	4
5	5.29	5	7.65	3
6	4.15	7	6.22	4
Average	4.38	6	6.01	4

[a] °B = mL of 1 *N* NaOH to neutralize a 100-mL beer sample, measured after 7 days fermentation at 30°C.

Source: Data courtesy of the Atomic Energy Corporation, Pretoria.

occur during home and reef-type factory brewing. However, because home brewers cannot control temperature, pH, or input quality as carefully as factory producers, home brewing results in more overlap among stages and less consistency in brew quality. Because of the wild yeasts and enzymes present in the malt, starch and protein breakdown as well as alcoholic fermentation begin to some extent even during souring. Similarly, souring continues during mashing and alcoholic fermentation. Moreover, spoilage organisms develop earlier in home brewing because of the brewers' inability to maintain the sequence of mashings and worts outside the dangerous low-temperature range as effectively as factory brewers can.

The inability of home brewers to rigidly control temperature and humidity during malting results in uneven development of diastatic power of the malt. This, in turn, leads to uneven diastatic breakdown during mashing. Although home brewers initially heat the malt and water mixture to be soured, they cannot maintain the high temperature during souring as easily as factory brewers. Consequently, the undesirable mesophilic lactobacilli develop more quickly in home brews leading to earlier spoilage. Home brewers also depend on wild cultures of lactobacilli and yeast. Although many regular brewers in effect inoculate through repeated use of the same brewing vessels, they cannot control the purity of cultures to the extent factory brewers do through laboratory-aided inoculation and temperature control. Hence, home brewers suffer more frequent failed brews than factory brewers. Of course, not all home brewers are alike. The professional home brewers, who produce 200-L batches several times per week and use commercial malt, can inoculate both lactobacilli and yeast cultures and standardize procedures so as to closely approximate factory optima. However, the irregular home brewers who malt their own sorghum or millet face the most irregularity in input quality and the greatest variability in product quality and produce beers with the shortest shelf life.

Factory brewers producing iJuba- and Kimberley-type sorghum alter the standard sequence of mashings and fermentations undertaken by home and factory brewers of reef-type beer (Fig. 10). In both the iJuba- and Kimberley-type beer production, brewers begin not by souring but by boiling the grain adjunct. To avoid excessive viscosity during gelatinization, iJuba brewers add a small quantity of premalt to the grain adjunct, whereas Kimberley brewers add commercially produced α-amylases. After gelatinization, both sets of brewers add the main quantity of conversion malt. Amylolytic breakdown then takes place at the natural pH, occurring more rapidly than in the acidic conditions of reef-type beer production.

In iJuba-type beer production, brewers sour the entire mash by lowering the temperature to 50°C to favor the development of thermophilic lactobacilli. After souring, brewers heat the mash to 100°C to stop both lactic acid

fermentation and starch breakdown. They then cool the mash to 40°C, add a final portion of malt to regulate viscosity, and then strain and pitch with yeast to initiate alcoholic fermentation.

Simultaneous with starch conversion of the main mash, brewers of Kimberley-type beer ferment separately for lactic acid. Like brewers of reef-type beer, they induce lactic fermentation by holding a mixture of sorghum malt and water at 49°C. They combine the sour with the converted mash, reduce the temperature to 28°C, and pitch with yeast to launch alcoholic fermentation. Novellie and de Schaepdrijver (64) and Watson (111) have described these procedures in more detail.

C. Alternative Brewing Technologies

In addition to the full-brewing procedures just described, there exist three alternative technologies for the production of sorghum beer. The first, use of powder beer, is an old technology aimed at supplying a covenient, premix packet primarily to consumers who wish to brew for themselves. However, the remaining two, pasteurizing and production of beer concentrate, represent state-of-the-art attempts to overcome the major problem still facing sorghum beer producers (i.e., the short shelf life). These two alternative technologies have the potential to dramatically affect the structure of the industry, although in very different directions.

1. Powder Beer*

The first alternative to full-fledged brewing involves the use of beer powder, a dry mixture of precooked corn meal, malt, and yeast. The corn meal is normally dry-cooked by steam injection, a procedure which presolubilizes the starch and allows for extremely rapid conversion of starches to sugar when water and malt are added. The production of beer powder is a very simple process. It is most often manufactured by maltsters who, with the purchase of only a steam gun for use along with the rest of their equipment, are able to produce beer powder without difficulty.

Sorghum beer can be produced from beer powder in a simple one-step procedure by merely adding warm water. With the addition of warm water, the presolubilized starch from the corn meal is available for rapid breakdown into sugars by the enzymes in the malt. At the same time, lactobacilli in the malt produce lactic acid, which gives the beer its sour taste. As the sugars form, yeasts convert the sugars to alcohol. Thus, mashing and both fermen-

* This section draws from the work of Haggblade (16).

tations take place simultaneously. The production of sorghum beer from beer powder requires only a 4–8-h fermentation period compared to the 18 h required for full-scale factory brewing.

Compared with fully brewed beer, that produced from powder has an extremely short shelf life (i.e., only 1 day). The powder beer spoils rapidly because the preserving powers of the lactic acid are not at full strength throughout mashing and alcoholic fermentation. Unwanted micro-organisms are not fully controlled by the initially low-strength lactic acid and they grow in the sugary wort, leading to early spoilage of the beer.

Powder beer is used by some home brewers, small factory brewers, and large mining companies. Beer powder is readily available to home brewers, as it is distributed in small packs through normal retail outlets. Women who brew powder beer do so most commonly as an easy method of brewing for family consumption. In rare instances, cash home brewers will produce powder beer for sale, although normally only when grain and malt are either unavailable or very expensive, usually just before harvest time.

Powder beer is also brewed on an industrial scale. Large mining companies throughout southern Africa frequently use it to supply the sorghum beer they distribute as part of their food rations. Small factory brewers occasionally also find it sensible to brew powder beer, normally as a low-cost means of testing remote markets before setting up a full-fledged brewery. At least three breweries in Botswana, two in the early 1970s and one in the 1980s, were of the powder mix variety (16). The attraction of powder mix brewing lies in its very low initial costs; it requires little sophisticated temperature control equipment, a far less skilled technician to run the brewing operation, and only fermentation vessels rather than additional souring and mashing tanks. Of course, the product quality attained lies substantially below that of fully brewed (home- or factory-brewed) beer. Perhaps for this reason, at least two of Botswana's three commercial powder mix breweries abandoned operations after less than a year (16). In Botswana, the only country for which we have information powder beer accounts for well under 5% of SMM beer production (16).

2. Sorghum Beer Concentrate

A recently developed alternative sorghum beer-brewing technology involves the production of a beer concentrate. This process, developed by the Sorghum Beer Unit in Pretoria, underwent its first nonlaboratory production trials in the summer of 1982 (16) and is now being produced in Bloemfontein, South Africa. The procedure follows standard South African factory brewing procedures up through the end of the mashing stage. Then, instead of pitching with yeast and straining, brewers dehydrate the mash, producing a thick, syrupy mixture of concentrated sugars and solids. This wort concentrate can

be inexpensively transported to remote locations where warm water can be added along with yeast to induce fermentation. It contains all of the typical ingredients of the wort, including lactic acid and fermentable sugars, in concentrated form. Because of the low water activity, even osmotolerant microbes are inhibited, thus ensuring a considerable shelf life of the concentrate. After fermentation, the beer is ready for distribution; it is a high-quality sorghum beer indistinguishable from fully factory-brewed sorghum beer.

Much like powder beer, one potential gain to factory production of beer concentrate lies in the servicing of remote or sparsely populated regions. These outlying areas are generally too small to support a full-sized brewery and are difficult to service from existing large breweries because of the low value for weight of sorghum beer, high transport costs, and the short shelf life of the beer. In these remote locations, the concentrate can be mixed with warm water and yeast in very simple fermenting vessels and then distributed to consumers.

The beer concentrate also addresses the crucial problem of short shelf life by arresting fermentations and mashing through dehydration. The beer can be reconstituted and fermented for alcohol at the time and location desired. Unlike pasteurization, this solution drastically reduces transport and distribution costs. Because the B vitamins are relatively heat stable, concentration of the product will not reduce their content significantly. Even so, because a large part of the B vitamins are entrapped within the viable yeast cells, only a part become nutritionally available.

Looking to the future of the industry, the sorghum beer concentrate could have a potentially huge impact, especially on the market share held by home brewers. With access to a factory-produced sorghum beer concentrate, home brewers could produce beer of a guaranteed consistent quality and absolutely indistinguishable from factory-brewed sorghum beer. Rather than continued erosion of the marketshare in the face of factory brewers expansion, home brewers could maintain or even increase their marketshare by acting as fermenter–retailers of factory-produced concentrate. Given what appear to be important income distribution implications of home brewing (97), such a strategy might be optimal on efficiency and equity grounds. Although the newly developed sorghum beer concentrate has the potential to substantially influence future evolution in the sorghum beer industry, it it still too early to tell how prevalent it will become. Benefits such as increased shelf life and lower transport and distribution costs will clearly favor the adoption of beer concentrate, especially in the sparsely populated regions of Africa.

3. Pasteurization

Because spoilage arises as a result of microbial contamination introduced by conversion malt, heat inhibition of microbes through pasteurization holds

obvious appeal as a means of prolonging shelf life. Although South Africa's Sorghum Beer Unit has experimented for many years with pasteurization, their efforts to date have not been entirely successful (64). Early efforts involved batch or tunnel pasteurization. These attempts failed because they caused additional starch gelatinization and, consequently, excessively viscous brews. They also destroyed active yeast cells, producing an uncharacteristically flat brew unacceptable to consumers.

Later efforts revealed that extensive thickening could be avoided by flash-pasteurizing the wort by means of in-line heat exchangers. Unfortunately, flash-pasteurizing of the wort did not completely eliminate the contaminating microbes, as they were protected by the substrate solids.

Pasteurization of the fermented beer—rather than the wort—proved more successful; it reduced contaminants and had fewer adverse effects on the taste and body of the beer. However, it did cause a loss of CO_2 and, hence, the effervescence of the product, producing a flat beer. Subsequent attempts at carbonation were unsuccessful because of suspended solids in the beer.

Although the pasteurization experiments have thus far failed to produce a standard sorghum beer with increased shelf life, they have resulted in the development of two novel, nonconventional sorghum beers with shelf life in excess of 3 months (64). Both are already under commercial production. One, a noncarbonated beer packaged in Tetra/Brik aseptic cartons, has only limited potential in areas where conventional effervescent beer is readily available. Factory brewers produce this beer in Potchefstrom, South Africa and distribute it in the sparsely populated areas to the west and northwest. Another version of the long-life beers is produced in the eastern Cape and Ciskei areas where pineapple juice, a product of the region, is added for flavoring.

Production of the second nonconventional beer begins with pasteurized sorghum beer. After adding a specific quantity of sugar, brewers inoculate with *S. cerevisiae* and fill the beer into 500-mL beer flasks. The bottle fermentation lasts 5–12 days, causing internal CO_2 pressure to rise up to 30 psi. This procedure results in a final product with a very long shelf life. Although clearly different, it compares favorably with conventional sorghum beer and is distributed under the brand name "Tugela Beer" in Natal, South Africa (64).

D. Nutritional Considerations

Indigenous beers enjoy an outstanding nutritional reputation. Since at least the 1930s, observers have promoted them as good sources of calories, B vitamins, and proteins (3,12,14,98). Novellie (14) asked whether sorghum beer is a food or a beverage, and Platt (13) used indigenous beers as a primary

example of "biological ennoblement," the enhancement of nutritional value through biological processes harnessed during food preparation. Moreover, many studies have commented on the nutritional superiority of sorghum beer over western "clear" beer (14,64,116).

The conclusion of several decades of research is that, although prior claims must be tempered somewhat, indigenous beers are indeed an important source of calories, riboflavin, niacin, and protein. The SMM beers provide these nutrients in sufficient quantity to constitute a valuable nutritional supplement to standard African diets. These beers contain about 5–7% solids, mainly as carbohydrates, hence their substantial caloric contribution. They contain about 11% protein in their dried solids (75) and B-vitamin concentrations as described in Tables 14 and 15. Moderate drinkers commonly consume 2 L (about half a gallon) of sorghum beer per day, a quantity sufficient to supply 40% of their daily protein and calorie requirements, and if all vitamins could be assimilated by humans, 200% of thiamine, 100% of riboflavin, and 70% of niacin requirements (calculations based on data in Ref. 1).

The nutritional value of indigenous beers is influenced by three factors: (a) the extent to which biological ennoblement takes place during brewing and malting, (b) the ingredients used in brewing, and (c) the degree to which nutrients can be assimilated by human consumers. Each is examined in turn.

1. Biological Ennoblement?

Tables 9–11 examine the case for biological ennoblement. As Table 9 shows, the malting of sorghum and millet synthesizes some B vitamins but not others. Substantial amounts of riboflavin are indeed synthesized by malting both sorghum and millet, whereas nicotinic acid remains about the same as in the original sorghum grain but increases in malted millet. For both grains, thiamine content actually drops during malting. In addition to vitamin synthesis, the malting of sorghum has also been found to improve protein digestibility by about 7% (22,118). During brewing, yeasts produce still greater quantities of riboflavin and nicotinic acid, although thiamine content drops slightly as a result of assimilation. Table 10 displays the magnitude of these changes.

Using maize beer as an example, Platt (13) summarized the net effect of nutritional changes accompanying the brewing of indigenous beers. He concludes, as shown in Table 11, that consumers drinking maize beer as opposed to porridge made from the same amount of grain lose only 1% of the caloric value of the grain, an insignificant loss considering the other improvements in nutritional value that occur during fermentation. Riboflavin and nicotinic acid content increase by 75–100% in the maize beer diet, potentially very important because these two B vitamins play key roles in combatting

Table 9 Effect of Malting on Vitamin Content of Sorghum and Millet (mg/g Dry Basis)

	Grain	Malt	Percent change
A. Thiamine			
1. Sorghum			
(a) South Africa, 1946	3.34	1.73	−48
(b) South Africa, 1961	5.5	5.2	−5
(c) Togo, 1959	5.6	4.4	−20
2. Finger millet			
(a) South Africa, 1946	1.9	1.6	−16
3. Pearl millet			
(a) South Africa, 1946	3.4	2.1	−38
B. Riboflavin			
1. Sorghum			
(a) South Africa, 1946	1.29	2.41	+87
(b) South Africa, 1961	1.2	2.0	+67
(c) Togo, 1959	0.8	2.5	+213
2. Finger millet			
(a) South Africa, 1946	1.1	2.0	+82
3. Pearl millet			
(a) South Africa, 1946	1.5	3.2	+113
C. Niacin			
1. Sorghum			
(a) South Africa, 1946	35.1	34.1	−3
(b) South Africa, 1961	40	37	−8
(c) Togo, 1959	49	71	+45
2. Finger millet			
(a) South Africa, 1946	9.5	13.7	+44
3. Pearl millet			
(a) South Africa, 1946	23.6	30.2	+28

Source: South Africa, 1946: Ref. 117; South Africa, 1961: Ref. 7; Togo 1959: Perissee et al. (1999) cited in Ref. 22.

pellegra and other vitamin deficiencies common in maize-based diets (7,22). The thiamine content of the beer, however, is less than that of the original grain.

Protein availability increases during malting and, to a lesser extent, during souring and mashing (64). The germination of sorghum results in a 10-fold increase in FAN with a several-fold increase for all the essential amino acids (119). As Tables 12 and 13 show, yeast fermentation does not contribute to any further change in the protein content of the beer. One liter of the sor-

Table 10 Effect of Factory Brewing on the Vitamin Content of Sorghum Beer, South Africa 1966

	Vitamin content (mg/100 ml)		
Time of fermentation (h)	Thiamine	Riboflavin	Nicotinic acid
3	41	21	175
9	40	27	160
24	30	34	210
30	32	41	210
48	38	34	220

Source: Ref. 98.

Table 11 Nutritional Impact of Consuming Maize in the Form of Maize Beer

	Amount of food eaten		
Food item	Diet without beer	Diet with maize beer[a]	
Maize, whole meal	350 g	137.5 g	
Maize, 60% extraction	350 g	137.5 g	
Maize beer	—	5 pints (2840 ml)	
Vegetables, leafy green and yellow	130 g	130 g	
Sweet potatoes	470 g	470 g	
Kidney beans	30 g	30 g	
	Nutrient values of diets		
	Diet without beer	Diet with maize beer	Percent change
Calories	3016	2979	−1
Thiamine	2.00 mg	1.95 mg	−2.5
Riboflavin	1.13 mg	2.32 mg	+105
Nicotinic acid	11.70 mg	20.30 mg	+73

[a] Substitutes 5 pints of maize beer for the maize used in its manufacture.
Source: Ref. 13.

Table 12 Nutritive Content of Raw Materials and Intermediate Products in Sorghum Beer Brewing, South African Factory Brew 1985

Sampe	Fat (g/kg)	Crude fiber (g/kg)	Protein (g/kg)	Ash (g/kg)	Carbohydrate (g/kg)	Food energy (kJ/kg)	Thiamine (mg/kg)	Riboflavin (mg/kg)	Nicotinic acid (mg/kg)
Maize grits	8.0	11.0	78.0	6.0	772	14,750	1.10	0.50	10.70
Sorghum malt	11.0	60.0	90.0	16.0	716	14,120	4.60	4.50	69.30
Brewer's yeast	4.0	9.0	337.0	54.0	511	14,570	28.80	42.50	391.50
	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(kJ/L)	(mg/L)	(mg/L)	(mg/L)
Mash	0.6	2.8	10.9	1.1	123	2,290	0.31	0.25	3.63
Wort	0.2	0.9	6.6	1.1	112	2,040	0.31	0.22	2.99

Source: Ref. 118.

Table 13 Nutritive Content of Eight Samples of Factory-Brewed Sorghum Beer, South Africa 1985

Beer sample no.	Total solids (g)	Alcohol % (m/m)	Fat (g)	Crude fiber (g)	Protein (g)	Ash (g)	Carbohydrate (g)	Food energy (kJ)	Thiamine (mg)	Riboflavin (mg)	Nicotinic acid (mg)
1	66	1.6	1	1	5	1	58	1570	0.36	0.42	3.30
2	45	3.2	1	1	9	2	32	1660	0.23	0.58	4.32
3	41	2.9	1	1	6	1	32	1530	0.25	0.41	2.95
4	40	3.1	Trace	1	5	1	33	1550	0.22	0.42	3.26
5	62	2.4	Trace	1	4	1	56	1720	0.19	0.23	2.65
6	64	2.2	Trace	Trace	6	1	56	1690	0.27	0.34	2.98
7	61	2.9	Trace	1	4	1	55	1840	0.12	0.27	2.21
8	65	2.0	Trace	Trace	4	1	59	1650	0.16	0.23	2.33
Mean	55.5	2.54	Median	Median	5.4	1.13	47.6	1651.3	0.225	0.363	3.000
SD	11.4	0.58	Trace	—	1.69	0.35	12.7	102.5	0.073	0.12	0.665

Source: Ref. 118.

ghum beer itself contains only a small contribution to daily protein requirements. However, practically all essential amino acids are present in the beer (Table 14).

Because they are insoluble, much FAN leaves the beer with the dregs during straining, making the residue a valuable nutritional supplement (64). Home brewers feed the dregs to children or to livestock, and factory brewers sell their residue as commercial livestock feed. Given the valuable nutritional properties of the strained solid material, some nutritionists have suggested that it could form the basis for production of infant or weaning foods. Both sorghum beer and its dregs have a protein value superior to that of the grain from which it is produced.

Overall, the case for biological ennoblement seems well founded. It rests on the increases in niacin and riboflavin that occur during malting and

Table 14 Amino Acid Composition of Sorghum Beer

	Sorghum beer						
	Sample No.						NAS[a] high-quality protein
Amino acid	1	2	3	4	5	Mean	
Aspartic acid	8.7	8.0	8.3	8.2	8.6	8.4	NE[b]
Threonine	3.8	4.0	4.0	3.9	3.9	3.9	3.5
Serine	4.2	4.5	4.4	4.3	4.1	4.3	NE
Glutamic acid	16.0	16.5	15.5	16.6	16.6	16.3	NE
Proline	8.5	8.6	9.4	8.2	8.4	8.6	NE
Glycine	4.5	4.0	4.3	4.0	4.5	4.3	NE
Alanine	8.5	9.2	8.9	9.7	9.3	9.1	NE
Valine	5.9	5.7	5.6	5.9	6.0	5.5	4.8
Cystine	Trace	0.7	0.6	0.7	0.8	0.6	2.6
Methionine	1.7	1.7	1.7	1.6	1.5	1.6	
Isoleucine	4.6	4.6	4.5	4.4	4.4	4.5	4.2
Leucine	10.9	11.9	11.2	11.4	10.2	11.1	7.0
Tyrosine	3.4	3.2	3.2	3.0	3.2	3.2	7.3
Phenylalanine	4.9	5.0	5.1	5.0	5.3	5.1	7.3
Histidine	2.6	2.6	2.7	2.7	2.3	2.6	NE
Lysine	4.8	4.1	4.4	4.2	4.6	4.4	5.1
Ammonia	2.7	2.0	2.0	2.0	3.4	2.4	NE
Arginine	4.3	3.7	4.2	4.2	3.4	4.0	NE

Note: Results are the mean of duplicate analyses.
[a] National Academy of Sciences (1974) pattern of high-quality protein for human consumption.
[b] NE = nonessential amino acid.
Source: Ref. 120.

brewing as well as the increase in protein occurring during malting and mashing.

2. Effect of Alternative Ingredients on Nutrition

Research has shown that the composition of ingredients used to produce indigenous beers plays a crucial role in the nutritional value of the final product. Maize contains lower concentrations of B vitamins than does sorghum, and any refined grain furnishes fewer nutrients than those ground with husk and all (7,98). For both of these reasons, observers have documented with great concern how the development of high-diastatic malt has allowed brewers to progressively substitute maize grits for sorghum malt in factory brewing. As Table 15 shows, the ratio of sorghum (malt plus grain) to maize has fallen drastically over the past 30 years among South African factory brewers of sorghum beer. The rapid displacement of sorghum products by refined maize resulted in a deterioration of the B-vitamin content of factory brews. Although the malt-to-adjunct ratios as well as the B-vitamin content have stabilized since about the mid-1960s, a move to brewing with α-amylases will undoubtedly result in further erosion in the sorghum malt and, hence, in the B-vitamin content of factory-brewed sorghum beer. Recent studies have documented this erosion, which is especially important in the case of thiamine (75,118) (Table 15). Table 5, which allows a comparison of factory-brewing recipes with (in Botswana) and without (in South Africa) commercial α-amylases and lactobacilli, suggests that sorghum-to-maize ratios could drop a further 35% in South African factory brewing.

Beers with the highest total solid content show the highest mineral and trace element concentrations. As Table 16 shows, 1 L of sorghum beer can supply a large part of the adult male recommended daily allowances of Fe,

Table 15 Trends in Vitamin Content and Sorghum-to-Maize Ratio in South African Factory-Brewed Sorghum Beer

Year	Average vitamin content of factory-brewed sorghum beer			Ratio of sorghum to maize ingredients
	Thiamine	Riboflavin	Nicotinic acid	
1954	153	95	494	4.0
1959	80	34	194	1.15
1963	32	36	189	0.8
1977	24	39	293	0.8

Source: Refs. 1, 11, 32, 98, 112, and 118.

Table 16 Mineral and Trace Element Composition of Five Samples of Factory-Brewed Sorghum Beer, South Africa 1987

	Minerals (mg/L of beer)								
Sample No.	Cu	Fe	Zn	Mn	Ca	Mg	K	Na	P
1	0.14	1.7	1.4	1.5	56	123	286	10	181
2	0.11	0.9	1.0	0.8	22	69	145	8	96
3	0.17	1.2	1.4	1.1	25	96	203	7	146
4	0.13	1.0	1.3	1.0	33	71	180	8	102
5	0.44	5.3	3.7	3.2	128	340	568	12	565
Mean	0.20	2.0	1.8	1.5	53	140	276	9	218
RDA[a]	2.5	10.0	15.0	3.8	800	350	3750	2300	800
% Contribution	8.0	20.0	12.0	39.0	7	40	7	0.4	27

[a] RDA = recommended dietary allowance (mg) for an adult man, 23–50 years of age.
Source: Ref. 120.

Mn, Mg, and P. Because the beer contains no detectable phytate, it appears that practically all minerals in the beer are available to the consumer.

Home brewers, because of the inputs they use, produce a beer with several nutritional advantages over factory-brewed sorghum beer. First, home brewers use a higher ratio of sorghum malt to grain adjunct (Table 5), and unlike factory brewers, they frequently use unrefined sorghum or millet rather than refined maize meal as adjunct. Hence, home brewers deliver both more sorghum and more unrefined cereal products in their beer. Moreover, home brewers' crude strainers (Figs. 16 and 17) cannot remove as much solid material as the factory brewers' centrifuges and mechanical strainers (Fig. 31). Consequently, home brewed SMM beers likely contain more calories, more B vitamins, more protein, more minerals, and more trace elements than do unfortified factory-brewed substitutes.

3. Human Assimilation of Nutrients

The availability of nutrients to humans is a third factor affecting the nutritional quality of SMM beers, and one that has only recently received close attention. Because many of the key nutrients found in SMM beers are contained within living yeast cells, they are not all available to humans. Tables 17 and 18 summarize recent work by Van Heerden (nee Zammit) on this question. Yeast cells contain about 90% of all thiamine in sorghum beer and about half of the other two B vitamins, riboflavin and niacin (123). As a result of rapid uptake by yeasts, the B-vitamin content, especially thiamine, in the soluble fraction of the beer drops drastically.

Table 17 Availability of B Vitamins in Factory-Brewed Sorghum Beer, South Africa 1978–1979

	Percent of vitamin in soluble fraction of sorghum beer	
B Vitamin	Normal	Pasteurized
Thiamine	9	76
Riboflavin	49	69
Niacin	52	74

Source: Calculated from Ref. 121.

Pasteurization can improve B-vitamin availability substantially. As Van Heerden's work demonstrates, pasteurization of the beer results in killing a large proportion of yeast cells, thereby making the B vitamins they contain available to human consumers of the beer. Hence, pasteurization could play an important role in enabling SMM beers to deliver the full value of B vitamins that they can potentially offer to human consumers. Van Heerden concludes that sorghum beer still offers a valuable nutritional supplement to

Table 18 Vitamin B Content of Untreated and Pasteurized Sorghum Beer Samples Supplemented with 1 mg/L Thiamine, 1 mg/L Riboflavin, and 15 mg/L Nicotinic Acid

	Days after vitamin addition	Untreated sorghum beer plus B vitamins		Pasteurized sorghum beer plus B vitamins	
Vitamin		I[a]	S[b]	I	S
Thiamine	0	32	68	14	86
	3	81	19	17	83
	6	82	18	17	83
Riboflavin	0	34	66	18	82
	3	26	74	22	78
	6	26	74	22	78
Nicotinic acid	0	27	73	15	85
	3	36	64	15	85
	6	35	65	16	84

[a] I = insoluble fraction.
[b] S = solube fraction.
Source: Ref. 122.

consumers, but one that could be substantially enhanced by improved brewing procedures such as pasteurization (75,121).

Vitamin fortification would also improve the B-vitamin contribution of sorghum beer. Recent experiments have shown that enrichment with thiamine concentrations of at least 2.5 mg/L are required for the thiamine loss to be less than 30% (123). Because thiamine deficiency is commonly found among consumers of relatively large amounts of alcohol, the availability of this vitamin in sorghum beer is of primary importance to consumers of volumes in excess of 2 L/day (124). In a study of thiamine deficiency in black male hostel dwellers, Van der Westhuizen et al. (125) found that this hypovitaminosis was related to both inadequate diet and excessive alcohol consumption. They conclude that fortification of sorghum beer with thiamine might reduce this deficiency and the cost would not significantly affect the price of the beer. Fortification with vitamin C also appears feasible, as concentrations are not reduced by fermentation and storage and has no adverse effect on the taste of the beer (126).

IV. PROSPECTS FOR THE FUTURE

A. Demand

The prospects for an indigenous beer industry depend to a large extent on the evolution of demand. Demand, in turn, is a function of population growth, incomes, income distribution, pricing policy relative to competing products, and shifting tastes and preference. The trends in sorghum beer demand are summarized in Table 19 for South Africa, the country with the highest per capita income in all of Africa.

Continentwide, all available evidence points to a substantially increasing demand for SMM beer over the medium run, probably over the next 20–30 years, followed by an eventual decline. These predictions are founded on several commonly observed demand parameters. First, the limited evidence on income elasticity of demand indicates that, at least at low-income levels, elasticities are positive; that is, as incomes grow, so does demand for SMM beer (16,128), hence, the likelihood of substantial growth in demand over the medium run. It is probable that the demand for packaged beer will grow especially fast, given not only its convenience but also the modern image the attractively decorated cartons convey (16).

However, several features of typical consumption profiles suggest that demand will peak and eventually decline over the long run. Many studies indicate that it is the young, the urban dwellers, and the very high-income groups that switch first from indigenous to clear beer (16,34,45,128). Thus, the parallel trends of growing urbanization and increasing drinking by young

Table 19 Trends in Sorghum Beer Consumption, South Africa (in Millions of Liters)

	Volume of sorghum beer consumed		
Year	Home brew	Factory brew	Total
1946	—[a]	33	—
1950	—	55	—
1955	—	159	—
1960	1552	249	1771
1965	1491	565	2056
1970	1017	985	2002
1975	1057	1098	2156
1977	1306	1004	2310
1984	1800	1015	2815
1985	—	990	—
1989	—	1030	—

[a] Indicates information not available.
Source: Refs. 1, 6, 11, and 127.

adults will combine to dampen sorghum beer consumption. Moreover, the income elasticity of demand for indigenous beer appears to turn negative beyond some moderate level of income, about $4500 per year in Botswana, the only country for which we have information (16). Thus, it is not surprisig that in South Africa, where incomes are both high and increasing; officials have predicted only a 1% increase in SMM beer consumption between 1985 and 1989, well below the rate of population growth (Table 19). They project an increasing shift in demand for more expensive Western-style clear beers, given the obvious consumer preference for clear beer as opposed to wine and spirits (Table 20). Overall, demography, rapid urbanization, and gradual income growth will, in the long run, lead to a shift from sorghum to clear beer. However, the data from South Africa, the country with by far the highest per capita income on the continent, suggest that any eventual declines in indigenous beer consumption will be far in the future elsewhere on the continent. Thus, other countries, where incomes are much lower, can in all likelihood expect strong continued growth over the next several decades.

Estimates of the price elasticity of demand for indigenous beers are extremely rare, although one very rough calculation, based on data from Zimbabwe, places it about -0.4 (16); that is, a 1% increase in price will lead to a 0.4% drop in demand. Thus, demand appears to be only moderately price responsive. Even so, given the significant nutritional benefits of sorghum, millet, and maize beers compared to those of clear beer (64), it will be prudent

Table 20 Annual Consumption (%) of Wine, Beer, and Spirits by Black Men and Women in South Africa 1982

Annual amount consumed	Wine		Beer		Spirits	
	Men	Women	Men	Women	Men	Women[a]
Less than 26 liters	46	55	5	35	87	—
26–75 L	31	23	9	6	13	—
76–499 L	21	20	56	43	—	—
500 L or more	2	2	30	27	—	—

[a] Very few black women indicated that they consumed spirits.
Source: Ref. 129.

for governments to continue, as most do, to minimize taxation of SMM beers relative to lesser nutritional products consumed by wealthier consumers.

B. The Future of Production Technology

The future supply structure of the indigenous beer industry will depend crucially on which alternative factory brewers adopt as the next-generation production standard. Two options, pasteurization and production of sorghum beer concentrate, appear most promising for resolving the brewers' perennial principal problem of short shelf life. If research permits necessary refinements in pasteurization and if factory brewers adopt this as their production standard, consumers will enjoy important nutritional benefits in the form of increased B-vitamin availability. However, over the long run, factory brewing will likely become increasingly concentrated in large, mega-breweries; because product perishability, low value for weight, transport costs, and population density will no longer combine to determine optimal brewery sizes. Small factory brewers and home brewers, even in very remote regions, will face keen competition, and their marketshares will undoubtedly diminish.

On the other hand, a factory brewer shift to sorghum beer concentrate will afford substantial possibilities for enhancing incomes of existing retailers, small factories, and home brewers by enabling them to convert, if they wish to fermenter–retailers of factory-produced concentrate. Given the important employment and income distribution considerations associated with home brewing, a move to the concentrate would be highly beneficial in promoting employment, equity, and balanced regional economic growth (97). Important nutritional improvements—such as fortification with thiamine, vitamin C, and certain essential aminos—may be achieved under either factory-brewing technology.

Home-brewing technology, too, may be considerably improved in the next 10–20 years if the commercial malting technology developed in southern Africa can be transferred to regions where factory brewing has not emerged and where commercial malt is, consequently, not presently available. In western, central, and parts of eastern Africa, the introduction of commercial malting for home brewers holds considerable promise. Because it generates higher diastatic power than homemade malt, commercial malt allows home brewers to reduce considerably total grain required for production of a given quantity of beer (Table 5). At a national level, figures from Burkina suggest that country's total grain usage could be cut by about 5% without reducing sorghum beer or sorghum grain consumption (97). In addition, commercial malt's more consistent quality and standardized, premeasured packaging allows greater consistency in beer quality and, hence, improves home brewer competitiveness.

Thus, the two important features of supply-side evolution involve the transfer of trade malting technology outside of southern Africa, and the choice between beer concentrate and pasteurization of factory-brewed sorghum beer in eastern and southern Africa where the factory brewers currently operate. Each of these potential changes will have significant impact on incomes, employment, and nutrition. The stakes are large, given the importance of indigenous beers in the diets and the employment structure across the continent.

Government policy across the continent will be crucial in governing the future course of evolution in the SMM beer industry. The current call for devolution of South Africa's sorghum beer industry to the private sector offers both flexibility and uncertainty. There, as everywhere, retailing and production laws will affect both the distribution of income accruing to home and factory suppliers as well as the nature of the market served by indigenous beers. Tax regulations will affect market demand and, of course, research on brewing technology and its dissemination will continue to play a key role in shaping the future of the industry.

C. Contribution to New Biotechnology and Genetic Engineering

Given the low cost of sorghum beer, its important nutritional, and employment-generating properties, as well as the low-wage rates prevailing across much of the continent, the introduction of sophisticated, capital-intensive new technologies may be undesirable in many countries. Clearly, though, the transfer of what is a very simple, low-capital commercial malting technology outside of southern Africa promises to yield considerable benefits.

In addition, in the southern African countries where factory brewing is most fully developed, there is ample room for improving both fermentation

stages of the brewing process. During lactic acid fermentation, brewers currently control the activity of thermophilic lactobacilli by maintaining both a high temperature and a low pH. Further improvements of this fermentation may be achieved in three ways. First, further selection and adaptation may produce thermophilic *Lactobacillus* strains that produce the pure L(+)-lactic acid isomer in sorghum malt substrate. Second, the supply of lactic acid starter cultures to the industry in concentrated, frozen, or lyophilized forms will make it possible for factories to practice direct inoculation in a factory-based prefermenter. Third, genetic manipulation of lactobacilli may make several improvements possible—for example, (a) increased lactic acid production under typical substrate and temperature conditions; (b) the ability to produce amylases that will ensure starch conversion simultaneous to lactic fermentation at elevated temperatures (in the range of 50°C) but inhibit conversion and lactic fermentation at room temperature; and (c) synthesis of bacteriocins which are active against the typical contaminants, mesophilic lactobacilli, introduced by the final addition of conversion malt.

Alcoholic fermentation could potentially be improved as well by cloning favorable properties into yeast strains. Improvement in sensoric quality of the beer could be achieved by closer control of the type and quantity of metabolic products formed. For example, yeast mutants have been found that actually excrete thiamine during ethanol production (130). Efforts in this direction could potentially overcome one of the most important nutritional deficiencies of the product and obviate the need for thiamine fortification. In a similar vein, yeast strains may be selected for their ability to readily release other B vitamins and even essential amino acids into the beer, thus making it available for human nutrition.

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7

Industrialization of Mageu Fermentation in South Africa

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I. INTRODUCTION

In southern Africa, mageu is a traditional sour maize beverage. It contains little or no alcohol, has a pH of about 3.5, and is popular among the black people of this subcontinent, where it may play an important role in the diet. Mageu is prepared by boiling a maize porride of about 8–10% solids into which, upon cooling, a small amount of wheat flour is mixed. In the traditional process, the wheat flour serves as inoculum of lactic acid bacteria, and a final acidity of about 0.4–0.5% (lactic acid) develops within 1–2 days at room temperature (1,2).

The term *mageu* (or *magou*) is synonymous with "amahewu" (Zulu) or "amarehwu" ("aramrewu") used by the Xhosa people. Other synonyms include "emahewu" (Swazi), "metogo" (Pedi), "machleu" (Sotho), and "maphulo" (Venda) (3). Mageu and the shortened Zulu version, mahewu, appear to be the most commonly used terms for this product.

Although mageu is considered typical for southern Africa, it may be related to traditional lactic fermented cereal products from Nigeria, such as

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ogi and uji. These products may contain maize as one of the ingredients and are described as "yugurtlike products" (4). (Steinkraus, 1983).

II. CONTRIBUTION TO THE DIET

Mageu is consumed mainly as a beverage during the course of a normal working day. Its popularity during the hot summer months is attributed to its refreshing sour taste and thirst-quenching properties. This also explains the strong decrease in the demand for the commercial product during the winter months and also during sudden cold weather spells in the summer. In addition to its use as a beverage, a thickened fermented "porridge" product with a higher solids concentration may have played a role in the traditional diet of the African people in earlier times.

Being a cereal-based product, conventional mageu has relatively low biological value, especially with respect to the proteins (7–9% of the total solids), which are deficient in essential amino acids such as lysine. One liter of mageu (containing 10% solids) produces about 4000 kJ, derived from the carbohydrates in the maize meal (5). If mageu is consumed by laborers as a part of a fully adequate diet, its nutritional value is of relatively little importance and no need for its fortification exists (2,6). However, in situations where laborers are undernourished or where conditions of malnutrition or an insufficient diet exist, fortification of mageu may solve these problems. This need is especially acute for people who rely on mageu as a traditional midshift beverage and who often do not pay sufficient attention to their nutritional needs and even commence work without having eaten a substantial meal (7). It has been shown that mageu can serve as a vehicle for supplying additional nutrients (2). The protein content as well as the biological value may be enhanced by the addition of skim milk powder, whey protein, soy flour, food yeast, or fish meal flour (6). One liter of this fortified product will provide about 30% of the daily assimilable protein requirement of an adult human male, as well as between 20% and 30% of the requirement for thiamine and nicotinamide (8,9).

Information obtained by interviewing 150 randomly selected rural women in Zimbabwe with children under 5 years old showed that 97% of the women had knowledge of some traditional fermented foods. Mageu (mahewu) was the most common fermented food known by the women, and most of the women (94%) indicated that mageu was consumed by all family members. The majority of these women gave mageu to infants from the age of 4 months. With the exception of mageu, other fermented foods were not given to infants frequently. A practical storage period of 1–3 days was

generally reported. Altogether, the study showed that mageu has a good potential for use as weaning food (10).

Mageu appears to be not only a safe and nourishing food for the young and old, but observations indicate strong protective features against different food-borne pathogens. This has been shown convincingly, for example, for the inhibition of *Bacillus cereus* in actively fermenting mageu prepared by controlled fermentation after inoculation by back-slopping, and in which the pH dropped to < 4.0 within 24 h (11).

During a recent outbreak of cholera in Burundi and Zimbabwe, a case-control study has been conducted to identify risk factors and potential control measures. One particular food, mageu, showed definitive protective properties. In laboratory studies, mageu has inactivated a variety of enteric bacterial pathogens, including *Campylobacter jejuni*, and strains of *Salmonella*, *Shigella*, and *Aeromonas* (12,13). More information may be obtained from the website www.cdc.gov/epo/mmwr/preview/mmwrhtml/00020745.htm.

According to Nceba Gqaleni, project leader and director of the Center for Occupational and Environmental Health at the University of Natal, Durban (South Africa), mageu (or amahewu, as it is called in Zulu) has an important role and place in biotechnology and business community development and in health promotion. A comprehensive review of mageu (mahewu) and other traditional fermented foods and their role and importance in society has been published by Gadaga et al. (14).

III. ANNUAL PRODUCTION AND CONSUMPTION

The consumption of mageu is seasonal and peak sales are registered during the summer months from October to March. The winter months can bring a decline of 50–60% in mageu consumption, with the worst months May through July. The urban and suburban markets are being supplied by the following principal producers of mageu: Mageu No. 1 (as far as known, the largest producer), Phuzimpilo (a franchise group, with factories known under different company names), Clover (also including the former "Creamline" and producing banana- and pineapple-flavored mageu, which proved to be the most popular flavors), and Madu. Another company, Awethu Beweries, has been listed on the Johannesburg Stock Exchange since 1997. It was founded in 1997 and has as major clients in the mining and catering industries, where deliveries are mainly in bulk, comprising 20-L drums and 2-L plastic bottles, through its franchise outlets in Gauteng townships. The Phuzimpilo brand is manufactured under a license and, as a group, it is the largest supplier of mageu.

The total market for mageu was about 146 million liters in 1984, of which about 97 million liters was packed and 49 million liters delivered in bulk. Bulk sales are mainly to mines and other industries where large numbers of black people are employed and provided with daily meals at canteens (15).

Mageu is not consumed on a regular basis by all black people in southern Africa. Laborers in the urban communities may consume up to 200 L of commercially produced mageu per year. By contrast, people living in the country will provide for their own needs by a home-fermented product. The annual per capita consumption of the commercial product can be estimated at 12–14 L per black adult.

IV. HISTORICAL BACKGROUND

Maize is indigenous to Central and South America and was used by the Aztecs of Mexico as a staple food. Although maize was extensively used and cultivated in Africa for centuries, it is not indigenous to this continent. It may have been introduced to southern Africa before the establishment of a Dutch colony in 1652. Portuguese vessels calling at the Cape for water probably left maize grain with the Cape colonists or even earlier with the natives (16). The Afrikaans word "mielie" for maize seems to be derived from the Portuguese word "milho," meaning grain. However, no documentation prior to 1900 on "sour," fermented maize could be found.

In addition, it is of interest to note that the earliest known description of a (sour) fermented maize beverage was given by Dampier in 1676 [quoted by Burtt-Davy (16)] for a product of Mexico:

> This country is very fruitful, yielding plentiful crops of maiz, which is their cheapest subsistence. After it is boiled, they bruise it in such a rubbing stone as chocolate is ground on. Some of it they make into small thin cakes, called Tartilloes. The rest is put into a jar till it grows sour; and when they are thirsty, mix a handful of it in a Callabash of water which gives it a sharp, pleasant taste, then, straining it through a large Callabash pricked full of small holes to keep out the husks, they drink it off. If they treat a friend with this drink, they mix a little honey with it; for their ability reaches no higher. And this is as acceptable to them as a glass of wine to us.... It is so much esteemed by the Indians, that they are never without some of it in their homes.

One of the earliest known descriptions of mageu was given by Bryant in 1907 (17):

> Amahewu is common to mealie porridge (i Palishi) to which, when cool, a small quantity of [wheat] flour [or other ferment] has been added.

> After fermentation has well set in, perhaps after 24 hours, it is eaten cold, and is deemed an excellent substitute for Amasi.

("Amasi" is a sour milk product common to the eastern parts of southern Africa.) Wheat is alien to the traditional diet of the black people in southern Africa, and Bryant therefore regards mageu as a "modern invention" (18).

Quinn (17) also describes mageu as a relatively new addition to the diet of the black African people. He considers it alien to the traditional Pedi diet and states that it was "recently introduced by labourers returning from the reef (gold mines) where this beverage plays an important role in the feeding of mine labourers." According to Quinn, mageu has gained wide popularity among the Pedi people.

V. SUBSTRATES

Because wheat and even maize are not indigenous to Africa, the preparation of "typical" mageu can only feature in recent history, perhaps dating back to the second part of the 19th century. Lactic-fermented sour milk products are well known and constitute an important part of the diet for many African people. This, as well as the fermentation of cereals indigenous to Africa, can most probably be dated back far beyond the written history of the subcontinent. Sorghum or kaffir corn (*Sorghum caffrorum* and *S. vulgare*), as typical indigenous products, may well have served as a substrate four a sour (lactic-fermented) beverage in ancient times. Together with pearl millet or m'nouti (*Pennisetum spicatrum*), sorghum may have been the staple foodstuff of temperate southern Africa (16). Sorghum-based fermented beverages are still common among the Pedi people (17). Today, malted and unmalted sorghum (partly substituted with maize) are fermented in a two-stage process to sorghum beer. The first stage typically involves a lactic fermentation. According to Platt (19), even cassava and plantains can be used for this process.

Traditionally, mageu is prepared by boiling a thin maize porridge containing 8–10% solids. Depending on the maize meal concentration, a fairly thin gruel or, thick mash is formed, which is then cooled to room temperature (25–30°C). Thorough cooking for longer periods of time will enhance thickening as a result of gelatination. After cooling, a small quantity of wheat flour or bran (2–5% of the maize meal) is added as "inoculum" and thoroughly mixed into the porridge. The mixture is then left standing in a warm (sunny) place to turn sour. The lactic acid bacteria constitute heterofermentative and mesophilic homofermentative strains that are capable of rapid growth in this substrate. Sugars derived from the enzymatic hydrolysis

Table 1 Average Annual Consumption of Industrial Food Components by the South African Mageu Industry

Industrial food component	Tons	% of total
Water	125,560	86.0
Maize	11,680	8.0
Sugar	7,592	5.2
Wheat flour	220	0.2
Additives[a]	146	0.1
Milk powder[b]	146	0.1
Others[c]	584	0.4

[a] Flavorings.
[b] Used only for cream-flavored mageu.
[c] Various chemicals.
Source: Ref. 15.

of starch are fermented to lactic acid and other metabolic products such as CO_2 and acetic acid or ethanol.

For the commercial production of mageu, maize meal is still the principal component. Between 11,000 and 12,000 tons of maize flour is used annually by the mageu industry (see Table 1).

A concentration of 8% maize flour is considered as the optimum for the beverage. After cooking at 85°C and cooling to about 50°C, between 0.1% and 0.2% wheat meal ("bread flour") and 1–2% sugar are added. Dibasic calcium phosphate and permitted edible preservatives (e.g., benzoic acid, potassium sorbate, calcium propionate) may also be added. The final product should comply with standard specifications (20), according to which the minimal total solids content should be 8% (m/m), not more than 0.25% ethanol (m/m) should be present. Other additives, including flavoring and coloring agents, should comply with the relevant requirements of the current Foodstuffs, Cosmetics and Disinfectants Act. These additives, including an additional 2–3% of sugar, are mixed into the product after fermentation. The product must be free from *Escherichia coli* and *Clostridium*.

VI. MAGEU PRODUCTION IN ANCIENT TIMES AND AS A COTTAGE INDUSTRY

In private households, mageu is produced by spontaneous fermentation of a readily available substrate maize meal, which is boiled into a thin gruel containing 8–10% solids. Maize meal is the main component in the diet of most

black people in southern Africa. However, apart from the possible introduction of maize to coastal dwelling tribes by merchant vessels during the 17th century, the large majority of the black people (living in the northern parts of southern Africa) only came to know this foodstuff during the 19th century.

This is obviated by historical documentation indicating the role missionaries played in introducing maize in areas such as (formerly) British Bechuanaland, Griqualand West, Swaziland, and Zululand (16). The traditional method of mageu preparation is schematically shown in Fig. 1 and further illustrated in Figs. 2–5.

VII. INDUSTRIAL PRODUCTION

During the 1960s, the first attempts were made to produce mageu on an industrial scale. A blend of pregelatinized maize powder and wheat flour was placed on the market by Jabula Foods Ltd. early in 1964. This easily fermentable product was well accepted in the institutional market where limited cooking facilities were available. During the period 1967–1970, a beginning was made by industrial concerns to produce liquid mageu fermented at room temperature. Starters were pitched daily from one ferment to the next. The product was packed mainly in 1-L cartons; it had a limited shelf life and was meant for immediate consumption (18).

As a result of the increasing urbanization of the black people, a rapid growth in the mageu market was experienced during the 1970s. After remaining fairly static during the late 1970s, mageu sales took off in 1980 and have shown steady growth since then (Table 2).

Various flavors of packaged mageu are available on the market. The "plain" mageu, however, is still the most popular, followed by the flavors banana, cream, pineapple, strawberry, and guava.

During the early 1970s, attempts were made to use mesophilic (30°C) fermentation processes for commercial production of mageu. The "initial" method (Fig. 6) used by Jabula Foods Ltd. produced a "thick and slimy" porridge as a result of immediate sterilization after mixing (18). A more acceptable product was obtained by a "modified" process, involving a soaking period of 1.5 h before sterilization (Fig. 7).

A team of scientists from the Council for Scientific and Industrial Research (CSIR), Pretoria, evaluated different parameters considered to be important for the production of mageu on a commercial scale (see also Ref. 21). The major findings were as follows:

- The use of a pure culture of *Lactobacillus delbrueckii*, adapted to the maize substrate, will improve the manufacturing process.

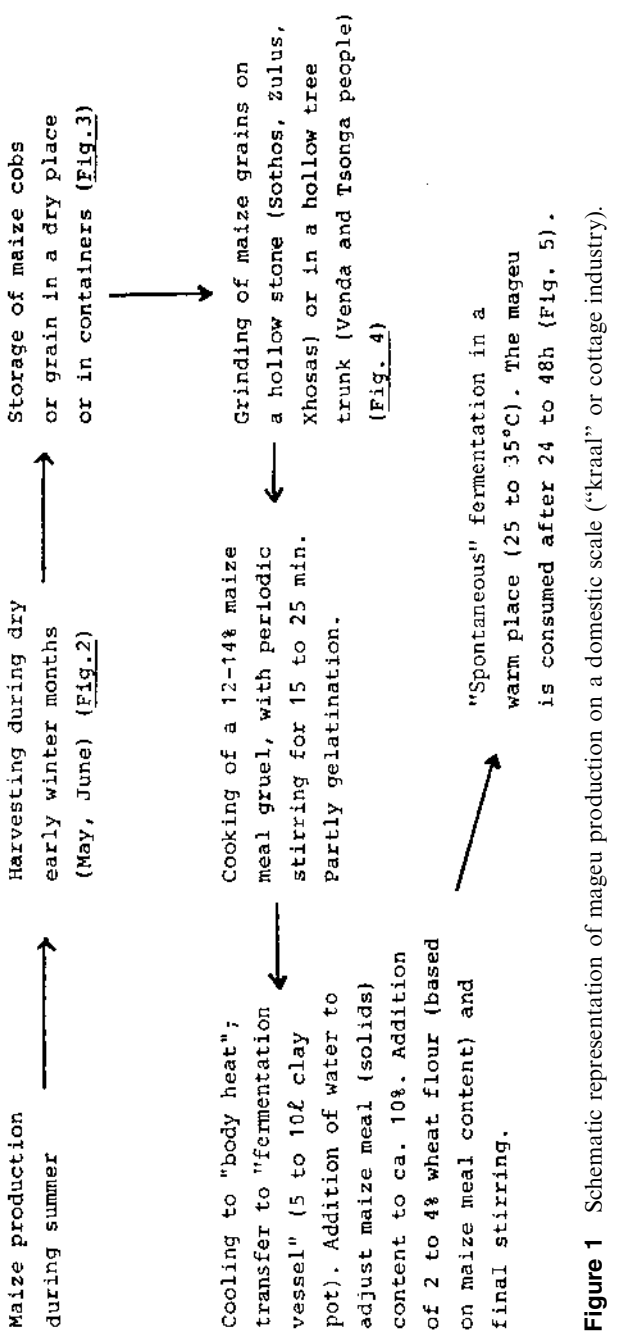


Figure 1 Schematic representation of mageu production on a domestic scale ("kraal" or cottage industry).

(a)

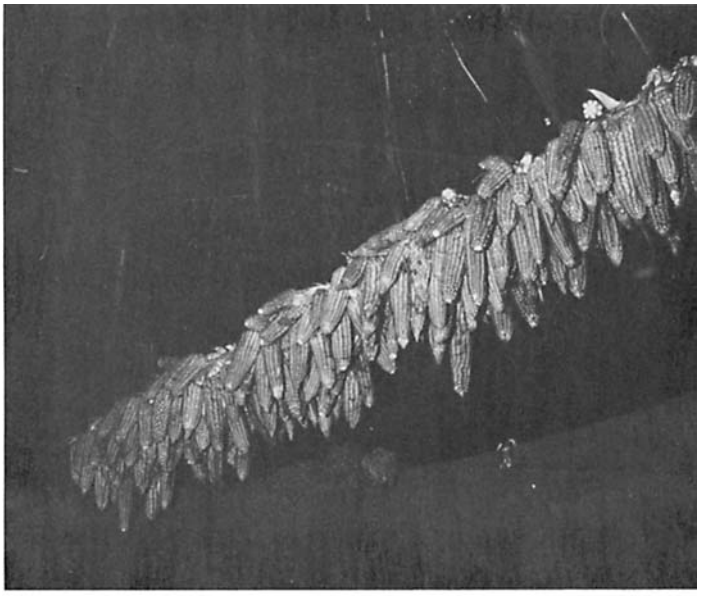
(b)

Figure 2 Threshing of maize after harvesting: (a) by central Sotho (Pedi) and (b) by Tswana (Ngwato) near Palapye, Botswana. (Photographs courtesy of National Cultural History and Open Air Museum, Pretoria.)

(a)

Figure 3 Storage of maize cobs in (a) a tree (Swazi people: Mananga, KaNgwane) and (b) a hut (Venda people: Khakum, Vendaland) and of maize grain in dry containers in (c) a "dulu" (Venda) or (d) "sefala" (Tswana, Phiring). (Photographs courtesy of National Cultural History and Open Air Museum, Pretoria.)

- The manufacturing process could further be improved by (a) adding either skimmed milk powder or whey powder, (b) by adding wheat bran instead of wheat flour, and (c) by using a incubation temperature of 50–51 °C.
- Mageu must have a clean, sour taste, produced by the fermentation of glucose (and other available sugars) to lactic acid. The presence of volatile acids and/or yeastlike flavors is not preferred.



(b)

(c)

Figure 3 Continued.

(d)

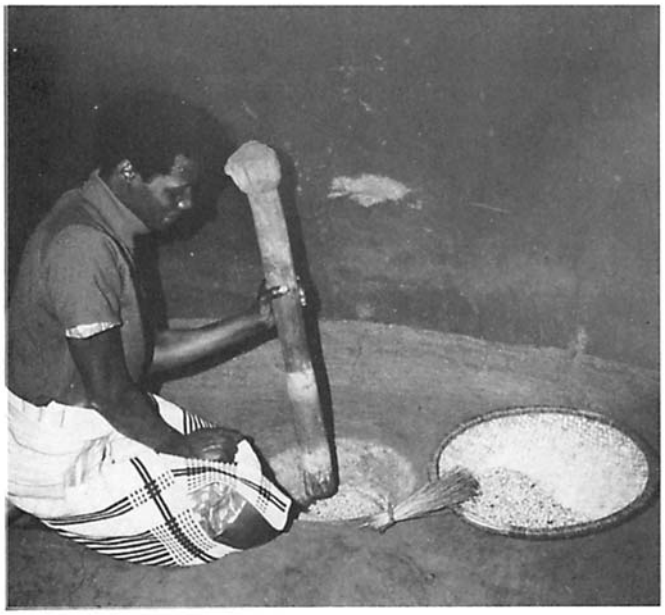
Figure 3 Continued.

- The additional of sugar does not promote acid formation.
- The majority of consumers prefer fine-textured maize flour.

A flow diagram of a modern mageu-producing plant is shown in Fig. 8. The "porridge" is prepared by first mixing 8% of maize meal in water at ambient temperature (Fig. 9) and then pumping this mixture to the stainless-steel cooking pots (2000–3000 L) where it is steam-heated to 85–90°C for 20–

Figure 4 Grinding of maize grains to maize meal (a) on a grinding stone ("tlapasila") in Lebowa (northern Sotho, Molepo), (b) in a pounding block out of wood or stone ("mutuli wa fhasi") in Vendaland (Thamba), or (c) in a wooden pounding block ("tsuri") in Gazankulu (northern Tsonga). (Photographs courtesy of National Cultural History and Open Air Museum, Pretoria.)

(a)



(b)

(c)

Figure 4 Continued.

30 min (Fig. 10). The substrate is cooled to about 50°C before it is transferred to the bioreactors (Fig. 11) (stainless-steel or fiberglass tanks) where 1–2% sugar and 0.1–0.2% wheat flour is added, followed by 7–12% of a thermophilic *Lactobacillus* starter culture. Initial starter preparation takes place on a laboratory scale, whereupon porridge at 50°C in the prefermenter (Fig. 12) is inoculated with 10% of an active culture. During the following 18–24 h, the temperature gradually drops to about 30°C and the pH reaches 3.0–3.4. Production cultures for large-scale inoculation are prepared in 1500–2000-L stainless-steel bioreactors (Fig. 13). The production stage of mageu is carried

(a)

(b)

Figure 5 Large clay pots typically used for sorghum beer brewing and for mageu fermentation: (a) in Vendaland (called "nnkho") and (b) in Lebowa (northern Sotho: Kgaga) (called "motsegana"). (Photographs courtesy of National Cultural History and Open Air Museum, Pretoria.)

Table 2 Consumption of Packaged Mageu in South Africa

Year	Sales (million liters)	% Change from previous year
1980	80.0	—
1981	82.4	+3.0
1982	95.0	+15.3
1983	96.0	+1.0
1984	97.0	+1.0

Source: Ref. 15.

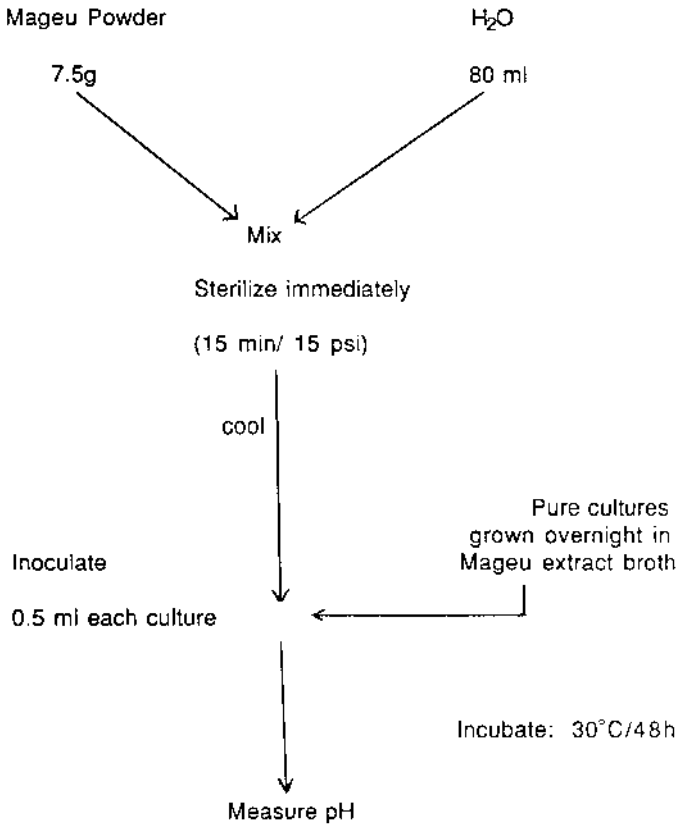


Figure 6 Flow diagram of the "initial" small-scale mageu process used by Jabula Foods Ltd. (From Ref. 18.)

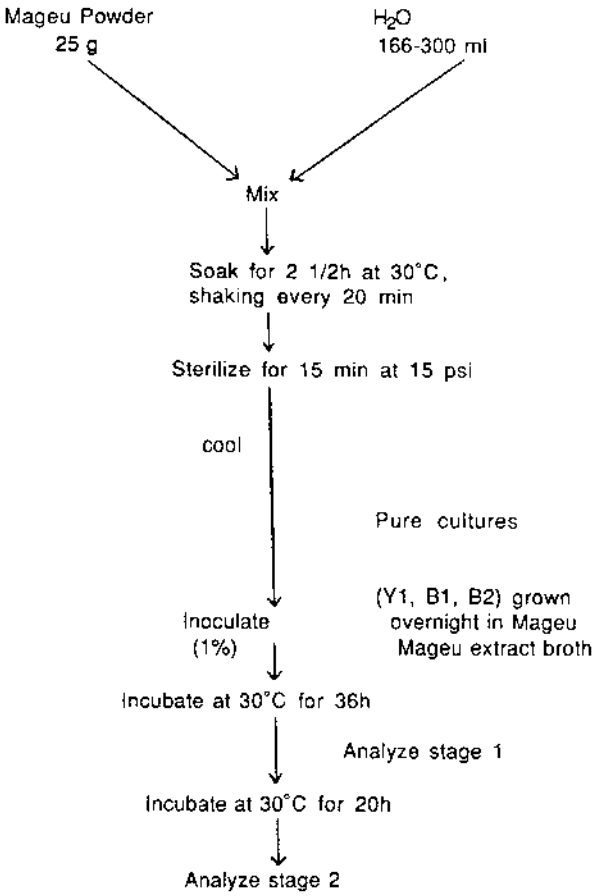


Figure 7 Flow diagram of "modified" mageu process used by Jabula Foods Ltd. (From Ref. 18.)

out in bioreactors with capacities ranging from 3500 to 10,000 L. After inoculation at 50°C with 3–5% of an active culture and thorough mixing, the lactic fermentation takes place under static conditions. Because no more heat is applied, the temperature gradually drops to about 30°C. Within 17–29 h, a pH of 3.4–3.8 should be reached. A sharper decline in temperature and culture activity is experienced during the winter months, when the final pH ranges from 3.5 to 3.9, as compared to 3.1–3.6 during the summer. This coincides with consumer preferences for a higher acidity (and better thirst-quenching properties of the product) during the summer months. When the desired pH

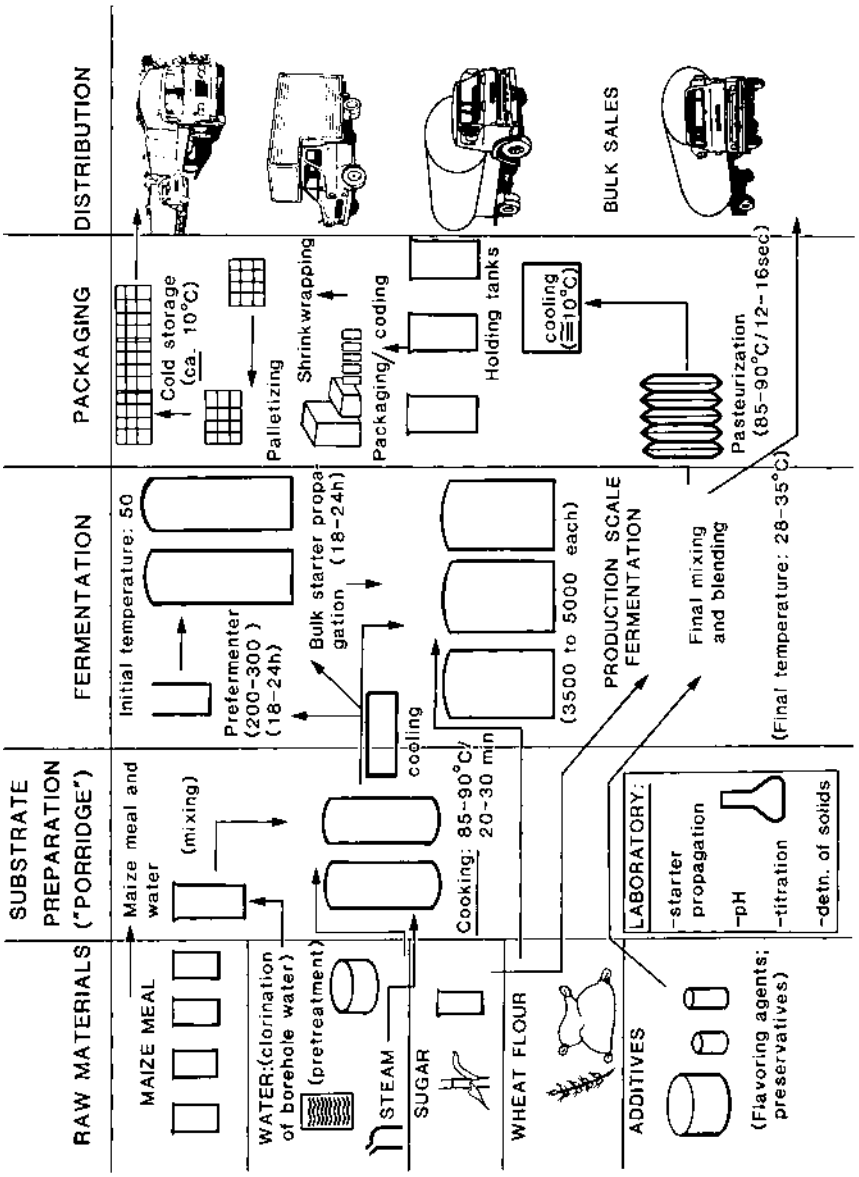


Figure 8 Flow diagram of a modern mageu fermentation plant.

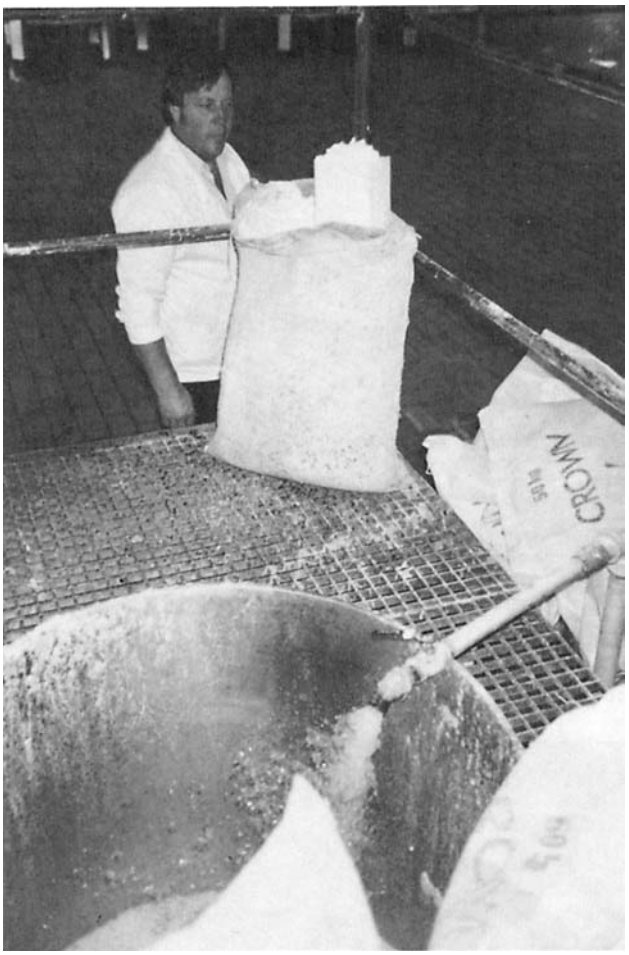


Figure 9 Preparation of maize meal "porridge" by mixing 8% maize meal in water at ambient temperature.

had been reached, about 2% sugar (based on the total volume) is added. Additional components such as approved flavoring and even coloring agents may be used in the final blending of the product. Sodium benzoate, potassium sorbate, or calcium propionate are often used as preservatives, mainly to control yeast growth.

The final product may be pasteurized in order to obtain a reasonable shelf life for retail distribution. The pasteurized mageu is cooled to 10°C and

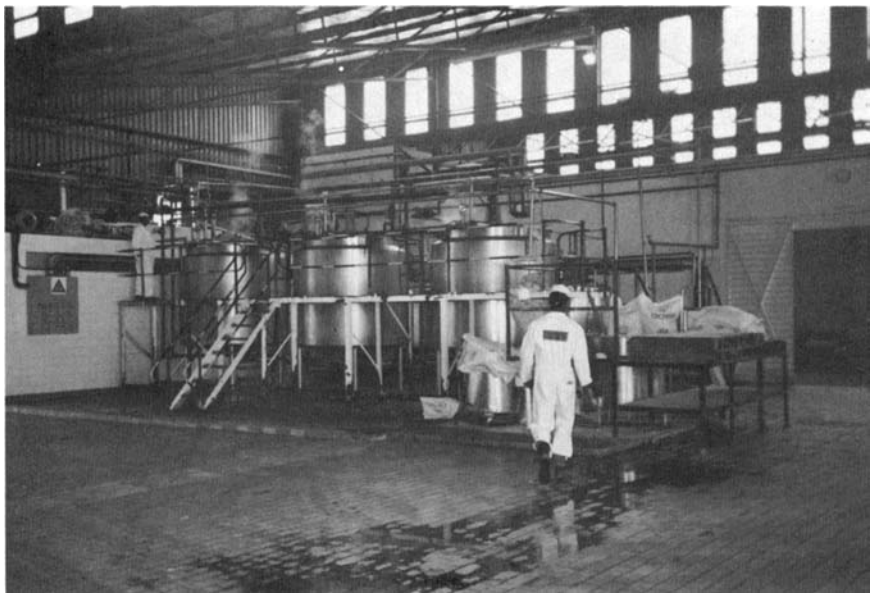


Figure 10 Cooking of maize meal porridge in stainless-steel vessels at 85–90°C for 20–30 min.

filled into 0.5- and 1-L cartons (Fig. 14). After sealing and encoding, the packages are palletized (Fig. 15) and stored at about 10°C (Fig. 16) until distribution in insulated trucks (Fig. 17). About one-third of the commercially produced mageu is distributed in bulk by tankers to mining companies and other industrial concerns.

VIII. INDIGENOUS VERSUS MODERN PROCESSING

Commercialization of mageu production probably went through different stages since the beginning of the 20th century. Initial steps toward large-scale production (e.g., on a mine compound) closely followed the domestic method of mageu processing (17) (see Fig. 1). The mainly mesophilic lactic acid bacteria introduced with the wheat flour multiply rapidly in the maize porridge. Normally, no sugar is added. The sugars, derived from amylolytic conversion of maize starch, are fermented to lactic acid, thereby reducing the pH to between 3.5 and 4.0 (0.4–0.5% titratable acidity). This traditional souring process is spontaneous and was soon found unreliable and unsatis-

Figure 11 Transfer of maize meal porridge (substrate) to stainless-steel or fiberglass bioreactors.

factory for large-scale industrial production of mageu. According to Van der Merwe et al. (9), the main reasons were as follows:

1. It took too long (36 h or more).
2. It proceeded too irregularly.
3. It permitted the development of undesirable bacteria, causing secondary fermentations.
4. Spoilage resulted from the metabolic products formed by these undesired bacteria.

In the late 1950s and early 1960s, attempts were made by Schweigart et al. (1) to introduce a thermophilic (50°C) industrial process for the preparation of mageu, by adapting *L. delbrueckii* and *L. bulgaricus* to maize meal as a substrate. According to Van Noort and Spence (18), this method did not find industrial application. This, however, should only be seen in the context of these two bacterial species. A thermophilic homolactic fermentation has become established for one of the more satisfactory fermentations for the commercial production of mageu. Perhaps in analogy with the use of single-strain *L. delbrueckii* cultures by Schweigart and co-workers (1,2,6,9,22–24), the conception of *L. delbrueckii* as the predominant thermophilic mageu

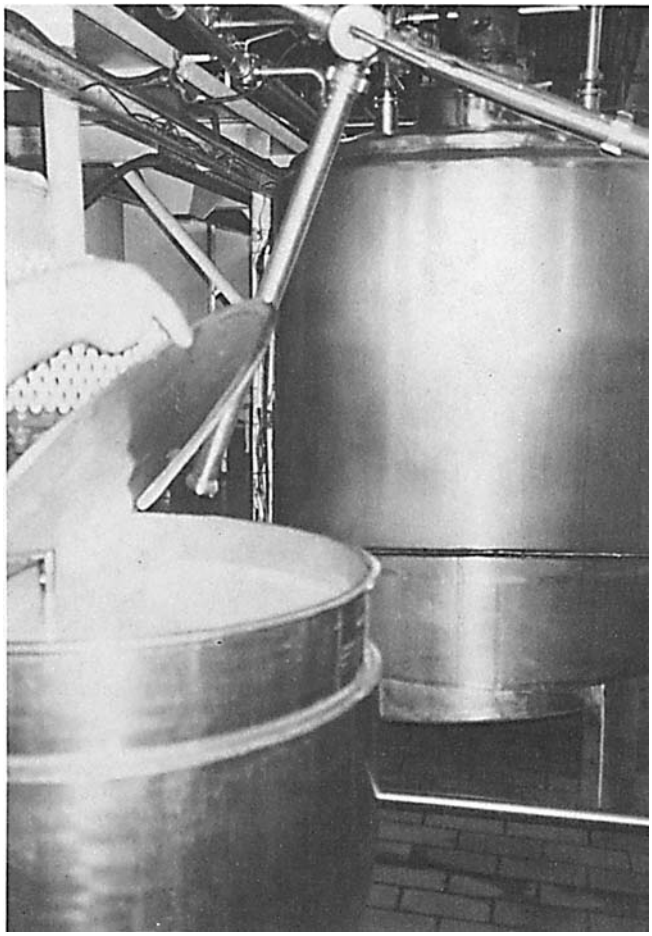


Figure 12 First stage of industrial-scale thermophilic starter preparation in a "prefermenter."

organism became generally accepted. However, no conclusive taxonomic evidence of the identity of the typical thermophilic mageu strains has been given yet. This is discussed later.

Apart from the use of specially selected thermophilic lactobacilli in modern (thermophilic) mageu processing, the addition of an easily fermentable carbon source and sweetener (sugar) is also typical of the commercial product at present. In the indigenous process, amylolytic enzymes introduced by means of flour and/or sorghum malt served this purpose (in addition to its function as an inoculum source of lactic acid bacteria).

Figure 13 Second stage of industrial-scale preparation of thermophilic starter in 2000-L bioreactors. Small-scale prefermenters in foreground.

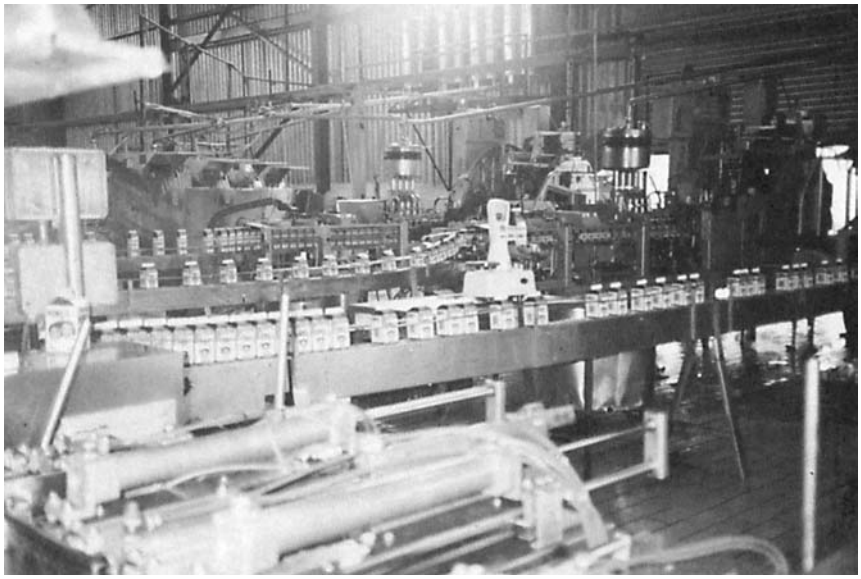


Figure 14 Packaging of the pasteurized mageu in 0.5- and 1-L cartons.

Figure 15 Palletizing of the packaged mageu.

Figure 16 Cold storage (10°C) of mageu in cartons before distribution.

Figure 17 Distribution of mageu in large trucks.

IX. CRITICAL STEPS IN MANUFACTURE/FERMENTATION

Mageu is presently produced by means of a standardized and well-controlled process. Although practically all commercial plants utilize a thermophilic fermentation process, information on several steps used by the largest group ("Phuzimpilo") is not available. Furthermore, one specific plant does not utilize a fermentation process, but instead relies on acidulation of the unfermented maize porridge. Qualitywise, this product does not seem to compare well with the fermented versions on the market. By way of generalization, the more important critical steps in modern-day thermophilic mageu processing may be summarized as follows:

1. Culture preparation, both on laboratory and factory scale. Optimal activity is reached only after (a) adaptation to the maize meal substrate, (b) regular transfer of subcultures, and (c) incubation at 50°C until a pH of 3.0–3.5 has been reached.
2. The "cooking" process whereby sufficient gelatinization is achieved in a maize porridge with about 8% solids.

3. Control of the large-scale fermentation process, especially concerning (a) pH and acidity, (b) the initial fermentation temperature, which should be maintained at $> 45°C$ for at least 2 h, and (c) prevention of airborne contamination in open bioreactors, especially in the lower temperature range around 30°C.
4. Final mixing and blending of the fermented product. Contamination may be introduced from the air and utensils and via additives.
5. The quality of the product prior to packaging. Although not all plants make use of a pasteurization process, it does contribute significantly to shelf-life extension. Because of its high viscosity, the typical mageu may easily be underpasteurized, leaving yeasts and fungal spores (in addition to thermophilic lactobacilli) to survive. Holding conditions (e.g., time, temperature, hygiene) before packaging are extremely critical and will play a major role in the shelf life of the product.
6. Maintenance of temperatures below 10°C during storage and distribution. Several plants do not have cold-storage rooms, and refrigerated trucks are used for distribution only in the rarest cases. It is, therefore, vitally important that the product reach the retail outlets within the shortest time possible after packaging.

X. MAJOR PROBLEMS IN INDUSTRIALIZATION

A. Technical

During the 1950s and 1960s, attempts were made to introduce a spontaneous mesophilic fermentation process for industrial preparation of mageu (discussed earlier). Although optimal acid production was at 35°C (1), it was still unpredictable and often insufficient for the commercial product. Furthermore, the dominant microbial population during fermentation was determined by their quantities present in wheat meal (1) and also by their adaptational abilities to the maize meal substrate.

The main disadvantages of the spontaneous fermentation process may be summarized as follows; (1,6,9,21): (Kriel and Wood, 1977):

1. Development of undesirable organisms, including yeasts, *E. coli*, and even clostridia.
2. Acidulation (lactic acid production) takes too long.
3. Acidulation does not proceed regularly and predictably as a result of nonlactic microbes and also because of heterofermentative lactic acid bacteria with lower lactic acid-producing abilities.

4. Production of volatile fatty acids (acetic and butyric) and other off-flavors.

Initial attempts to introduce single- and multiple-strain thermophilic fermentations apparently did not find industrial application either (18). Pure (authentic) cultures of *L. acidophilus*, *L. bulgaricus*, *L. delbrueckii*, and even *Streptococcus lactis* were adapted to the maize meal substrate at 51°C (*S. lactis* at lower temperatures) (1,9). In addition to maintaining the high temperature, factors such as wheat flour or bran, buffer salts (KH_2PO_4), and proteins (yeast extract, soya, whey powder, etc.) were found to be stimulatory to lactic acid production. *Lactobacillus delbrueckii* gave the best results under these conditions, although *L. bulgaricus* appeared satisfactory when whey was used as the additive (9). However, it seems as if pure *L. delbrueckii* cultures have not found general application in industry. The reasons may be as follows:

1. Dependence of the "adapted" cultures on added growth factors for optimal metabolic activities.
2. By virtue of their strict thermophilic nature, the inability to grow actively at reduced temperatures (25–35°C) was reached during later stages of the industrial process.
3. Commercial availability of "natural" thermophilic *Lactobacillus* starter cultures, adapted to a relatively simple maize meal (porridge) substrate. (These lactobacilli, which differ from typical *L. delbrueckii*, are discussed later.)
4. The inability of manufacturers to maintain active and pure lactic acid producing cultures.

Spoilage and restricted shelf life is another problem associated with commercially produced mageu. Among these spoilage microbes (discussed later), the yeasts typically predominate and may reach 10^7/mL within 7 days, especially under insufficient refrigeration.

B. Commercial

In spite of the positive image of mageu (i.e., nonalcoholic, healthful, thirst-quenching, etc.), it is still considered solely a beverage for black South Africans. As a result, factors which affect several other industries will especially influence the mageu market:

1. Unavailability of money in recessionary times
2. Political unrest
3. Competition from other (partly novel) beverage products, including "Maas" or "Amazi" (a fermented sour-milk beverage) and

"Tropica" (a novel whey-based orange drink with sales of 50 million liters expected for 1986).

XI. OPTIMAL ENVIRONMENTAL CONDITIONS FOR FERMENTATION

The thermophilic lactic acid fermentation has become generally established for industrial mageu production. Although authentic *L. delbrueckii* cultures apparently did not find their way to industrial fermentation, several production parameters have been assessed by Schweigart and co-workers using this organism (1,6,9). They considered the following environmental factors to be of prime importance for the fermentation process:

1. The effect of stimulating agents
2. The buffering capacity of the medium
3. The methods of cultivating and propagating the cultures
4. The age of the cultures when they are transferred to new nutrient medium
5. The effect of stirring during fermentation
6. The composition of the nutrients available to the bacteria

Valuable information was gained and the results emphasized the importance of the above-mentioned factors described in the following (1,6,9):

1. The addition of high-quality protein was beneficial to culture activity. The deficiency of essential amino acids (especially lysine) in maize meal was overcome by the addition of milk and whey proteins (through milk and whey powder). Good results were also obtained with food yeast, soya flour, and fish meal. Growth was further stimulated by addition of 5% wheat flour (based on the maize meal content) or 0.5–1% of wheat bran. The active agents were considered to be amylolytic enzymes, which were denatured by heat. It was, in fact, found that a bran extract prepared at 50°C had no stimulatory effect (as compared to an extract produced at 3°C).
2. Due to the low buffering capacity of the substrate, lactic acid production is accompanied by a rapid fall in the pH, resulting in the inhibition of microbial activities. Ideally, a titratable acidity of 0.5% lactic acid (17–22 SH) should be reached. Phosphate-buffering salts ($CaHPO_4$ or KH_2PO_4) gave satisfactory results, enabling continued production at pH levels below 4.0 and, in addition, were found to have physiological advantages. Presently, between 0.1%

and 1% of phosphate-buffering salts are used in industry. The addition of whey or milk powder will also add to the buffering effect of the substrate.

3, 4. Pure *L. delbrueckii* cultures develop their greatest activity in mageu when they are inoculated into fresh medium every 8 h. Very little activity occurred after 24 h. The following maize meal medium was found satisfactory for propagation of *L. delbrueckii* culture (1):

Maize meal broth (8% solids)	1000 mL
L. delbrueckii pure culture	50 mL
Glucose	10 g
Peptone (high quality)	1.0 g
Yeast extract	0.5 g
KH_2PO_4	5.0 g
$MgSO_4$	1.25 g
NaCl	0.25 g

5. After thorough mixing of the culture into the porridge, no further stirring is necessary during the fermentation process of an 8% mageu porridge. This will ensure maintenance of a low Eh during the process, favoring growth of thermophilic lactobacilli. Furthermore, the initial Eh is relatively low as a result of the cooking process and the hot filling of porridge into bioreactors. For the production of concentrated mageu (20–25% solids), however, continuous stirring is necessary.
6. Sufficient fermentable sugars are available for growth and lactic acid production. These sugars are provided by the initial addition of sugar (about 2%) and also as a result of wheat enzyme action.

Most production plants currently rely on the following parameters for mageu preparation:

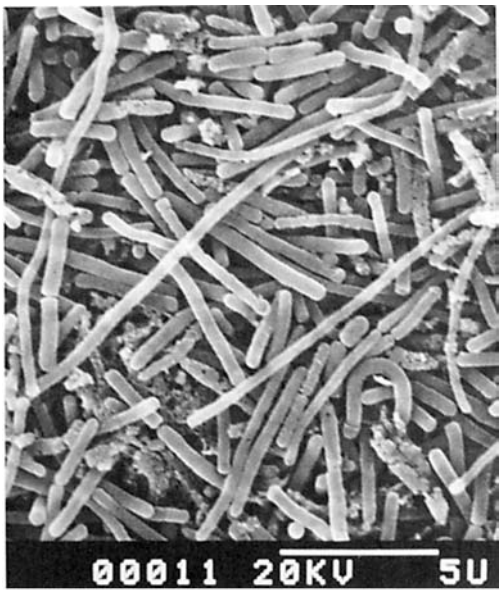
1. Addition of an active thermophilic *Lactobacillus* culture, adapted to maize meal substrate
2. Addition of wheat meal or bran (1–5% based on total solis) to the substrate at 50°C
3. Addition of 2–3% sugar prior to fermentation
4. Addition of 0.1–1% of phosphate ($CaHPO_4$ or KH_2PO_4)
5. Maintaining the temperature above 45°C during the first 3–4 h of fermentation
6. Final pH of 3.5–3.7 to be reached within 24 h during which the temperature has dropped to about 30°C (the titratable acidity should be at least 0.5%)
7. Continuous stirring avoided

XII. ESSENTIAL MICROORGANISMS FOR FERMENTATION

As a result of the work of Schweigart and co-workers (1,2,6,9,24), *L. delbrueckii* was generally accepted to be the typical mageu organism.

Lactobacilli presently used in thermophilic mageu starter cultures were derived from spontaneous thermophilic fermentation of mageu enriched with proteins, sugar, wheat flour, and even sorghum malt. These organisms have hitherto not been subjected to systematic taxonomic and pure-culture physiological studies. These cultures, in general, appear to be heterogeneous and may include *L. delbrueckii*. However, at least three characteristics of these cultures are "atypical" of *L. delbrueckii*:

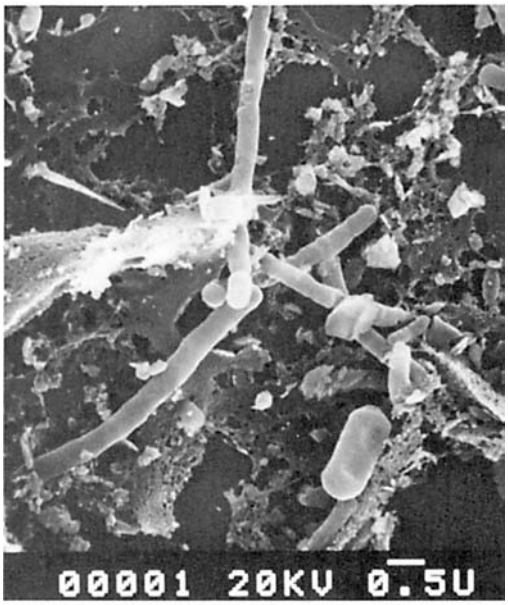
1. They produce DL-lactic acid. By contrast, *L. delbrueckii* should produce *D*(−)-lactate (25).
2. They do not grow well on MRS or other typical elective media for lactobacilli.
3. They retain their activity at 50°C even after several days.



(a)

Figure 18 Scanning electron micrographs of typical thermophilic mageu lactobacilli: (a) in pure culture (bar = 5 μm); (b) in pure culture on maize porridge substrate (bar = 5 μm), and (c) in mixed mageu culture (bar = 5 μm).

(b)



(c)

Figure 18 Continued.

In addition, our investigations on these thermophilic cultures have shown them to be highly aciduric. A pH of 2.5–2.8 is typically reached at 50°C without significant loss of activity. Although these cultures appear to be heterogeneous, the dominating population shows the typical slender, long rod morphology of the thermophilic lactobacilli ("subgenus" *Thermobacterium*) (Fig. 18). Transmission electron micrographs of thin sections of the cells revealed a typical Gram-positive cell wall (Fig. 19).

No suitable semisynthetic medium has been found yet that efficiently supports the growth of these typical thermophilic mageu lactobacilli. The following mageu-based medium was formulated to give acceptable recovery probably of most of these lactobacilli:

Filtrate of 5% maize meal porridge	500 mL
Tween-80 (Merck)	2 mL
Whey powder	10 g
Wheat flour	5 g
$MgSO_4$	0.05 g
$MnSO_4$	0.025 g
Triammonium citrate	2 g
Sucrose	10 g
Cysteine, HCl	0.5 g
Agar-agar (Merck)	7.5 g
pH before sterilization	6.0

Incubation was at 50°C for 3–5 days under reduced atmosphere (Anaerocult, Merck). Inclusion of triphenyltetrazolium chloride facilitated the enumeration of single colonies against the white medium. The majority of isolates failed to grow on semisynthetic media (e.g., MRS-agar + 0.1% cysteine, HCl). A few isolates could be subcultivated successfully, some of which were found to ferment melibiose, trehalose, sucrose, and raffinose and to hydrolyze esculin. None fermented lactose, but starch was fermented by some strains.

Because of the inability of several mageu lactobacilli to grow on conventional media, their numbers in the product have thus far been grossly underestimated. These numbers reach at least 10^8/mL in an active growing culture within 2 h, producing up to 1.2% lactic acid, of which 40–60% is present as the L(+)-isomer.

In addition, it appears as if a heterofermentative, single-strain of *Lactobacillus* culture is being used by some companies. The use of this culture is protected by a franchise, and no more information could be obtained.

Our investigation on spontaneously fermented mesophilic mageu has indicated *Leuconostoc mesenteroides* and *Lactobacillus brevis* as the major organisms. All strains investigated grew at 15°C but not at 45°C; active growth was also detected at pH 3.9 (in MRS broth) and in the presence of 10% sucrose. A final pH of about 4.0 was reached in MRS broth. These strains

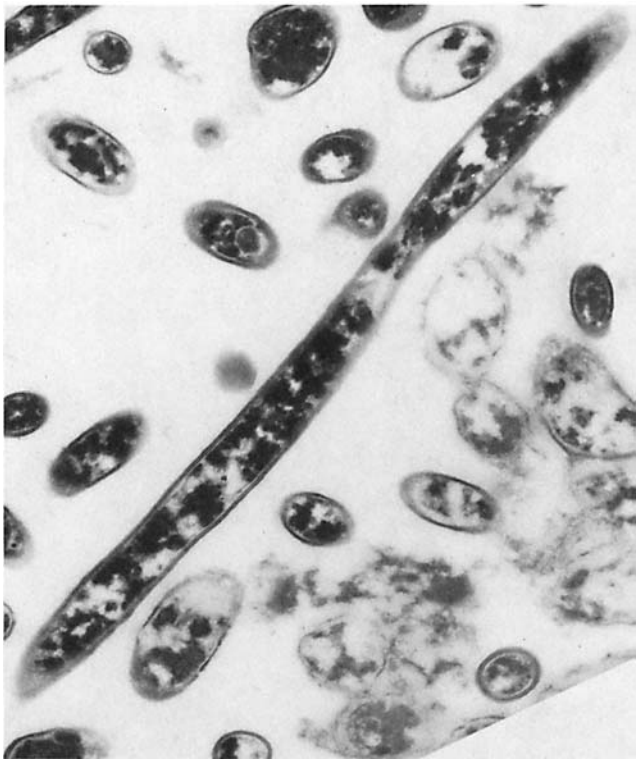


Figure 19 Transmission electron micrograph of a typical thermophilic mageu *Lactobacillus* showing a typical Gram-positive cell wall. (Magnification: 16.7 mm = 1 μm.)

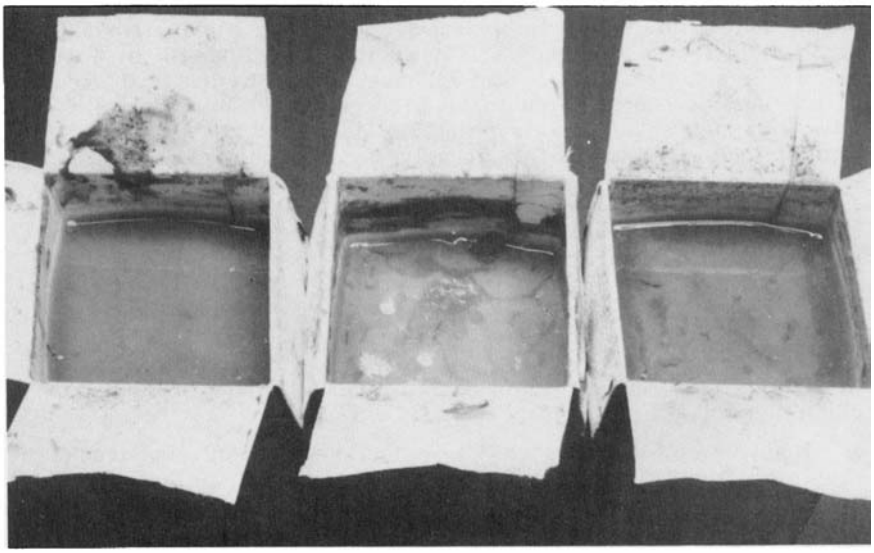
hydrolyzed esculin and fermented maltose, fructose, and arabinose, but not lactose, ribose, or xylose. Schweigart (24) described the drying of thermophilic mageu cultures (presumably *L. debrueckii*). Higher activity was retained when cultures were partially neutralized with $CaCO_3$ before freeze-drying (pH 4.6–4.8) or spray-drying (pH 5.1–5.2). These dried cultures could be readily activated when transferred to fresh medium.

XIII. MICROORGANISMS THAT CAUSE SPOILAGE

Conditions that may contribute to microbial spoilage of mageu include the following:

1. Inactive culture (also as a result of low fermentation temperature) resulting in insufficient acid production

(a)



(b)

Figure 20 Spoilage of packaged mageu by *A. liquefaciens*.

2. Contamination during fermentation in open bioreactors
3. Underpasteurization (or lack thereof)
4. Insufficient cleaning procedures, especially of pipelines
5. Recontamination after pasteurization (in holding tanks, pipelines, and during packaging)
6. Insufficient refrigeration (or lack thereof) during storage, transport, and/or on the retail level

The most important spoilage organisms are yeasts, sometimes reaching 10^6–10^7/mL in the product. The yeast population is dominated by *Pichia* spp. It appears, however, as if numbers of around 10^6/mL do not affect the quality of the product adversely and the presence of alcohol is even accepted by the consumer as "typical." Moreover, a total maximum alcohol content of 1% is allowed for in the standard mageu specifications (20).

Bacteriophage appears in some instances to be the cause of an inactive culture.

Acetobacter spp. may cause spoilage of the packaged product, sometimes resulting in a significant loss of products after retail distribution. *Acetobacter liquefaciens* was found as the major acetic acid bacterium involved in mageu spoilage and may reach numbers of 10^7/mL. This organism not only metabolizes lactic acid and produces off-flavors, but it also causes a red-brown discoloration of the product (Fig. 20).

Complete anaerobiosis does not exist inside the package and, also as a result of airborne contamination, fungal growth may be encountered on the inside.

An inactive or slow-acid-producing culture may be the cause of spoilage by clostridia. Under well-controlled conditions, however, this problem is rarely encountered.

XIV. CHEMICAL/BIOCHEMICAL CHANGES IN SUBSTRATES DURING FERMENTATION

A. Flavor, Texture, and Aroma

Mageu produced with homofermentative *Lactobacillus* cultures has very little flavor or aroma, but does have a distinct and refreshing sour taste. Because lactic acid is a nonvolatile acid, this sour taste is relatively neutral and thirst quenching. This kind of mageu is perhaps the most suitable for the addition of flavors such as banana, pineapple, cream, and so forth.

Heterofermentative lactic acid bacteria (typical for traditional and mesophilic-fermented mageu) produce metabolites such as CO_2, acetic acid, and ethanol, in addition to lactic acid.

The presence of yeasts in spontaneously fermented mageu and (as contamination) in the commercial product (in numbers up to 10^7 mL) normally does not have adverse effects on the product (apart from numbers $>10^7$/mL, causing excessive CO_2 production and "blowing" of the cartons). The actions of heterofermentative lactic acid bacteria and contamination yeasts result in a more piquant product.

B. Changes in pH and Total Acidity

Because maize meal porridge has little buffering capacity, the pH of the product will quickly drop to <3.5 during fermentation, especially when thermophilic cultures are used. The addition of buffering salts (e.g., $CaHPO_4$) or protein-rich products (e.g., whey powder, soya flour, etc.) will improve the buffering properties, thereby enabling the organisms to produce more lactic acid. These aspects have been thoroughly investigated by Schweigart et al. (1) and summarizing data are presented in Table 3. The choice of a $CaHPO_4$ buffer seemed beneficial from a physiological viewpoint whereby a better calcium/phosphorus ratio is provided to a substrate relatively poor in calcium.

C. Sugar Content and Sweetness

Traditional mageu was not prepared with added sugars, and fermentable sugars were derived from wheat flour enzymatic action on the maize starch. According to Schweigart et al. (1) between 0.3% and 0.6% reducing sugar (expressed as glucose) was found in spontaneously fermented mageu, as compared to 1.5% for mageu produced with selected cultures.

Furthermore, according to Schweigart et al. (1), the addition of glucose did not appear to enhance the speed of souring or the quantity of lactic acid produced by *L. delbrueckii*, and they concluded that the sugar supply was not a limiting factor for this organism.

In present-day commercial production of mageu, between 1% and 2% of sugar was found to favor rapid fermentation. An additional 2–3% of sugar is added at blending of the final product.

D. Ethanol Content

Although mageu is claimed to be alcohol-free, the contrary is true for most commercially available products. Even in pure or mixed lactic culture studies, Van Noort and Spence (18) found between 0.32% and 0.7% ethanol in the final product. This may be attributed to metabolic activity of heterofermentative lactic acid bacteria present in mesophilic cultures.

Table 3 Fermentation of Maize Meal Porridge (8% Solids) by Spontaneous Souring and with *L. delbrueckii* Starters Showing the Effect of Buffering and of the Addition of High-Quality Proteins (Whey Powder)

	Spontaneous souring by addition of 5% wheat flour (35°C)		Control		*L. delbrueckii* culture					
					1% $CaHPO_4$		20% whey powder		1% $CaHPO_4$ and 20% whey powder	
Fermentation time (h)	pH	1% lactic acid	pH	1% lactic acid	pH	1% lactic acid	pH	1% lactic acid	pH	1% lactic acid
0	6.70	0.02	5.70	0.09	—	0.09	5.75	0.09	5.80	0.09
5	—	—	5.50	0.11	5.40	0.14	5.40	1.13	5.40	0.05
12	6.68	0.14	3.95	0.27	4.10	0.33	3.95	0.31	4.25	0.36
22	4.01[a]	0.28[a]	3.45	0.40	3.75	0.52	3.70	0.53	3.75	0.60
53	3.42[b]	0.57[b]	3.27	0.73	3.39	0.93	3.0	0.99	3.39	1.23

[a] Data after 24 h.
[b] Data after 53 h.
Source: Ref. 1.

Yeasts can be considered as the major contaminants of commercial mageu and may produce up to 1% ethanol during retail distribution.

Most consumers consider the presence of ethanol acceptable and even "typical" of this product. Perhaps for these reasons, provision was made in the Standard Specifications for mageu (26) for a maximum acceptable alcohol content of 1%.

E. Enzymes

A stimulating effect on fermentation was attributed to enzymes present in wheat bran and flour (1). These enzymes were heat sensitive, even at 50°C. No further work was undertaken on these enzymes, but they can be considered amylolytic. The stimulation influence may, among others, be related to their hydrolytic action on the maize starch, thereby making fermentable sugars available.

XV. NUTRITIVE VALUE AND GENERAL COMPOSITION OF MAGEU

The approximate chemical composition of different mageu products is given in Table 4. These data represent analyses performed by two different groups and emphasize the possible fluctuations in the chemical composition and nutritive value of commercially produced mageu.

The addition of 2% food yeast to spray-dried mageu powder significantly increased the biological value of the proteins (9). Results obtained by tests on growing rats indicated a digestibility of 91%, a biological value of 72.4%, and a net protein utilization of 65.9 and a net protein value of 10.3 per 100 g of powder. It was also shown by Van der Merwe et al. (9) that fermentation with *L. delbrueckii* did not influence the nutritive value of the proteins and that the ability of the rat to utilize these proteins had not been impaired by the presence of lactic acid. These workers calculated that 1 L of reconstituted enriched mageu (104 g of solids) can supply 31.5% of the daily protein requirement of an adult male.

Schweigart et al. (1) found a 30% decrease in the thiamine content as a result of the cooking of the mash, and a similar decrease in riboflavin in the normal but not the enriched mageu. However, they showed that a considerable proportion of the nicotinic acid, present in a bound form in the maize (unavailable to the body), was liberated by the cooking process. Lactic acid fermentation did not have any influence on the B-group vitamin content of mageu (1).

Table 4 Approximate Chemical Composition of Normal and Enriched Mageu

Component	Van Noort and Spence (18)		Schweigart and Wehmeyer (23)	
	Mageu liquid	Mageu powder[a]	Mageu powder	Enriched mageu
Moisture % (m/m)	90.0	9.0	3.5	4.6
Protein % (m/m)	0.8	8.0	9.6	13.9
Fat % (m/m)	0.1	1.0	3.7	3.2
Carbohydrate (by diff.)	9.07	81.5	80.1	73.6
Fiber % (m/m)	—	—	1.0	1.0
Ash % (m/m)	0.03[b]	0.05[b]	2.1	3.7
Lactic acid % (m/m)	0.4	—	—	—
Alcohol % (m/m)	0.4	—	—	—
Thiamine (mg %)	0.08	0.8	0.38	0.33
Riboflavin (mg %)	0.05	0.5	0.09	0.44
Niacin (mg %)	0.2	2.0	1.40	1.75
Carotene (mg %)	—	—	—	590.0
Calcium (mg %)	0.13[b]	1.3[b]	416.0	724.0
Iron (mg %)	0.50	5.0	7.7	14.7
Magnesium (mg %)	10.3	10.3	117.0	136.0
Phos. (mg %)	3.50[b]	35.0[b]	418.0	589.0
Potassium (mg %)	4.0	40.0	195.0	—

[a] Constituents used for enriched mageu powder: 7.7% maize flour, 1.2% whey powder, 0.3% skim milk powder, 0.3% fish flour, 0.3% soya flour, 2.0% fresh carrots, 0.2% food yeast, 0.2% wheat bran, 0.1% $CHPO_4$, 88% water.

[b] Presumably without any phosphate buffer being added (e.g., $CaHPO_4$).

It can therefore be expected that the intensive cooking process used in industry may have a detrimental effect on only some vitamins in the final product. The high cooking temperature is considered necessary to obtain optimal gelatinization of the 8% mash.

Because of its relatively low nutritive value, several studies have been undertaken to improve the product by enrichment (1,2,6,8,9,22). According to Van der Merwe et al. (9), mageu lends itself well to enrichment. The type of enrichment will depend on the needs of the consumer. About 1.1 L of mageu enriched with soya meal and fish flour would provide 4000 kJ as well as 31.5% of the daily protein requirement of an adult male: 30% of the thiamine, 13% of the riboflavin, 26% of the nicotinamide, and 77.5% of the calcium requirement, with a favorable Ca/P ratio of 1 : 1.2.

XVI. FUTURE ROLE OF MAGEU AS A FOOD

Among the traditional fermented foods typical of Africa, those prepared by lactic fermentation of cereals such as maize and sorghum are of special importance. Apart from its preserving action, lactic acid renders a refreshing and thirst-quenching taste to these products that is extremely acceptable to the palate of the black South African.

The present South African black population of around 25 million is expected to increase to 45 million toward the end of this century. As a result of several drought spells, South Africa is sometimes forced to import maize, which serves as staple food for the largest percentage of its population. In addition, however, maize is also one of the main feed ingredients for cattle and nonruminants.

Because 7–10 kg of plant protein is required to produce 1 kg of meat, it can be expected that more maize will be gradually made available for human consumption. As a result of the continuing high inflation rate, the lower income groups will find it increasingly difficult to afford meat as part of their daily diet.

The traditional role of maize in the African diet may be extended through novel maize-based products and especially by the modification of lactic-fermented beverages and high-solids porridges related to mageu. The improved keeping quality of lactic-fermented enriched mageu products will facilitate distribution even to the rural population.

Presently, a 1% annual increase in the consumption of packaged mageu is expected. It is also expected that more industries will start supplying mageu to their workers (15). This will affect sales in bulk form favorably. From the viewpoint of the mageu manufacturer, bulk sales are encouraged because "bulk sales do not experience the same drastic dip during winter as packaged sales; complaints and returns are reduced to a minimum" (15).

The pioneering research work of Schweigart and co-workers during the 1960s (1,6,9,23) has laid a firm foundation for future modifications and improvement of mageu. Against this background and with the fast-growing black population in view, mageu may play a significant role in satisfying the increasing demand for well-balanced, high-protein foods in one or more of the following forms:

1. Enriched mageu beverage with 2–3% high-quality protein fortified with vitamin B12 and carotene.
2. Concentrated mageu (25% solids) containing whey and other high-quality proteins, $CaHPO_4$, and 1% lactic acid after fermentation (9).
3. Fortified mageu powder, prepared with adapted cultures before spray- or roller-drying (9). According to the SABs specification

(26), the powder should have a shelf life of at least 6 months under normal storage conditions and is reconstituted by adding 150 g to 1 L of water. Furthermore, it must comply with the requirements given in Table 5.

4. Mageu paste that can be consumed as a porridge, or diluted to the normal consistency of mageu, taken as beverage (9). Again, this form of the fermented product can be fortified with proteins and vitamins to meet the needs of the lower-income group. Special flavors may be added according to preferences of different consumer groups.
5. Low-priced mageu/yogurt mixtures as an alternative to popular traditional fermented (and expensive) milk products (cultured buttermilk, maas or amazi, and yogurt). The continuous increase in the retail price of milk puts it out of reach for many people in the low-income group. In a mageu/yogurt or mageu/amazi mixture, the milk component may be partly or fully substituted with soya or whey powder. Specially adapted mesophilic or thermophilic lactic cultures may enable the preparation of buttermilk- or yogurt-flavored fermented products.
6. High-solids, low-fat product prepared through concentration of fermented enriched mageu paste by adsorption and description procedures. In this way, a long-shelf-life, "intermediate-moisture," or "shelf-stable" mageu food may be produced. The keeping quality will be determined by a combination of parameters including reduced *aw* and low pH (through lactic acid).
7. A commercial, ready-to-mix, unflavored mageu powder, marketed under the name "Funa Classic Mageu" and which is described as "a

Table 5 Composition of Mageu Powder to Meet SABS Specification

Constituent	Min	Max
Fermented cereal % (m/m)	60	—
Protein[a] % (m/m)	12.0	—
Vitamin B_2 (riboflavin) (mg/100 g)	2.5	—
Niacin (mg/100 g)	9.0	—
Vitamin C (mg/L of the beverage)	70	—
Fat % (m/m)	—	3.0
Fiber % (m/m)	—	1.5
Sucrose % (m/m)	—	15.0

[a] Derived from natural sources (e.g., milk products, food yeast, soya products, and the cereal itself).

Source: Ref. 26.

nutritious maize drink". It is packaged and distributed in 25-kg white, woven polypropylene bags with a liner; a shelf life of 12 months under normal storage conditions is claimed. The ingredients consist of pregelatinized starch, sugar, wheat flour, minerals, stabilizer, sodium saccharin, sodium cyclamate (as non-nutritive sweeteners), and yeast. Per 100 g of powder, the products contain 8 g of moisture, 7.3 g of protein and 79.0 g of carbohydrates providing 1515 kJ of energy. According to the recommendations, it is suspended in water by adding 1 kg of powder to 10 L of water at temperature between 20°C and 35°C. After the addition of 0.5 L of starter obtained from a previous brew (by "back-slopping"), it is allowed to ferment for 24 h. It should be consumed within 3 days or may be stored for up to 10 days in a refrigerator. More information is available from the website www.mbendi.com/nutfoods/data/p1451.htm.

XVII. MAGEU FERMENTATION IN THE FUTURE

Very little scientific information is available on "typical" mageu bacteria. Extensive studies have only been undertaken on improvement of the technology and nutritive value. In these studies, pure cultures of (adapted) *L. delbrueckii* and *L. bulgaricus* (1,9) or mixed mesophilic (heterofermentative?) lactic acid bacteria (18) have been used. None of these cultures seems to have found general application in the present day mageu industry.

Basically, three types of commercial starter culture are currently in use:

A heterofermentative (?), single-strain (?), thermophilic culture, the use of which is protected by a franchise

A multistrain homofermentative thermophilic *Lactobacillus* culture producing DL-lactic acid

Mesophilic (heterofermentative) starter cultures used by a few minor industries to produce a "traditional" type of mageu with fluctuating quality

This situation clearly indicates a lack of important microbiological information, basic for a large fermentation industry in southern Africa.

To fill this gap and in order to enable the mageu industry to meet future needs for product improvement and modification, as well as further mechanization, additional research should be directed at the following:

1. Screening and selection of lactic acid bacterial isolates from spontaneous mesophilic and thermophilic fermentations as well as authentic cultures for suitability in "traditional" and novel

commercial mageu fermentations. Rapid methods should be applied as far as possible, to improve screening procedures for properties such as the following:

Resistance to bacteriophage
Production of flavor and taste preferred by the consumer (use of taste panels)
Inhibitory action (in early stages of fermentation) against spoilage microbes (especially clostridia and yeasts) and pathogens
Predictable and constant acid production ability under a given set of conditions
Relatively simple substrate requirements, based on maize porridge medium
High acid resistance, even in nonbuffered systems
Ability for growth and acid production in concentrated substrates
Production of pure L(+)-lactic acid, which is preferable from a health point of view (27)

2. Culture improvement by mutagenesis followed by further selection procedures, as well as by amplification or insertion of "ideal" properties (mentioned earlier by means of in vivo and in vitro genetic manipulation. Production of an amylase enzyme, operative only under thermophilic conditions, may increase the fermenting ability in the maize starch substrate. Unnecessary reduction of viscosity will be prevented by strict temperature control. Attention should also be given to adaptation and genetic manipulation of cultures with therapeutic value, such as *Lactobacillus acidophilus*.
3. Technological improvements especially with the focus on continuous fermentation.

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8
Industrialization of Ogi Fermentation

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I. INTRODUCTION

Ogi is a Nigerian name given to a traditional fermented and often sour starch cake processed exclusively from maize, sorghum, or millet; never from rice or wheat. Ogi usually has a smooth texture and is boiled into a porridge called pap before consumption. Fermented ogi pap has a mild to strong sour flavor, resembling that of yogurt and a characteristic aroma which quickly differentiates it from starch and corn flour. The color of ogi depends on that of the cereal—slightly cream for white maize, cream for yellow maize, light brown for sorghum, and greenish to gray for millet (1,2).

In Nigeria, the name depends on the locality and the cereal. Ogi is the generic name in the western states of the country where the product was previously processed from white maize only but recently also from yellow maize. Ogi processed from maize is simply called "ogi"; however, it is known as "ogi-baba" and "ogi-gero" when sorghum and millet are used, respectively. In northern Nigeria, "kamu" is the equivalent of maize ogi when it is consumed as a thick pap by adults. "Kunu" is the name given to the maize pap by the Hausa-speaking people when it has a very light consistency suitable for children and for adults who prefer it so. In the republics of Togo, Benin, and Ghana, all of which are members of the Economic Community of West

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African States, ogi from maize is known as "koko." The consistency of "koko" is similar to that of kunu. When prepared for adults only, kunu may taste spiced hot and is known as "kunun zaki" because it contain various spices—ginger (65%), red pepper (25%), cloves (10%), black pepper (10%), and ground sugar. Onuorah et. al. (3) reported a heavy contamination of "Kunun zaki" by staphylococci and coliforms because of lack of hygiene during processing. The equivalent of ogi is uji in Kenya (4) and mahewu in Southern Africa.

The Igbo people and other ethnic groups living east of the River Niger in Nigeria consume only maize ogi, which they call "akamu." The Igbo "akamu" is slightly sour, for they do not cherish sour gari or sour ogi. Although "furah" has been reported to be the name given to ogi from sorghum grain in northern Nigeria (1), its method of production does not make it an ogi product, because it is not fermented. Furah is prepared by grinding or beating dry sorghum in a mortar, dry sieving, wetting the meal with a small quantity of water, and forming balls about 2 in. in diameter. The balls are steamed to gelatinize and dusted with dry flour to a semidry consistency and sold as such. For consumption, the furah balls are crumbled by hand into sour liquid milk (nono) and eaten with a large wooden spoon or from a piece of calabash. It is the sour liquid milk that gives furah its fermented sour flavor, which is a distinguishing feature of ogi products.

II. CONSUMPTION IN DIET

Ogi is rarely eaten as a main meal; it is essentially a breakfast cereal eaten by adults and convalescents, and a weaning food eaten by infants and children. Indigenous Nigerian ogi is sold as a raw, wet starch cake wrapped in thin polybags, having about 55% water content, which makes it amenable to rapid deterioration. To cook, a little water is bought to boil in a kettle. Next, the ogi cake is made into a smooth slurry in a bowl using an appropriate quantity of water at ambient temperature. Then, the boiling water is slowly added with stirring into the ogi slurry to make a pap. The ogi starch swells and more water may be added to obtain a proper thickness of the ogi as desired—thicker for adults and very thin, for infants. Sugar may be added to sweeten the ogi, especially for infants and children. When the pap is thin, it has about 5–6% solids content and this is for children in Nigeria; in other western African countries, it is the standard for koko. Except for children and convalescent adults, ogi is seldom consumed alone in the diet. It is eaten in conjunction with (a) wet milled cowpea (Vignia) flour fried in peanut oil or other vegetable oil (called akara balls), (b) with sliced ripe plantain (*Musa paradisiaca*) fried in oil, (c) with moin-moin or (d) heated further to a solid gel (eko, agidi).

Moin-moin is processed from cowpea (or sometimes maize) by wet milling, filling meal cups or wrapping in special leaves (etere), followed by steaming to cook. Meat, eggs, and fish may be added to moin-moin according to the purchasing power of the individual. Ogi hardly ever goes with tea or coffee. Ogi, having about 8% solids, could be eaten with a tablespoon, whereas koko and kunu are very thin ogi which may be consumed with a large kitchen spoon, a piece of native gourd with a handle, a piece of half calabash, or from the container, particularly when just warm.

The most common way of child feeding is from a clean feeding bottle. Ogi may also be boiled in water to a thick consistency, wrapped in an "etere" leaf which imparts flavor and allowed to set to a gel known as "eko" or "agidi," or eaten with a stew (5). This is hardly a main meal, but it suffices for those who do not wish to eat heavily. It is also excellent for convalescent adults.

III. IMPORTANCE IN DIET

Several years ago, ogi was the major traditional infant weaning food from maize. It was discovered that children fed exclusively on ogi, with deficient breast milk, suffered a nutritional protein deficiency disease called kwashiorkor (6). For this reason, the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos developed "soy-ogi", a soybean-enriched ogi, specifically as infant weaning formula to upgrade the traditional ogi (7). The soybean component raised the protein level to 15% from 7–8%. A child whose mother has gone to the farm or business may be fed ogi four or more times until the mother returns. The Yoruba adults cherish maize ogi and "ogi baba" and can consume ogi every morning up to five times a week, usually accompanied with cooked whole cowpea, garnished with red palm oil stew. This is simply known as beans (agwa-Igbo, ewa-Yoruba). They also often take the ogi with hot akara balla (beans). Ogi is therefore an important breakfast cereal for adults. Currently, its importance in infant feeding has diminished considerably because of the emergence of a commercial multinational product based on an unfermented maize–soybean blend called "Nutrend" processed by extrusion cooking. Soy-ogi was also produced commercially under the brand name "Mama-joy," but because of microbiological problems with the health authorities, its production was stopped. Adults to date look forward to adult soy-ogi, having the cherished flavor and nutritional advantages. However, Nutrend has fulfilled the much needed instant baby weaning food with maize, and although it is not fermented, infants cannot differentiate ogi taste from other pleasant tastes that are common in infant foods. In addition, Nutrend is never in short supply as long as one has money to purchase it.

Furah is, on the other hand, the major lunch meal of adults in northern Nigeria and other neighboring Saharan African countries. Furah is consumed every afternoon of the week and is coarser and more filling than sorghum-based ogi (ogi baba) or kunu. It is unlikely to be changed in the places and countries that traditionally consume sorghum as furah and as tuwo meal. The method of child feeding is crucial to the child's welfare. In some places, particularly in western Nigeria, weaning children were fed ogi by holding the child's head upside down on the mother's lap and, by means of the mother's palm, guiding the light pap into the mouth as the child makes a gagging, choking noise due to a sort of forced feeding. At least it was reasonably hygienic. However, when the trend changed to bottle feeding, feeding bottles were rarely properly washed or not washed at all by the rural folk, causing increased incidences of infant diarrhea in villages because of ignorance. However, most people in Nigeria use a teaspoon to feed the child.

IV. TOTAL PRODUCTION

Total production of maize in Nigeria for the years 1993, 1994, and 1995 was 6,290,000 metric tons, 6,902,000 metric tons, and 6,931,000 metric tons, respectively (8). For millet, it was 4,602,000, 4,751,000, and 5,563,000 metric tons for the 3 years, respectively, and for sorghum grain, it was 6,051,000, 6,197,000, and 6,997,000 metric tons, respectively. Although Adeyinka (9) estimated that up to 60% of the grains, particularly maize, was employed in the production of ogi, that claim is hardly tenable in recent times where traditional ogi processing is concerned because commercial traditional ogi production for weaning foods has become outdated in favor of a soybean-enriched, unfermented maize extrusion—a cooked commercial variant known as Nutrend, a product of Nestle Nigeria Plc. However, to maintain their massive production targets, it is likely that Nestle has to import some maize, including soybeans, for the baby food manufacture, not for ogi production. Traditional ogi producers, including furah production based more on sorghum and millet grains, produced the product using the traditional methods. It is not easy to estimate per capita consumption.

V. EARLIEST KNOWN REFERENCES

Maize (*Zea mays* Linn) is one of main grains of western Africa known to have originated in South America. It was introduced by the Portuguese to western Africa in the 16th century and first exported from Nigeria in the 20th century (10). The total production in Africa was estimated to exceed 39 million tons in

1989 and over 33 million tons in 1991 (11). Maize is grown all over the country but principally in the northern parts of the country, where spoilage is least and the heavy tropical rains are minimized. In addition, in the northern Saharan parts and many western African countries, millet and sorghum grains are consumed as staples. Wheat is grown to a limited extent and is marketed in the open market in the same way as other grains. Ogi is the first native food given to weaning babies of over 10 million Yoruba people in Nigeria (12). It is most likely that ogi consumption started soon after the introduction of maize in the 16th century by the Portuguese or by freed slaves who settled in Lagos, Liberia and other western Africa countries. Ogi is not the only major traditional product processed from maize. Others are snaks, tuwo, corn flour, brabusco, and couscous from dry whole grains, pito and burukutu drink from sprouted grain, and agidi and eko from fermented whole grain (13,14) (Fig. 1).

VI. SUBSTRATES USED

Ogi substrates are (a) maize (white variety) and, recently, the yellow variety, (b) sorghum, also called guinea corn, and (c) millet as far back as is known (2). However, industrially produced ogi is based wholly on maize and sorghum, probably because of the lower costs of sorghum and maize compared with that of millet, as well as the very small size of millet that hampers cleaning and handling (2).

VII. PRODUCTION IN ANCIENT TIMES

Ancient ogi production was a family business, the art being passed from the mother to the female children (Fig. 2). The housewife prepared the ogi in small quantities using maize (or sorghum and millet) harvested from nearby farms or that stored at home under smoke fire from the kitchen. She also purchased a basin or two of dried maize from the market on market days for the ogi processing.

It was dried to a reasonably low moisture content by the ancient people by tying unsheathed maize in bunches and hanging these over the fireplace in the mud kitchen. The combination of smoke and heat from the wood fires sterilized the cob against insect and fungal attacks as well as drying them to a moisture level of about 12%, suitable for storing them until the next planting season 4–6 months away (2).

Fresh or dried maize was shelled manually when required for processing. When the women produced ogi for the market, they purchased additional

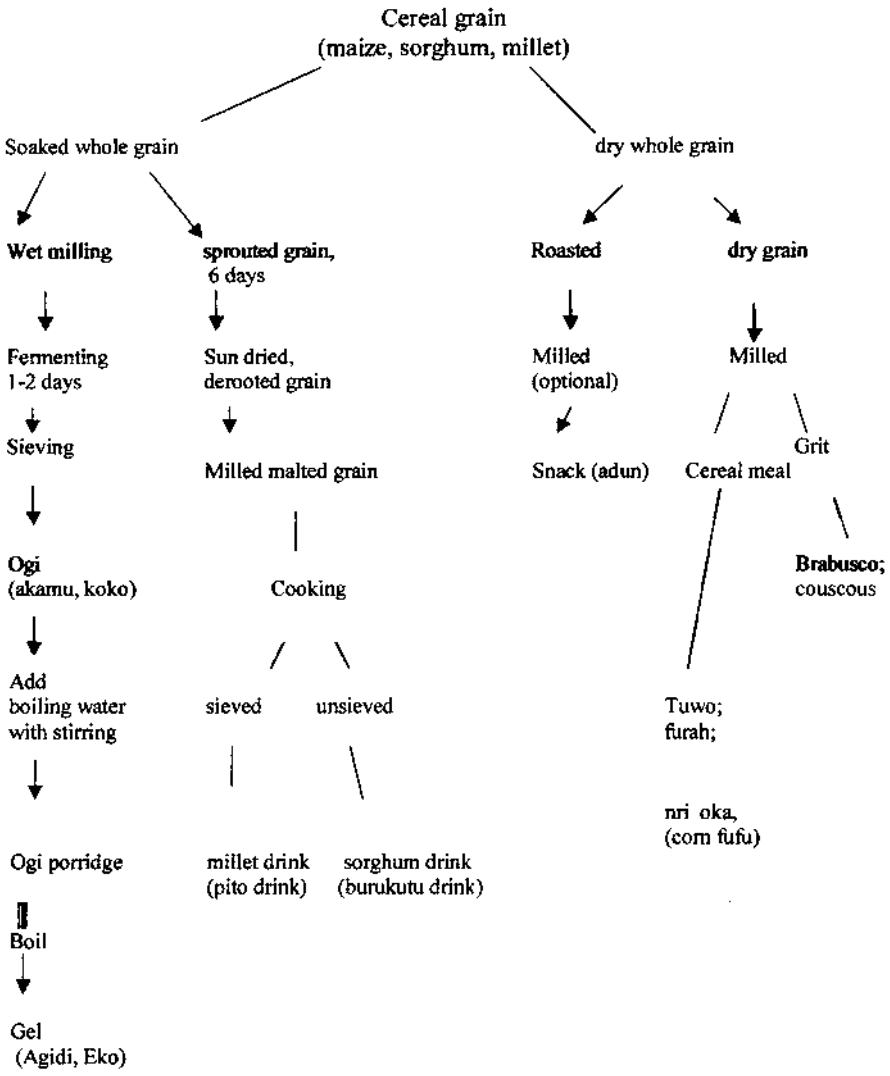

Figure 1 Flowsheet for various traditional food products derived from cereal grains in Nigeria. (Modified from Ref. 13.)

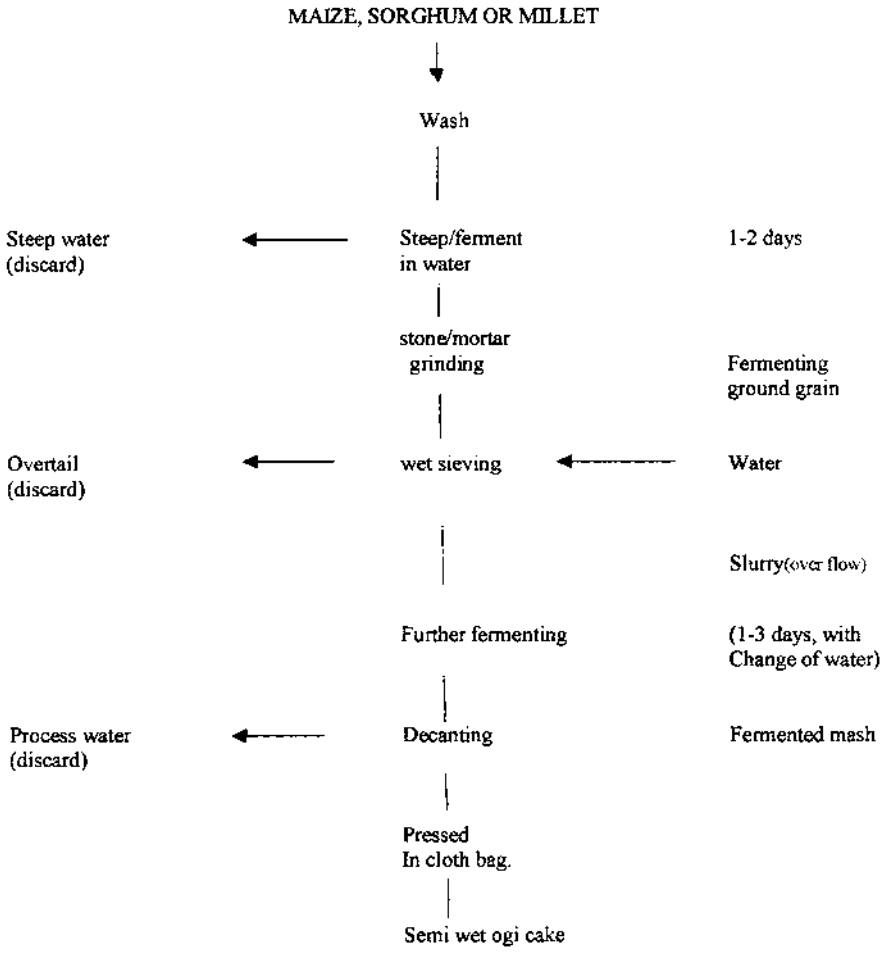

Figure 2 Flowsheet for ancient indigenous Nigerian ogi preparation. (From Ref. 2.)

shelled maize from local markets in basin measures but rarely in bagfuls. Dry maize was soaked in enamel basins containing water after initial manual scrubbing in water, such that the grains were fully covered. Occasionally, warm water was used for the steeping stage in order to quicken the process of water absorption by the grains, particularly in the cold, windy harmattan weather. Steeping softened the grain and enabled it to be ground on a stone slab having a smooth surface on one side. The grinding stone was previously

chipped to make it smooth so that it could be conveniently handled by the housewife. The same stone slab was used for various other products (e.g., tomato and pepper grinding). Sometimes, grains were beaten in a wooden mortar (Fig. 3). Ground grain was placed in a basin of water and agitated to wash off starch into the water. The chaff (pomace) was reground with a little water added until maximum starch yield was attained.

A dried maize grain was regarded to be superior to fresh maize in its yield of ogi. Sometimes, a local sieve made out of raffia palm frond was used to

Figure 3 Ogi preparation: ancient times method, showing size reduction of maize (or sorghum) in a wooden mortar.

facilitate the sieving process, using a liberal quantity of clean water to wash out the starch; the overtails were thrown to chickens roaming unrestrained in the village. The slurry in the basin was then covered with another basin and allowed to sediment and sour for 1–3 days with occasional change of water. The fermented mash was decanted and dewatered in cloth sacks with the aid of a heavy object compressing it to a slightly wet starch cake of about 55% water content, which was marketed in small mounds wrapped in dry banana leaves (2). The entire process was characterized by a lack of any quality control measures whatsoever.

VIII. COTTAGE/VILLAGE METHODS (FIG. 4)

The cottage scale of ogi processing is the major source of ogi production in all the ogi-consuming countries in western Africa. Even in Benin, where mawè (a fermented maize dough, slightly different from ogi) is a staple, the so-called commercial producers are still cottage-level producers (14). The cottage method is not very different from the ancient type of ogi production except for the following:

1. Raw material acquisition and storage
2. The equipment used in size reduction of the steeped grain
3. The wet sieving stage
4. The packaging of wet ogi cakes

In the dry northern parts of the country where the annual rainfall is as low as 50 cm, temperatures go up to 34°C, and relative humidity is as low as 45% between December and March, the grains (especially maize) are left on the farms to dry and are stored using village-level methods in maize cribs. Cribs are rectangular platforms having bamboo legs, which, because of their slippery nature, do not allow rats and other rodents to climb into the storage platform. The crib also is protected by wire gauze with a framework which supports the sides and ends up in a roof, made of aluminum or grass. Traditionally, the crib is constructed in the village square, clear of trees and bushes to enhance good ventillation of the maize in the crib and keep out rodents. The long side of the crib generally faces the direction of the prevailing wind and thus allows efficient drying. The dimension of the crib varies depending on the quantity of maize, but, generally, 1 ton of maize requires a crib 150 cm long by 120 cm wide and a 120–150-cm high storage space (15). Although the length of crib could be of any reasonable size, the width must be kept narrow, from 50 cm in heavy rainy areas of the forest zone to about 150 cm in dry areas. With respect to the southern rainforest area having an average annual rainfall of 430cm between April and October and a humidity

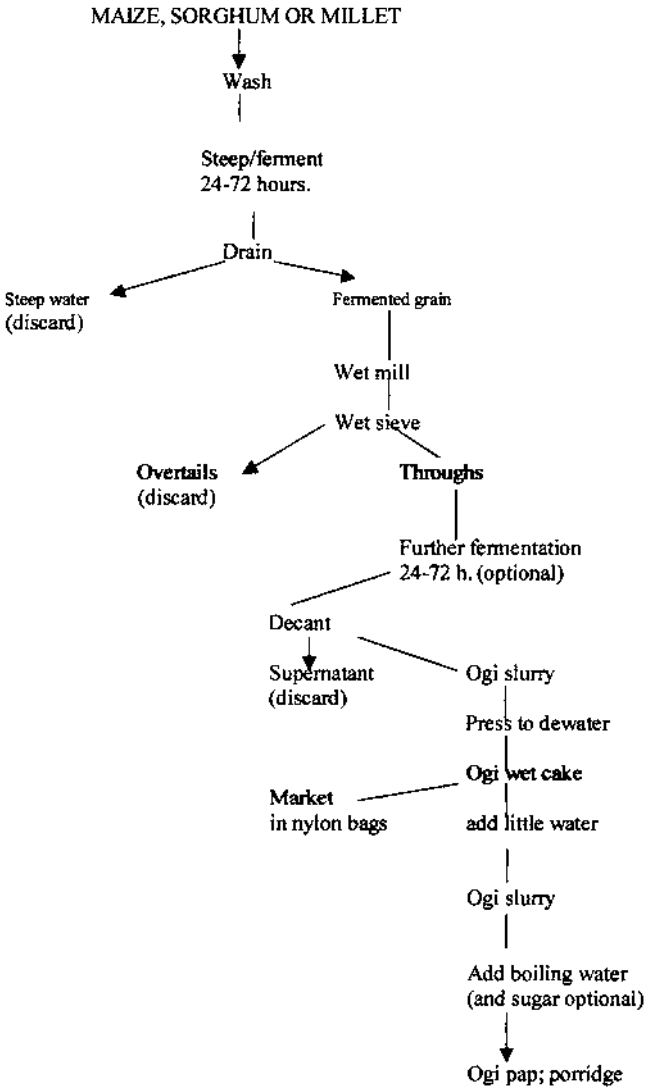

Figure 4 Present cottage Nigerian ogi production. (Modified from Ref. 1.)

of about 95% during the rainy season, maize is harvested while still wet (16) and there is no specific storage arrangement. Grains are purchased in Hessian sacks which weigh approximately 80–100 lbs or in basinfuls. Because the production is essentially a household affair, except for a few cooperative producers, not more than a couple of bags of grain are bought at a time.

As in ancient times, the cereals are scrubbed in water by hand to remove filth and grit and steeped in drums or basins of water at ambient temperature (29–32°C) for 1–2 days depending on the dryness of the grain. Where the maize grain is very dry, it will naturally take a longer time for it to absorb water to soften and initiate fermentation. There is no attempt in the cottage level to test the moisture content of the grain. Oguntunde and Abebawo (17) reported that most of the moisture uptake in white maize type TZPM, white and red types of sorghum and pearl millet, took place within the first 24 h, whereas the peak water uptake or saturation moisture content occurred at about 36 h, irrespective of the soaking temperature used. At the end of the soaking step, the grain is sufficiently softened and has become the fermenting grain, which is currently taken to nearby petrol-operated plate millers. It is no longer usual to pound the wet grain in a mortar or grind on grinding stones. Therefore, the equipment used in the modern cottage method for the size reduction of the steeped grain is mechanically superior to that of the ancient times of ogi production. Wet grain is poured into the hopper of the 1–2-hp disk attrition mill (Premier 1A type) corn mill, using a wooden rod to push down the grain into the grinding metal teeth of the mill, adding water to keep the grain wet and collecting the product in a receptacle below. Under special arrangement, the ground grain can go through a second round to ensure higher yield of fine slurry.

Wet milling of grain is followed by wet sieving. Here, the processor carries home the ground grain slurry which she places in an aluminum bowl, having perforated bottom and sides which serves as a sieve. The grain slurry is transferred using another bowl, via the sieve (sometimes a white cotton cloth is used), and this sieve is held over a basin of water. With manual agitation, starch washes out of the sifter and the pomace is left on the sifter bowl or cloth sieve. Washed pomace is fed to the chickens and the container is covered to prevent flies and other contaminants; this assists in creating a semianaerobic condition in which fermentation is ideal, otherwise oxygen causes foaming and eventually results in an off-flavored ogi (2). Fermentation is allowed to continue further for 1–2 days.

The longer the fermentation after wet milling, the greater the sour taste of the ogi. Generally, sorghum and millet must be given 48 h to sour further, and where a less sour taste is preferred in maize ogi, the old supernatant should be discarded and fresh water added before the dewatering process, which is the next stage of processing.

Dewatering in the modern cottage process is facilitated by placing the cloth bag containing the sedimented fermented starch slurry under a village-made screw press or, as in the ancient times, using heavy objects to place over the bag to expel the liquor to yield a harsh starch cake having about 55% water content. The ogi is sold wrapped in thin nylon bags which may prevent contamination from the environment but insufficient to prevent spoilage within 2 days, unless stored under its sourced liquor until required, with occasional change of water, or under refrigeration. The price of ogi fluctuates according to the season—highest during the period of maize scarcity, the same as for the other grains.

IX. INDUSTRIAL/COMMERCIAL OGI PRODUCTION (FIG. 5)

Industrial ogi production starts with the acquisition of the maize and other cereals which are not abundant all the year round because of inadequate storage facilities and dependence on rain-fed fields. Therefore, the cereals are purchased at the time they are abundant and relatively low priced. They are purchased in Hessian bags each weighing nearly 100 lbs for maize and 80–90 lbs for sorghum and millet. Experience has shown that at 12% water content, the grains are storable in fumigated, rodent-free warehouses rather than in silos. The reasons are twofold. First, silo technology is too sophisticated for Nigerians (16), not to mention the huge investments required in foreign currency. Therefore, only transnational companies which can afford to retain silo engineers have silos attached to production factories. Second, grains are purchased in 100-lb bags and additional human labor would be required to remove the bags from trucks and open them for sale. Nonetheless, fairly shallow fiberglass silos are being used in our pilot plant for storage (Fig. 6) and fumigated with phostoxin tablets.

A. Cleaning

Grains are weighed on an Avery scale of 100 kg maximum capacity and dry cleaned first by aspiration to remove dust, using a Buhler aspirator (type MVSB) (Fig. 7). Then, gravel and grit are removed by a locally fabricated destoner equipment and are washed in water in a corn-washing machine which finally produces clean grain.

B. Steeping

The grains are soaked in water in aluminum drums (Fig. 8) using soak water one and a half to double the weight of grain, basically similar to the village

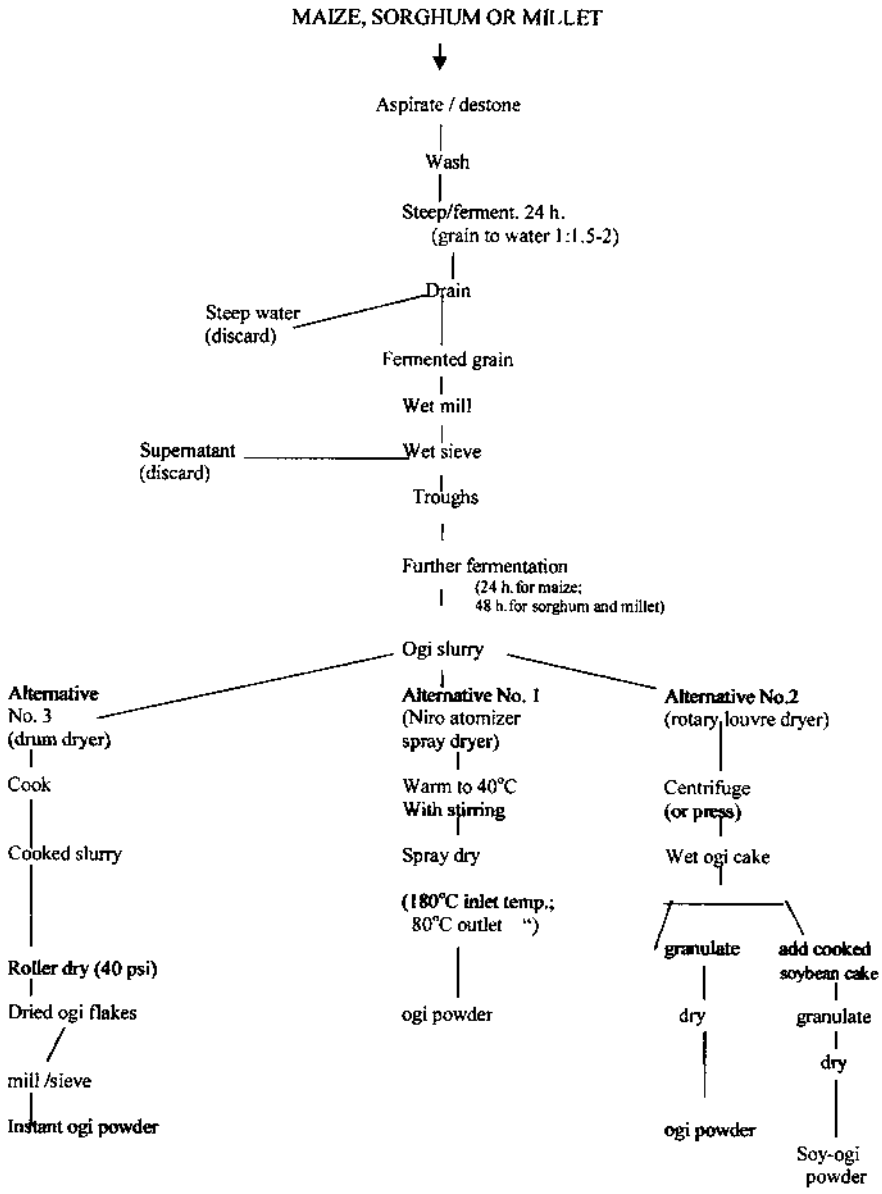

Figure 5 Flowsheet for industrial ogi production, showing three alternative drying methods (Modified from Ref. 2.)

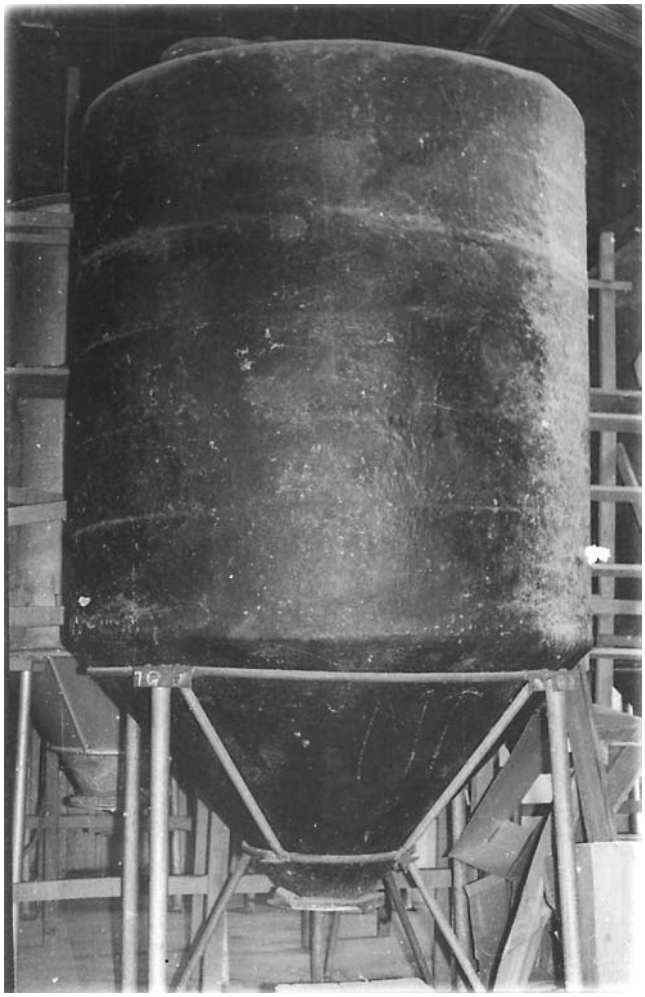

Figure 6 Storage of grain in a fiberglass silo. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

method. Fermentation of the grain is initiated during steeping and the grain softens sufficiently for wet milling after 24–36 h.

It appears that the ratio of steeping water to grain weight and the temperature of steep water are both significant. For example, Krochta et. al. (18) found that the yield of corn starch decreased from 70.3% to 67.5% when the steep water-to-corn ratio was reduced from 2 : 1 to 1 : 1 at the same sulfur

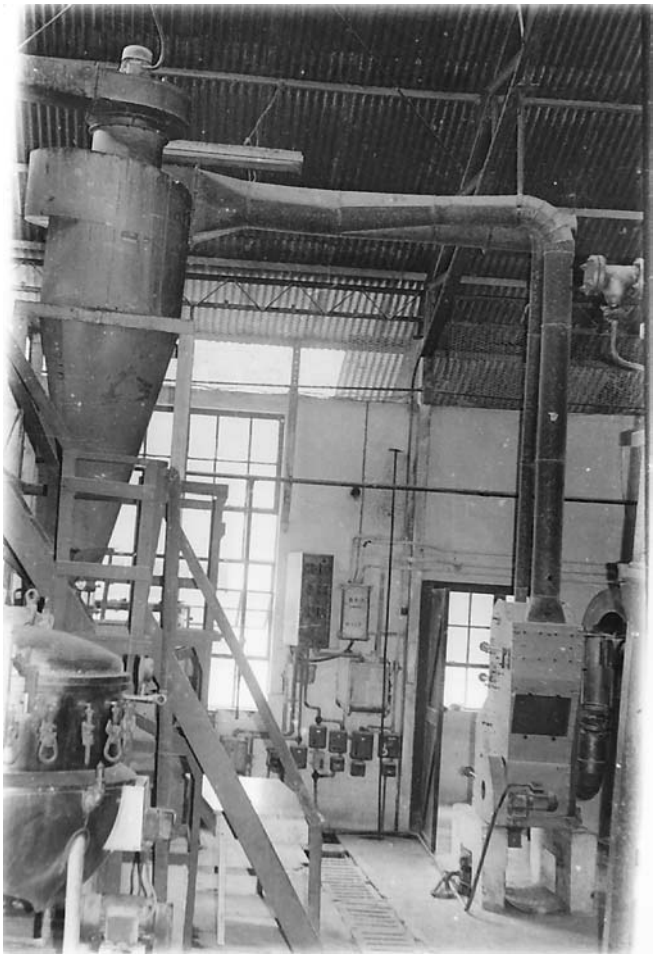

Figure 7 Maize cleaning with Buhler aspirator machine. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

dioxide concentration in the steep water. It was also reported elsewhere (17) that the peak moisture content increased when the soaking temperature was raised from ambient (29–32°C) to about 45°C.

C. Wet Milling (Fig. 9)

Like the ancient and cottage-level ogi processing methods, the softened, fermenting wet grain is wet milled in a Sprout Waldron disk mill (Paper and

Figure 8 Grains fermenting in soaking tanks. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

Pulp, Pennsylvania) mounted with a 25-hp electric motor which has coarse and fine adjustments for the grinding teeth, such that the teeth are widely separated to give coarse grinding at the first passage. Grains and water are fed into the hopper of the mill while an operator turns a handle that lets the grains onto the disk-grinding surface. The roughly ground grain is collected in a vessel and returned to the hopper for finer milling after the necessary fine adjustment (2). Compare this procedure with the double machine grinding of the grain in the cottage-level production. The double grinding ensures that most of the available starch materials are harvested. The total weight of water to be used is roughly twice that of the initial weight of the grain and this yields approximately 30% of the total solids after sieving and fermenting (2).

D. Wet Sieving (Fig. 10)

The milled product is sieved on a vibrating sieve known as vibroscreen. It has a 310-μm sieve mesh [British sieve size (BSS) 50], which generally retains the pomace while allowing the slurry to pass through into a stainless-steel lower deck of the vibroscreen, where the throughput is diverted via a spout into a collecting 200-L aluminum vessel. Several batches are collected in large aluminum vats mounted on wheels that enable them to be wheeled to the

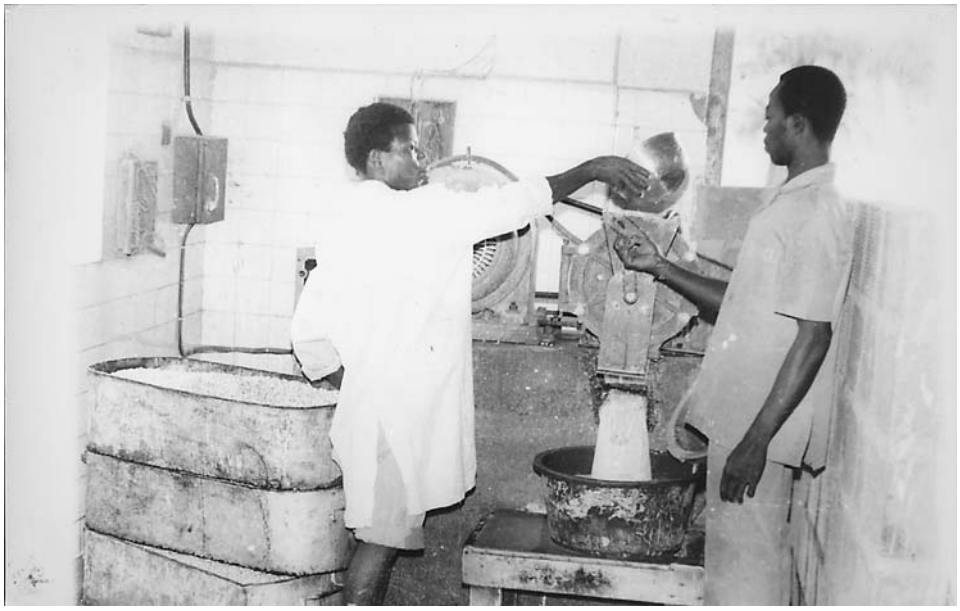

Figure 9 Wet milling of softened fermenting grain in a Sprout Waldron electric disk mill. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

fermentation area. The pomace is further dewatered in a basket centrifuge (Broadbent, UK) lined with cheesecloth and the liquor is collected and added into the slurry.

E. Further Fermentation

Fermentation of the grain started at the steeping stage but is insufficient to give the desirable slightly yogurt-type flavor. Therefore, further fermentation is necessary and usually 1 day is sufficient for maize slurry, but in sorghum and millet, the required sourness cannot be reached until 48 h later. By this time, the slurry has sedimented and a clear supernatant fluid is available for quality control. The pH of the maize slurry should have gone down from an initial pH of 6.5 to about a pH of 4.0–4.2 or about 0.6% lactic acid (19). The pH and total titratable acidity of the supernatant liquid determine the point of termination of the fermentation. These are therefore monitored carefully to yield uniform products.

Figure 10 Sieving of grains slurry in a vibrating sieve (vibroscreen) having a 310-μm mesh aperture. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

F. Drying

The primary and best method of drying the slurry into ogi powder is by the use of a spray-dryer (2) of the Niro atomizer type (Holland). The fermented slurry is pumped to a steam-jacketed, stainless-steel vessel having a stirrer (Fig. 11), where it is held for a few minutes to raise its temperature from the ambient temperature (29–32°C) to 40°C. By holding the slurry at the higher temperature, the drying time of the product in the spray-dryer is considerably reduced. The slurry is stirred continuously at a slow speed, and by means of a monopump, it is elevated to the drying chamber of the Niro atomizer.

The drying chamber is made of stainless-steel and has an atomizing nozzle at the top center (Fig. 12) connected to the feed slurry tank via the monopump. The ogi slurry is brought into contact with the atomizer nozzle by means of the pump. The atomizer generates small droplets which create a large surface area for moisture evaporation and, in addition, acts as a metering device to control the flow rate of the product for drying. It distributes the slurry into the airstream in a relatively uniform manner, producing droplets of the proper size that results in the desired product size after the process is

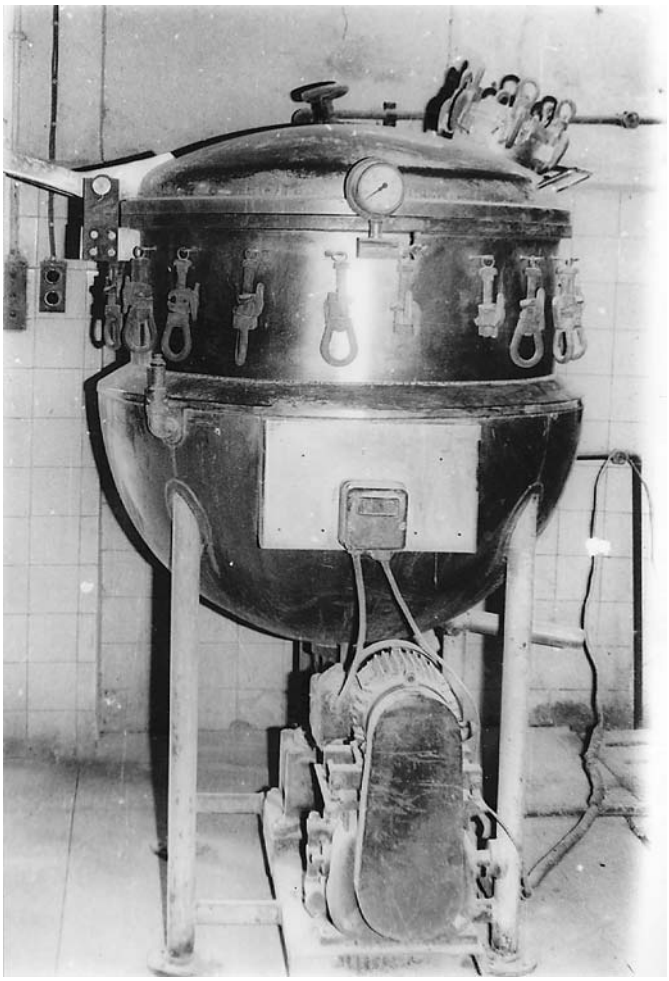

Figure 11 Holding of ogi slurry in a stainless-steel, steam-jacketed kettle at 40°C for spray-drying. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

completed. Unique features of the spray-dryer include a rapid drying rate, producing a final product which is ready for packaging as it leaves the dryer. Air is heated externally by means of fuel oil and is mixed with newly formed droplets at the atomizer. Then, the product and air move concurrently as dehydration proceeds. The inlet temperature at the control panel is 180°C and the outlet temperature is 80°C. The product and most of the air leave the

Figure 12 Drying of ogi slurry in a Niro atomizer spray-dryer showing the atomizer spray nozzle. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

drying chamber at the lower end and move to a cyclone separating system where powder particles are stripped out of the air and fall into a receiving container (Fig. 13). Additionally, three pairs of electrically operated hammers knock against the wall of the drying chamber intermittently and force it to release dried product sticking on the wall into another collecting clean aluminum drum. The ogi powder is thus collected at two points, one at the drying chamber and the other at the cyclone (2). The two product lines are blended together in a twin blender, sieved and waterproof packaged.

Two other methods of drying the ogi slurry were used in our pilot plant (see Fig. 5) and these might not have been more energy saving than spray-drying. The first was the use of a roller (drum) dryer. The ogi slurry was cooked in a stainless-steel, steam-jacketed vessel with stirring, and the cooked slurry was dried on a laboratory drum dryer at a steam pressure of 40 psi. When soybean flour was added to process into soy-ogi, drying was done at 60 psi and ogi flakes were obtained which were milled into microflakes in a disk

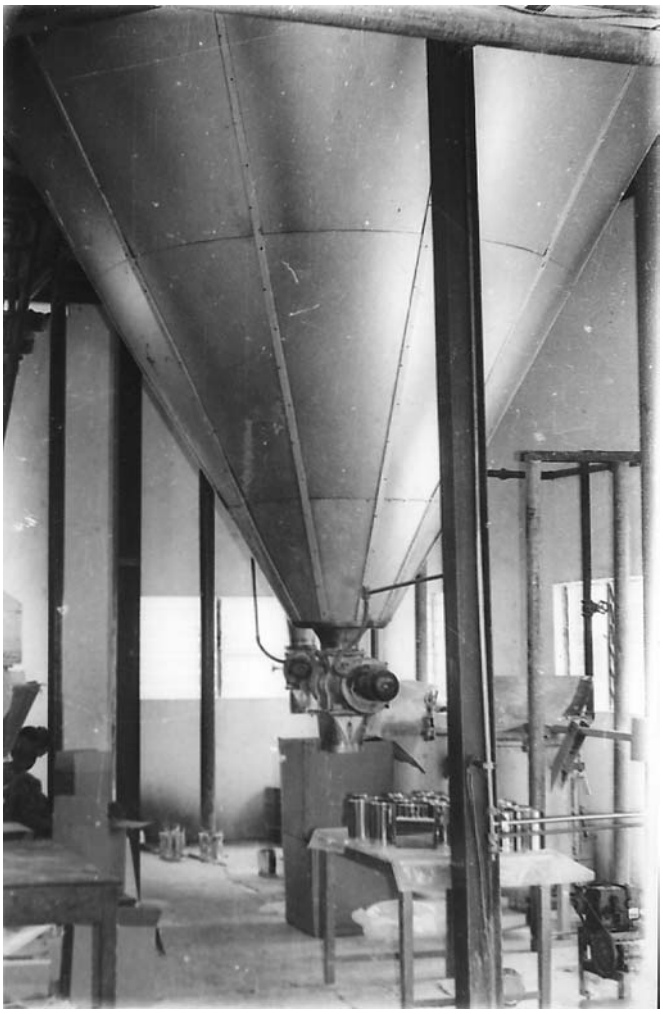

Figure 13 Drying of ogi slurry in a Niro atomizer dryer, showing collecting point of dried ogi. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

mill (Apex mill) and packaged in polythene bags. Hot water was used to reconstitute the microflakes. The problem with this method was the high cost of steam generation and loss of the ogi characteristic flavor because of the rather high steam temperature (2) and only about 78% of the original acidity remained (20).

The other optional method of ogi drying is the use of a rotary louvre dryer (Fig. 14), which is the current method for the soy-ogi production because of the breakdown of the other facilities in the pilot plant. The ogi slurry is dewatered in a Broadbent-type stainless-steel basket centrifuge lined on the inside with reinforced cheesecloth. The resulting starch cake containing about 40% water is granulated in a Kek granulator and fed into a tabular louvre-type (Newell Dunford, UK) rotary dryer of dimension 1 m long $\times$ 0.78 m wide heated indirectly by hot air from a diesel operated burner. A high-pressure fan blows the hot air across the length of the louvre dryer while a cyclone system at the end of the rotary dryer strips the dried ogi powder from the air. The ogi is elevated onto a decked sieve as a hot ogi cake which when cool is milled into ogi powder in an Apex disk mill and packaged in polythene bags. Rotary-dried ogi is rehydrated into a slurry and heated for about 3 min

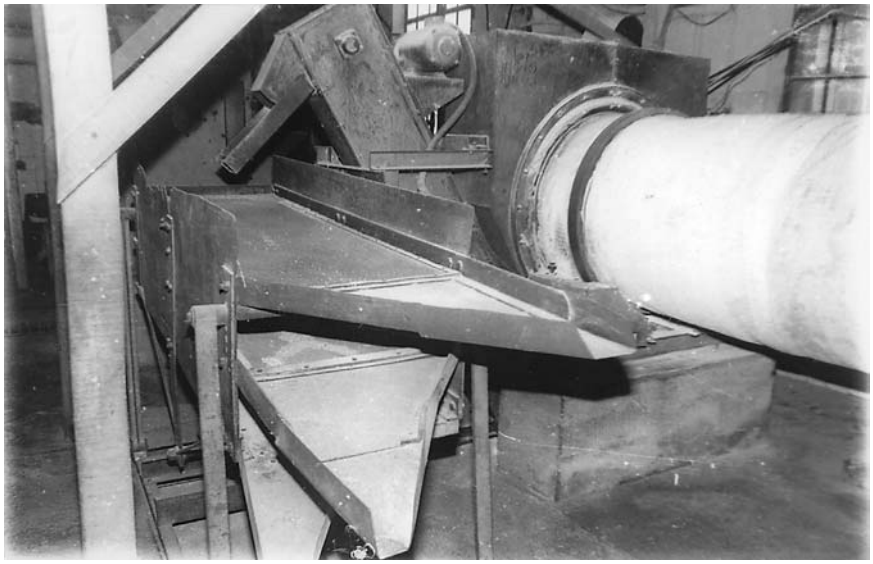

Figure 14 Newell Dunford (U.K.) louvre rotary dryer, suitable for drying ogi and soybean granules. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

into a pap for consumption. Traditionally, ogi slurry is not heated; boiling water is usually added to it to form the pap as it is stirred.

G. Soy-Ogi Production

Soy-ogi production for infant feeding as well as adult soy-ogi for adults is the area of interest and focus in the industrialization of ogi fermentation in Nigeria. Unfortunately, the production has been restricted to the pilot plant level at FIIRO, Lagos because the institute for years negotiated with Nestle of Switzerland to manufacture ogi in Nigeria and so commercialize the institute's research result in nutritional improvement of ogi, the traditional infant weaning food. The problem of ogi flavor has always been overflogged, but Nestle company, rather than produce soy-ogi (a soybean enriched ogi variant), has been producing a maize-soybean blend for infants exclusively without fermentation of the maize. It is decorticated and blended with soybean, using extrusion cooking methods to manufacture this baby product called Nutrend. Soy-ogi is yet to be seen in commercial quantity, but we now describe the pilot-plant production.

Soy-ogi production has two vital lines: (a) a fermented maize or sorghum line (called ogi line) and (b) a cooked soybean line (the patented process by spray-dryer is shown in Fig. 15). We have already described the ogi line which stops at the slurry level. Now, the soybean line proceeds as follows. The soybeans are weighed to reflect the ratio 70 : 30 of maize-to-soybean by weight. The soybean seeds are aspirated and the meat is washed in water and cooked for 1 h in a steam retort or 2 h on a domestic cooking stove using butane gas. When the Niro atomizer is to be employed for drying, then the cooked soybeans are wet milled in a Sprout Waldron electric mill to fine slurry first by coarse grinding, and followed by fine grinding, through careful adjustments of the grinding disk plates. The wet-milled soybean is filtered through a 310-μm sieve on the vibroscreen and mixed with the already prepared ogi line, warmed to 40°C, and pumped into the Niro atomizer drying chamber. After drying, calcium carbonate, potassium iodide, multivitamins, and flavor (optional) are blended into the soy-ogi flour ready to be sieved and packaged.

The final alternative method of soy-ogi production with the rotary louvre dryer is as follows (Fig. 16). Ogi slurry from the ogi line is dewatered in a basket centrifuge or with a hydraulic press after putting the slurry in double-layered polypropylene sacks. The boiled and milled soybean is also dewatered separately. Then, the two (i.e., ogi and soybean lines) are blended together using the Kek granulator, which produces 1-cm-sized granules of the ogi–soybean mixture, previously calculated at the ratio of 70:30 maize to soybean. Drying of the granules of soy-ogi takes place in a louvered tunnel cylindrical

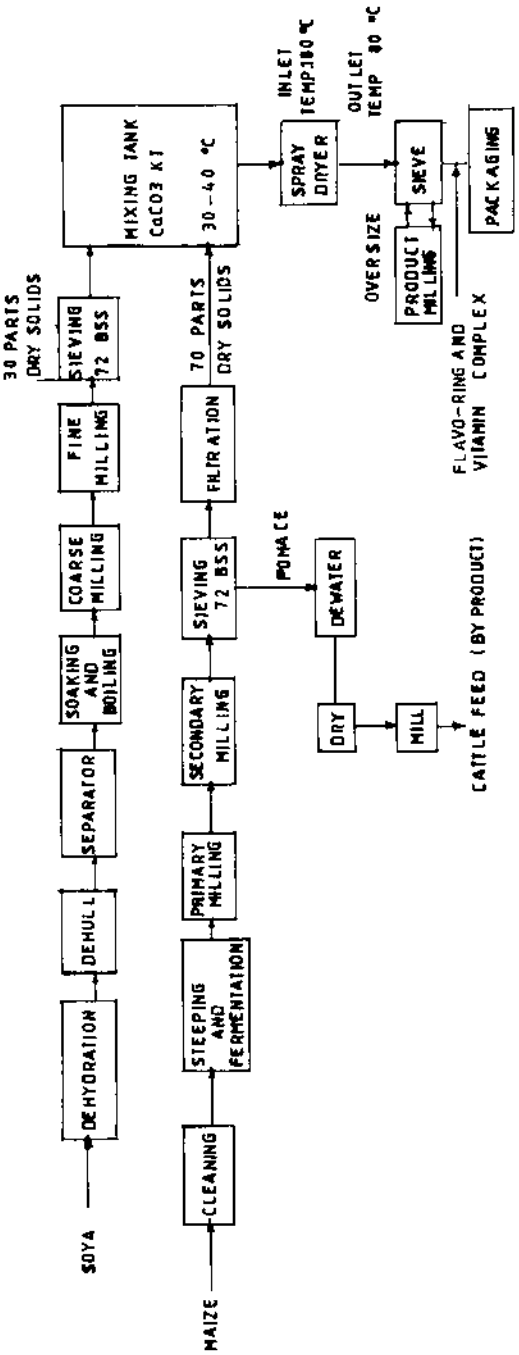

Figure 15 Patented soy-ogi processing layout for the spray-drying method. (From Ref. 32.)

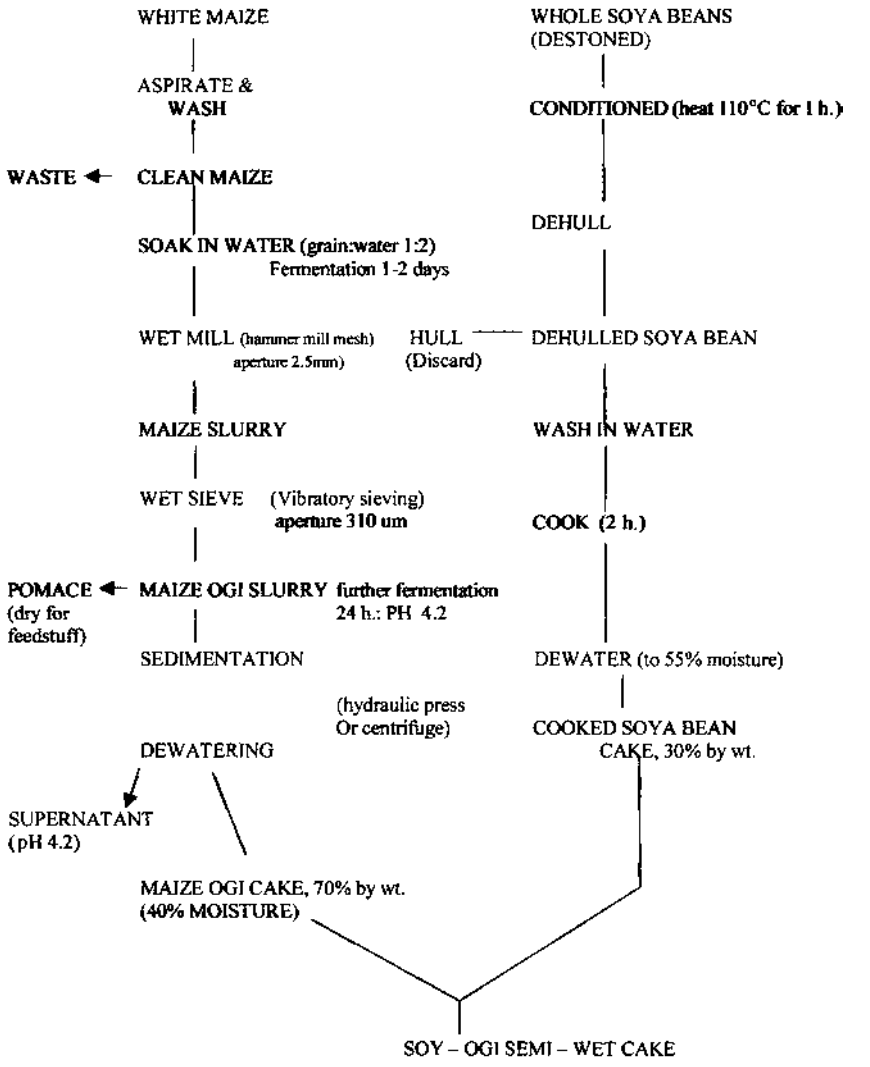

Figure 16 Flowsheet of ogi and soybean production lines to soy-ogi wet cake for the rotary drying method.

dryer (Fig. 17) rotating horizontally at a slow speed of 3 rpm and having a special alloy material inner lining and a good heat-resistant lagging material on the outside . The louvered dryer is heated by incoming hot air from a heating chamber, which is separated from the dryer by a wall. Diesel oil in a tank outside the building flows through a pipe into an atomizing chamber, where it is atomized by incoming compressed air. The highly charged oil particles are lit with a match to provide a large fire within the heating chamber. The

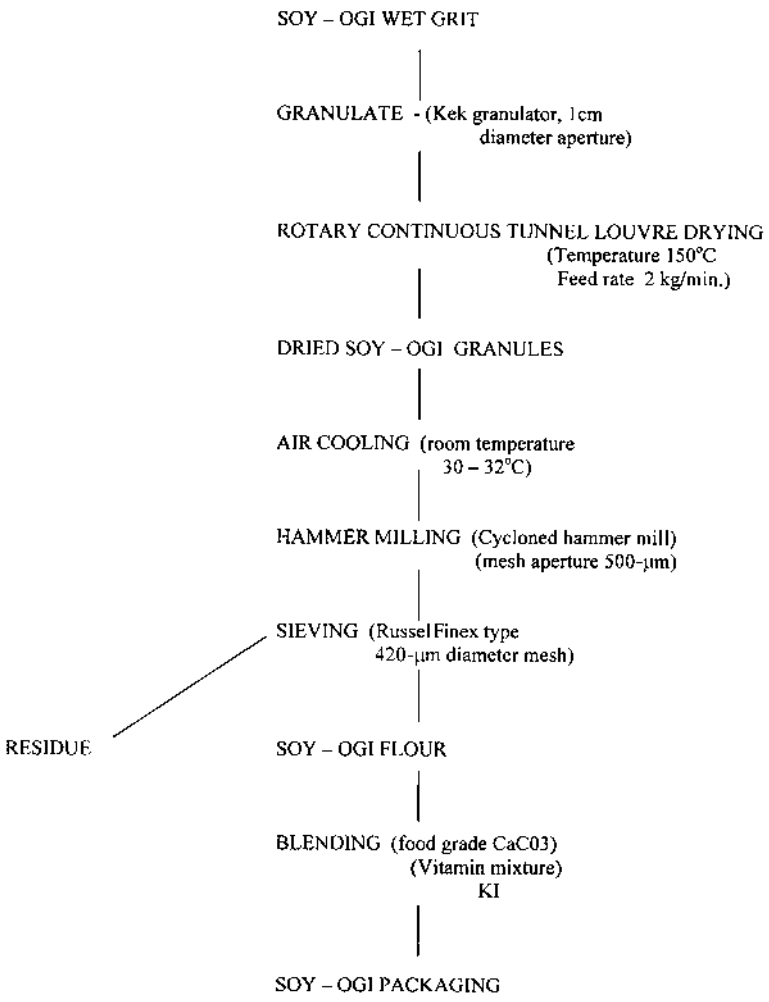

Figure 17 Flowsheet of mechanical drying of soy-ogi wet granules to ogi powder by N.D. rotary dryer. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

heated air is transferred into the louvered dryer, by a high-speed suction fan situated at the opposite end of the heating chamber, at an initial inlet temperature of about 150°C. The soy-ogi granule having an approximately 40–45% moisture content is fed via a vibrator at the hopper end into the horizontal tunnel, window-type dryer, where it takes 8 min to pass through at an average temperature of 130–150°C. The dried soy-ogi appears as small lumpy cakes, which are elevated via basket elevators at the discharge end, into a collecting aluminum pan. There is also a door at the discharge end of the tunnel dryer for observation of the drying process as it progresses and a cyclone to strip fine flour from the hot lumps.

H. Sieving, Blending, and Packaging

After the drying process, further treatment depends on the drying process. Specifically, spray-dried ogi (or soy-ogi) does not need further size reduction by milling. Drum-dried products are flaky and are reduced for flour with a hammer mill (Apex mill). The rotary-louvre-dried ogi (soy-ogi) is granular and lumpy, so special treatments are needed in the final stage. The final stage of the soy-ogi (ogi) process using the rotary dryer involves milling the lumps in a hammer mill built by Fober Engineering Company (Ibadan, Nigeria). This mill has built-in 500-μm aperture sieves and a cyclone system that facilitates the milling. This is followed by blending in a twin blender where flavor, minerals, and vitamins are added to the infant soy-ogi as already described. Finally, the product is sieved on a Russel Finex-type sieve having a 420-μm-diameter sieve and the soy-ogi is packaged in food-grade, water-resistant polythene bags. In conclusion, the most convenient method of ogi and soy-ogi production is the use of the Niro atomizer, especially because it reduces handling.

Another alternative for ogi fermentation advocated by Banigo et al. (21) and also suggested by some international baby food manufacturers in Nigeria for soy-ogi is the so-called controlled fermentation. No one has adopted it in the industry, for this would involve the maintenance of microbial bank cultures and returns on investment might be negative. Akinrele (19) identified several molds as associated with fermenting maize during ogi manufacture, the most important of which were *Cephalosporium, Rhizopus, Oospora, Cercospora, Fusarium, Aspergillus,* and *Penicillium*. Fears that some disease-causing fungi might be present (22) could be justified and this might result in the alternative use of steam-sterilized grain followed by inoculation of wetted meal with known approved micro-organisms (22). Whether the final flavor of such ogi will be acceptable to adult taste is yet to be seen, and, which local industrialist would dare make an investment in this area? We have already seen the fate of soy-ogi with respect to its anticipated commercialization by Nestle Company in Nigeria in favor of its unfermented variant,

Nutrend, which has been in the market for about 20 years now. We have, however, found that well-fermented ogi slurry is self-sterilizing and inhibits the growth of pathogenic *Escherichia coli* (23). For years, research results aimed at altering the texture of Nigerian ogi have failed to be adopted. For example, it was attempted (21) to increase the protein content of ogi by using dry-milling methods but ended up with somewhat particulate ogi, changing the usual smooth texture. Other workers (24,25) also processed ogi from dry-milled maize but had particulate products which also failed to gelatinize when preparing the ogi pap. To the contrary, particulate ogi is the normal texture in Ghana.

We carried out a taste-panel evaluation in our pilot plant of particulate ogi prepared from decorticated maize by dry milling and found that it was totally rejected (2). However, this is not to say that the adoption of traditional ogi pattern for industry is easy. The long processing steps are amenable to contamination.

X. CONTRAST BETWEEN INDIGENOUS AND MODERN PROCESSING

Modern ogi processing differs from indigenous methods mainly in better mechanization, better hygiene, and improved control measures. This results in a final product having better presentation and longer shelf life, but not necessarily of better nutritive value unless when ogi has been enhanced with soybean to produce soy-ogi. Otherwise, the basic processes are identical.

Grains are purchased in small quantities, from a few basins up to a few bags of 100 lbs each, in indigenous processing and stored in covered basins to keep out rats. In other occasions where traditional cribs for grain storage exist, the crib is the source of the grain for the indigenous ogi processing (16). On the other hand, bulk purchase and storage of grains in warehouses contained in Hessian sacks or in fiberglass silos is the usual practice in modern processing. In the village method, grains are scrubbed manually in water and gravel is removed when observed, whereas in modern methods, air aspiration, destoning machines, and corn washing machines are utilized for cleaning (2). Milling of grain is done with a petrol-operated wet disk mill commonly placed in front of residential houses and marketplaces, principally by women who are modern ogi cottage-scale producers utilizing the disk attrition mill (Premier 1A type) for wet grain milling. Rarely is the wooden mortar and pestle used nowadays, as the resulting wet meal is usually much coarser than that from an electric disk mill used in modern industry, despite the more tedious and time-consuming procedure with the traditional methods. Generally, electrically operated wet grinding mills, like the Sprout Waldron type, meant for wood

grinding, give much greater ogi yield on the commercial ogi scale than in the other. Again, in the traditional method, the white cloth is the sieve, whereas in the modern method, a mechanical vibrating steel sieve having a fine aperture of 310 μm facilities the more satisfactory removal of trash. Fermentation in the modern methods is monitored by pH changes and total titratable acidity of the steep liquor and the milled fermenting grain liquor (2); quality control, is absent in indigenous procedures.

The primitive palm frond sieve used in ancient times could not remove the finer particles as perfectly as that of the modern processes using the 310-μm sieve that removes virtually all coarse particles from the slurry (2). The lesser refinement in the traditional process is not intentional, because ogi is not expected to have particles. As stated earlier, furah is precooked and, even to date, the wooden mortar is employed frequently by housewives who pair themselves to beat sorghum grain in traditional mortars. When reconstituted in sour milk, furah is actually lumpy to the palate, unlike ogi.

In the modern process, the final ogi product is a dried powder that is packaged in cardboard and polythene (Fig. 18) and this improved packaging has given the ogi a shelf life of up to 6 months, whereas the ogi sold as a cake of

Figure 18 Packaged soy-ogi variant in cardboard showing adult and infant brands. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

40% water using traditional methods spoils within 2 days. Even when under refrigeration, the sour flavor is soon lost, and yellowish discoloration sets in within 7 days. However, the best way of storing traditional ogi is to leave the cake in its sour liquor, wherein it may remain in good condition for up to 14 days and for nearly 39 days if refrigerated before discoloration destroys the entire product (26).

The various optional drying methods (spray, roller, rotary drying) used in commercial practice are large departures from the traditional procedures. Especially revolutionary is the choice and control of the fermenting organisms, by dry milling the cereal, moistening the flour, and inoculating same with *Lactobacillus* and yeasts (21). This method has not yet been commercially adopted, and it may not be profitable because of the maintenance of microbial banks, which may be contaminated, after all and the evil circle continues over a product whose major consumers are the poor (27).

XI. CRITICAL STEPS IN MANUFACTURE/FERMENTATION

A. Input Grain Storage

The first critical point to begin with is the purchase and storage of maize and sorghum grains. Millet is bought whenever needed. Maize is purchased when it is most available and least expensive. The highest level of moisture content for storage is approximately 12%. Fresh maize grain or moldy grain is not acceptable. The grain is cleaned by air aspiration and the Hessian bags in which they are delivered are opened and the grain is dumped into inverted fiberglass silos carried on metal frames, being properly cleaned and fumigated with phostoxin tablets placed randomly within the maize from the top to bottom of the silo. Up to 10–20 bags of the maize weighing about 100 lbs each may be contained in one silo. Several tons of healthy maize are stored for a few months according to the plan of production. The top of each silo has a lid and a padlock to prevent pilfering, and each silo has a platform to climb to the topmost height of the silo. Samples are taken from time to time to check for moisture content and moldy grains. The silo storage is the source of grain for the manufacture of ogi and soy-ogi, and in order to aerate the silos, grains are transferred periodically from one silo to another. Soybean grains are also stored separately in other silos, all under a roof in the warehouse, with occasional monitoring for moldiness.

B. Grain Soaking

Apart from removal of gravel, filth and moldy grain by the use of a destoner and corn washer, the next critical step in the manufacture is grains soaking in

water to soften and initiate fermentation (28). First, the critical ratio of 2 : 1 for steep water to grain in the ogi process is not negotiable. For instance, Krochta et al. (18) found that the yield of corn starch decreased from 70.3% to 67.5% when the steepwater-to-corn ratio was reduced from 2 : 1 to 1 : 1 at the same sulfur dioxide concentration in the steepwater. Although no sulfur dioxide was used in ogi steepwater, it was reported (29) that sulfurous acid, apart from disrupting the protein matrix during corn steeping also acted to limit undesirable fermentations. For instance, Akinrele (19) discovered a large number of mold contaminants which remained up to 6 h of soaking, but these could have been eliminated much earlier had it been that the maize steepwater contained sulfur dioxide in acidic environment as was suggested (30), although nobody knows how it could have affected the ogi taste.

Because, from literature (17), white maize-type TZPM absorbed the most moisture within the first 24 h and the peak water uptake was at 36 h, white maize should normally be soaked for a minimum of 24 h to a maximum of 36 h, but sorghum is best used after 48 h steeping. Other workers (30) also reported that the highest level of solubilization of corn insoluble proteins was achieved in steepwater at pH 3.0 and 3.5 under sulfur dioxide acidic conditions, with protein degradation ceasing after 20–25 h. Therefore, the soaking time and temperature are important, for in cold harmattan weather, warm water should be used, as has been suggested (17).

C. Wet Milling

Finely ground maize, especially dried maize which normally has a high starch content, will naturally yield a considerably greater quantity of starch when ground with a sharp-toothed mill than using a blunt disk mill for the wet milling. In industry, the grinding teeth of the maize mill are sharpened frequently to obtain optimum yield of slurry and starch.

D. Wet Sieving

It has already been stressed that particulate ogi is not usual in Nigeria, and according to the method of ogi into pap, it is impossible to use it for infant feeding. Adeyemi et al. (25), who processed ogi from dry-milled grains, found, to their dismay, that the particulate product failed to gelatinize readily into ogi pap. Furthermore, panelists in our pilot plant rejected ogi having particles prepared in the pattern of Ghanaian ogi. Therefore, a fine cloth sieve used in traditional ogi or a stainless-steel sieve as fine as 310 μm is needed to remove all of the grain particles, leaving only the fermented carbohydrates to pass through.

E. Fermentation

Fermentation of the grain starts in the first 24 h during steeping, being initiated by naturally selected microflora from the environment, especially *Lactobacillus* (19,31). The sourness of the milled and sieved grain is never sufficient to reflect the mild yogurt-type flavor. Therefore, additional fermentation of the starch slurry for 24–28 h to a pH of about 4.3 ± 0.2 is the usual practice in the industry (7).

F. Soybean Preparation

For soy-ogi production, healthy grains are the ones fit for use in production. Soybean is screened to remove gravel and then heated at 110°C for 1 h to facilitate its cracking, using the Bentall disk mill just before it is required for use. Long storage of the meal causes rancidity to develop because of the release of lipoxidase enzyme in the bean. In addition, the cooking of the meal is critical. Sufficient cooking should be allowed to produce softened soybean meal in order to ensure that antinutritional factors, particularly trypsin inhibitors, lipoxidases normally present in uncooked soybean, are totally eliminated. Improperly prepared soybean is unsafe for soy-ogi preparation. Again, once the boiled soybean is wet milled into a slurry, it is ready to be blended into the ogi line and dried by spraying without additional fermentation. It is pertinent to note this point in that Nestle, the company that was expected to commercialize soy-ogi, faulted Akinrele's patent, (32) which gave an additional 2–3 h period to ferment the soybean–maize slurry mixture, postulating that the increase of pH would hamper the antimicrobial effect present in ogi. A problem of unstable viscosity of cooked soy-ogi when raw soybean was added was reported (33) and the incomplete destruction or regeneration of the amylolytic and lipolytic enzymes present in the soybean component was suspected. The answer is that freshly cracked soybean not having high levels of lipoxidase or amylases through exposure to moisture following cracking and storage for considerable length of time should be the one fit for soy-ogi processing. To add raw soybean to soy-ogi was ridiculous and defeated the need to cook the bean. Again, fears that the rise of pH from pH 4.0–4.2 in ogi to pH 5.0 in soy-ogi liquor, according to Nestle company, might reduce the antimicrobial factor inherent in well-fermented ogi against pathogenic micro-organisms, as reported by Onyekwere and Solomon (23), and thus permit disease-causing micro-organisms, especially fungi, to multiply. Hence, the further fermentation following the blending of the ogi line with soybean reported earlier (34) has been modified in the pilot plant for soy-ogi processing. What happens currently is that the ogi slurry, blended with the soybean slurry, is dried immediately without further fermentation. As already

explained, liquefaction of soy-ogi has its source from insufficient cooking of soybean or use of soybean meal that has been dehulled and improperly stored.

Secondary fermentation was reported to vary from 24 to 72 h (9,31), but experience has shown that maize ogi fermented for more than 24 h in which the steep liquor is to be used in its spray drying is unacceptably sour.

G. Drying Method

The choice of drying method is critical, although each has its merit according to what is available. The most acceptable soy-ogi is by spray-drying using the Niro atomizer. The color, flavor, aroma, and granulation are excellent but the cost of the Niro atomizer is prohibitive. Drum-dried ogi, although less expensive-saving than spray-drying, is not as acceptable (9). Rotary drying of ogi cake shows the greatest appeal because of its low-level technology, but it is apt to contamination because of many intermediate steps. Extrusion cooking is being used by the company manufacturing a nonfermented maize and soybean blend exclusively for infants instead of soy-ogi. Whichever drying method is used, the moisture content of the product should not be above 6%. Soy-ogi dried with the spray-dryer has a low moisture content of 5%.

H. Packaging

Unlike the cottage-level ogi, 450 g of industrial soy-ogi is packaged in water-resistant polythene in cardboard housing to prevent moisture from being absorbed from the environment (Fig. 18). Also, cartons are used as containers for the ogi in polythene so that flour weevils, which are among the causes of spoilage of ogi products, might have no access (Fig. 19).

I. Regular Monitoring of Critical Control Points

Critical control points (28) in ogi manufacturing should be regularly monitored to conform to set standards or quality. These points are relisted for emphasis:

1. Grains (maize, sorghum, soybean): Check moisture content; no moldiness.
2. Grains storage: Aerate the grains; fumigate the silo.
3. Steeping in water: Use the proper ratio of 2:1 for water to grain.
4. Grinding: Wet grinding until fine slurry is obtained.
5. Boiling soybean for soy-ogi: Proper cooking of healthy soybean grain in order to eliminate every antinutritional factor.

Figure 19 Packaged adult soy-ogi variant in tin. (Courtesy of Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

6. Final moisture after drying: Ogi (or soy-ogi) moisture kept as low as possible (certainly below 9%).
7. Dry-milling and dry-sieving points: The dry milling and dry sieving points where the ogi will pass for final packaging should be cleaned with a solution of hypochlorite about 30 min before use for microbiological reasons.
8. Laboratory control: The quality control laboratory should maintain the set standards of pH in soak water, pH of 4.0–4.2 in ogi slurry, standard flavor of the ogi, total plate count within the limits, general hygiene in the factory satisfactory; the operatives should wear nose masks and cover their hair.

XII. MAJOR PROBLEMS IN INDUSTRIALIZATION

The real interest in Nigeria as far as ogi is concerned is the commercialization of soy-ogi, not the plain ogi. Soy-ogi is the ogi variant meant for infants, as the

first solid food that should replace the traditional ogi commonly used widely, particularly in southern Nigeria for infants. The major problem in the industrialization of soy-ogi is the absence of a single company that can take responsibility for assembling all of the equipment into a turnkey system, readily available for the entrepreneur who has the money to invest in it. The Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, which pioneered the soy-ogi research, has failed in its leadership role to assemble the equipment into a continuous production system, unit by unit matching in capacity, with little or no manual handling. Because baby food manufacturing demands high level of hygiene and precision, the only indigenous company that commercialized soy-ogi under the trade name "Mama-joy" had to be disbanded because of some quality problems with the country's National Agency for Food and Drugs Administration and Control (NAFDAC). The products were declared unfit for consumption after a short period of operation. The company did not manufacture soy-ogi with the Niro atomizer. They attempted to use a flash dryer with the advice of FIIRO, which did not have adequate knowledge of this equipment, commonly being used for drying cassava starch. Luckily, as far as Nigeria is concerned, an international company, Nestle, has since produced, instead of soy-ogi, an infant's first solid food called Nutrend, which is a maize–soybean blend but without fermenting the maize. Because Nutrend is strictly for infants, suitable baby flavors are present, thus making unnecessary the so-called ogi sour flavor which only adults can fault if it is not present.

For soy-ogi to be successfully produced in Nigeria, the investor will have to go back to the purchase of a Niro atomizer, and other units of equipment will have to be fully synchronized with the full involvement of FIIRO as a technical consultant.

Another problem area is the availability of raw materials, especially soybean and maize. Backward integration failed with respect to gari industrialization and it will fail with soy-ogi. If farmers of maize and soybean are part of the company, then they will make sure that they keep their own portion to make the grains available at reasonable prices, otherwise the grains may not be regularly available and their prices will soar until the manufacturing company folds up. This is a common feature of the food manufacturing industry in Nigeria. Undoubtedly, the adult brand of soy-ogi will find a ready market, particularly in southern Nigeria, because soy-ogi will hardly outsell Nutrend for infants. In summary, the lack of turnkey equipment at reasonably low cost, lack of proper technical expertise, shortage of capital and poor infrastructure for industry in Nigeria, and fear of nationals to invest in manufacturing, even in the face of a large market in Nigeria and along the western countries of Africa, are some of the major problems in industrialization of ogi or its improved variant, soy-ogi.

Wet milling is tedious and more time-consuming than dry milling and seems to be another bottleneck in ogi industrialization. Technically, the adoption of basic traditional steps in modern processes tends toward batch production. For example, maize steeping and fermenting take approximately 2–3 days and other intermediate steps predispose the product to contamination, and if inoculation with mixed cultures of *Lactobacillus plantarum*, *Streptococcus lactis*, and *Saccharomyces rouxii* (21) is adopted by industry, the cost of maintaining a pure culture bank would be prohibitive and thus make the product out of reach of the native poor, who are the target group. In addition, the milled grain ought to be sterilized by steaming before inoculating if pure fermentations are the target, but preheated maize does not ferment as well as raw grain (35,36). As already mentioned, the particulate type of ogi, advocated by some workers (21,25,37,38) is not an answer to ogi industrialization, because it will have the problem of acceptance unless the ogi is precooked so as not to choke babies and even adults. Taste panel tests show that soy-ogi processed by dry milling of maize was not as acceptable as normal soy-ogi. Indeed, the level of wastage in input material and loss of nutrients in ogi are very discouraging points in its full industrialization. Thus, adults might as well consume corn flakes, tuwo, or beans (ewa) instead for breakfast. Other than as soy-ogi for infants, traditional ogi with its present level of presentation in nylon is quite acceptable even in its wet state.

XIII. OPTIMAL ENVIRONMENTAL CONDITIONS

Maize fermentation in steepwater is semianaerobic at ambient temperature (29–32°C) and a ratio of maize to water of 1:1.5 to 1:2 usually proves satisfactory for fermentation (2). The same ratio is used for sorghum when it replaces maize in ogi. In the cold, windy harmattan season (about 25°C ambient), fermentation is slow, and at 45°C, fermentation of grain is retarded (7). Steeped maize liquor falls from pH 6.3 to approximately pH 4.2 and the milled slurry changes from pH 6.35 to pH 5.25, both within 24 h of steeping (35). The pH of the steep liquor at pH 4.3 $\pm$ 0.2 is the standard for ogi (7,40). This is equivalent to 130 mg NaOH/100 g of product (31) and the most acceptable ogi flavor is reported to come from ogi of pH 3.6–3.7, but no less (7). Unfermented ogi has a bland, unacceptable taste, and aerobic fermentation, which is vigorous through increased production of volatile acids (especially acetic) by enterobacter, results in off-flavor (2). The addition of 30% cooked soybean shortens the fermentation of maize, reducing it to 3–4 h (33). A total solids content of 25–30% is used for soy-ogi spray-drying, so that the total water added after wet sieving is limited to give this range of total solid (2).

The processing factory for ogi (or soy-ogi) should be protected from birds by protective wire screening. Because there is a large amount of starchy slurry in the washwater entering into soakaway, the factory could be a breeding ground for rodents and cockroaches. Therefore, proper arrangements to evacuate the soakaway at regular intervals so as to eliminate fermenting, offensive odors, as well as fumigation from time to time against rodents and cockroaches should be in place. The wall of the building, up to 6 ft, particularly in the processing area, should be lined with wall tiles which can easily be washed and kept clean to keep the factory in proper hygienic conditions. Finally, the process vessels, utensils, and dry, milling points should be rinsed with a solution of hypochlorite 30 min before use each time, to reduce or eliminate microbial hazards in the ogi product (28) and operatives must wear protective clothing as consistent with good manufacturing practices. Those of the workers who are carriers of staphylococcus or who are suffering from the common cold should see the medical doctor.

XIV. MICROORGANISMS ESSENTIAL TO FERMENTATION

Most tropical indigenous fermented foods experience lactic acid fermentation and ogi is not an exception. Indeed, lactic acid fermentation could be a low-cost means of the preservation of the product (40) and, in fermented corn foods, e.g., kenkey, it improves the nutritive value (41). Akinrele (19) found that at the maize-soaking stage for ogi production, the surface of the steep water was contaminated with molds, namely, *Cephalosporium*, *Rhizopus*, *Oospora*, *Cerospora*, *Fusarium*, *Aspergillus,* and *Penicillium*, but these were all eliminated within 6 h of steeping. The naturally occurring fermenting bacteria reported by him were *L. plantarum*, *L. brevis*, *L. cloaca*, *Corynebacterium* sp., *Clostridium* sp., *Enterobacter cloaca*, and *Acetobacter*. The yeasts found to be in association with the maize-fermenting bacteria were *S. cerevisiae*, *Rhodotorula* sp., and *Candida mycoderma*. It was concluded that *L. plantarum* was the major fermenter in maize ogi, dominating in the production of lactic acid (and tolerant to it) and that this acid contributed to the acceptable sour taste of ogi flavor. Banigo and Muller (42) confirmed *L. plantarum* as the dominant micro-organism in association with some yeasts during ogi processing. Similarly, other workers (43) isolated *Lactobacillus* sp. and *Enterobacter* in steeped maize within a 24–48-h period. When sorghum was steeped in water for ogi, they isolated *Leuconostoc* within 24 h, but this was superseded by *Corynebacterium* sp. in 48 h. While using millet grain, they noted that the micro-organisms were identical to those isolated in maize in both periods of time (Table 1).

Table 1 Analysis of Bacterial Flora of Steeped Grain Liquor Grown on Nutrient Agar

Time for steeping	Cereal	Organisms identified	No. of cells/g
24 h	Maize	*Lactobacillus* sp.	4.6×10^7
		Enterobacter	3.0×10^8
	Sorghum	*Leuconostoc*	5.4×10^7
	Millet	*Lactobacillus* sp.	3.5×10^7
		Enterobacter	3.1×10^6
48 h	Maize	*Lactobacillus* sp.	5.0×10^7
		Enterobacter	4.3×10^9
	Sorghum	*Corynebacterium*	6.0×10^6
	Millet	*Lactobacillus* sp.	4.6×10^6
		Enterobacter	3.2×10^2

Source: Ref. 43.

The role of the dominant micro-organisms was investigated by Akinrele (19). After sterilizing steeped maize for 24 h using 5% sodium metabisulfite and following this with milling and sieving, the samples were inoculated with isolated dominant micro-organisms in combinations.

The samples inoculated with 24-h steep liquor produced the greatest souring. This was followed by a combination of *L. plantarum*, *E. cloaca*, and *Saccharomyces*. Single organisms were also studied and *L. plantarum* was found to produce the greatest amount of acid (lactic), probably because it can utilize dextrins of maize after the fermentable sugars have been depleted (19,44). *Corynebacterium* is said to hydrolyze the starch of maize to form organic acids, with *S. cerevisiae* and *C. mycoderma* contributing to the flavor acceptability.

Analysis of bacterial flora of steep liquors of maize, sorghum and millet at 24–48 h of steeping were similar, namely, *Lactobacillus* sp. and *Enterobacter* sp., with *Lactobacillus* occurring in greater numbers each day for maize and millet (Table 1). In sorghum, *Leuconostoc* sp. appeared at 24 h but was replaced by *Corynebacterium* sp. at 48 h (43). Odunfa and Adeyele (45) isolated *L. lactis* from sorghum fermenting for sorghum ogi (ogi-baba). Additionally, Adegoke and Babalola (46) isolated, during a 72-h period of ogi fermentation, the following predominant flora at the hours specified:

0 h *Klebsiella oxytoca*
24 h *K. oxytoca*
48 h *K. oxytoca; S. cerevisiae*
48 h *Bacillus subtilis*
72 h *S. cerevisiae*

Other workers writing on similar traditional fermented maize products have reported principally the presence of lactobacilli. Fields et al. (47) isolated *L. fermentum*, *L. cellobiosus*, and *Pediococcus acidilactici* from naturally fermented corn meal. A product similar to ogi is uji in East Africa which Mbugua (48) discovered to be fermented by lactic acid bacteria (*L. buchneri* and *P. pentasaceus*). Gashe (49) isolated *Leuconostoc mesenteroides* and *L. brevis* from tef. Nout (50) confirmed that the development of lactic acid bacteria in sorghum-based fermented infant foods was stimulated by the presence of yeasts which provided soluble nitrogen compounds and other factors. Hounhouigan (14), working on mawè, a fermented maize dough staple in Benin, reported that in the fermentation of both home-produced and commercial mawè, *Lactobaccillus* sp., constituted the majority (about 94%) of the strains of the lactic acid bacteria isolated, among which 89% represented the Betabacterium group. They included *L. fermentum* (biotype cellobiosus, 41%), *L. fermentum*, or *L. reuteri* (19%), *L. brevis* (26%), *L. confusus* (less than 2%), and others. Interestingly, no strain of *L. plantarum* generally reported as dominating lactic acid bacteria (and dominant in ogi) at the final stage of fermentation of most plant foods was isolated (14). It is indeed significant to note in the mawè production that a change from whole steeped maize and wet milling in ogi processing to decorticated maize and dry milling in Benin's mawè production makes a great deal difference in the interplay of micro-organisms involved in the early and final stages of natural fermentations of the two maize products. The inoculating micro-organisms could come from the environment, utensils, old slurry, and so forth, as was found with pozol, a traditional Mexican fermented maize dough (51).

Teniola and Odunfa (52) monitored microbial populations at different stages during steeping and souring of ogi fermentation using white maize. They noted that there was bacterial dominance throughout the fermentation process except at the very late souring stage (168 h). During the steeping stage (first 24 h), there was continuous increase in microbial population (see Table 2) and when souring started (28 h) with processing steps such as grain washing and sieving to remove the grain chaff, these led to a reduction in population. The highest total mesophilic aerobic population was observed at 72 h, but the yeast population was erratic and continued to increase to the end of fermentation (168 h). They suggested that the variation in the microbial population might be influenced by factors such as nutrient availability for growth (i.e., carbon and nitrogen sources), pH, oxygen level, and the type of micro-organism present in the samples. *Saccharomyces* was the dominant yeast during steeping and up to the early souring stage (72 h), wherein *Candida* was noted at low proportion but became dominant from 96 h to the end of fermentation. They implicated *Candida valida* and *C. krusei* as well as

Table 2 Changes in Microbial Counts (CFU/mL) of Fermenting Maize Grain During Ogi Fermentation

Fermentation period (h)	Mesophilic aerobic bacteria	LAB[a]	Yeasts
0	2.28×10^5	2.47×10^5	1.60×10^3
12	9.20×10^7	9.82×10^6	4.50×10^5
24	9.80×10^7	1.40×10^7	2.10×10^5
28	9.70×10^6	1.20×10^7	1.03×10^5
36	1.39×10^8	9.60×10^7	2.80×10^4
48	9.65×10^8	5.50×10^8	6.20×10^4
60	1.05×10^9	7.50×10^8	1.18×10^5
72	1.19×10^9	8.40×10^8	7.05×10^6
84	5.75×10^8	9.80×10^8	2.95×10^6
96	9.81×10^8	7.05×10^8	2.34×10^6
120	6.35×10^8	1.45×10^7	5.18×10^6
168	1.52×10^8	1.04×10^8	8.32×10^8

[a] Ogi prepared using bacteriocin-producing lactobacillus (53).
Source: Ref. 52.

Geotrichum candidum, which appeared at a lower dilution. *Geotrichum candidum* appeared to be the dominant fungal culture in a spoiled ogi sample but it was not previously reported to be linked with ogi fermentation, except in gari, a cassava product (54).

New attention is currently on the use of starter cultures in solving numerous problems associated with the ogi product. Ogi, which has the ability to prevent and treat many waterborne diseases and highly relevant to the African environment, was developed by Olukoya et al. (53) and Olasupo et al. (55,56) using bacteriocinogenic lactobacilli. The usually unhygienic traditional processing methods in ogi are liable to several bacterial and fungal contaminants and only properly heated ogi can ensure elimination of the micro-organisms. This is often the case in well-fermented ogi, being bacteriocidal against *Escherichia coli* (23). However, traditional ogi is not usually boiled, rather, boiling water is added to the ogi slurry, with stirring, to prepare the pap. When boiled, the pap changes to a gel (eko, agidi), consumed as a different maize light meal, used also for convalescent adults. Recently, Teniola and Odunfa (57) and Odunfa et al. (58) used lysine and methionine lactobacilli and *S. cerevisiae* strains as starter cultures to increase the lysine and methionine levels of ogi, both amino acids known to be deficient in maize ogi diet (59).

XV. MICROORGANISMS THAT CAUSE SPOILAGE

The most important microorganisms that cause spoilage in wet ogi stored as a wet cake outside its soured liquor are *Rhizopus nigricans*, *Aspergillus* sp., and *Penicillium* sp. and are similar to the spoilage micro-organisms isolated in market gari (60). These fungi cause a yellowish discoloration and blackish spots in the wet ogi, giving the product a fruity offensive odor. Onyekwere and Anibaba (26) studied changes taking place in ogi cake stored for more than 50 days at ambient temperature (29–30°C) and also under refrigeration (4–10°C). The ogi in its fermented liquor was also observed under similar conditions. It was noted that spoilage had set in as yellowish discoloration within 3 days in ogi cake wrapped in cellophane under 30°C, but when refrigerated, the discoloration was delayed until the seventh day. Still better, if the ogi was in its soured liquor, even without refrigeration, discoloration was not until the 14th day. Surprisingly, however, ogi in its liquor and refrigerated did not discolor until 39th day. Although ogi cake in cellophane discolored within 3 days, mold growth was not observed easily until the 24th day of storage. The products were totally acceptable up to 28 days for ogi cake in cellophane unrefrigerated, 41 days for of refrigerated ogi cake, 21 days for ogi in its liquor unrefrigerated, but up to 51 days when the ogi in its liquor was refrigerated (Table 3). In practice, ogi need not be stored very long, for the sour flavor is lost within 2 days outside its liquor.

Teniola and Odunfa (52), studying ogi fermentation until its spoilage, which took a period of 7 days, isolated the micro-organisms involved and developed some physical and chemical parameters to measure the extent of ogi spoilage after long fermentation. They observed that the ideal fermenta-

Table 3 Changes in Ogi Cake Stored at Various Conditions

	Days of Storage			
Condition of Storage	Yellowish discoloration	Mold growth	Fruity off-color	Totally unacceptable
Ogi cake in cellophane under 30°C	3	24	7	28
Ogi cake refrigerated 4–10°C	7	—[a]	15	41
Ogi in liquor, 30°C	14	18	16	21
Ogi in its liquor, refrigerated (4–10°C)	39	48	40	51

[a] Indicates not conducted.
Source: Ref. 26.

tion was up to 72 h, beyond which there was gradual reduction of the desirable sour, yogurtlike taste which was an indication of some deterioration. Ogi off-odor was first noticed on the fourth day of fermentation (including 24-h steeping). Yeast isolates such as *Candida valida*, *C. krusei*, *Geotrichum candidum*, and bacteria, such as *Lactobacillus brevis*, *L. plantarum*, *Pediococcus pentosaceus*, *Bacillus subtilis*, *Brevibacterium linens*, *Br. oxydans* and two other *Brevibacterium* spp., dominated the fermenting mash at the spoilage stage. The brevibacteria contributed most significantly to ogi off-odor. The study also showed that ogi samples which had been seeded with lactic acid bacteria increased in acidity and product acceptability over the time of fermentation. Changes in pH, titratable acidity, dissolved hydrogen sulfide, and dissolved ammonia level adversely affected ogi acceptability over the period of study. However, overall the H_2S level appeared to be the most useful of the parameters they studied, especially including the presence of brevibacteria. However, coliforms and clostridia were not identified during spoilage (52).

In the study (52), *Candida* dominance and proliferation was followed by an increase in pH and reduction in total acidity of the fermenting gruel. *Candida* sp. has also been implicated in decreasing the acidity of pozol, a Mexican fermented maize dough (61). *Candida* sp. in ogi contributes to ogi spoilage by reducing product acidity and promoting the growth of other spoilage microbes. Bacteriocin-producing *L. plantarum* and *L. casei* with activities against many food-borne pathogens such as *Pseudomonas*, *Aeromonas sobria*, *A. cavice*, enterotoxigenic *E. coli*, and *Vibrio cholerae* were also isolated from ogi and Kenkey (55). *Brevibacterium linens, Br. oxydans*, and two other *Brevibacterium* spp. have already been pointed out as spoilage organisms in ogi (52) but seem to exist normally in many cheeses and habitats with a high salt concentration. These *brevibacteri*a produce *S*-methylthioacetate and other sulfur compounds which contribute significantly to cheese aroma but are spoilage organisms in seafoods (52). Metabolites produced during fermentation are responsible for the physical and chemical indicators, which may be desirable or otherwise. The work of Teniola and Odunfa (52) showed clearly that large increases in total dissolved H_2S were good indicators of ogi spoilage. For example, they found that whereas the reference ogi gruel had only 17 mg/L total dissolved H_2S, fermenting in the presence of *Br. linens*, or *Br. oxydans* singly ranged between 324–372 mg/L H_2S, and *Br. linens* with *Br. oxydans* produced 434 mg/L H_2S. Also combinations of *Br. oxydans* and *C. valida* yielded 334 mg/L H_2S and so forth (see Table 4). One can also see the increasing fall in pH and percent total titratable acidity with low scores in acceptability, as indicated in Table. 4

In dried ogi powder, the granary weevil, (*Sitophilus granarius* L.) is the major infestation, and unless tinned soy-ogi (a soybean enriched variant) has

Table 4 Effect of Microorganisms on Ogi Spoilage Using pH, Titratable Acidity, Total Dissolved Hydrogen Sulfide, and Aroma as Indices

Sample	pH	Total titratable acidity %	Total dissolved H_2S (mg/L)	Acceptability[a] (aroma)
1. Reference	3.50	0.1141	17	-
2. Uninoculated	3.30	0.1328	63	3.70
3. *L. brevis*	3.40	0.1238	70	2.50
4. *L. plantarum*	3.30	0.1541	82	2.30
5. *Br. linens*	3.50	0.1149	372	1.80
6. *Br. oxydans*	3.40	0.1194	324	1.80
7. *B. subtilis*	3.45	0.0991	97	2.40
8. *C. valida*	3.45	0.0991	84	2.40
9. *Br. oxydans* and *C. valida*	3.40	0.1194	334	1.90
10. *B. subtilis* and *C. valida*	3.60	0.1049	128	2.20
11. *G. candidum* and *Br. oxydans*	3.60	0.1058	147	2.40
12. *Br. linens* and *Br. oxydans*	3.80	0.0968	434	1.40
13. *B. subtilis* and *Br. oxydans*	3.65	0.0991	348	1.60
14. *L. plantarum* and *Br. linens*	3.50	0.1239	113	2.20
15. *L. brevis* and *Br. oxydans*	3.50	0.1128	93	2.20

[a] The highest detectable acceptability (aroma) level as determined by the panelists in all samples was 5.00.

Source: Ref. 52.

been exhausted, or filled with nitrogen gas, weevils may be present within 3 months of storage. Rats at home will destroy soy-ogi packaged in nylon alone and so it needs to be safely stored, preferable in refrigerators.

XVI. CHEMICAL/BIOCHEMICAL CHANGES IN THE SUBSTRATE

The importance of the proportion of grain to its steep water has already been discussed and a ratio of 1 : 1.5 to 1 : 2 is used in processing. In fact, Krochta et al. (18) discovered a significant reduction in the yield of corn starch from 70.3% to 67.5% when the steepwater-to-corn ratio was reduced from 2 : 1 to

1 : 1 at the same sulfur dioxide concentration in the steepwater. The sulfur dioxide was said to disrupt the protein matrix of the corn at steeping and also acted in limiting the growth of undesirable fermentations (29). Most of the moisture uptake in white maize-type TZPM, white and red types of sorghum, and pearl millet is said to take place within the first 24 h, whereas the peak water uptake or saturation moisture content occurred at about 36 h, irrespective of the steeping temperature used (17). It was also found that the peak moisture content increased when the soaking temperature was raised from ambient (29–32°C) to 45°C (17).

In the commercial production of ogi, the pH of the maize during steeping decreased from pH 6.25 to 5.25 in 24-h steeping whereas the steepwater fell from pH 6.3 to 4.19 during the same period (35). It was reported (42) that in the traditional process, the fermenting maize during 72-h steeping fell from pH 5 to 4, whereas the corresponding titratable acidity increased from 80 to 300 mg NaOH/100 g of the grain. The moisture content of the grain increased from about 12% to approximately 33% in 24-h steeping, and if air was bubbled into it for up to 6 h, it rose to between 35% and a maximum of 38% in 48 h. Other workers (43) found that the yields of ogi from maize, sorghum, and millet were 61.5%, 59.0%, and 62.8%, respectively, on a dry-matter basis. For soy-ogi dried in the Niro atomizer equipment, the yields were 68.3%, 55.4%, and 69.9% based on the three respective cereal grains, whereas the lactic acid levels of the three samples were 1.22% for maize soy-ogi, 1.14% for sorghum, and 1.17% for millet soy-ogi, with their respective colors appearing as creamy, peach, and greenish-yellow. Banigo and Muller (31) showed that lactic acid and butyric acid were the major carboxylic acids in traditional ogi which appeared in the order 0.55% in maize ogi, 0.52% in sorghum ogi, and 1.52% in millet ogi. They also noted substantial loss of protein (40–50%) occurring during the processing of maize into ogi. Akingbala et al. (37), working on seven sorghum cultivars, found mean yields of ogi, bran, and solubles to be 72.9%, 15.9%, and 7.5%, respectively. Mean ogi composition was 84.3% starch, 8.3% protein, 2.5% fat, 0.59% ash, and 1.3% soluble sugars, and yields of ogi were significantly affected by the variety of sorghum. The yield of ogi was lowest with the bird-resistant sorghum, which contained high levels of tannin. However, Empere (20) discovered a means of effectively using the bird-resistant sorghum variety in ogi preparation by a thermochemical treatment of the grains with hydrochloric acid, which led to total inactivation of the effects of the tannins in the grain. It was followed by a special milling technique using an ultrarotor, which was capable of milling and drying simultaneously. After sieving, the flour was fermented, cooked, and drum-dried into a convenient ogi. The loss of the aleurone layer and germ in maize contributes to the high protein losses in maize ogi (19) and thus makes the biological value of the ogi inferior to the whole grain (12).

Fermentation of the milled grain brings about some enrichment, especially in thiamine and niacin, but the original grain levels are not attained. Drum-dried ogi is the least acceptable, possibly because of the loss of up to 22% of some volatile acids (20). Further, the lysine content in drum-dried ogi was found to be significantly lower than other dried ogi and still much less than that of raw corn (62). Adeyemi et al. (25) discovered that the variety of maize used in ogi manufacture had an effect on the product. Similar conclusions were reached by other workers (21,62). In ancient times and the immediate past, ogi was wholly processed from white maize, but, recently yellow, probably high-lysine, maize type is also being used in traditional ogi, but its sour flavor profile seems inferior to that of traditional white maize (personal observation). Amylases are released into the medium, increasing sugar levels in sorghum ogi fermentation (45).

The chemical composition of dried ogi and its raw materials, were reported (43) as follows: The protein contents of maize ogi, sorghum ogi, and millet ogi (on dry matter basis) were 6.92%, 7.42%, and 10.06%, respectively, and their corresponding raw materials' protein contents were 10.35% for maize grain, 11.84% for sorghum grain, and 12.66% for millet grain (Table 5). The crude fiber contents of the three ogi samples in the same order were 1.85%, 1.97%, and 2.38% and the total ash in the same order were 1.99%, 1.37%, and 1.17%. Regarding soy-ogi, the respective protein contents were 19.48% for maize soy-ogi, 21.60% for sorghum soy-ogi, and 21.45% for millet soy-ogi (Table 6) and the respective total ash contents were 2.83%, 2.49%, and 1.50%. The protein contents have since been reduced to 15% for infant soy-ogi to conform to the World Health Organization (WHO)

Table 5 Average Chemical Composition of Dried Ogi and Its Raw Materials (Percent of Dry Weight)

	Ogi			Raw materials		
Test	Maize ogi	Sorghum ogi	Millet ogi	Maize grain	Sorghum grain	Millet grain
Dry matter (%)	88.85	89.36	88.35	86.08	90.10	90.37
Crude protein (%)	6.91	7.42	10.06	10.35	11.84	12.66
Ether extract (%)	4.70	3.94	5.77	4.06	3.59	5.05
Crude fiber (%)	1.85	1.97	2.38	2.25	3.67	2.65
Total ash (%)	1.99	1.37	1.17	3.86	2.79	2.06
Nitrogen free extractives (%)	84.55	85.28	80.62	79.48	78.11	77.58

Source: Ref. 43.

Table 6 Average Chemical Composition of Dried Soy-ogi from Different Grains (Percent of Dry Matter)

Test	Maize soy-ogi	Sorghum soy-ogi	Millet soy-ogi	Soybean flour (cooked and dried)
Dry matter (%)	96.05	95.14	95.99	93.65
Crude protein (%)	19.48[a]	21.60[a]	21.45[a]	54.42
Ether extract (%)	5.25	6.83	6.58	24.02
Crude fiber (%)	3.55	3.86	4.15	0.56
Total ash (%)	2.83	2.49	1.50	4.85
Nitrogen-free extractive (%)	68.89	65.22	66.22	16.15

[a] The protein content is now about 15% to conform to WHO requirements.
Source: Ref. 43.

requirements for infant weaning foods, but for adult soy-ogi, the protein level is kept at 12%.

Onyekwere and Anibaba (26) studied the changes in traditional wet ogi cakes stored at ambient temperature (29–32 °C) for 20 days and monitored the changes in pH and titratable acidity (Table 7). On the first day of storage, the acidity obtained was 190.9 mg NaOH/100 g, which fell to 72.8 mg on the third day, but rose again slightly to between 64.5 and 69.7 mg on the seventh and ninth days, then fell again afterward. The loss of acidity in ogi liquor was more gradual, changing from 185.9 mg NaOH/100 g on the first day to 102.9 mg NaOH on the 11th day. On the other hand, the pH of the liquor increased from 3.7 on the first day to pH 4.7 on the 11th day and thereafter remained constant. It is likely that loss of volatiles caused the rise of pH and the fall of total acidity indicating spoilage as seen earlier (52). Banigo (1) and Banigo and Muller (31) found that lactic, acetic, and butyric acids were the major carboxylic acids in traditional ogi and that lactic was the more preponderant acid, appearing from about 0.55% and 0.52% in maize and sorghum, respectively, to 1.52% in millet (Table 8). Thus, the ratio of acetic to lactic is below 1 unit. Anaerobic fermentation favored the higher proportion of lactic acid, whereas exposure to air increased the proportion of volatiles, especially acetic and butyric acids. They also showed that the method of wet milling was responsible for loss of nutrients, notably lysine. Other workers found similar losses in riboflavin, thiamine, and niacin (19,63) and trypotophan (64). When cooked soybean was incorporated into milled sieved maize obtained from 24-h steeped whole maize during the production of infant soy-ogi food, the cooked soy-ogi porridge was unstable in viscosity within 2 h holding. However, by giving the steeped and milled maize a further 24-h

Table 7 Acidity and pH Changes in Wet Ogi Cake and Its Liquor Stored at 30–32°C

Time (days)	Acidity (mg NaOH/100 g)		pH
Initial	198.0[a]	196.0[b]	3.80[c]
1	190.9	185.9	3.70
3	42.8	185.9	3.50
5	47.8	192.4	4.80
7	64.5	156.0	3.70
9	—[d]	—	—
11	69.7	102.9	4.70
13	44.8	103.0	4.70
15	35.3	84.7	4.80
17	58.5	84.7	4.90
20	46.5	46.8	4.95

[a] Cake.
[b] Liquor.
[c] Liquor.
[d] Indicates study not done.
Source: Ref. 26.

fermentation in water before the addition of cooked milled soybean, the problem of rapid liquefaction was eliminated. This could be explained by the complete destruction of amylolytic enzymes in the soya ingredient during the second maize fermentation process (19). Akinrele et al. (33) had the problem of unstable viscosity of cooked soy-ogi when they added raw soybean and they suggested the incomplete destruction or regeneration of the amylolytic and lipoxidase enzymes present in the soybean component. Indeed, they were defeating the purpose of cooking the soybean in the first place—to destroy all enzymes (antitrypsin inhibitors, amylases, lipoxidases, ureases, etc.) which are often harmful to health.

Table 8 Principal Organic Acids in Various Types of Ogi

Ogi type	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)	Ratio of acetic to lactic
Maize	0.55	0.093	0.0065	0.176
Sorghum	0.52	0.10	0.01	0.195
Millet	1.52	0.09	0.01	0.06

Note: Average of one to three samples.
Source: Ref. 31.

The fermentation of the maize grain in water raised the level of amylases, but lipolytic enzymes were not secreted (44). Some of the starch was converted into fermentable sugars and organic acids such as lactic, acetic, and buytric acids, which are some of the components of ogi flavor (44). Ethanol and fusel oil were not formed because the fermentation was essentially an acid fermentation. Protein losses occur in ogi production and the losses are highest in millet, although the less digestible fraction of the grain is also removed (31) resulting in reduced ash content in the ogi. By producing a dehulled maize flour using a combination of a hammer and roller mill followed by fermentation of the dry-milled flour, imitating mawè production (14), an improved ogi which had a slightly higher protein content than the traditional ogi was made (21). Generally, the nitrogen, fat, crude fiber, and minerals are not significantly affected by fermentation (44), and vitamin content changes are not significant. Vitamins A and C are not present, however. Phakar and Leung (65), working on Ghanaian fermented maize meal, found the moisture content to be 49.6%, the starch content 37.5%, and total acidity 4.3 mg NaOH/g, pH 3.76, and using a fermented dough of 48% water content, they fortified it with defatted soy flour up to 10% on replacement basis and concluded that the enrichment did not affect consumer acceptance.

Hounhouigan (14) compared a home-made mawè sour dough from partially dehulled maize meal which had undergone natural fermentation for 1–3 days with commercial industrial mawè and found that the main difference between them was the higher removal of hull and germ from the commercial mawè. This was whiter than the home-produced mawè and had better swelling and thicker characteristics, but the nutrient loss was correspondingly also higher. This study showed that the physico-chemical changes occurring in the fermenting product depended on the processing method used, as well as the treatments applied. For example, Teniola and Odunfa (57) carried out organoleptic assessment of ogi they had prepared from (a) steeped whole maize grain, exposed to spontaneous fermentation, following the cottage traditional methods and (b) steeped dehulled maize grain inoculated with *Lactobacillus* and yeast. These were allowed to ferment for up to 96 h. Simultaneously, however, dehulled dry-milled maize flour slurry was divided into four portions and treated as follows; (1) One portion was given spontaneous fermentation, (2) another was inoculated with *Lactobacillus*, (3) the third was inoculated with *S. cerevisiae* yeast; (4) while the last was seeded with a combination of lactobacillus and yeast, the strains known to be lysine and methionine were excreted. Using a 7-man taste panel to assess the various ogi samples, the whole grain steeped following the traditional cottage method was the best in terms of color, aroma, sour taste, and smoothness to the palate (texture). With the highest possible score being 5, this ogi product scored as high as 4.286 ± 0.184 in color and 4.000 ± 0.300 in texture. The dehulled

Table 9 Organoleptic Assessment of Ogi Production from Whole Uninoculated Steeped Maize, Inoculated Dehulled Steeped Maize, and Dehulled Dry-Milled Flour, Seeded and Unseeded

Parameter	Whole maize grain (steeped) spontaneous fermentation (N)	Dehulled maize grain (steeped) *Lactobacillus* + yeast inoculation (P)	Dehulled dry-milled flour	
			Unseeded (C)	Seeded (YL)
Color	1.286 ± 0.184	2.571 ± 0.297[a]	2.857 ± 0.404[a]	3.143 ± 0.261[a]
Aroma	3.429 ± 0.369	3.143 ± 0.450	1.429 ± 0.202[a]	3.000 ± 0.436
Taste	3.643 ± 0.283	2.286 ± 0.174[a]	1.857 ± 0.459[a]	2.429 ± 0.297[a]
Smoothness (texture)	4.000 ± 0.309	1.286 ± 0.286[a]	2.143 ± 0.459[a]	3.143 ± 0.459

Note: N = normal spontaneous traditional method; P = seeded with *Lactobacillus* and yeast; C = spontaneous fermentation; YL = inoculated with *Lactobacillus* and yeast.
[a] Values were significantly different ($p < 0.01$).
Source: Modified from Ref. 57.

maize, although steeped in water, was inferior in all respects to the village method and the dehulled dry-milled maize flour prepared as a slurry, whether fermented spontaneously, inoculated with lactobacillus alone, or yeast alone, or both combined, was decidedly inferior to the traditionally based ogi and therefore very poorly assessed (Table 9). This shows that a people used to their traditional food products know what their choice is, and processors must endeavor to follow the best option to get the best value in financial returns by making reasonable efforts to upgrade the indigenous product, not to force unnecessary changes on the consumer. There is decrease in the amylase level of ogi baba (the sorghum version of ogi) with the subsequent increase in its sugar content during preparation of ogi baba gruel (66). Therefore, although the protein content with minerals is reduced in the product and less than in the raw material, there are other positive chemical improvements, in the final ogi product, in terms of flavor enhancement, stability improvement, and some enrichments in aroma and taste profiles.

XVII. NUTRITIVE VALUE CHANGES

A nutritional disease of childhood associated with infants who were weaned on ogi but deficient in breastfeeding was reported in 1933 by Williams (6). The process of wet grinding and wet sieving of maize during ogi production removes the protein-rich germ, thus making the fermented product inferior to

whole grain in protein content (Table 5) and in biological value, net protein utilization, and the protein efficiency ratio (12,44). The nutritive value of ogi per se has not been increased by the traditional method except by the following:

1. The use of dehulled maize milled into dry flour which was fermented. The protein content was slightly higher than in traditional ogi (21,24,42). The ogi was, however, altered because of its particulate nature.
2. By incorporation of cooked soybean into fermented whole maize grain following traditional methods and drying the ogi into an upgraded version called "soy-ogi" (32,67,68).
3. By the use of starter cultures of lactobacillus (52,53,55,56) and yeast (52,57,58).

Most of these techniques are recent. Compared with steeped corn, fermented ogi is slightly richer in its levels of thiamine and niacin, but these are still lower than those of the whole grain.

The most far-reaching attempt to evaluate and commercialize soy-ogi was pioneered by Akinrele and the staff of FIIRO (7,32,62) with many publications. Soy-ogi was prepared from maize, sorghum, and millet, with the incorporation of cooked soybean in the ratio of grain to soybean meat 70 : 30, resulting in maize soy-ogi, sorghum soy-ogi, and millet soy-ogi, respectively, according to the grain used. Early soy-ogi productions were programmed at 21% protein content for infant feeding (Table 6), but the recent level of protein is kept at 15%, in line with the World Health Organization standard (59). The nutrient level of "soy-ogi" is definitely superior to that of fermented ogi, also in biological value, net protein utilization, and protein efficiency ratio (Table 10). Usually, the adult brand of dried soy-ogi contains less soybean in the blend because its protein content is aimed at 12% compared with protein of 6.9% in dried ogi powder (Table 11); adults consuming soy-ogi have an enhanced traditional food to improve their well-being. The nutrient composition of soy-ogi was widely compared with similar weaning foods from all over the world, including lactogen, a dried commercial cow-milk powder (Table 12). Soy-ogi was comparable to most of them in protein level and in energy content (69), especially with that of corn-soya meal of the United States. Furthermore, biological evaluation of soy-ogi was compared with a commercial baby milk formula (lactogen) and with ogi powder. The results showed that for protein efficiency ratio corrected value for casein standard, casein was 2.5, soy-ogi was 2.2, lactogen was 2.1, and dried ogi was only 0.77 ± 0.01. Similarly, in net protein utilization, nitrogen digestibility coefficient, and biological value, soy-ogi was nearly as good as lactogen (69) (Table 13).

Table 10 Nutrient Values of Fermented Ogi and Soy-ogi

Feature	Nutrient level (dry weight basis)	
	Fermented ogi[a]	Soy-ogi[b]
Moisture content (%)	54.61	4.07
Crude protein (%)	9.2	17.59
Fat (%)	5.13	7.93
Crude fiber (%)	0.72	3.55
Soluble carbohydrates (by difference) (%)	84.28	72.27
Ash content (%)	0.66	2.20
Calcium (mg/100 g)	76.60	503.61
Phosphorus (mg/100 g)	183.50	308.45
Iron (mg/100 g)	16.70	13.79
Thiamine (B_1) (mg/100 g)	0.11	0.40
Riboflavin (B_2) (mg/100 g)	0.08	0.50
Niacin (B_3) (mg/100 g)	0.85	3.00
Pyridoxine (B_6) (mg/100 g)	0.01	0.01
Folic acid (mg/100 g)	0.05	0.04
Pantothenic acid (mg/100 g)	0.01	2.00
Biological value	43.57 ± 2.83	91.3 ± 2.86
Net protein utilization	42.13 ± 2.90	69.5 ± 3.79
Protein efficiency ratio (PER)	0.77 ± 0.01	2.3 ± 0.21
True digestibility coefficient	96.11 ± 1.00	91.6 ± 2.15

[a] Adapted from Ref. 12.
[b] Data from Ref. 67.

The use of soy-ogi in the treatment of children suffering from protein-calorie malnutrition was provided by Akinrele and Edwards (69). Using a total of 15 children (ranging between 1 and 3 years old) who were suffering from kwashiorkor at various stages, the test group was fed a soy-ogi diet and the control was maintained on a standard commercial infant milk food "lactogen" made from dried cow's milk. Blood samples were taken from each patient every 7 days for 28 days and analyzed for total serum protein, albumin, globulin, packed cell volume, and hemoglobulin by standard clinical methods. The response to dietary treatment was measured in terms of acceptance and tolerance of the food, disappearance of edema and apathy, and weight changes. After the first 5 days, the soy-ogi diet was found to be as acceptable as the lactogen diet. Generally, disappearance of apathy and increasing interest in the environment became noticeable in all children in both groups in the third week, showing that patients were as responsive to soy-ogi as lactogen. There were, however, greater weight gains among the lactogen group than the soy-ogi group. There were also indications that lactogen was

Table 11 Chemical Analysis of Infant and Adult Maize Soy-ogi Compared with Dried Ogi and Soy Flour

Features	Soybean[a] flour	Infant soy-ogi[a]	Adult soy-ogi[a]	Ogi[b] (dried)
Moisture (%)	7.92	6.26	5.45	11.15
Ash (%)	3.18	1.46	0.69	1.99
Fat (%)	23.73	9.17	8.32	4.70
Crude protein (%)	46.24	14.84	12.85	6.91
Crude fiber (%)	2.55	1.13	0.84	1.85
Carbohydrate (%)	18.93	68.27	72.69	84.55
Calcium (%)	0.09	0.088	0.16	—[c]
Phosphorus (%) as P_2O5	6.01	0.29	0.003	—
Iron (%)	0.006	0.009		—

[a] Courtesy of FIIRO pilot plant.
[b] Data from Ref. 43.
[c] Indicates not conducted.

more efficient in promoting serum protein recovery than soy-ogi (maize brand), although values obtained from soy-ogi were acceptable. Personal experience (67) also revealed that children intolerant to lactose sugar in their mother's milk who were placed on infant soy-ogi fared well exclusively on the soy-ogi diet.

The toxicological evaluation results of soy-ogi were provided by Oniwinde and Akinrele (70), who gave data on rats to show that the soy-

Table 12 Nutrient Value of Soy-ogi Compared with Other Similar Protein Foods

Constituent	Soy-ogi (Nigeria)	Pro-nutro (South Africa)	Incaprina (Guatemala)	Corn-soya meal (United States)
Protein % (Nitrogen × 6.25)	20.3	22	27.5	20.9
Moisture (%)	4.7	—	—	9.5
Fat (%)	6.3	12.9	4.2	1.2
Ash (%)	3.0	4.6	—	2.1
Calcium (%)	0.43	0.43	0.66	0.40
Phosphorus (%)	0.44	0.48	0.70	0.44
Carbohydrate (%)	63.7	56.5	—	61.0
Energy (kcal /g)	4.00	4.13	3.7	3.58

Source: Modified from Ref. 69.

Table 13 Protein Efficiency Ratio, Net Protein Utilization, Nitrogen Digestibility Coefficient, and Biological Value of Ogi and Soy-ogi Compared with That of Commercial Cowmilk Powder (Lactogen)

Analysis	Casein	Soy-ogi	Lactogen	Ogi[a]
1. Protein efficiency ratio				
Experimental[b]	2.6 ± 0.37	2.3 ± 0.21	2.2 ± 0.62	—
Corrected[c]	2.5	2.2	2.1	0.77 ± 0.01
2. Net protein utilization				
Experimental	85.5	83.6 (std)[d] 69.5 ± 3.79 (opt)[e]	86.6	42.13 ± 2.90
Corrected	72.0	70.4 (std) 58.5 (opt)	72.9	
3. Nitrogen digestibility coefficient				
Experimental	96.6 ± 0.61	91.6 ± 2.15	93.1 ± 2.59	96.71 ± 1.00
4. Biological value				
Experimental	88.5 ± 3.63	91.3 ± 2.86	93.0 ± 2.45	43.57 ± 2.83

[a] Data from Ref. 12.
[b] Exptl.* = experimental values with + SD
[c] Using standard value for casein.
[d] Operative.
[e] Standard.
Source: Ref. 69.

ogi diet was toxicologically safe by examining internal organs and that there was normal growth in experimental rats through three generations comparable to rats fed on a commercial diet. (Table 14) Ozumba and Onyekwere (71) showed that although maize soy-ogi and sorghum soy-ogi at the 15% protein level were adequate for maintaining growth in rats, food consumption and weight gain were higher among rats fed on maize soy-ogi. (Table 15).

Table 14 Toxicological Evaluation of Soy-ogi in Rat for Three Generations

Group	Diet	No. of generations	Litter size	Survival (%)	Birth weight (g + SD)	Weaning weight (3 weeks old) (g + SD)	Growth rate (g/rat/day)
Control	Commercial	3	7.7	88	5.6 ± 0.25	42.1 ± 0.62	1.73
Experimental	Soy-ogi	3	7.5	85	5.4 ± 0.32	40.9 ± 0.45	1.69

Note: Results were mean of 10 rats per generation.
Source: Ref. 70.

Table 15 Rat Performance on Infant Maize Soy-ogi and Infant Sorghum Soy-ogi Diets

Diet	Protein level (g)	Mean total weight change (g)	Mean total feed intake (g)	Mean total protein intake (g)
Casein	10	$63.61^{c} \pm 0.21$	$235.65^{b} \pm 0.80$	$24.28^{c} \pm 3.10$
Infant sorghum soy-ogi (operative)	15	$96.50^{b} \pm 0.20$	$238.56^{b} \pm 1.23$	$40.55^{b} \pm 5.18$
Infant sorghum soy-ogi (standard)	10	$51.40^{c} \pm 0.12$	$203.84^{c} \pm 0.49$	$22.44^{c} \pm 1.95$
Infant maize soy-ogi (operative)	15	$114.19^{a} \pm 0.39$	$301.69^{a} \pm 0.31$	$45.20^{a} \pm 1.32$
Low protein	2	$-10.00^{d} \pm 0.06$	$103.01^{d} \pm 0.30$	$5.16^{d} \pm 1.26$

Note: Values with different letters were significantly different from each other.
Source: Ref. 71.

Perhaps sorghum molecules are larger than that of maize. Whereas maize ogi contains about 6.9% protein, powdered soy-ogi has a minimum level of 15% protein for infants, in addition to the improvement of the amino acid profile from the soybean component. The fat from the full-fat soybean increases the energy value of ogi, raising it from about 200 to about 400 kcal/100 g.

Soybean has not been the sole vegetable source for ogi enrichment. Some workers (72) have used the black-eyed cowpea to produce the "ewa-ogi." formula or further enriched it with milk to give the "ewa-ogi-milk." Nutritional quality evaluations showed that the ewa-ogi-milk formula could counteract infantile kwashiorkor in 4 weeks; ewa-ogi was also effective but not as rapid.

Nutritive values of ogi have recently been enhanced by the work of Teniola and Odunfa (57), Odunfa et al. (58), who followed the pioneering work of Sands and Hankin (73,74), Haidaris and Blattacharjee (75,76), and other workers (77), who used lysine-producing lactobacillus and yeast for food and feed enrichment. This reminds us of Banigo et al. (21) in their utilization of high lysine corn for the manufacture of what they called a new improved ogi, at least in improving the lysine content of the ogi. Already noted is the excellent work of Olukoya et al. (53) in preparing ogi they called "dogik" which is said to possess the potential to control diarrhea in infants consuming it. Also noteworthy is the work of Olasupo and co-workers

(55,56), who used a bacteriocin-producing lactobacillus strain in ogi production, ensuring, we believe, the safety of the consumer. To our mind, the easiest method of fortification of ogi is through soybean enrichment and drying with the Niro atomizer spray-dryer. We have taken pains to go into the details of the potential advantages of full commercialization and industrialization of soy-ogi by reputable industries that can ensure the full safety of the end product. We challenge patriotic rich Nigerians and West Africans, where ogi is popular, including production of kunu, to awaken and invest in soy-ogi full industrialization.

The failure to commercialize soy-ogi to date is an unhappy trend in ogi industrialization, particularly for infants and sick adults in western Nigeria who still largely use ogi as a staple food, in spite of the changing times. Cereals grains are often low in lysine, and white maize, the type that is traditionally employed in ogi production, is deficient in lysine and methionine (62). The FAO/World Health Organizations report (59) on energy and protein requirements implicated lysine and sulfur amino acids as the most limiting essential amino acids in Nigeria and Egypt, being the only African countries reported. Amino acid analysis on ogi showed that there is substantial loss of lysine from the raw grain after processing (59).

Recently, Teniola and Odunfa (52,57) developed a starter culture for ogi production from *S. cerevisiae* OY4 and *L. brevis* XO43. They produced batches of ogi from maize flour slurry, dehulled maize grains and whole maize grain with the starter culture and demonstrated fermentation over a period of time using steeped whole maize grain, steeped dehulled maize, dehulled dry-milled maize flour slurry. The starters were used singly and in combination over each group of the specified maize samples. Changes in lysine and methionine contents during fermentation were monitored. Results showed that fermenting dehulled steeped maize with mixed starter cultures showed the highest lysine level. This was followed by spontanously fermented flour slurry and *L. brevis* inoculated fermentation in decreasing order. The traditional fermention method depicted the most erratic changes. The methionine level was highest in fermenting inoculated dehulled maize grain, followed by the spontaneous flour slurry. The fermentations involving mixed starter cultures on maize flour slurry and *S. cerevisiae* alone had the lowest methionine levels (59). With respect to the aroma and taste attributes, the traditionally processed ogi used as a control had the best quality and acceptability among all the samples, and the spontaneously fermented maize flour slurry had the least acceptability in aroma and taste quality attributes. It was concluded that ogi from dehulled maize inoculated with the starter culture showed the highest levels of lysine and methionine and that steeped grain brought about stronger ogi aroma than when maize flour was fermented alone as a slurry. *Lactobacillus brevis* contributed most to the taste of ogi, and

fermentation with starter cultures showed good prospects for improving the lysine and methionine levels of ogi.

In a follow-up work, Odunfa et al. (58) evaluated the lysine and methionine they had produced with their ogi starters. Lysine- and methionine-producing cultures of *L. brevis* and the yeast *S. cerevisia*e in batch fermentations of ogi were selected by growth in the presence of the analogs, *S*-2-aminoethyl-*L*-cysteine (thialysine) and ethionine, respectively. Their study revealed that 42.5% of the *Lactobacillus* and 83.3% of the yeast isolates tested were capable of lysine production, whereas 25.0% of the *Lactobacillus* and 87.8% of the yeast isolates produced methionine. The lysine and methionine yields of *Lactobacillu*s were significantly ($p < 0.01$) higher than that of yeasts. The majority of the yeast isolates excreted most of the lysine and methionine produced, and generally more lysine was produced than methionine in all tested isolates. Their results differed from the observations of Sands and Hankin (74) that no *Lactobacillu*s wild-type isolates produced lysine. The highest levels of lysine produced by *Lactobacillu*s and yeasts, they concluded, were 247 and 156 mg/L, respectively. More of the lysine produced was said to be excreted out of the cell than retained within; over 74% of the *Lactobacillus* that Odunfa et al. (58) examined produced more extracellular lysine than within the cell. With respect to the yeast isolates tested, none of them produced more intracellular lysine than extracellular. The selection of organisms as starter cultures may be genetically manipulated in the future to enhance their lysine and methionine production for ogi nutritional improvement. Another interesting approach to ogi nutritional improvement is the work of Olukoya et al. (53), who have developed an ogi which has the capability of preventing and treating many waterborne diseases by using bacteriocin-producing lactic acid bacteria cultures. Additionally, Olasupo et al. (55,56) increased the shelf life of ogi by also using a bacteriocin-producing *Lactobacillus* isolate. The high nutritional requirement of infants and convalescent adults who consume ogi frequently make the above research approaches very relevant to ogi as an important traditional food.

XVIII. FORECAST

Ogi and furah are important traditional fermented porridges that are unlikely to be eliminated from traditional Nigerian dishes, although it is coming closer to the option (27) that some traditional fermented foods belong to the poor. Because of its ready digestibility and pleasant fermented sour flavor as compared to whole-cereal foods, adults and convalescents are most likely to continue to use ogi, particularly the soy-ogi variant and modern cottage

ogi that is no longer wrapped in leaves but now in nylon. Because it has taken soy-ogi close to 30 years and it is still not fully industrialized, it is doubtful that soy-ogi will be successfully commercialized by FIIRO, which carried out the research. The commercial unfermented maize–soybean blend of infant food (Nutrend) from 4 months and older which has been in the market instead of soy-ogi makes the need for infant soy-ogi less pressing than previously.

Table 16 Nutritional Information of Nutrend, an Unfermented Weaning Food in Nigeria

Average Composition	100 g
Fat (g)	9.0
Protein (g)	16.0
Carbohydrate (g)	63.7
Sucrose (g)	19.0
Starch (g)	39.6
Oligo saccharide (g)	5.1
Dietary fiber (g)	5.0
Mineral (ash) (g)	2.3
Energy value kcal	400
Energy value kJ	1670
Linoleate (g)	3.5
Vitamin A (IU)	1500
Vitamin D (IU)	200
Vitamin E IU	3
Vitamin C (mg)	50
Folic acid (μg)	22
Thiamine B_1 (mg)	0.8
Riboflavin B_2 (mg)	0.3
Niacin (PP) (mg)	4
Vitamin B_6 (mg)	0.3
Vitamin B_{12} (μg)	0.75
Biotin (μg)	25
Panthothenic acid (mg)	1.5
Calcium (mg)	390
Phosphorus (mg)	260
Iron (mg)	10
Sodium (mg)	85
Potassium (mg)	570
Iodine (μg)	35
Zinc (mg)	7.0

Source: Nestle Foods Nigeria Plc., Lagos.

Nevertheless, some mothers still depend on FIIRO's pilot plant to supply small quantities of infant soy-ogi for their children who are allergic to lactose milk (67). It is unlikely that anyone would invest in ogi or soy-ogi only because of adults needs. The nutritional information of Nutrend is given in Table 16.

XIX. CONTRIBUTION TO NEW INTERNATIONAL INDUSTRY

Many indigenous traditional technologies are not easily adopted by transnational companies without altering the methods of preparation and perhaps ending up with a product of altered flavor and unacceptability. The international industries may want to produce ogi or soy-ogi from dry-milled maize or even from sterilized whole maize which is inoculated with known pure cultures of lactobacilli or combination of two or more micro-organisms. This would require the maintenance of pure bacterial culture banks. Although this might increase production cost, it could very well result in the development of new micro-organism(s) through genetic engineering that may hasten the process of ogi fermentation and produce ogi or soy-ogi of excellent flavor. This may eliminate the need for the present steeping in water, wet milling, and wet sieving, all of which do not readily yield to continuous production. The mixed culture of lactobacilli and yeast used earlier (21) in inoculating a fine meal of milled cereal for fermentation could be as selected and engineered as brewer's yeast in the beer-brewing industries. It is not impossible that new, valuable secondary metabolites will be produced from such micro-organisms in due course.

Additionally, ogi has prospects of not only soybean enrichment but also correcting deficiencies of lysine and methionine by the use of lactobacillus and yeast starter cultures as well as making the product possess bacteriocidal properties against diarrhea-causing micro-organisms. However, full commercial productions of soy-ogi and these new forms need to be hastened.

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9

Industrialization of Gari Fermentation

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I. INTRODUCTION

Gari (Nigeria) (attieke [Côte d'Ivorie]) is a fermented gelatinized, granular starch food product traditionally processed from bitter, poisonous cassava tuber (*Manihot utilissima* Pohl; *M. palmate*) (Fig. 1) but rarely from *M. esculenta* Crantz. Its processing begins with scrubbing the root manually in water to remove mud, then peeling away a top leathery nonstarchy portion with a 12-in.-long sharp kitchen knife, followed by dicing and washing. The clean starchy part is grated (macerated) on a local grater or a pulping machine filled into double-layered cloth bags, and placed under a screw press to expel cassava liquor and simultaneously ferment, within 1–5 days.

The fermented mash, now having a reduced moisture content of about 50–55%, is broken up with a wooden mallet, granulated manually or mechanically, and roasted in a thick iron pot on a hot wood fire to gelatinize and dry, ensuring that it does not stick and char on the heated pot by using a piece

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Figure 1 Cassava tuberous root, showing also the stem and palmate leaves. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos.)

of calabash to stir. Sometimes, a pinch of palm oil is added to the roasting mash to give a slightly yellow colored gari which is graded by sieving when cool. Three primary products derived from cassava root processing in Nigeria are gari, fufu, and cassava flour (lafun), as shown in Fig. 2.

Attieke processing somewhat resembles that of gari, in that the peeled root is also grated, then mixed with already fermented cassava pulp (3:1 ratio) as a primer, adding oil and salt, then dehydrating and sun-drying. The granules in attieke are more uniform and glossier than those of gari. Naturally,

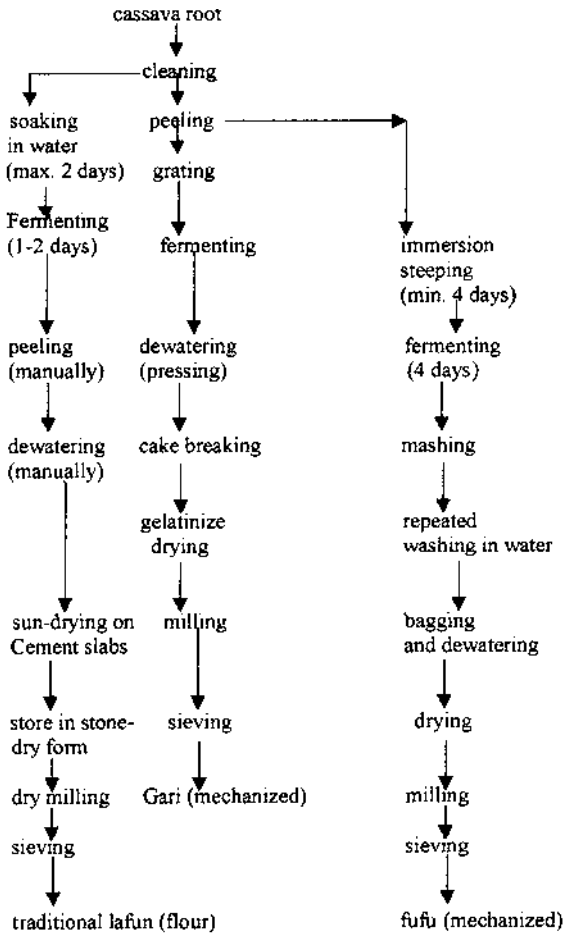

Figure 2 Flowsheet of three traditional Nigerian fermented food products from cassava processing. (From Ref. 1.)

mechanization has reduced tedium in its production. The processing steps in traditional fermented cassava products in Africa are shown in Table 1.

II. CONTRIBUTION TO THE DIET

Gari is consumed usually as a main meal but often also as a snack, particularly by office workers and traders who carry along with them a small quantity and a few lumps of sugar to soak in ice-cold water and is accompanied with roasted peanuts, relished for its cooling effect in hot weather. However, as a full meal, a gari meal is no longer regarded as poor people's food because a good traditional stew that goes with it could be expensive. A measure of dry gari is added to a bowl of boiling water and dirt is decanted: the gari is then allowed to swell and soften. For places where gari fermentation is in very short supply, (about 1 day), gari is prepared into eba using hot water only, instead of boiling water, otherwise the dough (eba) becomes very sticky due to excessive saccharification. The semistiff dough is uniformly blended in a mortar or in a bowl with a wooden spoon until the texture is uniform and can be rolled together into a ball with three fingers and swallowed with a dip of the stew. Because eba hardens when it cools, it is usually eaten warm, and the level of satisfaction and nourishment depend on the amount of money available for the preparation of the stew (3,4). Gari is a staple in southern Nigeria and consumed widely along western Africa, as attieke is the staple in Côte d'Ivorie. Gari is sometimes consumed in Ghana by wetting it slightly with cold water and blending in corned beef or eating it with fried fish like a rice meal. The majority of low-income group who used to consume "eba" up to 5 days a week cannot afford that now because of the high cost of preparing even a simple stew that may be enjoyed. Gari contributes up to 60% of the total calorie intake in western Africa (5), whereas cassava itself provides about 90% of the total carbohydrates from the other various sources as grains, yam, and cocoyam (6,7).

Cassava cultivation, at least in Nigeria, appears to be more favorable than other tubers, in terms of its lower cost. Specifically, as was quoted (8), yam (*Dioscorea* sp.) was sold about 1.9 times higher per kilogram than the same weight of cassava tuber, 2 and 2.6 times higher for sweet potato and Irish potato, respectfully, and 2.5 times for cocoyam (Table 2). Furthermore, the cassava plant can survive even in poor soils and can produce 250,000 cal/ha/day compared with 209,000 cal for maize, 176,600 cal for rice, and 114,000 cal for wheat. The devastating cassava blight disease in Africa is now under control, thanks to the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, which has developed improved varieties of cassava that mature before 9 months unlike the local breeds that took up to 2 years. Another added advantage is that in some of the cultivars, the hydrogen cyanide

Table 1 Processing Steps in Production of Various Cassava Products in Africa

	Processing Step						
Cassava product	i	ii	iii	iv	v	vi	vii
Chickwange (Central Africa)	Fermenting pulp (3 days)	Sieving	Steaming 45 min	Kneading	Boiling 1–2 h		
Ntuka (Zaire)	Fermenting pulp (3 days)	Steaming 2 h					
Fufu (Zaire)	Fermenting root (2 days)	Sun-drying	Milling	Cooking			
Gari (Western Africa)	Grating	Dehydrating	Fermenting	Frying	Storing (1 month)	Storing (4 months)	Cooking eba
Attieke (Côte d'Ivorie)	Grating	Mixing with fermented pulp (3:1)	Adding oil or salt	Dehydrating	Sun-drying		
Plakali (Côte d'Ivorie)	Grating	Mixing with fermented pulp (3:1)	Sun-drying	Pounding and cooking			
Oyoko (Zaire)	Grating	Mixing with fermented pulp (3:1)	Steaming				
Fufu (Ghana)	Boiling fresh roots	Pounding					
Fufu (Nigeria)	Soaking in water (1 day)	Dehydrating	Steaming	Pounding			
Fufu (Nigeria)	Soaking in water (3 days)	Pulping and extracting	Dehydrating	Steaming	Pounding		
Flour (Lafun, Nigeria)	Soaking 1 day	Dehydrating	Sun-drying	Milling			
Konkonde (Côte d'Ivorie)	Drying chips unfermented	Milling	Cooking				
Pondu (processed leaves)	Blanching	Pounding	Cooking				

Source: Ref. 2.

Table 2 Relative National Prices of Tuber Crops in Nigeria in 1990

Tuber Crops	Relative prices (per kg)
Yam (*Discorea*)	1.94
Sweet potato	2.03
Irish potato	2.63
Cocoyam	2.53
Cassava (fresh root)	1

Source: Modified from Ref. 8.

content in their finished product is reasonably low (2) (see Table 3). According to worldwide cassava production statistics of the United Nations in 1991, out of a total production of 161.5 million metric tons, Nigeria was highest, with 27 million ton (7) (Table 4). However, Nigeria's yield per hectare was lower than those of the four leading producer countries (Table 5), with Thailand topping them all, followed by India and Brazil (9). Traditionally, Nigeria produces the poisonous cassava varieties of *M. utilissima* Pohl and *M. palmate*, which, incidentally, are the varieties specified by the Nigerian Standards Organization for gari production. The use of the poisonous cassava for gari is understood to make better-tasting gari; in addition, wild rats do not consume the bitter cassava in the farm, avoiding terrible loss to farmers. More recent reports gave Nigeria cassava production in 1993–1995 at about 30.8×10^6 tons on the average and estimated for 1996–1997 to be nearly 33×10^6 tons per year.

In spite of the apparent high cassava production by Nigeria, there is still scarcity of gari, which leads to unreasonable high prices. The majority of low-income earners years ago consumed gari at least once a day every day of the week but they cannot afford to do so now because of the high cost of preparing the necessary stew to go with the eba. Indeed, even the fairly well-to-do persons resort to consumption of imported rice and local beans (cowpea) to reduce their cost of living.

III. PER CAPITA CONSUMPTION

Assume that Nigeria's total cassava production in 1991 was used as follows: 8×10^6 tons exported: 4×10^6 tons used for fufu, starch, and lafun (flour) production, and the remaining 15×10^6 tons available for gari production. Because a ton of gari is obtainable from about 4 tons of cassava root (10), 15×10^6 tons of whole roots would produce 3.75×10^6 tons of gari. Even if 23×10^6 tons of cassava all went for gari processing, we would expect 5.75×10^6

Table 3 Content of HCN in Traditional African Cassava Food Products Made from IITA Improved Varieties

	Total HCN, mg/100g of fresh weight[a]					
Product	TMS 50395	TMS 30572	TMS 30555	TMS 30001	Mean	Percent reduction[b]
Peeled, fresh tuberous root	23.48	15.85	13.53	10.68	15.89	0.00
Attieke	0.51	0.86	0.51	0.70	0.65	95.91
Boiled tuberous root	5.42	4.02	6.39	3.32	4.79	69.85
Chickwanga	c	—	—	—	—	100.00
Eba (from 4 months old gari)	—	—	0.02	0.04	0.02	99.87
Fufu (Ghana)	4.37	2.96	2.78	2.27	3.10	80.49
Fufu (Nigeria)	0.79	0.74	0.75	0.58	0.72	95.47
Fufu (Zaire)	0.04	—	—	0.03	0.02	99.87
Gari (freshly fried)	2.22	1.83	4.18	2.42	2.26	83.26
Gari (after 4 months storage)	0.30	0.32	0.32	0.21	0.29	98.17
Konkonde	0.70	0.78	0.78	0.60	0.72	95.47
Ntuka	0.05	0.02	0.04	0.01	0.03	99.81
Oyoko	1.11	1.48	1.98	1.28	1.46	90.81
Plakali	0.87	0.53	0.19	0.51	0.53	96.66
Fresh leaves	305.00	155.00	142.50	—	201.00	0.00
Pondu (cooked leaves)	10.63	6.82	8.15	—	8.53	95.76

[a] Initial weight of tuberous roots was 5 kg per replication per product.
[b] Reduction was expressed as a percentage of the total HCN in peeled, fresh roots and fresh leaves.
[c] No HCN was detected (less than 0.01 mg/100 g fresh weight); (IITA) International Institute of Tropical Agriculture, Ibadan, Nigeria.
Source: Ref. 2.

Table 4 Worldwide Cassava Production in 1991

Country	Tons ($\times 10^6$)
Nigeria	27
Brazil	25
Thailand	20.9
Zaire	18.2
Indonesia	17.1

Source: Ref. 7

Table 5 Cassava Yields in the Four Leading Producer Countries in the World

Country	Yield (kg/ha)
Thailand	17,821
India	16,437
Brazil	12,300
Nigeria	10,000

Source: Ref. 9.

tons, which should be a better figure to feed nearly 50×10^6 inhabitants of southern Nigeria than the mere 3.75×10^6 tons. In either instance, the per capita consumption of gari would be from 75 to 115 kg. The best option is to increase the yield of cassava per hectare and also mechanize its cultivation and with irrigation, so as to have excess for consumption and for export.

IV. EARLY REFERENCE TO GARI

There are no early references to gari other than that cassava was introduced into western Africa from tropical America by the Portuguese in the 6th century (11). It is possible that freed slaves popularized its cultivation and use.

V. SUBSTRATE

Cassava root is the only substrate known for gari and other cassava foods used in Africa, as shown in Table 1. The cassava plant is a perennial semishrub that grows under cultivation to a height of about 2–4 m (Fig. 1). The leaves are large and palmate, usually with five to seven lobes, borne on a long, slender petiole (11). Clusters of mature roots appear in the ground at a length of 30–120 cm and 4–15 cm in diameter. The outer part in the peel consists of two layers: a corky layer that is paperlike and easily peeled prior to processing and an inner fleshy portion generally used for all cassava food products.

Cassava has been classified into three categories as follows: bitter or very poisonous, when its poisonous component exceeds 100 mg HCN/kg of pulp; moderately poisonous (50–100 mg HCN/kg of pulp); nonpoisonous or sweet (less than 50 mg HCN/kg) of pulp (11). The very poisonous varieties are traditionally used in gari production, because they make a better sour-tasting gari. Although the sweet cassava can be consumed in the raw state or merely

Table 6 Distribution of Cyanogenic Glucoside (mg HCN/kg Fresh Weight) in Different Tissues and in Gari from 12 Cassava Cultivars

Cassava cultivar	Leaf	Peel	Pulp	Gari
1. 53101 (Oloronto)	2256	724	243	28.5
2. 60447	2101	1053	187	19.2
3. 60506	2060	756	114	21.7
4. Isunika kiyan	1288	621	93	23.6
5. Sweet cassava	1355	700	91	26.7
6. Ozu-nwangwe	1463	586	108	16.8
7. Imo	1422	157	61	11.8
8. Okobo	780	626	113	13.5
9. Uboma 11	1287	793	173	26.7
10. Nwugo	678	577	108	25.7
11. Ife	1152	177	155	19.5
12. Ogbomoso	2060	520	297	20.3
Mean	1490	607.7	145.3	21.1
SD ±	519	247.7	69.3	5.3
CV%	34.8	40.8	47.8	25.1

SD = standard deviation, CV = coefficient of variation
Source: Ref. 12.

boiled, the hydrogen cyanide occurring as a complex cyanogenic glucoside (linamarin and lotaustralin) being distributed unevenly in many parts of the plant must be reduced to tolerable safe levels by fermenting or soaking the tuber in water as important steps in their processing for food.

Obigbesan (12), working on 12 cassava cultivars, found that the leaf and the peel of the root had the highest concentrations of the cyanide (Table 6) and that within the root, the central fiberous core was very rich in prussic acid. Therefore, because of the toxic nature of the bitter variety, special detoxification processes such as peeling, fermenting, grating, immersion soaking in water, boiling, and drying are employed during processing and these account for the complex methods traditionally (and industrially) used in the conversion of the cassava roots to food products. Cassava is high in food energy, low in protein content and minerals, and contains no thiamine, vitamin A, riboflavin, niacin, or vitamin C.

VI. PRODUCTION IN ANCIENT TIMES

The earliest known gari production was a household affair undertaken by the wife and assisted by the children (Fig. 3). The basic equipment consisted of the

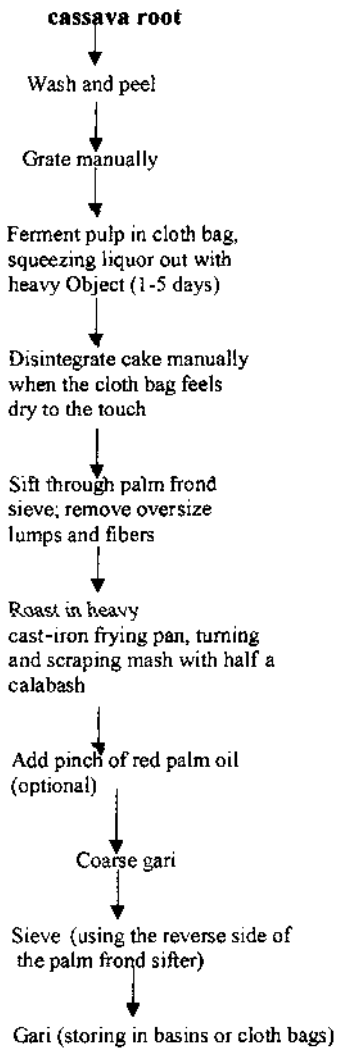

Figure 3 Flowsheet: ancient household production of gari in Nigeria. (From Ref. 10.)

following: a sharp knife for peeling cassava; a native grater, which was the reverse side of a roughly punched aluminum sheet about 2 ft long by 2 ft wide framed on wood; a cloth sack for a vat and filter; a heavy object or four to six lengths of wood tied with rope over the cloth bag as a press to dewater the cassava liquor; a sieve made from the back of an oil palm frond; a heavy cast-iron frying pan for a roaster; a fire hearth heated with firewood for a source of heat; a half-calabash for a stirrer (10). Cassava roots were harvested by uprooting from a farm, which took up to 2 years to mature, and carried home on the head in a rectangular basket having a wooden bottom or in enamel basins. Traditionally, the cassava was harvested on particular market days to avoid pilfering by unknown persons. The sides of the basket were made of wild bush rope woven together and passed through the edges of the rectangular wooden bottom to give it strength. Freshly harvested cassava root, the very poisonous variety, was washed in water, and the nonedible, leathery outer portion was carefully peeled off with a sharp kitchen knife, leaving the starchy part with the central fiber to be grated into a pulp (mash) by a to-and-fro manual pressure over the roughly punched surface of the aluminum sheet. The grating step characteristically distinguished gari processing from fufu and lafun (flour) products, and a well-grated root gave more yield than a lumpy product because it was likely discarded, because it did not pass through the traditional sieve. The grated pulp was transferred into a cloth bag and securely tied onto four or more lengths of firewood to expel the cassava liquor, or sometimes a heavy object was placed on the cloth bag, to dehydrate the mash gradually. The mash was left in a shaded place outside of the house to ferment for 18 h or up to 5 days, depending on the degree of sourness or when it was dry enough. By the time the liquor was no longer present, as determined by feeling the bag or by the absence of visible liquid, the mash was assumed sufficiently fermented and there were no quality control measures. Even in the 1-day quick process, dryness of the bag was the only index for terminating the fermentation stage, by which time the poisonous glucosides had been sufficiently hydrolyzed (2) and the moisture content of the grated mash had been reduced from about 65% to nearly 55% (10). The semidried harsh cake was disintegrated manually and sifted through the sieve (the reverse side of the grater) to remove large lumps, fibers, and oversized pulp. The sieve size was never specified, although it was determined by the closeness of the woven palm fronds. The smooth side of the grater served as a sifter for wet mash and gari. The throughput was roasted in an open iron pot heated on a wood fire to about 120°C and constantly turned using a half-calabash to prevent sticking and charring. In some places where gari was produced, it was characteristically more inviting than the white-colored well-fermented, usually sour gari traditionally known as Ijebu gari, but the Ijebu people will have nothing to do with it. Throughout the process, there were no known quality control mea-

sures whatsoever, but the women were experienced, and variable drying resulted in gari having a high moisture content—in some instances as much as 14–20%, too high for long storage (10). For this reason, ancient gari had to be consumed within 2–4 weeks.

VII. PRODUCTION IN VILLAGE/COTTAGE INDUSTRY

The cottage gari industry is an improvement over that in ancient times specifically in (a) collection of roots from farms by lorries, (b) mechanization of the essential grating step by using the services of village grating points for a fee, and (c) the employment of a village screw press to dehydrate the fermented cassava. Women still peel the roots manually (Fig. 4) and disintegrate the pressed cake by hand, which they sieve through wire mesh or on the smooth side of the double-acting ancient type sifter/grater to remove fibers and cassava lumps. Roasting of gari is still done in a heavy cast-iron frying pan using a half-calabash to prevent sticking and charrings, as in ancient times. The rest of the process is sieving to collect fine gari and standard gari because coarse gari is sold at a lower price than fine-grain gari. Incidentally, there has been an improvement in the hygienic standard of gari processing at the cottage level compared to ancient time.

Figure 4 Hand peeling of cassava roots. (From Ref. 10.)

The modern, improved cottage gari industries (Fig. 5) have simple grating machines. The grater consists of an aluminum sheet punched very roughly and mounted on a round wooden roller, which is rotated by a petrol-powered motor of about 1.5 hp. The roller is mounted on an iron frame, which imparts additional support. Peeled root is placed between the aluminum sheet and a wooden rectangular frame that narrows inward built over the aluminum-plate-covered roller. While the roller rotates, the root is pressed down with a wooden aid and the cassava is grated by the scraping action of the rough surfaces of the aluminum metal sheet. The root is pushed further down to make better contact with the metal surface as the grating progresses. One grating machine can serve about 30–50 persons, as the women carry their peeled and washed root to different privately owned grating and dewatering places in the vicinity. The screw press is used for dewatering grated cassava mash, and the principle of its design is based on the maximum efficiency of the mechanical driving screw used to move a pressure ram during operation. It is operated by using a rod with an adjustable lever for favorable mechanical advantage.

In every instance, the operator roasting the pulp suffers from excessive heat from the open fire. Several years ago, the Federal Institute of Industrial Research, Oshodi, (FIIRO), Lagos designed a low fireproof wall over the fire to protect the women (13). The range could have as many as 10 frying places, which are controlled from one end by bellows that keep the wood fires alive. Each roasting pan is manned by a woman operative, and this can still be seen in a few places, even outside Nigeria (Fig. 6). In the cottage gari level, no mill is used to reduce oversized granules before sieving, as will be seen later in the mechanized gari factory.

VIII. METHOD OF CONSUMPTION

Gari is prepared into "eba" for consumption. A measure of gari is slowly poured into a bowl containing an estimated quantity of boiling water, stirring with a spoon until all of the gari granules are fully immersed in water; the gari is then allowed to swell for a while before thorough mixing or pounding in a mortar to make the eba granules cohesive. Eba hardens when it cools, so that it is traditionally consumed warm, with a favorite stew. Traditionally, stews for gari are an important factor and should also be described. There are usually two varieties of stews: light stews and vegetable stews. In places where palm nut stew and groundnut (peanut) stew are preferred, palm nut and groundnut are used for the light stews, whereas vegetable stews contain thickeners and vegetables. Familiar stews with vegetables are okro stew (*Hibiscus esculenta*), bitter leaf stew (*Vernonia*), and pumpkin leaf stew (*Telferia*

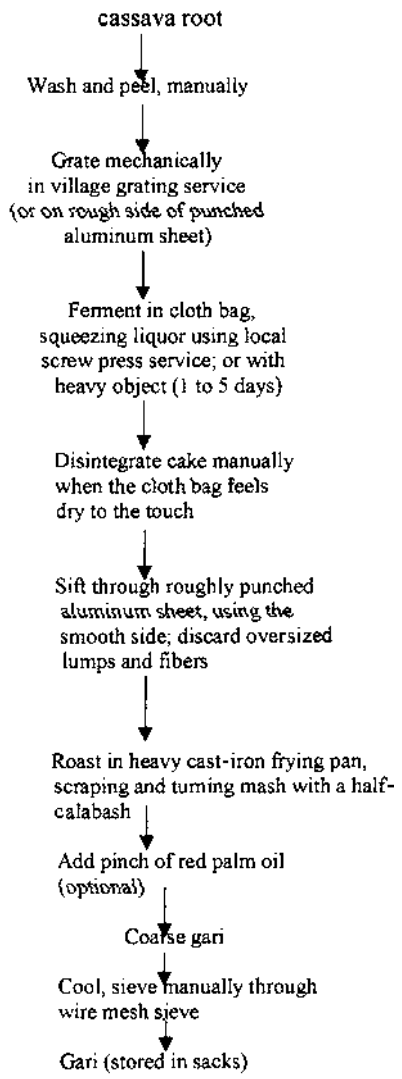

Figure 5 Flowsheet of gari production: modern village/cottage industry. (From Ref. 10.)

Figure 6 Village/cottage gari industry today, showing gari frying with a multiple frying range. (From Ref. 10.)

occidentalis). Among the stew thickeners are egusi (melon) (*Citrullus vulgaris* Shrad), ukpo (*Mucuna sloanei*), achi (*Breachystegia nigerica*), and cocoyam flour. It is important to mention that the traditional method of cooking the vegetables (by adding to the boiling stew and cooking for 5–10 min) causes the loss of ascorbic acid (Table 7), ranging from 32% to 68% (14). Interestingly, the stew thickeners can add as much as 19–35% protein, even if little animal protein is available (14). Typical egusi stew ingredients are presented in Table 8. In almost all instances, except where groundnut oil is preferred, palm oil is

Table 7 Loss of Ascorbic Acid Content of Some African Vegetables During Preparation of Traditional Stews for Gari Consumption

Vegetable in stew	Botanical name	Ascorbic acid, fresh (mg/100 g)	Ascorbic acid after cooking (mg/100 g)	Retention after cooking (%)
Pumpkin leaf	*Telferia occidentalis*	91.2 ± 2.3	36.4 ± 1.8	40
Okro	*Hibiscus esculenta*	98.8 ± 4.8	23.4 ± 1.5	48
Bitter leaf	*Vernonia*	38.6 ± 2.1	12.3 ± 1.8	32
Water leaf	*Talinum triangulare*	32.3 ± 2.1	18.3 ± 1.3	57

Source: Ref. 14.

invariably present to garnish the stew and this incidentally provides vitamin A in the diet.

The usual method of preparing the African egusi stew is as follows:

1. Dirt is removed from the melon (egusi) and this is ground into powder and set apart.
2. The meat (beef, fish, or stockfish) is cut, washed in water, and placed in a pot container 1.5 L of boiling water, to which salt is added.

Table 8 Typical Western Africa Egusi Stew for Gari Consumption for a Family

Item	Measurement	Cost (Naira)
1. Egusi (melon) (*Citrullus vulgaris* Shrad)	4 cigarette cups	120
2. Bitter leaf (*Vernonia*)	Already prepared; 2 wraps	20
3. Fish (dried)		200
4. Meat (beef or bush meat)	Variable	200–400
5. Palm oil	1/2 beer bottle	50
6. Crayfish		20
7. Stockfish		50
8. Commercial flavoring	1 wrap	5
9. Pepper (red; dry or fresh)		10
10. Onion	1 ball	10
11. Water	~1.5 L	—
		Total = $ 5–7

3. Then, ground fresh pepper, sliced onion, and measured palm oil are added to the pot containing the meat/fish, to boil further until the meat is tender.
4. Egusi is ground and then added as a paste and boiling is continued for 10 min, with intermittent stirring.
5. The addition of crayfish and commercial seasoning (or traditional seasoning; e.g., iru, ogiri dawadawa, fermented crab) follows, with stirring.
6. The already washed bitter leaf is checked for loose sticks and these are removed; it is washed again to reduce bitterness if necessary, before adding to the stew, stirring well with a long spoon.
7. Finally, the pot is covered and boiled briefly; the stew is tasted and served with eba (or fufu).

Other African stews that are very popular are: a vegetable drawing stew known as ewedu stew; an almost all-vegetable mixture of pumpkin leaf (ugu) and water leaf in palm oil exclusively, with plenty of beef, fish, and bush meat, traditionally called edikanikong, and an essentially boiled beef gravy containing spices for swallowing a sorghum cooked dough (known as tuwo). This is the standard stew in all Hausa-speaking African countries (in Nigeria, Niger, and other places bordering the Sahara desert) when tuwo is consumed in place of gari as a staple. In such places, the stew (called miya) is poured over the tuwo in a bowl; for eba, the stew is usually served in a separate bowl.

IX. INDUSTRIAL/COMMERCIAL PRODUCTION

Historically, FIIRO, Lagos, Nigeria in collaboration with the Newell Dunford, Engineering (NDE), United Kingdom designed and built a large-scale industrial gari plant capable of producing 6–10 ton gari per day based on earlier biochemical and microbiological investigations of traditional methods. The patent of the gari plant was issued in Nigeria and Brazil in 1972. Feasibility studies recommended a processing factory with backward integration of the cassava farming approach on a scale never heard of before in black Africa. Specifically, the gari plants required in 1 day, for 250 days a year, 30–40 tons of roots. The logistics of acquiring land from native owners, the high cost of importing the plant from the United Kingdom with scarce foreign exchange, the lack of mechanization of cassava planting and harvesting, inadequate reliable electricity source, lack of capital, and poverty among the gari-consuming nations unable to bear the higher cost of factory-processed gari over that of traditional gari indeed gave the new gari ventures no breathing space to succeed. In the 1970s, several factories were opened in

western Africa, three of these factories were opened in Nigeria at Iddo [Ibadan C.F. and P.M.S. Ltd Ilaju] near Ibadan; Texaco Agro-Industrial (Nigeria) Limited, Opeji, near Abeokuta; and Oke-Ayo Farms Ltd, Ondo. One factory was built in Ghana at Universal Cassava Product Ltd., Assin- Faso, Central Region, and one was built in Guinea at a site near Farcenah. Currently, the only surviving one is a pilot-scale plant built by FIIRO producing about 1 ton gari per day, a far cry from the original half ton per hour envisaged by NDE. Because of this failure, FIIRO conducted a national survey in 1989 to see how to assist the local small-scale gari equipment manufacturing companies to produce standard equipment which could stock and sell spare parts. With the expert collaboration of ARCEDEM (African Regional Centre for Engineering Design and Manufacturing. Ibadan, Nigeria) and under contract No. 91/004 of the United Nations Industrial Development Organization (UNIDO), five units of this low-technology gari processing equipment were shipped from FIIRO in 1990 to Bamenda in Cameroun, Togo, Ghana, Zaire, and Sierra Leone, all gari-consuming countries of Africa, with one unit in Enugu State, Nigeria. The technical drawings were left with those countries after undergoing instructions by staff of FIIRO, with the hope that the equipment would be replicated in the various countries to assist the communities. Based on the new developments, our discussion will now be on FIIRO's gari plant of NDE origin and the ARCEDEM/FIIRO/UNIDO gari plant (UNIDO gari plant). There are, of course, other gari plants still in operation, particularly those imported from Brazil. The national survey also revealed that cassava farms were small holdings and no group of processors could organize more than 2–3 tons of cassava per day. For this reason, the UNIDO gari plant was designed to produce a maximum of 0.5 ton of gari per day, and this was thought viable for a small-scale plant (15). The unit operation equipment are coupled together by belt-and-pulley drives using a diesel or air-cooled engine and gas, charcoal, kernel shell, or any waste of high colorific value as a possible heating medium (Fig. 7). These made the plant feasible in rural areas, and unlike other gari plants, this one was versatile in that it could be used to make fufu and lafun as well. The equipment had the following four components:

1. Grater/granulator: A hammer-type grater is most suitable for large-scale operation in terms of capacity and cost. In the UNIDO equipment, the abrasive type was suitable and was made up of a rotating disk having an abrasive periphery in a stationary enclosure, with its walls serving as the feed area. The quality of grating depends on the extent of abrasiveness of the disk and plate and the clearance between the disk and plate. The designed output was 500 kg/h and

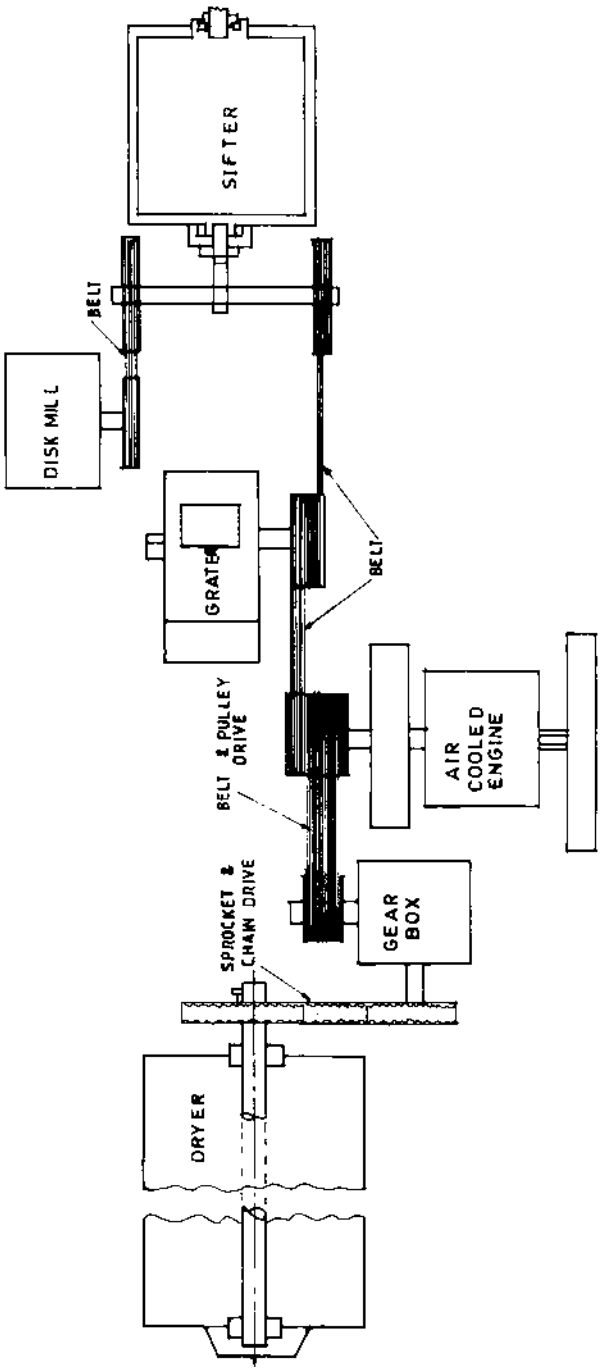

Figure 7 Industrial commercial production. Schematic arrangement of low-technology ARCEDEM/FIIRO/UNIDO gari plant installation. (From Ref. 15.)

served the dual purpose of grating cassava and granulating the fermented pulp after dewatering.

2. Screw press: This was used for dewatering grated cassava mash and was operated using a rod with an adjustable lever for mechanical advantage. The designed capacity was 2 tons per batch.
3. Gari sifter: The principle of the design was based on vibration theory with one degree of freedom. The important design parameters were eccentricity of the rotor cam on the main rotation shaft, lowest natural frequency or speed of rotation that would not reach catastrophic values, and stiffness of the loading spring required to damp the vibration (15).
4. Gari fryer/dryer: This was a long tubular arrangement made of mild steel, 8 ft. long on the gari gelatinization side and a terminal 8 ft. long, which was the drying end. A central rotating shaft carried wooden paddles with more paddles on the drying end than at the frying portion. The fryer/dryer was mounted on insulating bricks and heated with gas or charcoal as available.

In summary, the gari machinery at our disposal spans the Newell Dunford large-scale capacity of 6.5–10 tons of gari per 24 h period to a low-technology cottage-based outfit having a maximum daily output of gari of 0.5 ton per 8-h period. Whereas a daily input of 40–60 tons of tubers, depending on the variety, type of soil, rainfall, and time of year, was required with the NDE factory with its attendant problems already highlighted, the extreme unprogressive minimal output of about 0.5 ton of gari based on a daily input of cassava roots of 2–3 tons per day commercial scale was the next option. To date, no successful duplication of the UNIDO plant is reported. However, the attieke factory in Côte d'Ivorie has not suffered this catastrophe and is still successfully organizing the required cassava to feed its factory. This is not say that all other factories for (e.g., PRODA, FABRICO) are not in production. The commercial processing of gari follows the flowsheet in Fig. 8.

A. Peeling

The processing of cassava tubers to edible gari begins with peeling. To date, it is still largely manual, because mechanical peelers have not proved to be of wide practical application. A major problem with mechanical peeling of cassava root is its irregular shape, unlike that of potato, so that there is always a possibility of overpeeling of up to 30–40%, because the skin near the inner part cannot be removed unless a certain amount of material has been removed at the thicker portion. Except for a patented French version being employed at the attieke factory in Côte d'Ivorie, none in Nigeria is of much significance.

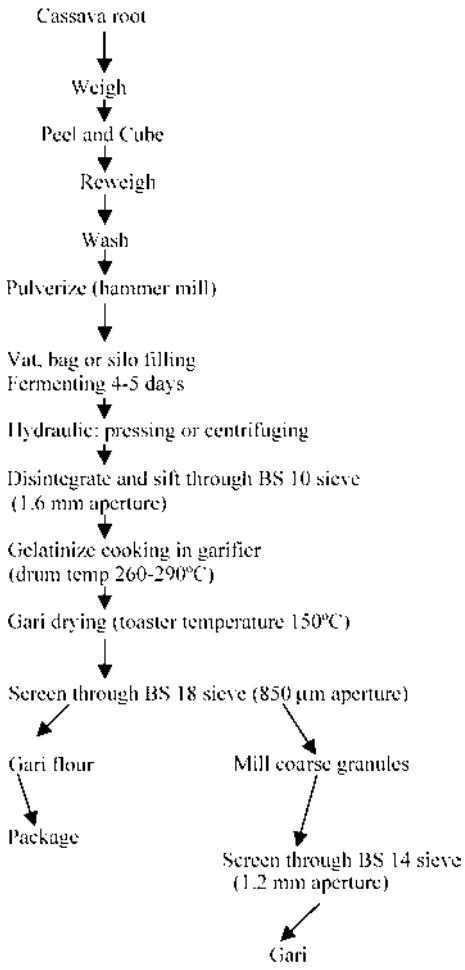

Figure 8 Flowsheet: Nigerian gari commercial industrial production. (From Ref. 16.)

Even with the Côte d'Ivorie peeler, only the papery outer layer of the peel is removed and this would not be satisfactory in gari processing because of discoloration of the final gari. The NDE peeler is a revolving eccentric drum resembling a conventional concrete mixer (Fig. 9), except that it has abrasive inner linings. While the drum revolves, the combined action of the lining and the roots rubbing one another accomplish some limited peeling effect; and

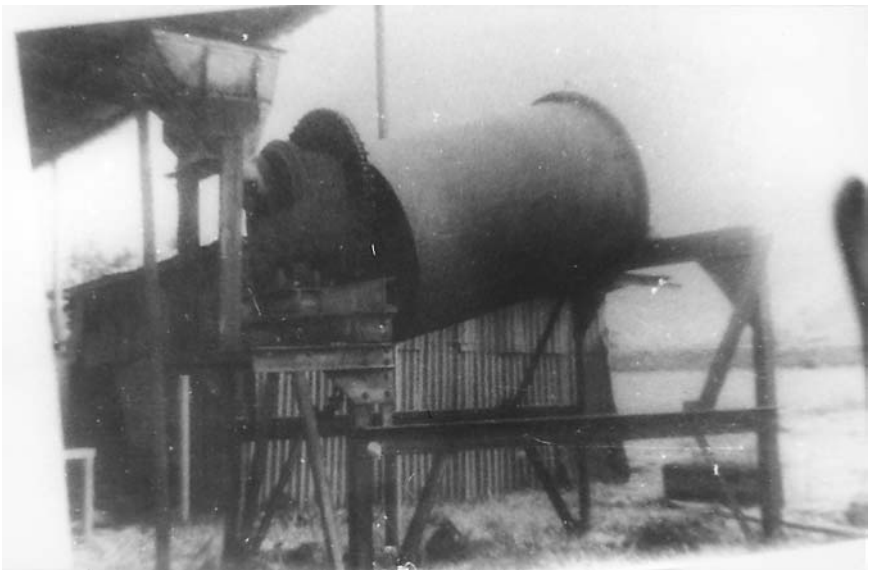

Figure 9 Commercial industrial gari production. Newell Dunford mechanical cassava peeler of a concrete mixer type. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

water being continuously pumped into the peeler washes out the peels (10). In a case study by Newell Dunford using the Texagri gari plant, it was found that 20 men, if properly supervised, could operate the ND peeler to have a throughput of 2400–2500 kg peeled roots per hour. In the mechanical peeler–crusher of Bertin–Palmindustrie Technical Association of France, cassava root is split vertically into four long pieces prior to peeling (17). Ezekwe (18) reported that a conveyor-belt peeler, when fed sliced roots 2.5 cm in length, could peel with an overpeeling of not more than 2.4% and maximum underpeeling of 3.5% of the correct depth. However, the convey-or-belt peeler was never commercialized nor is there a published concerted effort to develop others. Lye peeling with hot caustic soda has proved unsatisfactory because fermentation was retarded. Two other peelers (PRODA and FABRICO) were also in operation, but the factory owners, including Texagri all subsequently reverted to hand peeling because of the excessive peeling losses. Traditionally, cassava for gari has to be peeled to remove the inner leathery portion of the peel, which is left by the Ivorian peeler, as this causes a mauve discoloration in fermenting mash due to the

oxidation of leucoanthocyanins present in fresh cassava root (10,19). Therefore, cassava peeling is still a bottleneck in industrial gari processing, and the average rate of peeling by skilled operators is 22 kg per man per hour (13).

B. Grating

Roots are trimmed to remove the top and tail, diced into short pieces, washed in water (Fig. 10), and grated in a fast-revolving electric hammer mill [Robert Fries K-G Landmachinefabrik, Stuttgart (Figs. 11 and 12)] such that at least 70% of the cassava pulp will be retained on a 0.058-cm-aperture sieve but will pass through a 0.25-cm-aperture sieve (1,20,21). The mash is transferred into an inverted fiberglass silo or vats (Figs. 13 and 14) and covered for 4 days to ferment. For the 0.5 tons gari per day, the mash is filled into cloth bags (Fig. 15) and placed on the stationary ram of the screw press and pressed to continuously dewater the mash (Fig. 16) as at the cottage/village level as fermentation is taking place.

Figure 10 Commercial industrial gari production. Peeled cassava tubers being transferred into wash tanks. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

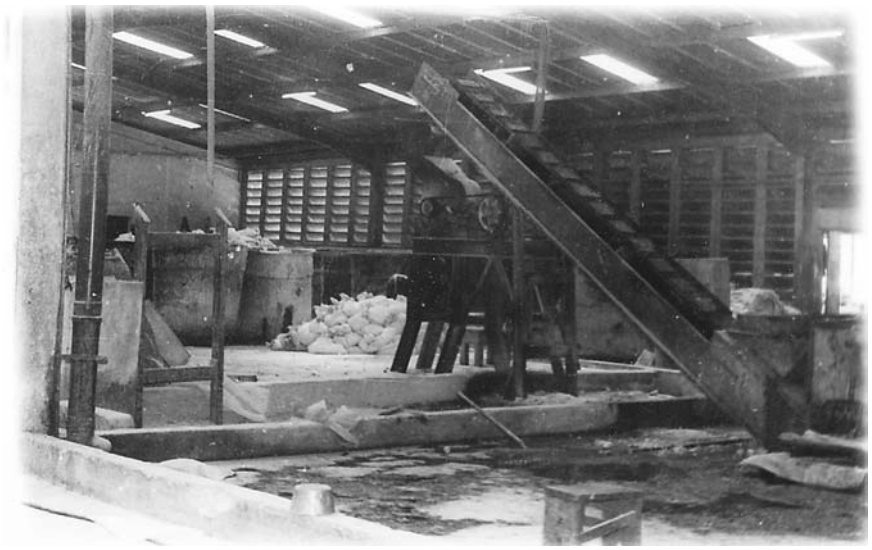

Figure 11 Commercial industrial gari production. Washed roots being elevated onto an electric pulper. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

C. Fermenting

The stage of fermentation is very important, first, to detoxify the mash for the safety of the gari product (10,22) and, second, for development of acceptable taste and aroma profiles. We stated earlier that the very poisonous cassava varieties are traditionally used for gari, with the assumption that the sweet varieties do not sour well. The fermenting stage is not controlled by seeding known micro-organisms, and, so far, research on the use of starter cultures in gari fermentation is only an academic exercise (23). Current literature has the following view. Grating of the root parenchyma releases an endogenous enzyme, linamarase, from the cell sap. The enzyme hydrolyzes its substrate, linamarin, in the presence of water in the cell sap at a low pH optimum of 5.5 (24) and a temperature below 62°C. Through this process, the cyanogenic glucosides of cassava are hydrolyzed and acetone, glucose, and various organic acids are formed with the release of HCN, CO_2, and other gases. Also, significant reduction of the original level of the prussic acid level occurs in the fresh root from about 300 mg HCN/kg to about 20–30 ppm in the finished gari (10). A pH of 4.0–4.2 $\pm$ 0.15 or 0.85% total acidity (as lactic acid) indicates that fermentation is complete (10). Where sour gari is not tradition-

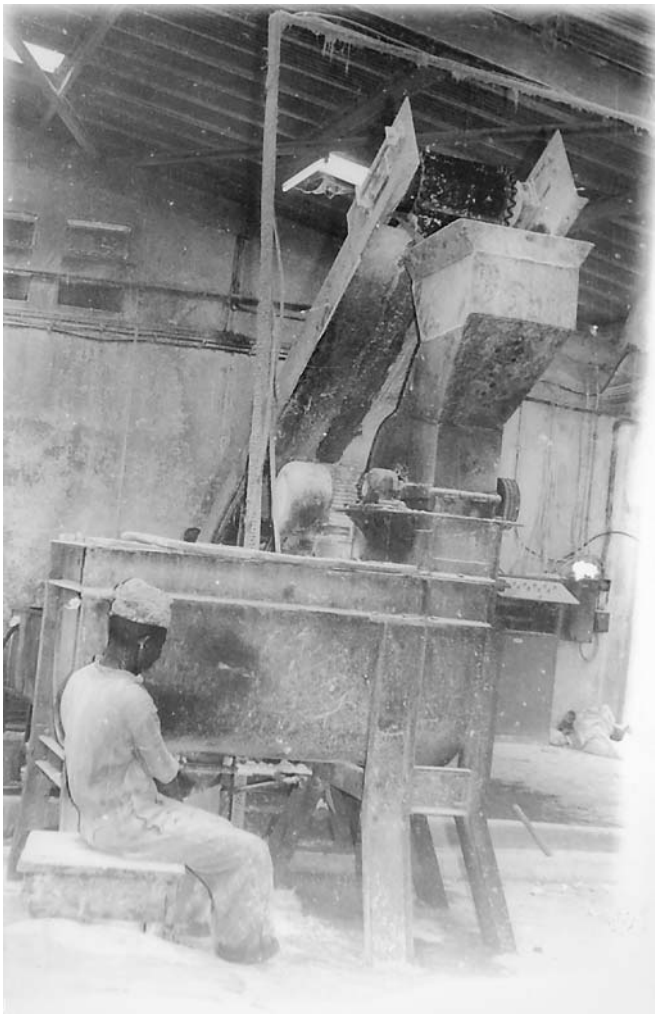

Figure 12 Commercial industrial gari production. A cassava elevator dumping root pieces unto a hammer mill. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

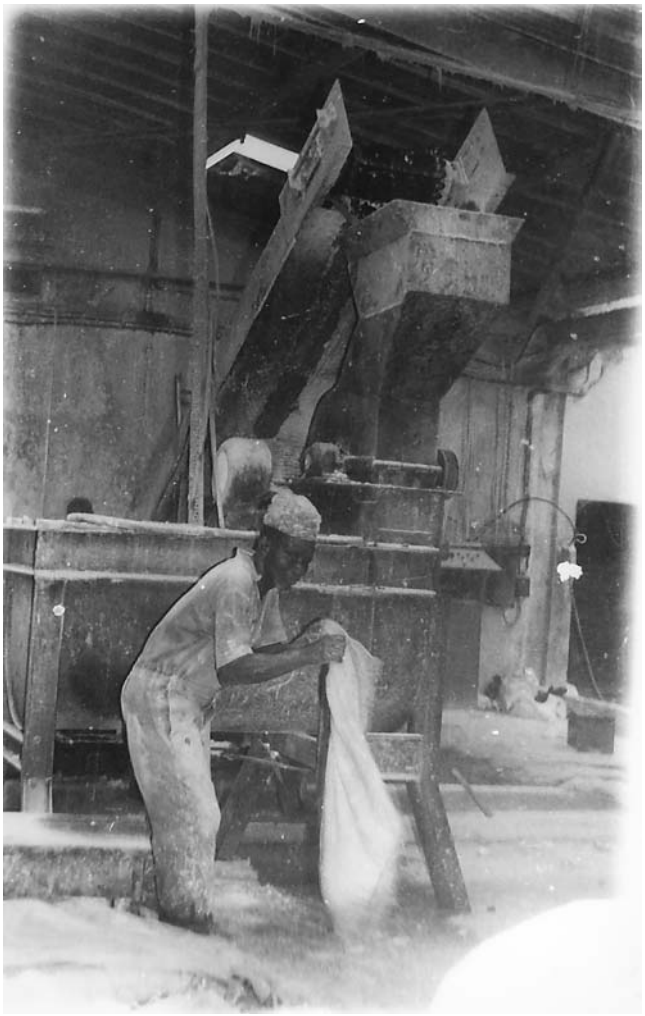

Figure 13 Commercial industrial gari production. Pulped cassava being bagged for fermentation. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos.)

Figure 14 Commercial industrial gari production. Bagged pulped roots fermenting in plastic open-bottom modern vats. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

ally prepared, fermentation as little as overnight in warm weather is often adequate, but the gari may be soft and sticky. Micro-organisms in various successions add to the acceptable slightly yogurtlike sour taste and the aroma is most prominent in gari fermented for 4 days or more. Whether at the commercial or rural level, fresh roots are used for gari processing because dried roots by themselves do not ferment properly. Recently, however, Onyekwere et al. (25) succeeded in using cottage sun-dried cassava tubers in the pilot plant to process acceptable gari by soaking the pulverized root overnight with approximately 1.8 times its weight of water and priming the mash with 10–15% (w/w) grated fresh cassava root (Fig. 17). This transferred the linamarase fermenting enzyme. After mixing and allowing fermentation for 2 days, the gari process successfully entered the next logical stage of dewatering (pressing) and roasting.

D. Dewatering

Where nondraining metal vats are used to ferment the mash in the NDE plant, there is an accumulation of cassava liquor which has to be expelled by the use

Figure 15 Commercial gari production. Pulped cassava roots fermenting in a fiberglass silo. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

of NDE hydraulic presses or a basket-type centrifuge, (type 86, Broadbent, Huddersfield, England). In other cases (e.g., where the fermentation occurs in a fiberglass inverted silo or cloth bag), continuous loss of the liquor significantly brings about reduction of the liquor content when fermentation is terminated. In either case, the pulp has to be pressed further to bring the mash to a cake with about a 50% moisture content (10). The screw press is used in the low-technology gari plant to accomplish this task. The residual moisture

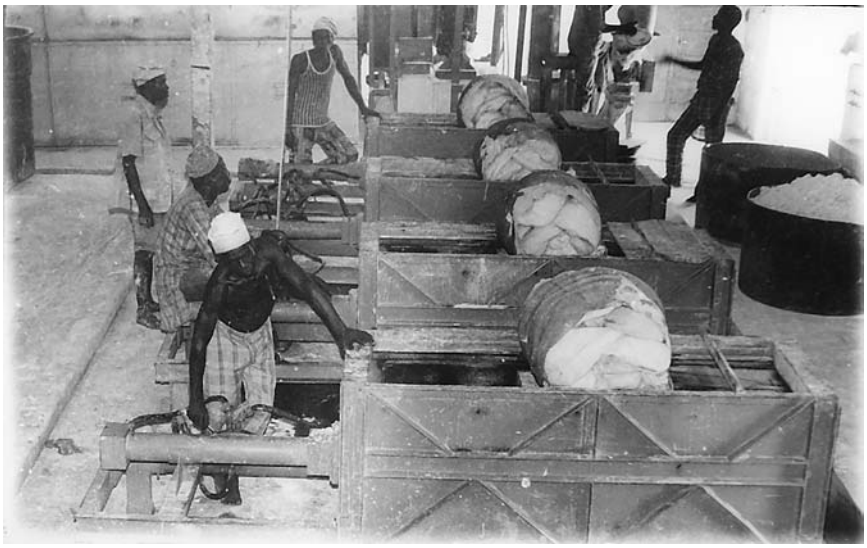

Figure 16 Commercial industrial gari production. Fermented cassava mash being dewatered in hydraulic presses. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

content is crucial before the next stage in that water content above 55% causes lumpiness during drying, whereas a very low moisture of below 47% results in the production of cassava flour and not gari (10). The NDE hydraulic press (Fig. 16) consists of a rectangular frame from whose top a series of bags are hung; between each is a piece of metal that stops the bags from actually touching, which might impede liquor drainage. The bags are filled individually as the vat is emptied. After filling, a large flap on the top of one side of the bag is pulled down to prevent mash from squeezing out of the bag under pressure. A metal ram at one end of the hydraulic press moves forward, pressing each bag against a guard; pressure builds up as the last bag presses against the solid end piece. The bags, having been pressed, are lifted off the hangers and emptied onto a table where the caked mash is broken up into small pieces with a mallet.

E. Granulating

The small pieces of cassava mash are fed onto an elevator that feeds a small hammer mill to completely granulate the pieces. In the pilot plant, a Kek

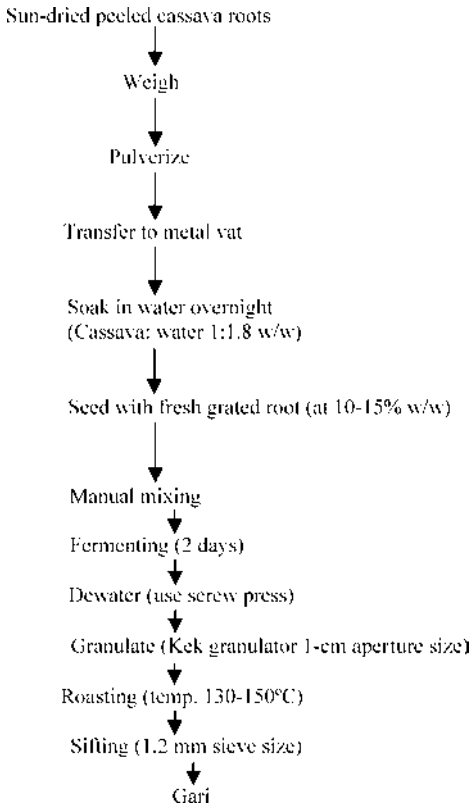

Figure 17 Flowsheet for pilot-plant-scale demonstration of gari production from cottage peeled sun-dried cassava tubers. (From Ref. 25.)

granulator having a BS 10 sieve to remove lumps and fiber trash is fed manually, whereas in the UNIDO plant, the grater serves also as granulator. After granulating, the pulp falls onto a moving belt that feeds the cassava cooker drum (the garifier) for roasting.

F. Roasting (Garification)

Roasting of the granulated cassava is done in the garifier, which is a stainless-steel drum, about 1 m long by 0.78 m wide and is heated by three burners to a temperature of 260–290°C. It takes about 10 min for the mash to travel from the inlet end of the steel drum to the outlet, during which time the starch becomes gelatinized as the mash core temperature reaches 80–85°C. There is a

rake in the garifier that rakes up the gelatinized mash as it travels down the length of the drum (10). This is a mechanized improvement over the action of the calabash scraper used in the ancient and village/cottage methods (Figs. 18–20). (In the FABRICO plant, the fryer is a planetary type one.) The gari falls out of the end of the drum and with the assistance of a vibrating machine situated at the outlet end of the garifier, the cooked mash falls onto a conveyor

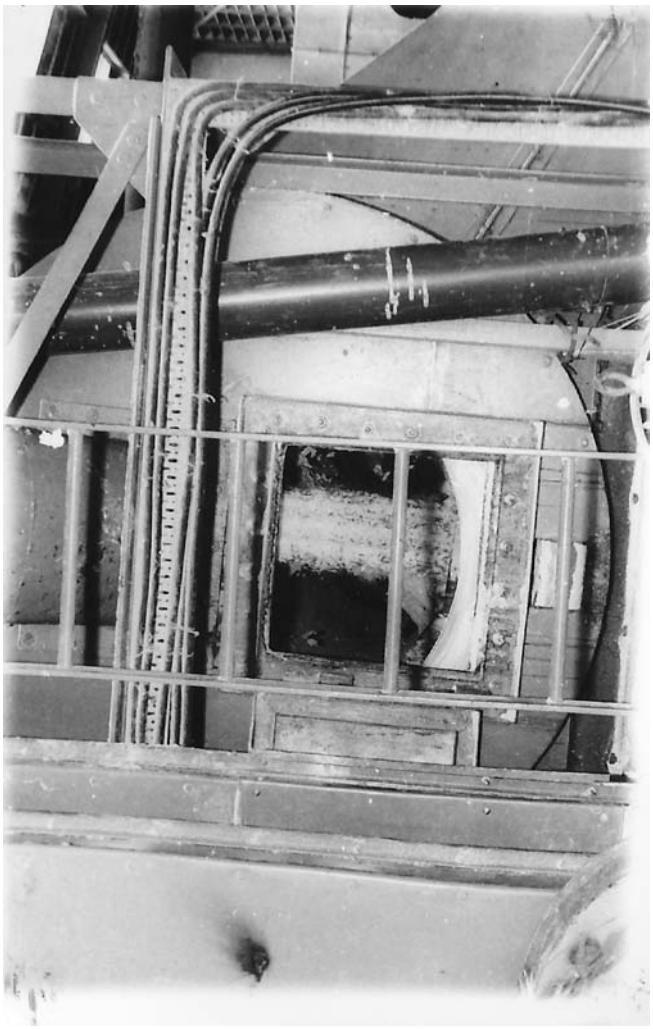

Figure 18 Commercial industrial gari production. Front view of garifier. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

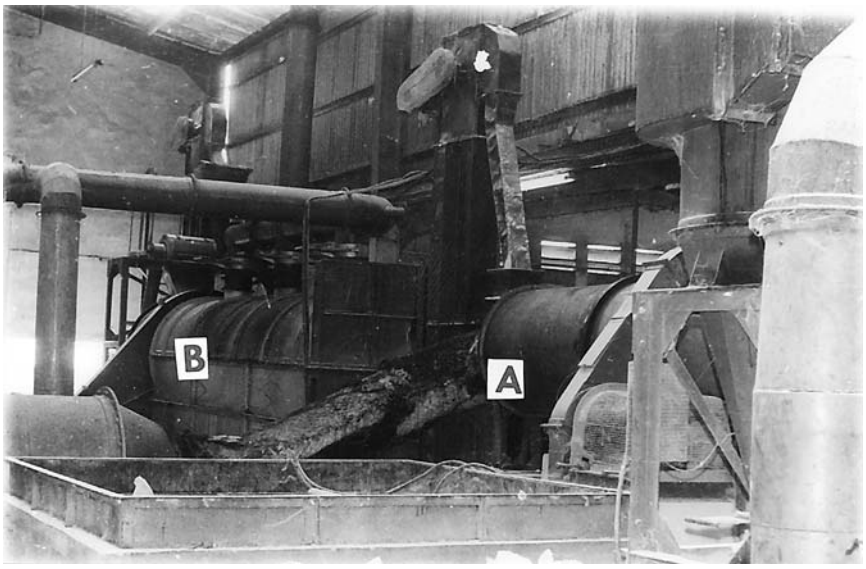

Figure 19 Commercial industrial gari production. Side view of the garifier (A) and dryer (B). (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

belt by which it is again elevated and fed into a rotating louvered cylindrical dryer for drying.

G. Drying

The louvered dryer is heated externally by an oil-fired burner connected to a high-pressure fan which carries the hot air along the length of the dryer, where it takes 8 min to pass through at an average temperature of 130–150°C. The window-type arrangement in the dryer enables only a drying effect to take place (Fig. 21). Previous laboratory investigations showed that gari frying is, in fact, not a single process. Rather, it consists of two antagonistic processes, one following the other. First, cassava starch gelatinization within the garifier requires a high heat transfer and a low mass transfer (10). The drying operation follows, which requires a low heat transfer and a high mass transfer, antagonistic to the first stage. This finding was a breakthrough in that it enabled FIIRO to effectively design the appropriate machinery in which the gelatinization chamber was separate from the drying chamber. This principle is again repeated in the UNIDO gari plant (15), where rotating paddles which

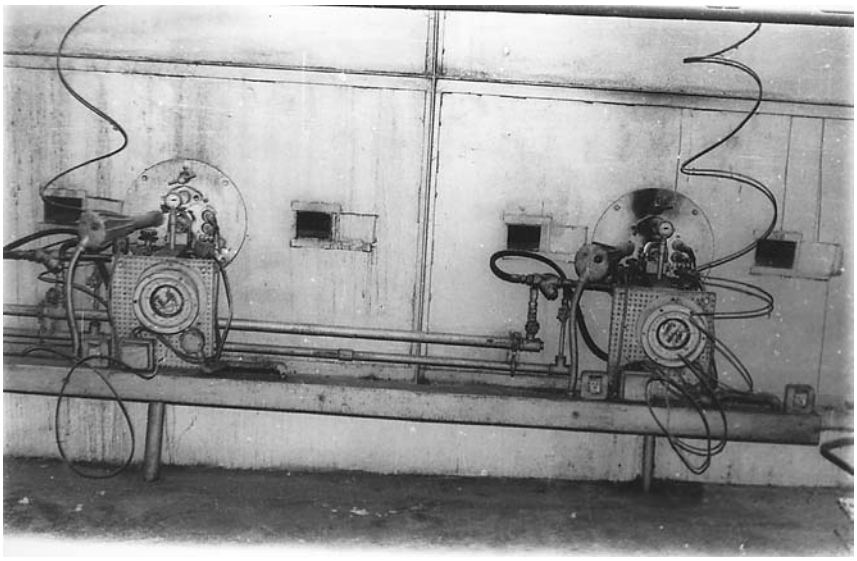

Figure 20 Commercial industrial gari production. Side view of garifier showing burner chambers. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

actually push the mash from the cooking side to the drying end are fewer in the garifier than in the drying part, in order to increase the resident time within the garifier and allow gelatinization, whereas an increased number of paddles quickly complete transfer in the drying. However, in various other commercial plants such as the Brazilian gari plant, PRODA, and FABRICO, as well as the village open pan-frying, a one-stage operation is assumed, which is not correct (10). Finally, the hot dried gari in the NDE factory is elevated onto a three-deck screen, having a BS 18 sieve (850 μm) through which the hot gari is sifted. Small-sized gari granules (flour) go through and are collected (Fig. 22); coarser gain gari is also collected and both are stacked separately to cool. Gari flour can be consumed in the same way as gari, but preferably by sprinkling cooked ewa on it (cowpea).

H. Milling and Packaging

Coarse-granule gari when cool is milled in a disk mill and sieved through a dry vibrating BS 14 sieve (1.2-mm aperture) to collect standard gari and other grades if so desired (Figs. 23–25). Industrial gari is packaged in 2-kg bags made of propylene, 10 bags to a carton.

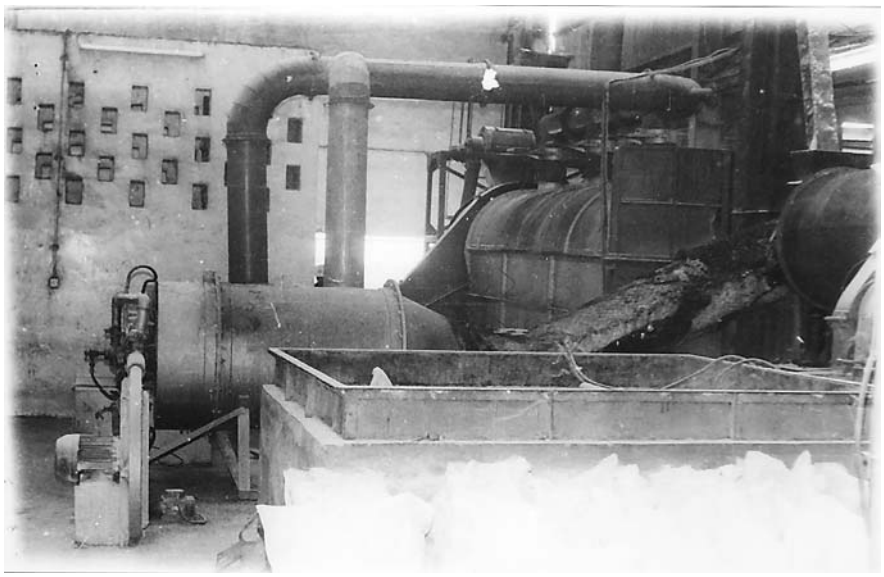

Figure 21 Commercial industrial gari production. Burner chamber of NDE gari dryer. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos.)

X. NIGERIAN STANDARD FOR GARI

The Standards Organization of Nigeria (26) approved the following standard for commercial gari:

1. Extrafine-grain gari. Not less than 80% by weight will pass easily through a sieve having an aperture less than 350 μm.
2. Fine-grain gari. Not less than 80% by weight will pass easily through a sieve with an aperture of 1000 μm, but less than 80% of the weight will pass easily through a sieve with a 355-μm aperture.
3. Coarse-grain gari. Not less than 80% of the weight will pass easily through a sieve having aperture of 1.4 mm, but less than 80% of the weight will pass through a sieve with of aperture 1 mm.
4. Extracoarse-grain gari. Not less than 20% of the weight is retained on a sieve with an aperture of 1.4 mm.
5. Unclassified gari. Gari which has not been classified by the sieve method to determine its category according to grain size.

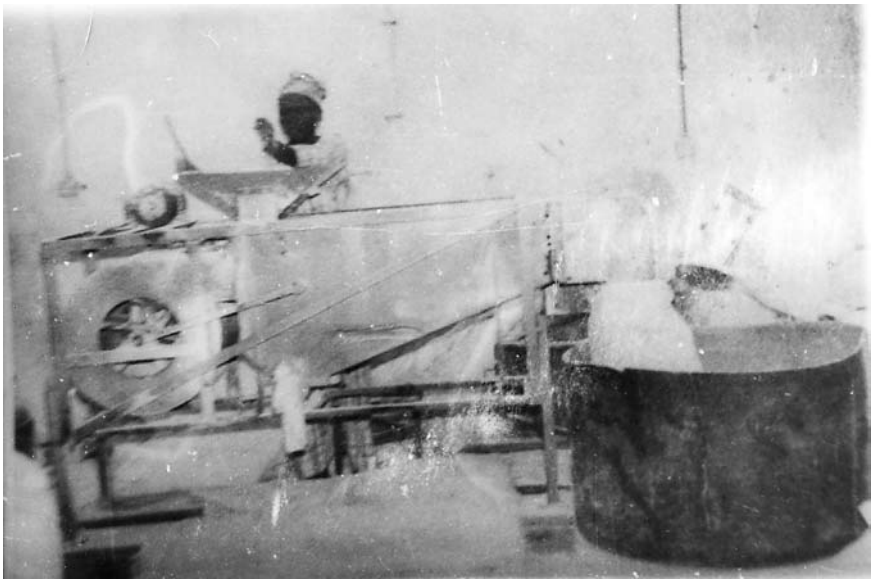

Figure 22 Commercial industrial gari production. Presifting of unmilled gari to remove fines (flour). (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

The essential composition and quality factors are as folllows:

- Raw materials. Clean cassava tubers of *M. utilissima* and/or *M. palmate* in wholesome condition.
- Organoleptic properties. The taste and odor of gari shall be characteristic of the product as is acceptable in a given locality.
- Analytical characteristics: Total acidity of gari shall not be more than 1% m/m, measured as lactic acid (no lower value, as some localities do not consume sour-tasting gari).
- HCN and its glucosides. HCN contents not more than 20 mg/kg weight.
- Moisture content. Not more than 10% m/m.
- Crude fiber. Not exceeding 2% m/m.
- Ash content. Not exceeding 1.5% m/m.
- Food additives. Gari may contain one of several or the following ingredients food-grade palm oil; enrichments with vitamins, proteins, and other nutrients shall be in conformity with the Public Health Regulations.

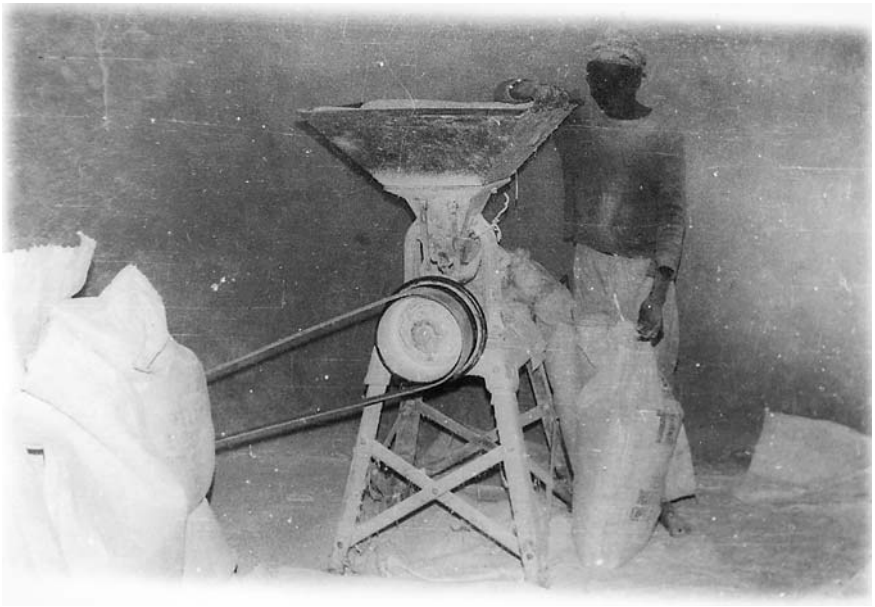

Figure 23 Commercial industrial gari production. Milling of course gari. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

- Contaminants. Maximum residual limits for pesticides shall be in conformity with the Food and Drug Regulation in pesticide residue or as recommended by Codex Alimentarius Commission.
- Hygiene. Gari should be prepared in accordance with International Code of Hygiene Practice; free from pathogens, poisonous or extraneous and deleterious substances in amounts which may represent health hazards.
- Labeling. Name of products, list of ingredients, net weight, name and address of manufacturer of packer, and date of manufacture to be declared.
- Packing. Packing materials should be such to protect the product against infiltration of moisture and micro-organisms or other contaminants.

XI. RAW MATERIALS STORAGE

Rickard and Coursey (27) reviewed cassava storage by various methods and noted that rapid deterioration occurred in poorly stored roots. From the

Figure 24 Commercial industrial gari production. Deck sifter grading of milled gari granules. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

abundant literature on the subject, the following methods had been used: storage in clamps, in boxes lined with moist sawdust, in plastic bags or films: cold storage at reduced temperatures of below 4°C; freezing, waxing, and chemical treatment (notably with the fungicide benomyl). The efficacies of these treatments varied and remained limited. Okoye and Ikeorah (28) reported that sweet and bitter cultivars of cassava tubers were successfully stored in two humidified galvanized metal sheets for 15 weeks. They observed storage losses of 2.3% and 4% in the sweet and bitter cultivars, respectively, and the jute materials they used for lining the chambers were treated with a 0.1% aqueous solution of thiabendazole to prevent mold infection. They also observed an average temperature reduction in the chambers of 2°C, whereas the reduction in humidity of the storage environment fluctuated between 93% and 100%. Moisture contents of the roots had a direct relationship with the relative humidity of the chambers during storage. Finally, there was no significant difference ($p < 0$–0.05) between the carbohydrate contents of processed from the stored cassava and initial cassava samples.

Traditionally, cassava roots are left in the soil until needed, but this ties up extensive acres of farmland. Recent studies on the processing of gari from sun-dried roots in a pilot plant using freshly grated roots at 10–15% by weight

Figure 25 Commercial industrial gari production. Gari in 2-kg bags. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

as primer proved positive (25). Its main objective was that rural communities could organize themselves to dry peeled cassava roots for gari, starch, and other industrial products. It was established that cassava chips dried in our laboratory were also successfully used for gari, but the technology of the two differed. Whereas the chips milled into flour were blended with the fresh grated primer, fermented 2 days, and dried into gari, the whole dried roots had to be milled into flour, soaked overnight in nearly twice its weight of water before priming with fresh primer and with 2 days' fermentation, followed by dewatering and roasting into gari (Fig. 17).

XII. CONTRAST BETWEEN INDIGENOUS AND MODERN PROCESSING

The differences between the traditional and modern mechanized processing of gari are more in scale and machinery, quality control, and improved hygiene than in the process itself.

A. Scale of Production

Cassava farms are in small family holdings in the indigenous process, but an attempt was made on an industrial commercial scale to organize farmers into cooperatives to supply the factory with sufficient roots. However, the quantity was not easily available because of fluctuating weather, changing cassava costs, and unreliability among the cooperative farmers. The processing steps are more mechanized in the modern processing than in the rural processing.

B. Cassava Peeling

Hand peeling of roots is used exclusively in the indigenous process, but an attempt to mechanize it by commercial gari factories was not altogether successful, with the result that most factories have reverted to hand peeling (10,29).

C. Grating

The modern gari factory used a fast revolving electric hammer mill as a grater that is more efficient and of higher output than the traditional grater, not to mention the ancient manual grating methods of using a punched aluminum sheet.

D. Containerization

The grated pulp is transferred into inverted fiberglass silos or vats for fermenting, whereas cloth bags are used in rural methods, with continuous dehydration facilitated by heavy piece of concrete on top of the cloth container or by means of a screw press. In modern processing, the pulp is dewatered in a hydraulic press or screw press or in a basket centrifuge. In both methods, the moisture content is reduced from about 65% to nearly 50%.

E. Fermenting

In both cases, fermentation is spontaneous, with no starter cultures being used. However, souring in traditional cloth bag is faster and is completed within 2 days (see Figs. 26 and 27).

F. Quality Control

Quality control tests (pH, total acidity as lactic acid) are used in modern processing to determine completion of the fermentation stage before dew-

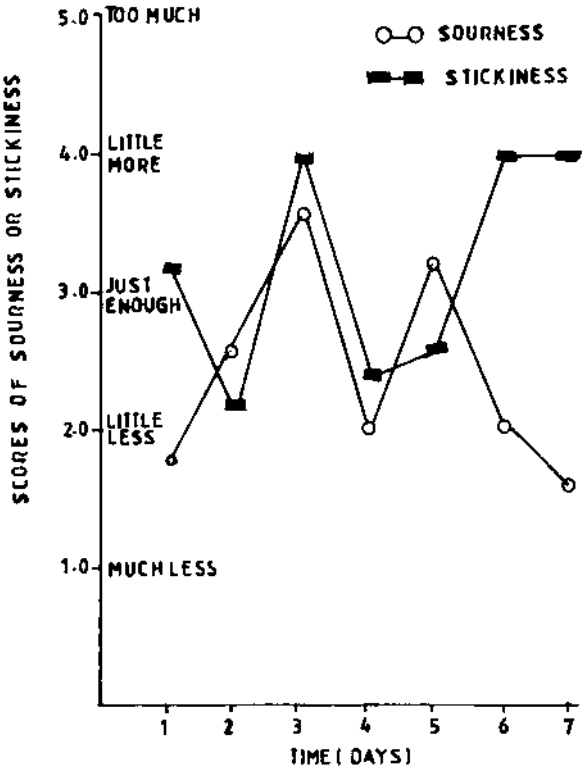

Figure 26 Taste panel scores of gari production from cassava fermented in nondraining modern vats. (From Ref. 30.)

atering and drying. This quality control assessment is completely absent in traditional methods.

G. Drying

Drying is inefficient using village methods, which results in producing gari of high moisture content (sometimes up to 14–20%). In modern factories, the highest moisture allowed is 10%.

H. Packaging

Waterproof packaging is used in modern factories, and with the combination of the low moisture content of the gari, a long shelf life of up to 1 year or more is often the case versus a maximum storage of 6 weeks for traditional gari.

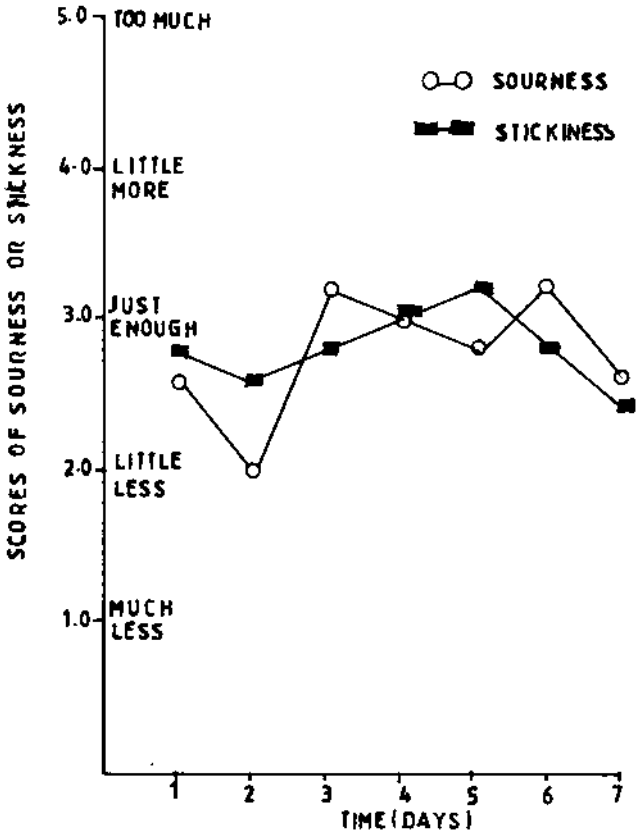

Figure 27 Taste panel scores of gari production from cassava fermented in a traditional cloth bag. (From Ref. 30.)

Traditional gari is often exposed and continues to absorb moisture, dust, and fungal spores in the high tropical humidity and progressively gets moldy with storage in unhygienic circumstances (31).

XIII. CRITICAL STEPS IN MANUFACTURE

In gari manufacture, the following inputs and practices should be carefully observed, particularly where very sour gari is cherished.

1. **Substrate**. Usually only good fresh cassava roots can produce good gari; moldy, rotten roots or overaged dry roots are not acceptable. Overaged roots are fibrous and almost impossible to

dewater and they ferment poorly. Roots beyond 2 days after uprooting should no longer be used.

2. **Peeling**. A high peeling loss of up to 30–40% can occur when inexperienced peelers are used, because peeling is more or less manual in most instances. Experienced peelers maintain a peeling loss within the 20–25% range.
3. **Grating**. Proper grating exposes the parenchyma and root cells to activity of the endogenous enzyme, initiating fermentation and detoxification processes (10). Increased processing loss occurs with large lumps of ungrated chunks, as these will be sieved out as waste.
4. **Vessel**. Cloth bags under continuous pressure to expel cassava liquor while fermenting result in complete fermentation and acceptable souring within 2 days, whereas cassava fermenting in deep, nondraining vats and silos fails to develop the desirable sour flavor and acceptable eba properties until 4 days. The best option is, therefore, to fill the grated mash in cloth bags and use a hydraulic press similar to the village method. The residual cyanide is reasonably low (13–20 ppm) and within the maximum level for nutritional safety (30).
5. **Fermentation**. Quality control of the fermenting mash sets the pH of correctly fermented mash to about pH 4.0–4.2, total acidity of 0.85–1% lactic acid, and optimum temperature of 32–35°C. Covered vessel to reduce oxygen and inhibit foaming (10,29). Cassava ferments exclusively in its natural liquor in gari production and not under water, as in fufu and lafun (Fig. 2).
6. **Ventilation.** Because of the loss of gases, especially HCN, H_2, and CO_2, there should be adequate ventilation in the building to prevent cyanide poisoning (20,32). The total minimum space requirement for peeling, grating, fermenting, and garifying operations is 25.5 m^3 (Table 9).

Table 9 Minimum Space Requirements for Large-Scale Cassava Processing

Processing operation	HCN liberated from roots (mg/kg root)	Volume of space requirement (minimum in m^3)
Peeling	120	11
Grating	80	7
Fermenting	38	3.5
Garifying (toasting)	42	4
Total	28	25.5

Source: Ref. 20.

7. **Dewatering**. The fermented pulp is to be dewatered to between 50% and 55% moisture extent for proper drying. A moisture level from 47% and below is too dry and will produce cassava flour, without being gelatinized to make gari. A mash moisture content of over 55% may produce a lumpy, undried product due to excessive water content (10).
8. **Drying**. Proper gelatinization of the cassava starch requires an outside temperature of at least 120°C with the traditional frying pot and 260–290°C for mechanical fryer to attain a starch gelatinization temperature of 80–85°C. A standard well-gelatinized gari is supposed to swell in water at ambient temperature to three times its original volume. Improperly dried gari having over 12% moisture content bagged in porous bags cannot be stored.
9. **Packaging**. Industrial commercial gari is packaged in waterproof polypropylene bags. Cottage gari is sold in Hessian sacks or jute bags, which affect its shelf life when stored in poorly ventilated market stores.
10. **Hygiene**. Fermented cassava liquor should not be poured on a concrete floor; it causes corrosion of the surfaces. Such wastes should be collected in a waste pit, to be emptied from time to time. Finally, the factory should be fumigated frequently against rats and insects.

Akinrele (19) carried out various studies in the 1960s to shorten the usual 5–6 days' fermentation period needed to produce the very sour gari, so-called Ijebu-type gari, which was a bottleneck in the factory.

1. He premixed freshly grated cassava with 4-day-old cassava liquor, in an effort to transfer fermenting micro-organisms. Although this produced sour-tasting gari within 24 h, it proved impractical due to the difficulty of mixing liquor into semisolid pulp. In addition, laboratory tasters still preferred the taste of gari by the natural, long method.
2. Another method was to quickly remove the cassava liquor within 24 h with a screw press (33). Although this also effectively reduced the residual cyanide content in the mash more than when the pulp was slowly dewatered for 3 days, the 1-day-old gari was not sour and was acceptable only to those who did not naturally consume sour gari.
3. Fermenting at a high temperature of 45°C was said to be effective in accelerating fermentation (19) but was outside ambient temperature to be useful. We found in our laboratory (34) that to reduce the time of effective satisfactory souring within 2 or 3 days, the

liquor had to be continuously reduced with a hydraulic press or screw press similar to the village press bag method (Fig. 27). The taste of such gari was found to be more acceptable than gari produced in nondraining vats and the residual cyanide level was even less than 20 ppm.

XIV. MAJOR PROBLEMS IN INDUSTRIALIZATION

The problems associated with industrialization of gari using the experience of Texaco Agro-Industrial Nigeria Limited (Texagri) are numerous. First, the cost of the gari plant is prohibitive. The Newell Dunford/FIIRO 10-ton/day gari plant was imported from the United Kingdom at a high cost in foreign currency. Second, as recommended (35), Texagri went into backward integration and acquired extensive acres of land for cassava plantation. Land acquistion was a difficult and expensive problem, especially on the large scale needed by the company to cultivate cassava in order to obtain close to 60 tons of roots per day. Having acquired the land near Abeokuta for the factory and farms, actual cassava cultivation and harvesting were not mechanized. Although harvesting of roots and the factory operations took place year round, the remainder of the work was seasonal, demanding large numbers of workers during the planting season to prepare the land, plant the cassava sticks, apply fertilizer, and for hand spraying (36). The company employed a permanent staff of 18, including 9 professionals in the senior cadre. The remainder of the workforce, amounting to as many as 500 during the peak season, were employed at a daily wage rate.

The factory costs were as important as the plantation costs (10). To find and arrange for third parties to supply 50 tons or more tubers a day to feed the factory was a difficult task, not only in arranging an even, regular supply but also in the logistics of collecting the tubers from subsistence farmers who probably never farmed more than 1–5 acres. The ratio of cassava to gari, although generally assumed to be 4 tons of cassava roots to 1 ton of gari, was variable depending on the time of year and rainfall, because the cassava farms were not under irrigation. For instance, in 1982 when there was a normal rainfall of 1225 mm, the ratio of tubers to gari was 5.8 : 1, whereas in 1983, a particularly dry year with 580 mm of rainfall, the ratio was 7.0 : 1. Strangely, the water content of tubers in a dry year appears to be higher than in a normal year when rain falls consistently through the wet season.

Another large investment for factory operations required 32,000 L of water per day (36). The factory was situated within the farmlands, away from the city, and, of course, was not supplied with municipal water. For this reason, Texagri built a water reservoir of its own and had invested in a

Table 10 Factory Cost of Gari Production in Texagri, Nigeria

Component	Cost per ton (U.S.$)
Peeling	68
Processing	39
Diesel	44
Packing bags	43
Spares and repairs	63
Fixed charges/overhead	56
Tuber costs explantation	595
Total	908

Source: Ref. 36.

280-kV-A electric generator plant to supply its electric requirement of 150 kV-A. Its diesel fuel consumption per day for electricity was 600 L, whereas diesel fuel consumption per ton of gari produced was 276 L. About 9000 tons of cassava tubers were required to reach the maximum ouput of 1625 tons of gari per year based on 250 working days. With the various problems of factory inputs (Table 10) and plantation costs (Table 11), lack of spare parts, and market competition between small gari producers who contributed up to 90% of the total gari sold and who undersold the large-scale producers, the fall of the Texagri gari business was predictable, as was other large-scale gari factories of NDE design. Well-processed, reasonably priced gari will be patronized by the houswife, whereas traditionally gari is not purchased from supermarkets.

XV. OPTIMUM ENVIRONMENTAL CONDITIONS FOR FERMENTATION

The major objectives for fermenting cassava root in gari processing are as follows:

1. To activate the endogenous enzyme linamarase under optimum conditions to hydrolyze its substrate linamarin after breaking the cell tissue
2. To cause the cassava cyanogenic glucosides to break down and thus detoxify the cassava pulp to a safe level of residual HCN in the gari
3. To develop a desirable sour taste through unconscious selection of fermenting microorganisms thence utilizing the free starches or the

Table 11 Cassava Plantation Cost in Texagri, Nigeria

Component	Cost per acre (U.S.$)
Labor	
Clearing/piling (heavily wooded land)	24
Clearing/piling (lightly wooded land)	11
Surveying and bunding	9
Land preparation: plough, harrow ridge	3
Planting	17
Cutting cassava	6
Cutting transport	1
Supplying	7
Fertilizing/application ×2	13
Application residual herbicide	2
Slashing (hand)	20
Knapsack spraying	3
Harvesting	44
Workshop labor	13
Contract labor charge, 15%	20
Inputs	
Herbicide knapsack application	16
Residual herbicide (tractor application)	35
Herbicide imperata control	100
Fertilizer/application ×2	36
Fuel	15
Spare parts and repairs	60
Fixed charges	20
Overhead	75
Rent	2
Total	415

Note: Only a portion of the plantation is treated in any one year.
Source: Ref. 36.

increasingly detoxified pulp by the loss of HCN and other gases as the liquor is being expelled

4. To reduce excessive free starch in the pulp that would otherwise cause stickiness in the texture of the gari when prepared into eba with boiling water

The following environmental conditions are most favorable. Freshly harvested cassava roots having a water content of about 65% and not beyond 2 days after uprooting are desirable. Depending on the cassava variety,

cassava between 9 and 12 months is recommended. Spoiled roots do not ferment optimally; overaged roots are woody, difficult to dewater at the end of fermentation, and have poor gari yields. A fast electric-type hammer mill is more efficient than manual grating in that it exposes a maximum surface area of the parenchyma and cell surfaces to linamarase activity, ensuring more effective fermentation. In addition, because cassava pulp ferments in its natural liquor, fermentation proceeds best at 35°C, particularly when the pulp is mixed intermittently (10). In the cold harmattan weather at about 25°C, fermentation is retarded. As we have seen earlier, continuous forced dewatering during fermentation of mash as in the rural method removes a greater proportion of the inhibitory effects of HCN than a silo or nondraining closed vat, thus enabling the rapid proliferation of aerobic lactic acid-forming bacteria, especially *Leuconostoc*, which produce the most desirable sour-tasting gari. This is notably so when the mash is fermenting in shallow surface containers, such as cloth bags. The higher fermentation efficiency was measured as higher levels of total acidity with the cloth bags within 2 days compared to pulp contained in deep, metal, nondraining vats (10,34). This would explain such predominant bacterial isolations as *Leuconostoc,* which Okafor (37,38) associated with the fermentation of forced, village-type, draining cassava pulp during a 6-day experiment.

Maduagwu and Oben (33) used the loss of nonglucosidic (free) cyanide and bound cyanide as a measure of the fermentation process by the use of a screw press to dewater fermenting cassava pulp. The pulp that was dewatered quickly within 24 h lost a higher proportion (98–99.7%) of its free cyanide than the mash draining slowly for 3 days, which had a range of loss between 92.2% and 98.8% hydrogen cyanide. The reverse was the case when the bound (glucosidic) hydrogen cyanide was considered. There, the quick screw-pressed pulp and the slow dewatered pulp lost 97.6% and 99.7% HCN, respectively. Because the breakdown of the bound cyanide is actually of more importance to gari consumers than the free cyanide, slow continuous dewatering of the mash for 2–3 days is recommended both in terms of food safety and for the development of flavor, eba-forming properties, and aroma profiles in well-fermented gari.

Of major consideration in optimum environmental conditions for gari production with regard to large factories is the safety of the workers. First, the factory must not be totally enclosed but should have three-quarters of the wall raised and the rest covered with strong wire netting. This will allow poisonous gases, HCN, and H_2 to escape to the environment, otherwise there will be danger of cyanide poisoning. The hydrogen cyanide content of bitter cassava root is displayed in Table 12. The whole root contains as much as 306 ppm HCN, the peeled root pulp has 184 ppm HCN, the peel has 660 ppm HCN, and the pressed out juice has 86 ppm HCN (20). The grating operation

Table 12 Hydrogen Cyanide Contents of Cassava During Gari Production

Material	HCN (ppm)
Whole cassava root	306
Peeled root (pulp)	184
Peeled root (grated)	104
Pulp mash pressed after fermentation	52
Pressed juice	86
Cassava peels	660
Gari	10
Total	908

Source: Ref. 20.

released about 104 ppm HCN and all these must be expelled quickly outside of the factory. In fact, the HCN contents of air around some key processing equipment have been measured (39) and given as follows: Around the peeling machine, it was 2 ppm HCN, around the grating machine, it was 10 ppm HCN, and none was detected around the fermentation tanks and granulator (Table 13). Ideally, exhaust fans should be installed in the factory to assist in the expulsion of the poisonous gases, Onyekwere and Akinrele (20) showed that 120 mg HCN/kg root was liberated during cassava peeling, 80 mgHCN/kg root liberated in the grating operation, 38 mg HCN/kg liberated in the fermentation, and 42 mg HCH/kg root liberated in toasting, with their respective minimum space requirements of $11m^3$, $7m^3$, $3.5m^3$, and $4m^3$, giving a total minimum volume factory requirement of 25.5 m^3 (Table 9).

It stands to reason that there is a minimum acceptable standard for a cassava factory handling as much as 25 tons or more of cassava roots. Cassava toxicity is a real possibility to operators.

We have earlier mentioned the importance of the fermenting vessel and noted that forced removal of the cassava liquor at the same time as

Table 13 Hydrogen Cyanide Concentrations around Various Pilot-Plant Machines during Gari Production

Process operation	HCN content of air (ppm)
Peeling	2
Grating machine	10
Fermentation tanks	0
Granulator	0

Source: Ref. 39.

fermentation of the mash progresses hastens not only removal of the inhibitory effects of HCN but also enables rapid invasion and proliferation of lactic acid bacteria that provide the desirable sour taste. Using a taste panel, the taste of gari in terms of its sourness and texture (or stickiness) were evaluated from cassava fermenting in nondraining vats and compared with the scores from cassava fermenting in traditional cloth bags for 7 days (10) (Figs. 26 and 27). Using a 5-point Hedonic scale where a 5.0 represented too much, 3.0 represented just enough (which is the desirable score point), and 1.0 denoted much less, gari made from traditional pressed cloth bag proved superior within 2 days in all respects to the nondraining deep vat. The latter vessel showed irregular scores throughout the experiment, even up to the termination of the fermentation. However, for a large factory, the use of a series of hydraulic presses as shown in Fig. 16 is recommended. Cassava fermenting in an inverted fiberglass silo (Fig. 13) is interesting because of the continuos loss of liquor from the fermenting pulp, through gravity. When the three fermenting vessels (i.e., traditional cloth sack, nondraining vat, and inverted draining silo) were compared using pH 4.2 $\pm$ 0.2 as standard for gari fermentation, the silo was the slowest. It took the silo content fermentation 4 days for the pH to fall to pH 4.00, whereas the cloth sack had fallen to pH 3.74 on the second day, followed by the nondraining modern vat, which also recorded a low pH of 3.92 on the second day (Table 14). In terms of total acidity, the traditional bag had recorded an impressive level of 4.6 mg NaOH/

Table 14 Changes in pH and Titratable Acidity in Cassava Pulp Fermenting in Traditional Cloth Bag, Modern Nondraining Vat, and Inverted Draining Silo Methods during Gari Production

Traditional pressed bag			Modern nondraining vat		Inverted draining silo	
Days	pH	Titratable acidity (mg NaOH/g)	pH	Titratable acidity (mg NaOH/g)	pH	Titratable acidity (mg NaOH/g)
0	5.83	1.4	5.83	1.4	5.80	—[a]
1	4.39	1.7	4.40	2.2	5.00	—
2	3.74	4.6	3.92	3.7	4.32	—
3	3.81	4.7	3.99	4.2	4.32	—
4	3.89	4.5	3.89	4.5	4.00	—
5	3.80	4.1	3.90	4.3	3.80	—
6	3.80	3.9	3.80	4.1	3.62	—
7	3.93	3.1	4.07	3.2	3.81	—

[a] Not conducted.
Source: Ref. 34.

g on the second day of fermentation compared with 3.7 mg NaOH/g for the nondraining vat. It was suggested (10) that the kind of fermenting vessel had an influence in the micro-organisms associated with cassava fermentation. Specifically, where Okafor (37,38) isolated *Leuconostoc* as the dominant fermenting organisms using shallow surfaces of the traditional cloth bag, showing that the fermentation was basically aerobic, early workers (19,40) used deep nondraining vats associated with anaerobic conditions. Therefore, the fermentation of cassava pulp in deep vats or with the pulp lying in its liquor which exudes from its cells after grating is essentially anaerobic at ambient temperature (29–32°C) with involvement of *Corynebacterium* and *Leuconostoc* bacteria. Fermentation is said to proceed best at 35°C, particularly if the pulp is mixed intermittently (41). As much air or oxygen as possible should be excluded in the fermenting mash because oxygen causes a bluish discoloration due to the oxidation of leucoanthocyanins (cyanidin and delphinidin), which are always present in fresh cassava root (19). Iron metal causes discoloration during fermentation of the pulp and the acids in the mash cause rapid rust formation (10). Therefore, the hydraulic presses must be thoroughly washed, including all other processing equipment that can tolerate washing. Plastic surfaces and stainless-steel equipment are therefore recommended, and fermented liquor should not be poured on concrete floors to avoid corrosion of the surfaces. The pH for correctly fermented cassava pulp has been reported to be about 4.2 ± 0.2 (19,21) and pH 4.0 and total acidity of 3.4 mg NaOH/g in 3 days (42) and reducing sugar of 2.8 mg/g in the same period (Table 15). However, some of these figures are only relative, because those who prefer nonsour, yellow colored, and bland gari will not use them. For this reason, the standards for gari elaborated in 1988 recognize both fermented sour and nonfermented gari. Therefore, only the highest level of acidity is mentioned (not more than 1% m/m as lactic acids) and no minimum value. Finally, we had discussed in Section XI and in the beginning of this section that freshly harvested cassava tubers are prime sources of gari

Table 15 Changes in pH, Titratable Acidity, and Reducing Sugar in Fermenting Cassava Mash for Gari Production

Time	pH	Titratable acidity (mg NaOH/g)	Reducing sugar (mg/g)
Fresh tuber	6.2	1.2	3.1
1 day	5.5	1.6	6.2
2 days	4.5	2.2	4.4
3 days	4.0	3.4	2.8

Source: Ref. 42.

processing and also noted the demonstration of sun-dried peeled cassava roots for gari production. It was mentioned that the presence of 10–15% freshly grated cassava root was used to introduce linamarase enzyme as a primer without which this technology would not be possible (25) (Fig. 17). This finding is yet to be utilized, but it is an avenue for making raw materials for gari production available almost all year round, because in the dry season, cassava is difficult to uproot by hand and so it is usually scarce and expensive, because the farms are not under irrigation.

XVI. MICROORGANISMS ESSENTIAL FOR FERMENTATION

The fermentable carbohydrates usable by micro-organisms for energy occur in cassava, particularly in the poisonous variety commonly used for gari production, such as the poisonous complex cyanogenic glucosides (linamarin and lotaustralin). The cassava plant has an endogenous enzyme (linamarase) which remains inactive until the cell wall with the parenchyma of the root is disintegrated (43) through the grating process, which is an important and distinguishing step in gari manufacture. From the grating of the root into a pulp, linamarase enzyme is released and immediately begins to hydrolyze its substrate linamarin (the poisonous glucoside) with the release of HCN and other gases. This process brings about gradual detoxification which is hastened by continuous loss of the cassava liquor through pressing with a hydraulic press, or the screw press, or a heavy object. The quicker the loss of the toxic liquor, the faster it is for the glucosides to be made nontoxic for the fermenting micro-organisms. In nondraining vats, where the liquor remains until the fermentation is terminated before dewatering, the fermentation is not aerobic like that with the cloth bag being pressed all of the time to dewater while fermenting.

The nearly detoxified glucosides of the pulp are invaded by micro-organisms for an energy source, especially *Leuconostoc*, which produce the sour flavor characteristic of the gari product. Okafor (37,38) elaborated the succession of the dominant organisms which he associated with the cassava acidification following the action of the endogenous enzyme linamarase (Table 16). The isolated bacteria were *Leuconostoc*, *Lactobacillus*, *Alcaligenes*, and *Corynebacterium* and the yeast was *Candida*. *Leuconostoc* predominated, appearing in increasing numbers up to the sixth day of the experiment. The yeasts were second in number and also appeared increasingly toward the end of the experiment. Alcaligenes was the third in importance, but was totally eliminated at the end of the second day. The author observed that *Corynebacterium*, which had been implicated by early workers as the major bacterium fermenting cassava, was isolated only once and in low

Table 16 Succession of the Major Micro-organisms Encountered in Cassava Pulp Fermenting for Gari Production

Days	*Leuconostoc*	*Alcaligenes*	Yeasts
0	7.2×10^6	2.4×10^5	1.2×10^5
1	4.0×10^8	4.8×10^5	2.0×10^5
2	6.4×10^8	5.4×10^5	3.5×10^5
3	9.2×10^8	0	4.8×10^5
4	1.4×10^9	0	4.6×10^5
5	1.2×10^9	0	5.2×10^5
6	3.6×10^9	0	5.6×10^5

Source: Ref. 38.

numbers. It is important to reiterate that the micro-organisms isolatable from the fermenting vessel could vary according to the fermenting vessel, (e.g., self-draining inverted silo, nondraining vat, and pressed cloth bag). The cloth bag has the widest shallow surface area among the three containers and aerobic micro-organisms will proliferate most readily in the cloth bag because it is being pressed continuously and losing its HCN content during the period of fermentation.

It is likely that the micro-organisms associated with cassava fermentation are actively releasing extracellular enzymes into the medium which enhance the fermentation process. For example, Okafor and Ejiofor (44) reported a highly active linamarase produced by *Leuconostoc mesenteroides* when linamarin was added to the growth medium.

Early workers (19,40,41,45) mentioned nothing of the enzyme breakdown of the poisonous cyanogenic glucoside (linamarin), but claimed a two-stage microbial fermentation of the cassava occurring through a combined action of two micro-organisms, one dove-tailing into the other. Specifically, they believed that a bacterium, *Corynebacterium manihot* at a neutral pH, first attacked the cassava starch, resulting in the production of organic acids and a fall of pH in the first 48 h. The acid medium caused a spotaneouns hydrolysis of the cyanogenic glucosides with the release of hydrocyanic acid.

In the second stage of the twin microbial activity, the early workers claimed that from organic acids, which included lactic acid, acetic acid, and others, *Geotrichum candidum*, a fungus, produced aldehydes and esters. The activity of *G. candidum* is still valid. For instance, Banjo (7), working with the fungus spores to enrich cassava pulp for gari by the use of solid-state fermentation, observed that a larger inoculum size produced a higher titratable acidity value. This agreed with early workers (19,45) and with Amund and Ogunsina (46) that *G. candidum* brings about further acidification of the

fermenting pulp. It is the organic acids, especially lactic acid (47) and lactic acid with pyrazines (48), that give gari its peculiar desirable sour flavor, whereas aldehyes and esters from *G. candidum* activity produce its characteristic aroma (10). The said aroma is also peculiar and pleasant but absent in poorly fermented, nonsour gari. It seems to be most noticeable in gari from deep, nondraining vats and 5–6-d Ijebu-type very sour gari, but is hardly noticeable in yellow gari containing palm oil with little fermentation.

In summary, the grating of cassava, which is an important step in gari production, breaks up the cassava parenchyma, thus releasing an endogenous enzyme linamarase which then attacks its substrate linamarin. Linamarin is the cyanogenic glucoside in cassava, which must be hydrolyzed by fermentation to toxify the root to a tolerable level of hydrogen cyanide content (20–30 ppm). The substrate–enzyme activity releases HCN and other gases and permits fermenting micro-organisms to colonize free starches released by the initial grating and linamarase activities. For surface fermentation in cloth bag aerobes, particularly *Leuconostoc* and yeast, quickly multiple within a few days, and with respect of deep tanks and silo, the process is nearly anaerobic. *Lactobacillus plantarum* and *Candida* yeast are the major fermenters, utilizing released starches for energy source and converting fermentable sugars into mainly lactic acid to give acceptable flavor to the gari product. Meanwhile, the fungus *G. candidum* under low pH condition (pH 4.0–4.2) of the pulp also colonizes the mash and brings about the aroma profile of the gari, usually very prominent in 5–6-d fermented gari and Ijebu-type very sour gari.

XVII. STORAGE OF GARI

Cottage-processed gari is the major source of gari being transported to urban places and is usually bulked into 80–100-1b Hessian and jute bags although purchased in small quantities from various local markets on market days or along selling points on major motor roads. Because gari is very hygroscopic, it easily absorbs moisture from the air if not properly stored in sacks, which should keep out adventitious micro-organisms and moisture. Ideally, gari in bags should be laid on wooden pallets such that at most five to six bags of 100-1b weight will be on one stack, with several rows well separated in a medium-sized warehouse, ventilated with windows covered by wire gauze to keep out rodents and weevils.

Igbeka (49) studied the efficiency of gari storage in two kinds of bag (Hessian and jute) traditionally used by middlemen to purchase and store gari in urban places for sale. Using a mathematical model, by the finite-difference method, he predicted moisture at the peripheral wall (Table 17) and the center of the gari in the two types of bag (Table 18). Results from the model showed

Table 17 Predicted Moisture Contents on the Walls of Two Gari Storage Bags under Varying Environments

Environment		Moisture content at the wall (% w.b.)	
Temperature (°C)	Relative humidity (%)	Jute bag	Hessian bag
30	60	12.8	11.8
	75	13.6	12.3
35	60	13.8	12.4
	75	15.4	13.2

Source: Ref. 49.

that gari under storage in the Hessian bag maintained lower moisture content than that in the jute bag, whether at the peripheral wall or at the center, under environmental conditions of 30–35°C and relative humidity between 60% and 75%. He used moisture of up to 12.7% as the "still safe" level for gari storage on the basis that 12.7% moisture content was the equilibrium moisture content under the environmental condition of 27°C and 70% relative humidity used earlier by Adeniji (31). When he compared the moisture of gari at the peripheral walls, he predicted that gari stored in the jute bag having a range of 12.8–15.4% moisture on the wall would spoil before the 180-day period of the experiment, whereas gari in the Hessian bag being at the 11.8–13.2% moisture level would be quite storable beyond that time. With respect to gari at the center of the two bags, the moisture in the jute sack was

Table 18 Predicted Moisture Contents at the Centers of Two Gari Storage Bags under Varying Environments

Environment		Moisture content at the center (% w.b.)	
Temperature (°C)	Relative humidity (%)	Jute bag	Hessian bag
30	60	10.5	10.15
	75	10.7	10.3
35	60	10.8	10.38
	75	11.2	10.52

Source: Ref. 49.

only 10.5–11.2% and that in the Hessian bag was even much less (i.e., 10.15–10.52%). The author therefore concluded that gari at the centers of the jute bag and Hessian bag could not be susceptible to fungal spoilage because they were far below the "safe level" of moisture. However, at the surface, the jute bag was very susceptible and inferior in its protection to the Hessian bag. This was because the jute bag at the peripheral wall showed moisture above the "safe level" of 12.7%, and fungal growth could spoil the gari within the period of study. The Hessian bag is better aerated than the jute bag.

Current industrial practice is to store gari in polypropylene sacks used for wheat flour. Because the ambient temperature lies between 29°C and 32°C most of the year, with relative humidity often above 70%, market gari can rarely be stored beyond 12 weeks without the problem of tainting (31). On the contrary, because industrial gari has a maximum water content of 9%, it can be stored up to a year. Naturally, gari which cannot be kept even for a month is of low quality. Ukpabi and Ndimele (50) evaluated the quality of gari in parts of eastern Nigeria where sour gari is not processed or consumed and noted that the moisture content ranged between 10.25% and 17.35% and this was related to aggregate size. For example, they found that where up to 50% of the gari weight had aggregate size up to or more than 1 mm in diameter, with the moisture level from 16% and above, the gari stored very poorly and also showed a decreased swelling index. The swelling index in gari is a quality measure that demonstrates that well-roasted and dried gari granules will swell up to three times the original volume when soaked in water at ambient temperature (29–32°C).

XVIII. MICROORGANISMS THAT CAUSE SPOILAGE

Rapid deterioration occurs in poorly stored cassava root and literature supporting this is abundant. To date, postharvest deterioration of cassava root is still a problem and the use of freshly harvested root is the best method so far.

The spoilage of fresh cassava roots involves two stages. First is a physiological enzymatic primary deterioration which is initially the cause of loss and is first manifested as fine, blue-black or brownish discoloration, usually more intense near the periphery of root. No single micro-organism can be isolated from the advancing margins of discoloration (10,27,51). A secondary discoloration occurs only after the commencement of the primary deterioration and involves microbial rotting by fungi and bacteria (27). Booth (51) isolated various species of *Pythium*, *Mucor*, *Rhizopus*, *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Glomerella*, *Gloesporium*, *Rhizoctonia*,

Bacillus, *Xanthomonas*, *Erwinia*, *Agrobacterium*, and many saprophytic bacteria from the surfaces of cassava roots but consistently failed to isolate any specific micro-organism from the advancing margins of the roots.

The spoilage of market gari was fully investigated and it was discovered that no market gari was free from molds (31). Eleven fungi were associated with market gari and stored gari according to Adeniji (31), and aspergilli, penicillia, and *Rhizopus nigericans* were said to be mostly responsible for the taste tainting of gari (Table 19). As many as 8.5×10^3 colonies/g gari of *Penicillium variabile*, 7.5×10^2 colonies/g of *Rhizopus* sp., and 8.1×10^2 colonies/g of *Aspergillus niger*; *A. tamarii*, and other aspergilli were in sizable levels of isolations using various growth media. For isolates of these micro-organisms when reinoculated into freshly roasted gari having a water content of 15%, the fusaria among the isolates had no effect (Table 20), whereas the other fungi grew and caused deterioration of the gari within 3–5 days. It was concluded that gari stored at low moisture (about 10–12%) will maintain good taste for up to 12 weeks and that gari meant for long-term storage should have a moisture content in equilibrium with a relative humidity of less than 65%. However, well-fermented gari, having a high moisture (14% or more), taints less rapidly than poorly fermented (i.e., 1-day type) with a similar water content, especially in a well-aerated store.

Invasion of stored gari by species of *Aspergillus* is a real danger to date. For instance, gari enriched with soybean was reported to be very susceptible

Table 19 Fungi Isolated from Nigerian Market Gari

	Fungus colonies/g gari $\times 10^2$		
Fungi	CDA[a]	MEA[a]	NU[a]
Penicillium variabile	85.0	6.5	4.1
A. niger	7.5	7.4	6.6
A. candidus	7.2	8.1	4.1
A. tamarii	1.4	1.3	1.0
A. versicolor	3.7	3.5	2.5
A. flavus	1.2	1.1	1.0
Fusarium solani	1.1	1.0	0.1
Fusarium sp.	1.0	0.5	0.1
Curvalaria sp.	0.5	0.3	0.1
Cladosporium sp.	0.01	0.1	0.01

[a] Agar media: czapecks dox agar (CDA); malt extract agar (MEA); nutrient agar (NU).

Source: Ref. 31.

Table 20 Effect of Fungi Inoculated on Moistened Oven-Fresh Gari

Fungi	No. of days to deterioration
Rhizopus sp	2
A. niger	3
A. tamarii	3
A. vesicolor	3
A. flavus	3
Cladosporium sp.	3
A. candidus	3
Curvularia sp.	5
Penicilium variabile	5
Fusarium solani	No effect
Fusarium sp.	No effect

Source: Ref. 31.

to aflatoxin by species of *Aspergillus* (52). Abba Kereem et al. (53) took 64 samples of cassava flour from different parts of Nigeria and 13 of them harbored *A. flavus*, which produced aflatoxin in yeast extract broth medium containing 20% sucrose. Ten of the aflatoxigenic strains produced aflatoxin B_1, and B_2 only. The amount of aflatoxin was relatively high and ranged from 3.0 to 14.2 μg/mL (B_1), 0.9 to 11.9 μg/mL (B_2), 0–12.5 μg/mL (G_1), and 0–9.2 μg/mL (G_2).

Contamination of gari by bacteria is also a threat to health. Cassava flour, gari, maize, yam, sorghum, and millet flours are commonly used in the preparation of staple foods in Africa. Traditionally, these dry produce are marketed in open basins in open-market environments and are variously contaminated, Umoh et al. (54) and Yesuf et al. (55) found that all traditional flours used as staples, including cassava flour and gari, were highly contaminated with *Bacillus cereus* and *B. mycoides*. Out of 300 samples taken, 295 (98.3%) were contaminated. Based on specific tests, 33 were toxigenic, 39 were nontoxigenic, and 113 other strains not tested for toxigenicity were subjected to antimicrobial sensitivity tests. A low percentage of the toxigenic and other strains were resistant to erythromycin, chloramphenicol, and streptomicin. Over 80% of the isolates tested were resistant to ampicillin, penicillin, cloxacillin, and co-trimoxazole. Only 2.0% of the flour samples tested were without detectable levels of *B. cereus* and 80–90% of the *B. cereus* strains were resistant to the common drugs of choice in the treatment of infections in Nigeria. The public health implications of *B. cereus* in gari and other flours must be recognized.

XIX. CHEMICAL/BIOCHEMICAL CHANGES IN SUBSTRATE

The major interest in cassava, the sole substrate for gari, is its content of cyanide and the appropriate processing method to make it safe as a food product. The cyanide can be considered to be of two types:

1. Bound cyanide (linamarin and lotaustralin), which occurs as complex cyanogenic glucosides.
2. free cyanide (nonglucosidic). Linamarin is a substrate for an endogenous enzyme, linamarase, which also occurs protected in the cassava plant. When linamarin comes into contact with the enzyme on tissue damage, such as peeling and grating, an appropriate cyanohydrin is produced which dissociates into HCN. The free HCN may be in equilibrium with cyanohydrins of other aldehydes and ketones present.

An enzymatic assay method was used to detect cyanide in cassava and its products (43,56) and the linamarase has been purified.

Although Collard and Levi (40) reported that a lowered pH was responsible for linamarin breakdown, Wood (24) found that the linamarin he had isolated from cassava was quite stable at very low pH, even at 100°C. Therefore, the effect of acidity was to enhance the action of linamarase, which has an optimal pH of 5.5 (57). It is the liberation of free cyanide that causes cassava toxicity in man by inhalation, especially during large-scale cassava processing (20,32). Activities of linamarase enzyme and its substrate linamarin have been thoroughly studied (24,43,44,56–58).

The bound cyanide is probably the major risk in the finished gari because the free cyanide is easily lost in the process of dehydration, boiling, or soaking in water. The processing method is therefore the determinant factor. For example, Pieris and Jansz (59) found that quick drying of cassava flour at a relatively high temperature prevents further enzyme reactions that could have broken up the bound cyanide. Thus, following moisturizing of the cassava flour, the enzyme system becomes reactivated. Unfortunately, they noted, that such flour was generally used for cooking purposes. They found this dangerous because of the possibility of the release of HCN in the stomach when consumed, leading to poisoning and nervous breakdown. For these reasons, grating, fermenting, and drying processes must be included in cassava for gari manufacture so as to reduce the poisonous cassava roots from about 350 mg HCN/kg in some varieties into a safer gari product having 20–30 ppm cyanide (10).

The water content of fresh peeled cassava root is about 71.5% and the carbohydrate content is 26.8%, whereas the protein content is 0.74% (Table 21). Village market gari has a water content of about 14–16%, whereas modern factory-processed gari has a water content of about 9%. Its protein

Table 21 Composition of Fresh Cassava Root (*Manihot esculenta* Crantz)

Constituent	Fraction of unpeeled root (% w.b.)	Fraction of peeled root (% w.b.)
Water	68.06	71.50
Carbohydrate (nitrogen-free extract)	29.06	26.82
Crude fiber	0.99	0.12
Crude protein (nitrogen × 6.25)	0.87	0.74
Fat (ether extract)	0.17	0.13
Ash	0.85	0.69

Source: Ref. 39

content is about 1.1–1.5% and its high fiber content of 1.4% makes it filling. The starches are hydrolyzed to glucose and other simple sugars while various carboxylic acids are formed during fermentation, which change its original bland taste to slightly preferred sour taste.

The composition of dry cassava is presented in Table 22 and the most noticeable change from wet cassava root is in the massive reduction of water content from 71.50% in the fresh peeled root to 12.6% in the dry tapioca root.

Table 22 Composition of Dried Cassava (Tapioca) (*Manihot esculenta* Crantz)

Dry cassava (tapioca)	Content in 100 g
Water	12.6 g
Food energy	352 cal
Protein	0.6 g
Fat	0.2 g
Carbohydrate (total)	86.4 g
Fiber	0.1 g
Ash	0.2 g
Calcium	10.0 mg
Phosphorus	18.0 g
Iron	0.4 mg
Sodium	3.0 mg
Potassium	18.0 mg
Thiamine	0
Vitamin A	0
Riboflavin	0
Niacin	0
Vitamin C	0

Source: Ref. 9.

The excessive bulk in cassava due to high water content is the major reason for its short storage properties. It is high in food energy (352 cal/100g), quite high in carbohydrate content, and still low in protein content (0.6%). Apart from the minerals calcium, phosphorus, iron, sodium, and potassium present in the tapioca, thiamin, vitamin A, riboflavin, niacin, and vitamin C are all absent.

We have already discussed the effect of the processing method of gari, the dominant fermenting micro-organisms, the taste, and final moisture content. Onyekwere et al. (34) monitored the free-cyanide and the total-cyanide contents in gari produced by the traditional pressed bag method and compared the trend against the result obtained from gari produced using the modern nondraining vat, in an 8-day experiment. To some of the samples, on the second day and on the fifth day, red palm oil was added to produce yellow gari, which is the choice in eastern Nigeria and parts of Sierra Leone and Bamenda (Cameroun). It was claimed (60) that yellow gari containing palm oil has practically no cyanide. We used Cooke's enzymatic method to test for cyanide in yellow and white gari processed from our pilot plant but failed to corroborate the results of Olarewaju and Boszormenyi (60). In fact, there was no reduction in HCN content of cassava mash samples fried with or without palm oil (Table 23). The important trend noted was that up to the second day of fermentation, the total cyanide in gari by the traditional bag method was

Table 23 Cyanide Contents of Gari Processed by the Traditional Pressed Cloth Bag Method and Modern Nondraining Vat Method, With or Without Palm Oil

Modern vat (mg HCN/kg gari)[a]				Traditional bag (mg HCN/kg gari)[a]		
Fermentation (days)	Sample no.	Free CN^-	Total CN^-	Sample no.	Free CN^-	Total CN^-
1	1	0.14	0.14	2	0.13	0.13
2	3 (oil)	0.17	0.17	4 (oil)	0.13	0.13
	5	0.19	0.19	6	0.13	0.13
3	7	0.14	0.14	8	0.17	0.17
4	9	0.17	0.17	10	0.19	0.19
5	11 (oil)	0.13	0.13	12 (oil)	0.19	0.19
	13	0.15	0.15	14	0.20	0.10
6	15	0.19	0.19	16	0.17	0.17
7	17	0.17	0.17	18	0.15	0.15
8	19	0.19	0.19	20	0.20	0.20

[a] Analysis courtesy of Chemistry Dept., University of Ibadan, Nigeria.
Note: Free cyanide is nonglucosidic cyanide. (Oil) = gari samples fried with palm oil.
Source: Ref. 34.

decidedly low (0.13 mg HCN/kg gari) and much lower than that gari processed from the nondraining vat, whose total HCN level was between 0.14 and 0.19 mg HCN/kg gari. However, the gari from the traditional bag method was not that low from the third day, recording a range of 17–20 ppm and similar to the gari from the nondraining vat. Generally, both methods of production were satisfactory, because the gari had cyanide that was within the maximum level of 20 ppm specified by the Standards Organization of Nigeria (26). Additionally, analysis of gari produced by the village method with FIIRO gari showed that the major difference was the lower water content of mechanized gari, which was 8.2% versus that of 14.4% in village gari (Table 24). With respect to HCN content, although mechanized gari had a very low content of 10 ppm HCN, village gari was within the permitted level of 20 ppm (26). With respect to the ability to swell in water at ambient temperature, village gari was superior, with a score of 320% as compared with 300% for mechanized gari and only 100% for fufu. Generally, well-dried gari swells up to nearly three times its original size, but cassava fufu does not display this ability, although its HCN content of 16 ppm appears satisfactory, as noted in Table 24. Generally, the low protein content of gari and its low mineral level is a nutritional concern, and enrichment to upgrade it nutritionally is of interest to all.

Table 24 Analysis of Traditionally Produced Gari and Mechanized Product Compared with Cassava Fufu

Constituent	Village gari	FIIRO gari	FIIRO cassava fufu
Carbohydrate content (by difference) (%)	81.8	87.0	73.0
Water content (%)	14.4	8.2	8.6
Oil content (%)	0.12	0.1	0.44
Crude protein (%) ($N \times 6.25$)	0.9	1.5	1.26
Crude fiber (%)	1.4	2.3	1.6
Ash (%)	1.4	0.9	0.15
Calcium (mg/100 g)	17.7	45.6	—[a]
Iron (mg/100 g)	2.0	2.2	0.0015
Phosphorus (mg/100 g)	57.2	56.9	0.02
HCN content (ppm)	19.0	10.0	16.0
Swelling (% vol)	320.0	300.0	100.0

[a] Not done.

Source: For village gari and FIIRO gari, Ref. 39; for FIIRO cassava fufu, Ref. 10.

Ofuya and Fiito (61) deviced a rapid method for determining the quality of gari based on the iodine reduction test. Ahonkai et al. (47) identified principally acetic, lactic, propionic, succinic, pyruvic, *i*-butyric and *n*-butyric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acids in gari and linked them with flavor compounds and linked ethyl methyl ketone with gari aroma. Similarly, Dougan et al. (48) found lactic acid and pyrazines to be the major flavoring constituents. Banjo (7) identified the organic acids in soybean-fortified gari meal (cassava : soybean ratio 70 : 30) with their corresponding response factor. The predominant organic acid was lactic acid, followed by succinic acid and then palmitic, stearic, citric, oxalic, malic, and fumaric acids. The major aroma compounds are aldehydes and esters and are related to the activity of *G. candidum* (19,40), which is prominent in well-fermented mash in deep vessels.

Generally, properly gelatinized gari is expected to swell in water at ambient temperature to three times its previous volume (10) and this test differentiates it from cassava flour, or fufu. Gari is also relatively denser than gari flour when equal volumes are compared. The pH of fresh cassava tuber is about 6.2 and falls to about pH 4.0 in 3 days (42). Initial titratable acidity is 1.2 mg NaOH/g mash, and after fermentation for 3 days, it rises to 3.4 mg NaOH/g (Table 15). Reducing sugars also rise to a peak on the first day of fermentation, doubling that of the raw fresh tuber, and thereafter it falls to a little less than that in the fresh tuber on the third day (42). Fermenting cassava pulp increases in volume by about 15% in 2 days but falls to 5.6% by the fifth day (62). Therefore, allowance for increase in volume should be made when designing a cassava-fermenting silo that does not drain its liquor. There is no change in the oil content of cassava root and white gari, except where palm oil has been added to produce the yellow gari.

Incidentally, yellow-colored gari is predominant in eastern parts of Nigeria and parts of Sierra Leone. Generally, it is more enticing and more expensive than white gari in Lagos. Ironically, it is the white gari that has gone through better fermentation with a better sour taste and longer shelf life because of its lower content of free starches that is of a superior quality, with a texture free from stickiness in boiling water for eba.

The problem of HCN concentration in cassava processed products had been fully highlighted by Mahungu et al. (2), and reductions in concentrations differed according to the product, varying from 69.85% to 100% reduction. They found that boiling peeled cassava roots failed to remove the poisonous factor and that pounding of the boiled root, as in Ghanaian fufu, decreased the concentration by only 10%. In spite of the extensive destruction of parenchyma cells resulting in increased contact between linamarase and the cyanogenic glucoside, fermentation for gari production was not as efficient as soaking in water. For example HCN was reduced at least 10-fold in other

African cassava products such as chickwange, ntuka, Zairois fufu, and Nigerian fufu whose fermentation took place under submerged soaking in water (Tables 1 and 3). Indeed, soaking of peeled tubers even for 1 day in fufu (excluding Ghanaian fufu) resulted in cyanide reduction from 15.89 mg HCN/100 g to 3.33 mg HCN/100 g. The effect of steaming or pounding was negligible because the final product still had as much as 0.72 mg HCN/100 g. The authors also found that for freshly fried gari contained a mean concentration of 2.66 mg HCN/100 g, storage for 4 months reduced the concentration to 0.29 mg HCN/100 g, and up to 2 years storage, HCN was no longer detectable. In practice, who stores gari that long? In summary, the use of sweet, low-cyanide-containing cassava is the best option.

XX. NUTRITIVE VALUE OF GARI

The protein content of gari in generally very low (1.1–1.5%), and in comparison to parboiled rice and soy flour, gari is far inferior to both (Table 25). Gari contributes mainly the carbohydrates of the meal for bulk and calories, but is also deficient in vitamins and minerals. It is therefore usually taken with a vegetable stew (often called soup) which provides the fat, protein, vitamins, and minerals, but this depends on the amount of money available for its preparation. (Table 8). Furthermore, the amino acid profile of gari is inferior to that of soy flour and below the FAO reference (Table 26).

Table 25 Nutritive Value of Gari per 100 g Commercial Productions Compared with that of Soy Flour and Parboiled Rice

Constituent	Gari	Soy flour	Parboiled rice
Water (g)	8.5	3.5	12.0
Protein (g)	1.3	42.0	7.5
Fat (g)	0.1	22.0	1.5
Carbohydrate (g)	87.0	29.5	77.0
Calcium (mg)	45.6	197.0	10.0
Phosphorus (mg)	56.9	552.0	100.0
Iron (mg)	2.2	12.0	1.0
Vitamin A (IU)	—	140.0	—
Thiamine (mg)	0.12	0.77	0.20
Riboflavin (mg)	0.04	0.28	0.07
Niacin (mg)	1.0	2.0	2.0
Calories	338.0	357.0	352.0

Source: Abridged from Ref. 4.

Table 26 Amino Acid Composition in g/g Total Nitrogen in Edible Portions of Gari Compared with That of Soy Flour

Amino acid	Gari	Soy flour	FAO ref.
Tryptophen	0.081	0.086	0.087
Threonine	0.172	0.242	0.175
Isoleucine	0.174	0.336	0.264
Leucine	0.257	0.482	0.300
Lysine	0.258	0.395	0.264
Methionine	0.039	0.084	0.138
Cystine	0.069	0.111	0.125
Phenylalanine	0.117	0.309	0.175
Tyrosine	0.117	0.199	0.175
Valine	0.190	0.328	0.264
Arginine	0.623	0.452	—
Histidine	0.096	0.149	—
Alanine	0.316	0.251	—

Source: Abridged from Ref. 4.

Fermentation of cassava meal does not improve the amino acid levels (Table 27), but its main advantage is in the reduction of the cyanogenic glucoside, with no significant improvement in its nutritional value.

Cassava has a high water content, up to 65% of unpeeled roots, and processing into gari reduces bulk and improves storability, handling, and flavor acceptability. The HCN content is reduced from over 350 mg HCN/kg root to less than 30 ppm. No one knows the exact safe level of HCN in gari, although the standard for gari in Nigeria is given as 20 ppm maximum (26).

Early efforts to enrich gari with vegetable sources to make it a more complete food product having a protein content of about 8% had one problem or another. Akinrele (4) used soy, sesame, and peanut flours, but none was found acceptable, as they altered the texture and reduced the sour flavor of gari. The lack of proper technology to incorporate the other flours into gari made the additives float and separate out when boiling water was added to prepare eba. Commercial peanut grits having a granulation similar to gari, when incorporated at a maximum level of 10%, raised the percent protein of gari to 7.3 (63). The gari texture, however, was adversely affected and the normal sour taste decreased by 8%, whereas in soy-enriched gari, the major organic acid was identified (7) as lactic acid, which had a response factor (R_f) of 11.87, followed by succinic acid, having an R_f of 2.14 (Table 28).

Fungal cells as single-cell protein have been repeatedly used to enrich cassava, but the increase was not above 4–5% protein. Strasser (64) incorporated *G. candidum* on a pilot-plant scale by culturing the fungus on cassava

Table 27 Amino Acid Composition of Raw and Fermented Cassava Meal

	Raw Cassava Meal		Fermented cassava meal	
Amino acid	mg/g of nitrogen	mg/100 g of food	mg/g of nitrogen	mg/100 g of food
Isoleucine	175	46	141	22
Leucine	247	64	231	37
Lysine	259	67	162	26
Methionine	83	22	77	12
Cystine	90	23	56	9
Phenylalanine	156	41	163	26
Tyrosine	100	26	106	17
Threonine	165	43	150	24
Tryptophan	72	19	46	7
Valine	209	54	168	27
Arginine	683	178	210	34
Histidine	129	34	72	12
Alanine	235	61	332	53
Aspartic acid	406	106	331	54
Glutamic acid	1009	262	693	111
Glycine	160	42	167	27
Proline	172	45	135	22
Serine	204	53	157	25

Source: Ref. 9.

Table 28 Organic Acids Identified in Proteinized Cassava Containing Soybean (Cassava : Soybean 70 : 30) with Their Corresponding Response Factor

Organic acid	Response factor
Lactic acid	11.87
Succinic acid	2.14
Palmitic acid	1.197
Stearic acid	0.086
Citric acid	0.372
Oxalic acid	1.101
Malic acid	1.199
Fumaric acid	0.084

Source: Ref. 7.

starch liquor waste and raised the protein of gari from 1.2% to 4–5%. Trevelyn (65) used *Rhizopus oryzae* to ferment cassava flour, to which he had added nonprotein nitrogenous substances and achieved a protein level of 4.3%. Banjo (7) confirmed the results of the two previous workers by using the solid-state fermentation technique with *G. candidum* and *Candida tropicalis* and achieved a protein content from 4.5% to 5.8% (Table 29).

Perhaps the most acceptable enriched gari to date is with soybean, but the method of incorporation is critical and decisive. Banjo (7) used a cassava mash to soybean ratio of 70 : 30 incorporated by the following three methods:

1. Wet mix method. The cassava mash and cooked soybean pulp were fermented together for 4 days, dewatered, and roasted.
2. Dry mix method. Dried gari and dried soybean flour were blended together.
3. Soak–mix method. Wet cassava mash was fermented for 96 h and then blended with cooked soybean paste dewatered overnight and roasted to gari (Fig. 28).

Table 29 Nutrient Composition of Fungal Enriched Gari Produced from Fermenting Cassava Mash Inoculated Separately with *G. candidum* and *C. tropicalis* Fungal Spores as Fungal-Enriched Gari

Parameter determined per 100 g sample	fermenting cassava mash					
	G. candidum		*C. tropicalis*		Control	
	50 kg	100 kg	50 kg	100 kg	50 kg	100 kg
Protein (g)	5.3a	4.1a	5.5b	5.1b	1.3c	1.25c
Fat (g)	0.75a	0.69a	0.85b	0.82b	0.6c	0.49c
Ash (g)	1.28a	1.25a	1.32b	1.30b	1.22c	1.20c
Carbohydrate (g)	96.67a	93.98a	92.35b	92.77b	96.6c	96.80c
Calcium (mg)	4.5a	4.2a	4.5b	4.4b	4.0c	3.9c
Phosphorus (mg)	16.0a	15.9a	17.5b	17.2b	11.5c	10.9c
Iron (mg)	2.8a	2.5a	3.1b	3.0b	4.4c	4.1c
Sodium (mg)	6.1a	6.0a	7.0b	6.5b	4.6c	4.5c
Vitamin A (mg)	0	0	0	0	0	0
Thiamine (mg)	18.8a	18.4a	16b	15.1b	17.6c	17.5c
Riboflavin (mg)	10.2a	9.5a	8.6b	8.3b	10.7c	11.0c
Vitamin C (mg)	25.0a	22.0a	49.0b	45.0b	17.0c	15.0c

Note: Any two values not followed by the same letter were significantly different ($p < 0.05$ as determined by Duncan's multiple range test).
Source: Ref. 7.

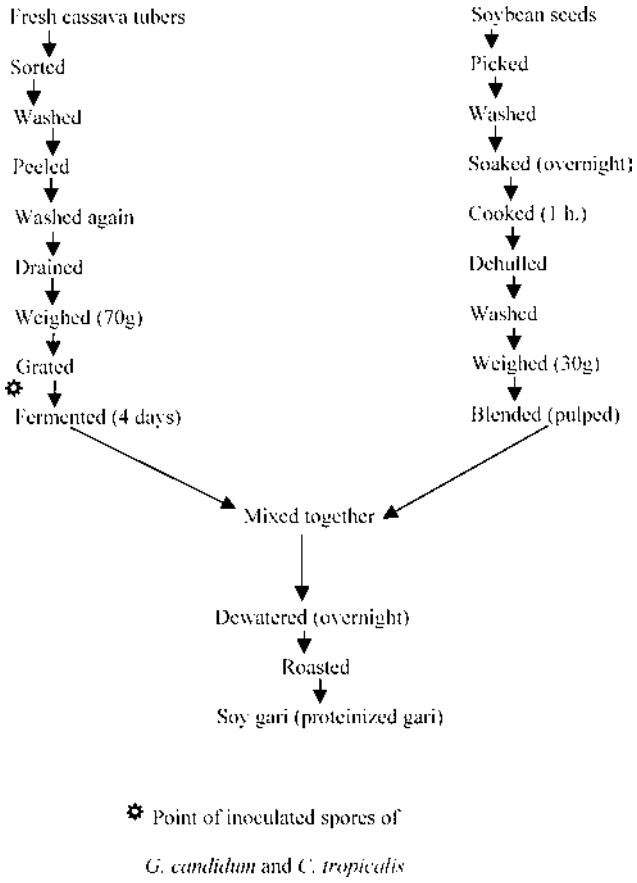

Figure 28 Flowsheet: production of soy-enriched gari (soy gari) using cassava mash to soybean ratio of 70:30, by the soak–mix method. (From Ref. 7.)

A taste panel evaluation of the three enriched gari samples showed that the method of choice was the soak–mix (Table 30). Organoleptically and chemically, soy gari prepared by the soak–mix method was acceptable on all points—in color, flavor, taste, swelling index, pH—comparable to the control gari sample. Once the soybean is properly processed, there should be no fear of toxicity in soy gari, unlike gari containing fungi, in which the fungal nucleic acid content was a concern to consumers (10). At the cassava : soybean blend of 70 : 30, soy gari protein was raised to between 7.9% and 8.4%, fat was 2.6%, and ash was 2.6% (7).

Table 30 Organoleptic Properties, Swelling Index and pH of Soy Gari from Three Production Methods Compared with Control Sample

Soy gari production (ratio 70 : 30 cassava : soybean blend)	Color	Flavor aroma	Taste in dry form	Taste in water at 28°C	Swelling index at 28°C	pH
Wet mix	Light brown	Beany	Sour with beany flavor	Sour with beany flavor	2.3 ± 0.03	4.5 ± 0.03
Dry mix	Brown	Strong beany	Slightly sour with beany flavor	Sour with beany flavor	2.5 ± 0.16	4.8 ± 0.05
Soak mix	Cream	Acidic	Sour	Sour	2.8 ± 0.01	4.2 ± 0.04
Standard (control gari)	Cream	Acidic	Sour	Sour	3.0 ± 0.27	4.2 ± 0.01

Note: Method of choice was to soak the mix.
Source: Ref. 7.

Following the seeding of *G. candidum* and *C. tropicalis* spores into the soybean-proteinised cassava mash, the overall protein content was further increased to between 10.15% and 10.40%, the fat content was 2.74%, and the ash content was 2.70% (Table 31). The point of seeding the spores was also critical and this was into the grated cassava before fermenting for 4 days (see Fig. 28).

The vitamins and minerals were also enhanced in soya-fortified gari. The total acids, fat, ash, calcium, phosphorus, iron, potassium, sodium, and free amino acids increased, whereas the pH, carbohydrates, and HCN decreased during fermentation. Lactic, succinic, palmitic, stearic, citric, oxalic, malic, and fumaric acids were identified. Vitamin A was absent in all samples of cassava mash as well as in cassava–soybean mash.

Thiamine (vitanin B_1) increased in *G. candidum* proteinized mash but decreased in *C. tropicalis*-proteinized mash. Riboflavin (vitamin B_2) decreased in both fungal samples. Ascorbic acid (vitamin C) increased in *C. tropicalis*-fortified mash but decreased with *G. candidum* (Table 31). The protein efficiency ratio (PER) of the three experimental diets was determined by feeding albino rats, all of which gained weight in each of the diets at the end of the study (7). For the high requirement of thiamine in gari, the study suggested that *G. candidum* should be used to fortify cassava mash, whereas soybean was the better option for producing gari with a high riboflavin content (66). For several years in the past, fears existed that cassava-based foods might have some chronic toxic effects in man and animals (67,68) and

Table 31 Nutritive Composition of Soy-Enriched Gari (Cassava Pulp to Soybean Ratio 70 : 30) Seeded Separately with *G. candidum* and *C. tropicalis* Spores for Fungal Soy Gari Production

Parameter determined per 100 g sample	Proteinised cassava-soybean mash					
	G. candidum		*C. tropicalis*		Control	
	50 kg	100 kg	50 kg	100 kg	50 kg	100 kg
Protein (g)	10.4a	10.15a	12.1b	11.8b	8.4c	7.9c
Fat (g)	2.75a	2.73a	2.85b	2.80b	2.6c	2.55c
Ash (g)	2.70a	2.65a	2.80b	2.77b	2.6c	2.56c
Carbohydrate (g)	84.15a	84.50a	82.25b	82.64b	86.5c	86.75c
Calcium (mg)	8.3a	8.0a	8.7a	8.5a	7.6a	7.3a
Phosphorus (mg)	16.2a	30.8a	32.5b	32.2b	251.2c	24.8c
Iron (mg)	3.8a	3.6a	4.4b	4.1b	2.8a	2.4a
Vitamin A (mg)	0	0	0	0	0	0
Thiamine (mg)	20.9a	20.2a	17.2b	16.7b	18.4c	18.0c
Riboflavin (mg)	15.2a	14.5a	14.0b	13.8b	17.8c	17.2c
Vitamin C (mg)	43.0a	40.0a	61.0b	59.0b	32.0c	27.0c

Note: Any two values not followed by the same letter were significantly different ($p < 0.05$, as determined by Duncan's multiple range test).
Source: Ref. 7.

those most exposed are communities that consume short-time or unfermented gari, especially if they utilise the bitter poisonous cassava variety. This is, of course, very dangerous. Circumstantial evidence had suggested that the residual cyanide content of gari was directly responsible for an ataxic neuropathy in Nigeria and indirectly for goiter (69). It is likely that increased intake of nitrogen in the diet, especially from animal protein and soybean, might remove the possibility of adverse effects of processed cassava foods. Indeed, the nutritive value of a cassava diet fed to animals was reportedly improved by supplementation with methionine (70). In summary, results of protein-enrichment experiments reported by Banjo (7) with soybean flour alone, with or without *G. candidum* or *C. tropicalis,* are as follows:

1. Plain gari alone: 1–1.3% protein content
2. Plain gari + *G. candidum*: 4.3% protein content
3. Plain gari + *C. tropicalis*: 5.5% protein content
4. Plain gari + soybean (70 : 30 ratio) (soy gari): 8.4% protein content
5. Soy gari + *G. candidum*: 10.4% protein content
6. Soy gari + *C. tropicalis*: 12.1% protein content

XXI. FORECAST

With the exception of a few African countries like Zimbabwe and Ghana, which consume mainly maize, cassava products, such as chickwange, ntuka, attieke, gari, fufu, oyoko, konkonde, and plakali form important food diets of the Africans, especially those along the western coast. Gari and fufu from cassava are major staples in southern Nigeria. Because the cassava plant grows even in poor soils where other crops will fail, it is apparent that Nigerians will continue to cultivate it in small family holdings, without

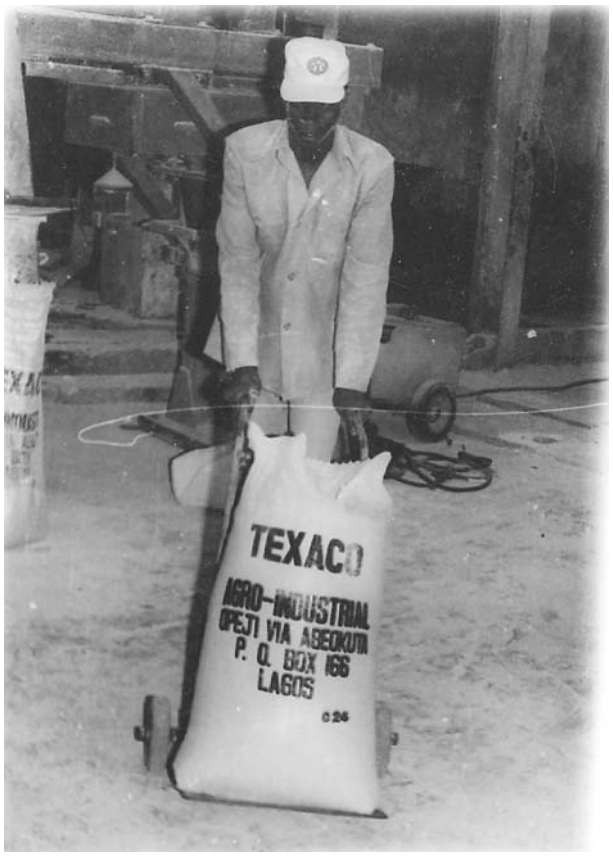

Figure 29 Bagged gari in 50-kg weights. (Courtesy of the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.)

irrigation, and the cottage-scale production will always far exceed the large, commercial producers (Fig. 29) because of the former's lower market prices. Because of research by the International Institute of Tropical Agriculture (IITA) based in Ibadan, Nigeria has wiped out the fear of cassava blight disease and, in addition, has selected early-maturing, higher-yielding, lower-cyanide varieties: it is up to the people to change over from their traditional cassava to the IITA varieties. The appropriate government agencies ought to promote this changeover by proper information and extension services to cassava-farming communities. In view of the apparent popularity of gari, especially among Nigerians raised on it, including those residing in Europe, it is certain that gari consumption will increase. It is no longer low-income food and almost everybody in southern Nigeria consumes it, almost on a daily basis, but the major constraint is the availability of the gari stew, which is costly to prepare. Gari is fattening, but so is rice, and compared with yam, plantain, potatoes, and cocoyam, gari is most available and its price is competitive in feeding an ever-increasing population. Incidentally, cassava-growing countries should use what they have to produce what they want. There is now available a booklet titled "New Food Products from Cassava" by Onabolu et al. (71) the International Institute of Tropical Agriculture. It contains recipes using cassava flour to extend the use of cassava just as corn-producing countries have various products from corn. Recipes from the booklet include cassava bread, cassava sausage rolls, cassava cakes, cassava cookies, and others.

XXII. CONTRIBUTION TO NEW INTERNATIONAL INDUSTRIES

Poultry feedstuff was prepared by substituting the maize component with cassava flour and enriching it with soybean cake in the pilot plant (72). Extensive tests showed that the poultry feedstuff was comparable to commercial poultry feed. The only problem might be the availability of the cassava flour in regular quantities year round. Breads and cakes are being produced from cassava flour and are now on sale. For years, FIIRO's all-cassava cake has been appreciated and this could be promoted by industrialists.

Cassava fermentation could contribute extensively to the development of other important industries. Rajagopal (73) reported developing an acceptable lager beer using a species of Nigerian cassava, and fungal proteins was obtained from cassava starch liquor during gari processing (64). *Leuconostoc* and, to a lesser extent, yeasts are the predominant micro-organisms in cassava fermentation. Various yeasts are used extensively in the fermentaiton industry for the production of industrial ethyl alcohol and, to a very small extent, glycerol, for the manufacture of malt beverages (brewing), wines and distilled

spirits, and for the production of miscellaneous products, such as leavening agents and vitamin concentrates. Depending on the actual yeasts that could be isolated further from cassava fermentation, special products could be made through culturing or biotechnology. It is also possible that *Leuconostoc* would be made amenable to genetic engineering to obtain a micro-organism with peculiar abilities to form valuable commercial products. The Brazilian breakthrough in producing ethy alcohol and using it in replacing a portion of gasoline in motor cars is well known.

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10
Industrialization of Mexican Pulque

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I. DESCRIPTION OF PULQUE

Pulque (1) is a milky, slightly foamy, acidic, somewhat viscous, alcoholic beverage made by fermenting (not distilling) the fresh or regular juice (aguamiel) of certain types of agave (Fig. 1) mainly grown in Central Mexico–Mexico, Hidalgo, and Tlaxcala states: agave *atrovirens* (*A. salmiana* or Pulque agave), agave *Americana* (century plant or Maguey cenizo), agave *mapisaga* (manso o Maguey mexicano) and agave *marmorata* (2).

For most of us, it is natural to think that the most popular Mexican drink is tequila. However, this high-alcohol-content drink, nowadays widely appreciated in international markets, has a relatively recent history. It was during colonization that Spaniards began to make it as a distilled spirit from a fermented juice of agave *tequilana weber*, another agave (maguey) that grows mainly in Tequila, a region within Jalisco, a southwestern Mexican state.

† Deceased.

Figure 1 Pulque agave plants.

Much before colonization, another drink derived from agave was already consumed in Mesoamerica: pulque, the tequila ancestor. Known as *octli* in prehispanic cultures, pulque had a mystical place in society, religion, culture, and nutrition in ancient Mesoamerican civilizations. For centuries, the mildly alcoholic, sour drink as beer has been regarded as healthy when consumed in moderation and possessed of medicinal qualities. Lindner (3,4) for example reported the therapeutic use of fresh and concentrated juice of agave in cases of renal and metabolic diseases.

The official Mexican norm [NMX-V-022 by Secofi (5)] regulates two kinds of nonadulterated juice (Table 1): a fresh juice (type I from a 30–60-day collection period) and a regular juice (type II after more than a 60-day period). The regular aguamiel's refractive index is at least 27 at 20°C with a density (Beaumé degree) higher than 4.5. Other important quality attributes are total solids higher than 7 g/100 mL, total reducing sugars (as glucose) higher than 6 g/100 mL, direct reducing sugars higher than 3 g/100 mL, gums (as glucose) higher than 200 mg/100 mL, proteins higher than 100 mg/100 mL, ash higher than 180 mg/100 mL, pH higher than 4.5, and total acidity (as lactic acid) lower than 4 mg/100 mL.

By official definition [NMX-V-037 by Secofi (1)], there are two specifications for pulque: a seed or premium quality (type I) and a commercial or handled in bulk quality (type II). Commercial pulque's refractometer degree is at least 25 at 20°C and its refractive index is between 1.3365 and 1.3380. Additional requirements include the following: pH from 3.5 to 4.0; alcohol content from 4.0% to 6.0% (v/v); total acidity (as lactic) from 400 to 700 mg/100 mL, total reducing sugars (as glucose) from 200 to 500 mg/100 mL.

Table 1 Mexican Agave Juice and Pulque Official Specifications

Average determination (g/100 mL)	Agave juice-hydromel (a)		Pulque-handled in bulk (b)	
	Fresh (Type I)	Regular (Type II)	Seed (Type I)	Commercial (Type II)
Refractive degree (immersion) at 20°C	—	—	32	25
Refractive index (Abbe) at 20°C	6.0	4.5	1.34	1.338
pH	7.1	4.5	4.0	3.8
Total acidity (as lactic acid)	0.095	0.04	0.58	0.55
Total reducing sugars (as glucose)	10.0	6.0	0.45	0.35
Direct reducing sugars (as glucose)	2.5	3.0	—	—
Gums (as glucose)	4.0	0.2	—	—
Total solids	15.0	7.0	2.2(c)	1.7(c)
Total protein	0.45	0.10	0.44	0.40
Ash	0.37	0.18	—	—
Ethanol	—	—	7.5	5.0

Source: (a) NMX-V-022 (Ref. 5); (b) NMX-V-037 (Ref. 1); (c) Tlachique is equivalent to a regular agave juice (type II) (Ref. 6).

Furthermore, other characteristics not regulated by the Mexican norms are the following: density (20°C) from 0.9960 to 1.000; total protein content ($N \times 6.25$) from 300 to 500 mg/100 mL, total solids from 1.7 to 2.2 g/100 mL (6); ash from 200 to 500 mg/100 mL; total acetic acid from 70 to 100 mg/100 mL; esters (as ethylacetate) from 11 to 22 mg/100 mL; aldehydes (as acetaldehyde) up to 2.5 mg/100 mL; higher alcohols (fusel oils) from 10 to about 50 mg/100 mL (7).

II. PRODUCTION AND CONSUMPTION

Mexico has an intermediate culture for alcohol consumption (i.e., modern elements mixed with ancestral customs): (a) drinking is a highly valued part of social life in a wet culture and (b) consumption typically is considered an aberrant behavior in a dry culture.

Compared with other countries (United States: 27 g/day; Chile: 44 g/day), Mexico has a low per capita consumption of alcoholic beverages in

people older than age 15 [i.e., 64 million (8)], consuming approximately 15 g (105 kcal) of alcohol per day. Indeed, a high proportion of the population in Mexico does not drink alcohol. For example, 47% of the population, from 18 to 65 years of age living in urban Mexico, abstained from alcohol consumption as reported by a national study conducted in 1988. According to the last 1998 addiction study, 79% of surveyed young men (ages 18–29 years) were presently drinkers, whereas among young women, the proportion was 54% (9). Only a small proportion of the Mexican population was responsible for most of the alcohol consumption: 75% of the available alcohol was consumed by only 25% of the drinkers (10). Yet, in contemporary Mexican society, including the modern urban (i.e., Mexico City) and rural areas, alcohol consumption is a facet of all aspects of social and family life. Pulque consumption is evident in some regions at all stages of the life cycle, from birth and baptism to wedding and funerals. Saints' days, community celebrations, and other special occasions are marked by drinking (10). In the next paragraphs, we briefly describe the geography of pulque production and consumption in Mexico.

Although the different varieties of pulque agave grow in a vast region of Mexican semiarid and arid areas, it is in the central region of Mexico where pulque is mainly produced and consumed.

Mexican agave plantations with 33,000 ha and 36 million plants have apparently remained constant in the last 30 years. According to the latest 1991 agricultural census (Table 2), there was a higher percentage of producing hectares in the northern states of Nuevo Leon, Hidalgo, and San Luis Potosi (74% of the total) than in the southern central states of Mexico, Guerrero and Puebla. However, the increased reported production for northern states included agaves destined to cattle and goat feed applications (11). It is in the central states of Hidalgo, Queretaro, Mexico, Puebla, and Tlaxcala where a higher percentage of developing plants (63% of the total) was observed.

Despite the constant number of agave plantations throughout the years, the annual percapita consumption of pulque has significantly decreased since the 1940s both at the national level and in Mexico City (Table 3). During the time of the Mexican Revolution (1910–1920), pulque was still being produced and distributed massively by several companies (2). However, slowly and progressively, the Haciendas Pulqueras (large-sized farms with many producing tinacales) were abandoned as beer began to be commercialized in Mexico in 1928. Still in 1972, 415,000 L of pulque were produced daily in the state of Hidalgo. However, in 1998, only 112,000 L/day were distributed in Mexico City, where pulque may be adulterated.

The total pulque production has decreased at least 60% in the last 20 years, and today, it represents 200×10^6 L with an estimated total value of more than US$10 million (> $0.5 peso: US$ > 0.05/bulk liter). In 1980, pulque production was 500×10^6 L [US$18 million; $0.035/bulk liter (15)].

Table 2 Mexican Agave Plantations and Pulque Production Trends (1940–2000)

	Year						
	1940	1950	1960	1970	1980	1990	2000
Agave plantations[a,b,c]							
Hectares (thousands)	31.8	47.6	54.8	33.6	—	33.0	—
Plants (millions)	10.2	30.0	27.6	35.8	—	—	—
Percentage under production	4.0	4.6	13.0	8.2	—	55.0	—
Pulque production[a,b,c] (million liters)							
Total	400	403	851	670	500	330	200
Taxed	—	180	200	200	170	112	8
Bulk price (US$/L)	0.020	0.025	—	—	0.035	—	0.050
Pulque factories[a,b,c]							
Traditional/tinacal (<700 L/day)	1390	1370	1250	1000	280	41	66
Modern/industrial (>700 L/day)	300	330	—	—	—	9	14
Canned-brands[a,d]	Miel-mex Crespomel	Xochitl Jicara	—	—	Magueyin	—	Nectar del Razo

Source: [a]Pulque factories paid a 30–35% production tax/SHCP (Ref. 6); [b]INEGI-SPP/1960–1980 (Ref. 12); [c]Census/1990–1998 (Ref. 13); [d]Nectar del Razo® brand (Ref. 14).

Also in 1980, the national beer consumption (25 L/year or 70 mL/day) was already significantly higher than pulque intake (6 L or 17 mL/day) (12). For pulque, this figure has evolved even more unfavorably since then.

Overall, Mexicans currently prefer beer and distilled alcoholic beverages (such as tequila). In fact, beer accounted for 89% of all alcoholic beverages consumed in Mexico (i.e., population of 100 million). Pulque and 96% proof alcohol are the next favorite beverages in rural Mexico, whereas distilled beverages and wine are more popular in urban Mexico (~60 million population). In the northern states, the beer preference (i.e., about 85%) is significantly higher than pulque preference (5%) (12). The trend will not change in the near future due to the Westernization of Mexican youths lifestyle. Hence, pulque will not become the type of beverage commonly used by the young population in Mexico. A preference study with urban college students aged between 17 and 25 years showed that 83% of the drinkers consumed coolers (86% preference), distilled beverages (like tequila, rum, and mezcal; 85%), beer (83%), and pulque (31%).

Table 3 Pulque Consumption Patterns in Mexico and Mexico City (1860–2000)

	Year						
	1860	1940	1950	1970	1980	1990	2000
Mexico[a,b,c,d]							
Consumption (million liters)	—	400	403	670	500	330	200
Population[a,e] (million)	—	22	28	50	80	90	100
Annual per capita consumption							
L/person/year	—	18.2	14.4	13.4	6.3	3.7	2.0
mL/person/day	—	50	40	37	17	10	6
Mexico City[a]							
Consumption (million liters)	39	154	191	60	30	—	23
Population[a,e] (million)	0.225	1.5	3.3	6.0	8.6	—	16.0
Annual per capita consumption							
L/person/year	173	103	58	10.0	3.5	—	1.4
mL/person/day	475	280	160	27	10	—	4

Source: [a]Ref. 6; [b]Ref. 15; [c]INEGI-SPP/1960-1980 (Ref. 12); [d]Census/1990–1998 (Ref. 13). [e]Census/1990–2000 (Ref. 8).

Pulque has practically lost its popularity compared to beer and other alcoholic drinks in Mexico. Taxed-pulque production and pulque factories (Table 2) have also been drastically reduced (about 95% and 75%, respectively), whereas the industrial beer factories have gained a marketshare for taxed-beer valued at more than US$2600 million (13). The relatively low taxed-pulque production is somewhat misleading, because it does not include the large quantity of illegal (tax evaded) and adulterated pulque (i.e., 30–70%) usually sold in urban areas (6). Loyola also estimated a 35–40% raw material cost and a 30–35% tax for the total puque production cost. Currently, the raw material represents about 50–60% of the total pulque value before tax (13). This unfavorable economical context (particularly for the small-sized pulque industry) instigates evasion and adulteration.

Who is consuming pulque then? Pulque is an important (sometimes crucial without a potable water supply) component of the diet of some communities in the central area of Mexico. For example, pulque consumption is common among pregnant women in Solis Valley, a highland region of the Estado de Mexico (a central state in Mexico). Most women there (73%) consume pulque during pregnancy and postpartum: 27% consume more than 400 mL/day and 6% were heavy drinkers (about 1400 mL/day or 63 g alcohol/day). In low to modest quantities (less than 400 mL/day; Table 6), pulque intake may have beneficial effect due to its vitamins and bioavailable iron content (16).

In some rural communities, heavy drinking patterns have been linked to pulque. A high cirrhosis mortality rate (27–32 deaths/100,000) was reported in 1988 in Mexico City and the surrounding states (Hidalgo, Tlaxcala, Puebla, and Mexico); on the contrary, the northern states have shown a considerably lower rate (7–11 deaths). The amount of pulque intakes (central Mexico: 60 mL/person/day and north Mexico: 2 mL/person/day in 1980) as well as environmental variables (rural versus urban areas) could explain this difference. The infectious hepatitis incidence (i.e., unsafe water supply), ethnic background (i.e., Náhuatl and Otomi), and nutritional deficiencies were also studied as possible risk factors (12).

In addition, there are different ways to prepare and drink pulque such as (a) mixing it with red pepper and corn leaf and sold as *charagua* in Tlaxcala, (b) mixing it with pepper, epazote, salt, and garlic, called *chiocle* in Guerrero, and (c) mixing it with *Opuntia* fruit juice in San Luis Potosi or mixing tropical/citric fruit juices as *pulque curado*.

In the arid regions of Northeast (Coahuila) or the central/south highlands (Tlaxcala and Michoacan) of Mexico, pulque and fiesta bread sometimes contain settlings from pulque fermentation, which gives the bread a distinct flavor and aroma.

A regional Mexican pulque bread (sourdough type) is locally produced in Saltillo, Coahuila. The La Mena brand (Pan de Pulque: Reg. S.S.A. No. 7609-A) has been made traditionally by Carlos Mena's family and includes the following traditional ingredients: wheat flour, pulque, vegetable oil, cane sugar (sucrose), and egg (see Fig. 2). A polyethylene-packaged bread can last about 2 weeks at room temperature without changing its textural, organoleptic, and shelf-life properties. Pulque bread can contain alcohol and lactic acid produced by pulque inocula, which can inhibit undesirable bacteria. This sourdoughlike fermentation process effected a superior quality and extended

Figure 2 Traditional bread in a Mexican market.

the shelf life of the resulting baked good without using commercial antibacterial and antifungal additives. The La Reina brand (Pan de Pulque dulce: Reg. S.S.A. No.102680-A) is also made with wheat flour, sugar (8%), egg, pulque (7%), milk, vegetable shortening, yeast, salt, and cinnamon.

A recent study by Oda and Tonomura (17) showed that *Zymomonas mobilis* subsp. *mobilis* (mutant strain from palm wine) had the highest leavening ability in wheat doughs containing either glucose or sucrose (i.e., 5%). When compared with baked goods made with compressed yeast, those made with the strain had similar specific volumes and proofing times, but they had a slight acidic flavor.

Pulque manufacturing still remains a predominantly traditional, self-consumption, barter, and small-scale industry which has not been able to massively reach urban markets in Mexico (other than Mexico City) or international markets. It is estimated that only 15–20% of the pulque factories can produce more than 700 taxed-liters/day, which are sold mainly to urban consumers. Three patented and canned pulque brands such as Miel- Mex/ Crespomel (MX patent No. 34441 in 1933), (MX patent No. 45035 in 1946) and Jicara (MX patent No. 56023 in 1954) are no longer produced industrially in central Mexico and Mexico City (16). However, Nectar del Razo® is industrially exported to the United States as a canned, natural pulque, or with added fruits, with a selling price of ~US$2.9 per liter or US$1.0 per 12 fluid ounce pulque can (14). Ironically, the market that can potentially save this ancient tradition of Mexico is outside of Mexico.

III. HISTORY OF FERMENTATION: EARLIEST REFERENCES

In Mesoamerica, people have eaten agaves for at least 9000 years (11). The Peabody Foundation archaeological expeditions to Mexico during the 1950s and early 1960s included agave as one of the principal items in the Mesoamerican diet from 7000 BC to AD 1500. From about 5000 BC, agave was found throughout the time scale in 25%–60% of mummified human feces. Since then, agave has been cultivated for use by humans for food, clothing, and herbal medicine (Fig. 3). The name maguey was brought by the Spanish and maguey remains in use today as the name for agave ("metl" by aztec).

Mesoamerican civilizations called pulque "octli". The term "pulque" was probably mistakenly derived by the Spanish from the aztec "octli poliuqui," which meant spoiled pulque.

In ancient times, pulque had a religious significance as an offering to the gods, particularly Mayahuel (Fig. 4), the aztec goddess of pulque. She is depicted naked, holding up a bowl of pulque and seated on a throne of a tortoise and snake. Night was her sacred time and she carried a cord that she

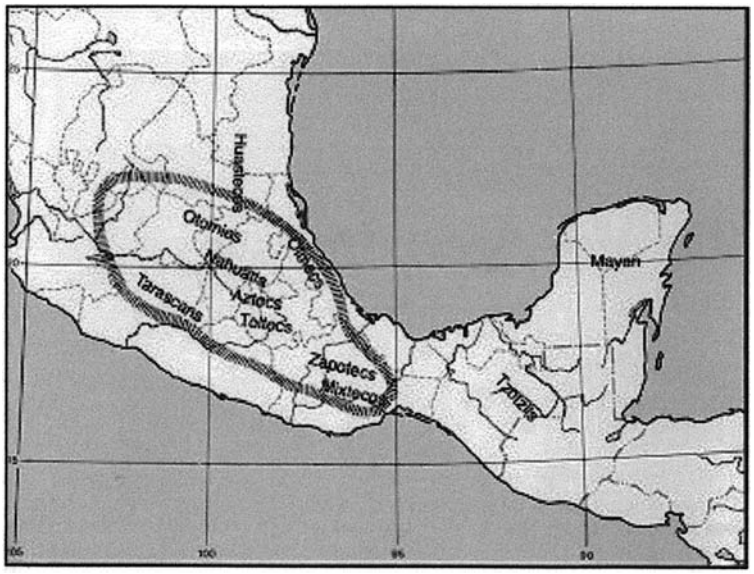

Figure 3 Region of intense agave culture with some of the principal mesoamerican cultures; olmecs were extinct before the Spaniards arrived. (From Ref. 11.)

used to aid women in childbirth. She is the goddess who discovered and introduced the gods to pulque.

Here, two versions about the origin of pulque are described. According to Indian history, during the reign of Tecpancaltzin (AD 990–1042), a Toltec noble named Papantzin discovered how to extract aguamiel from the maguey plant. In an attempt to interest the king in his daughter, Princess Xochitl, she

Figure 4 Mayahuel, the pulque goddess.

was sent to the king with an offering of the wondrous aguamiel (honey water). The king must have liked both aguamiel and Xochitl because they later had a son named Meconetzin (maguey boy). After the Spanish conquest, Fray Bernardino de Sahagun, a Christian priest gathered a group of native elders, his purpose being to create a written history of pre-Hispanic times. He named them "The Informants" and charged them to set down in writing all they could remember about life during those times. One of their essays was entitled "The Invention of Pulque." Although their description of the process was similar to that used today, it was dressed up and deified—the story going something like this. A woman named Mayahuel learned the art of scooping out the heart of the maguey and collecting its juice, but it was Pantecatl who made and discovered fermentation of the juice. Whenever an abundance of pulque was prepared on the hill of Pozonalteptl (the Hill of Foam), a call was sent out to all of the lords, high chiefs, and elders, summoning them to that place. There they paid homage to the gods and each drank four pots of pulque; however, the Chief Cuextecatl drank five and became very drunk, removing his clothes and acting in a shameful manner. Because of his shame, he was expelled and led his people off to a place near the sea which is now called Pantla. There they took the name of their chief and were called Cuextecas (today Huastecos), but the people followed in the footsteps of their chief and never abandoned their drunken ways and continued to run around naked until the Christians came.

Upon the collapse of the Aztec empire, pulque lost its preeminence for religious rituals, but it remained as a popular food beverage. During the colonial period (from 1521 to 1821), pulque was a popular drink in Mexico. Many Haciendas Pulqueras became famous during that period, being an important source of wealth or taxes for Spaniards in Nueva España (New Spain). Mostly used by the indigenous population to survive and forget their daily slavery and struggle for survival, pulque went from a religious elixir to a symbol of an oppressed culture.

IV. OUTLINE OF ESSENTIAL STEPS IN FERMENTATION

Pulque elaboration starts with cultivation of agave *pulquero* (pulque agave), where a plant can take of 8–10 years to achieve maturity for aguamiel production. An almost ritual process is performed to extract and collect aguamiel from agave (Sec. V). After such, aguamiel is transported daily to the fermentation vats (tinacales). Figure 5 presents a flow diagram of the traditional "fed-batch" process to ferment aguamiel (fresh and regular type) into pulque (seed and commercial or bulk type). The traditional method uses pulque samples from previous batches or fed-batches as natural inocula. Periods of maturation and "bouquet" development may follow before trans-

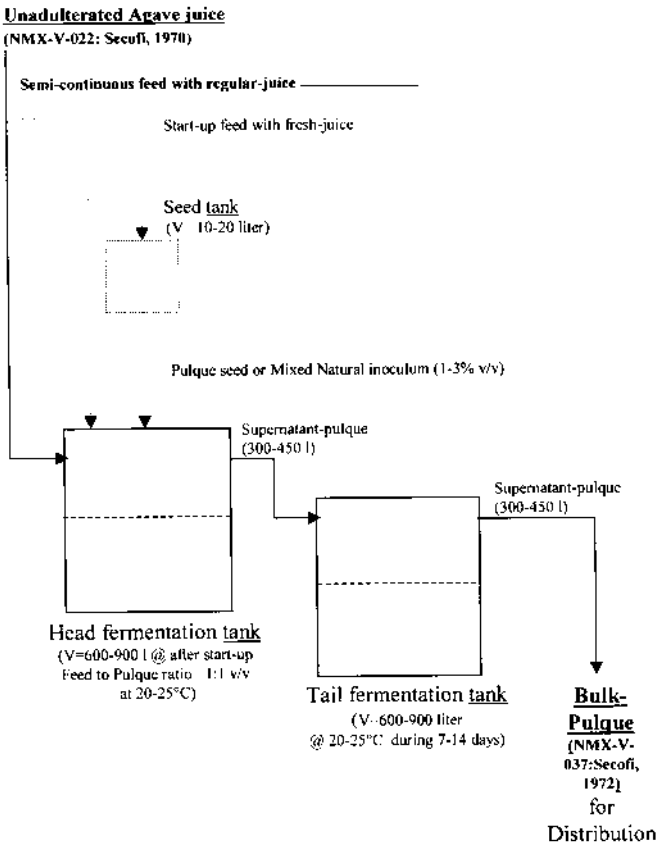

Figure 5 Flow diagram for traditional pulque fermentation. (Adapted from Ref. 6.)

portation to "pulquerias" (or pulque shops). Pasteurization of the bottled product is required for product stability preservation in the most recent and export version of the Nectar del Razo process.

V. INDIGENOUS FERMENTATION OF PULQUE USING RAW MATERIALS IN ANCIENT TIMES AND TODAY

A good pulque originates with a good agave plant. The substrate for pulque is agave juice (5) and it is extracted from the heart of mature plants (Fig. 1).

The pulque agave plant is itself a total icon from Central Mexico: agave *atrovirens* (*A. salmiana* or pulque agave), agave *Americana* (century

plant or Maguey cenizo), agave *mapisaga* (manso o Maguey mexicano), and agave *marmorata*. Agave is now mainly cultivated in 10 states nationwide with 5 central states having more propagating plants: Hidalgo, Querétaro, Mexico, Puebla, and Tlaxcala (6,13). Agave has historically provided drink, food, clothing and medicine to Central Mexico's communities.

Agaves are succulent plants or rosettes, often clumping, occasionally on short trunks (12 ft in diameter and 6 ft tall). The plants thrive best in the cold, dry climates of the central highlands north and west of Mexico City. They tolerate poor soil and drought and require excellent drainage. They have a sharp spine at the end of their leaves and each flower stalk blooms after 8–10 years. The blooming rosette uses its energy and stored sugar to produce a giant towering bloom (18–20 ft) and seeds (pups). Around the base of the adult plant, small pups grow, and when they are 1.5 ft high, they are detached and placed in a nursery to grow (i.e., for agave propagation or development). After 3 years, they are transplanted to the fields with a population density from 300 to 1000 plants per hectare (12). After 8–10 years, the plant reaches maturity and the top of the floral stem primordium (Figure 1: right photo) is cut with a special knife in an operation called "castration" of the floral bud. The fibrous pulp is removed, which leaves a deep depression (cajete) into which the aguamiel (agave sap) accumulates, similar to the sap of the rubber or maple tree which seeps into a collecting cup. An average plant yields about 2–3 L/day, and because of the presence of naturally occurring microoraganisms in the juice, aguamiel must be collected frequently (twice a day) to avoid a natural fermentation starting in the field. Collection is done by oral suction with an elongated bottle gourd (or squash) plant called an acocote (Fig. 6, left). A fresh agave juice (type I) drains out after a 30–60-d collection period, whereas regular juice (Type II) emerges after a 60-d period (5). After 4 months, the producing plant will have yielded around 230 L of sap, for pulque or syrup; without its nutrients, it finally dies.

After collection, aguamiel is transported in 50-L barrels to the tinacales (or places where pulque is produced). In order to start the traditional fermentation (Fig. 5), a 10–20-liter batch of fresh agave juice [type I (5)] is placed inside a closed seed tank (Fig. 6, right) for a first natural start-up fermentation. After a few days at ambient temperature (20–25°C), the microbial inoculum (Xinaxtli *or* xastle) not only stops growing but also forms a thick and floating layer which indicates that the pulque seed is ready for starting a second fermentation. Another batch of fresh juice (600–900 L) is then mixed with the natural pulque seed [~1–3.0% (v/v)] in the first tank (head fermenter). By keeping a uniform carbon dioxide production in this fermenter, the juice is gently agitated until the limiting sugar content controls microbial growth and flocculating biomass cells are finally allowed to settle.

Afterward, the first tank volume is kept constant by removing a 300–450-L batch of the supernatant pulque which is placed into a second tank (tail

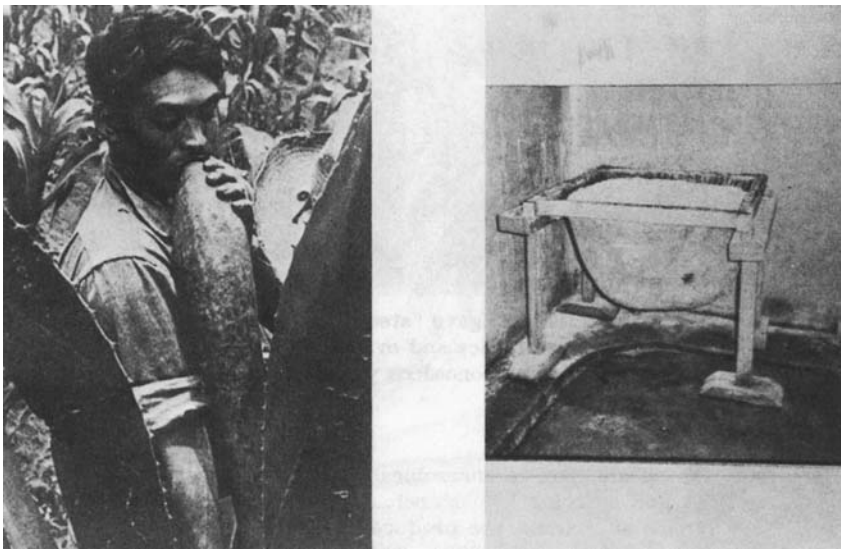

Figure 6 Acocote–agave juice collector and tinacal fermentation tank.

fermenter), leaving the same volume as inocula [~50% (v/v)] to ferment a batch of regular agave juice [type II; (5)]. This fed-batch process is sequentially repeated in order to keep the second tank with the same total volume of 600–900 L by withdrawing a batch of supernatant pulque (300–450 L) from the upstream tank. This process refers to an inoculated fermentor which has a semicontinuous feed, without pulque removal to the downstream fermentor except at the end of the desired processing time. After 7–14 days fermentation, a 300–450-L batch of pulque is finally produced from the second fermenter, comprising 50% of its total volume that is replaced with another batch of pulque from the upstream tank. A higher or lower pulque batch might be removed depending on the prevailing winter or summer temperatures, once the pulque master or "tlaquichero" can evaluate by taste that the final point (Table 1) of the fermentation has been reached.

These fed-batch tanks provide a rapid and low-cost technique for settling of microbial cells in both fermenters, so that when the pulque is withdrawn semicontinuously, the pulque biomass is retained for further sap fermentation. Another advantage may lie in the fact that semisterile conditions are readily kept inside the tanks without need for seed recycling. However, they must be cleaned after excessive biofilm growth inside the tanks, and the inoculum is sold for food or feed.

Pulque is finally barreled and distributed commercially in 250-L wooden or fiberglass containers to the pulquerias (urban pulque pubs). The shelf

life of pulque is usually 2 days after fermentation before becoming too sour to drink. There are street vendors who sell two types of bulk pulque (1): type II, commercial or tail pulque (also called tlachique) which is young and sweet, and type I, seed or head pulque which is old, strong, and sometimes sour. Because neither the optimum amount of seasonal juice (i.e., fresh/regular) nor the desirable time interval (i.e., inocula/temperature) between additions is maintained uniformly, the bulk pulque quality varies. Therefore, traditional pulque producers frequently resort to adulterations of the juice or the final product. It is estimated that at least 30% of the commercial bulk pulque or regular agave juice is adulterated with added sugar cane, alcohol, water, and plant gum or starch (6).

VI. MODERNIZATION OF PULQUE PROCESSING METHODS

In 1963, el Patronato Nacional del Maguey, a Mexican organization founded with the main purpose of fully and efficiently exploiting the potential of the agave plant in Hidalgo, Tlaxcala, and Mexico state (three central states in Mexico) sponsored a study to further explore an idea conceived by Sanchez-Marroquin and his research group. Sanchez-Marroquin and co-workers suggested using a combination of pure cultures (*Saccharomyces cerevisiae, Leuconostoc mesenteroides, Zymomonas mobilis,* and homofermentative and heterofermentative *Lactobacillus spp.*) to inoculate aguamiel in an attempt to better control its fermentation. As a result of this initiative, several articles on the microbiology of pulque were published from 1966 to 1977. One of these articles by Sanchez-Marroquin et al. (7) described the first pilot-plant scale implementation [1500-L/day capacity, in (15)] of pasteurized aguamiel fermentation to produce pulque using culture starters.

A schematic representation of such a process is presented in Fig. 7. In this process, the aguamiel is first passed through a heat exchanger in order to pasteurize it. Its sugar concentration and pH are adjusted to 8°Brix and 5, respectively. The propagation of the pure cultures to be used as inoculum is done by culturing at incremental volume stages in agitated tanks of 5, 10, and 20 L. For the propagation of *S. cerevisiae*, aerobic conditions are used. Sánchez-Marroquin examined experimental variations on the fermentation protocol in order to determine the best set of conditions to be used in order to achieve a robust and consistent industrial implementation. Strategies in which all starter pure cultures were added simultaneously at the beginning of the fermentation stage were compared versus sequential additions. A better control of the fermentation was achieved when a sequential addition of starters was implemented. Under this last protocol version, an agitated and aerated tank already charged with heat-treated aguamiel is inoculated

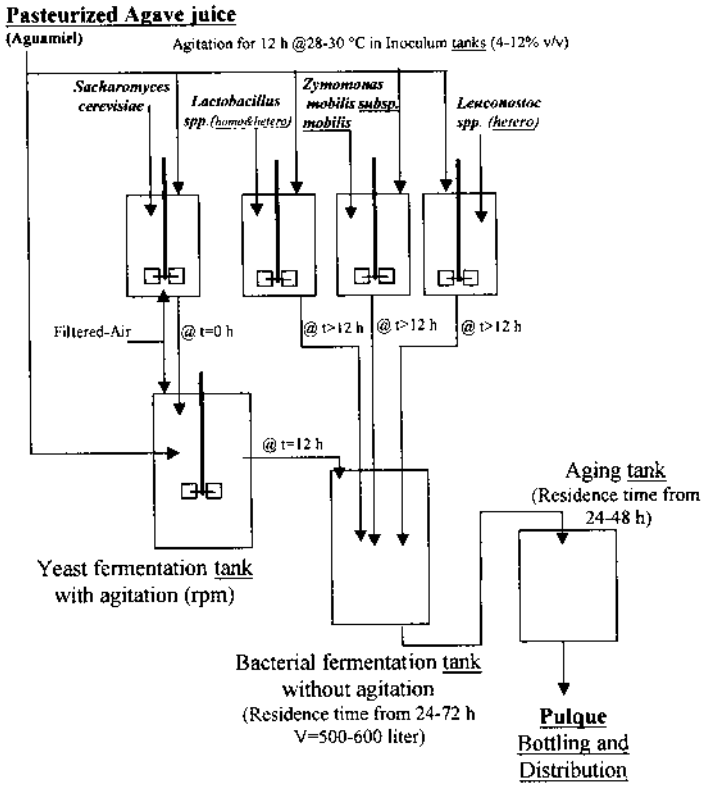

Figure 7 Flowsheet for modern pulque fermentation. (Adapted from Ref. 7.)

and fermented exclusively with *S. cerevisiae* for 12 h. After this initial stage, *Lactobacillus* spp. and *Z. mobilis* are inoculated to continue the fermentation, now under anaerobic conditions, for an additional period of 12–48 h. This "still-fermentation" stage is followed by aging for 24–48 h.

In a later communication, Sanchez-Marroquin and co-workers (18) designed three industrial production facilities for production of pulque based on the experimental data collected in their pilot-plant studies. These three pulque factories were also sponsored and constructed by the Patronato Nacional. Located in different states of Mexico, (Tlaxcala, Hidalgo, and Mexico State), each one had an approximated capacity of 50,000 L/day of pulque. The layout of each plant consisted of three levels. The area for reception and analysis of aguamiel and preparation of inocula was placed on the highest level. From there, 10 tanks (each with a capacity of 5000 L) were fed by gravity with

aguamiel and inocula. After a period of 24–48 h of still fermentation at 20–25°C, the product was transferred to aging tanks (5 tanks of 10,000 L each) for 16–48 h to complete its esterification and bouquet development. If aging is not completed during such a period, the product is transferred to a controlled temperature set at 15°C where pulque remains until all desired qualities are observed (Table 1).

Unfortunately, these three plants are not in operation today and, in fact, were never operated to full capacity. The main reason was a technical problem, not related with the process of fermentation itself but with the lack of successful strategies to preserve the quality of the final product for sufficient time to allow for distribution. Several attempts to bottle or can the product failed since 1967, and production of pulque for almost three decades continued to be merely artisan in nature, significantly losing market to beer, rum, and even tequila as one of the favorite alcoholic beverages among Mexicans.

Certainly at this point, the title and mere existence of this chapter would not seem reasonable to the reader. Indeed, the justification for a chapter on the industrialization of pulque is only found in very recent history.

In 1994, a small, family-owned company found a reproducible methodology to can pulque and began seeking out business not primarily in Mexico, but as far as California, Texas, and Germany. The export business of Productos Naturales de Agave (formerly Bebidas Naturales San Isidro) is grow-

Figure 8 Rodolfo del Razo holds a can of pulque, Nectar del Razo®. Del Razo is the founder and head of a successful family business that is now exporting canned pulque to American and European markets.

ing significantly. A total of 54,000 cans are distributed in the United States monthly, which represents a volume of approximately 18,300 L/month. The expectation is to shortly reach 200,000 cans/month (total sales for US$2,400,000/year).

The head of the company is Rodolfo del Razo (Fig. 8), a 75-year-old farmer from the high-plains state of Tlaxcala (township of Nanacamilpa). He does not reveal the family's secret for canning pulque, although he concedes it involves pasteurization (19). When the fermentation has reached 12 h, pulque is collected and natural flavors added before it is pasteurized, cooled, and packed in 12-oz (340-mL) aluminum cans for the U.S. export market. Del Razo's special processes have achieved pulque with a 12-month shelf life rating from the U.S. Food and Drug Administration (FDA).

VII. CHANGES FROM INDIGENOUS TO MODERN PROCESSING

The modifications made to the traditional pulque fermentation process in the last 35 years were due to (a) an attempt to improve quality consistency and (b) to extend pulque's short shelf life.

As in any mixed culture fermentation, the complex ecology or nature of the process determines the properties of the final product (20,21). The ecology of the process is determined by a set of conditions such as the microbiological nature of the inocula, the characteristics of the substrate, temperature, degree of mixing, amount of oxygen, processing time, and so forth. When pulque is produced the traditional way, some of these factors are uncontrolled, resulting in an inconsistent quality. For example, in the traditional process, where neither the aguamiel nor the inoculum is subject to any enforced guidelines (1,5,22), the selection of starters or the intrinsic factors of a particular aguamiel batch can drive the fermentation process in different directions.

Sánchez-Marroquin and co-workers focused their research on the understanding of some key issues that have an impact on the reproducibility of the fermentation process, precisely emphasizing aguamiel and inoculum quality. They introduced the idea of controlling pH and reducing sugars in aguamiel. Also, after studying the microbiology of the traditional process (see Sec. X), they recommended eliminating the natural flora in aguamiel to eradicate any undesirable microoraganisms and to use pure cultures as starters to better control the occurrence of ecological events during the fermentation. After determining the role that key microoraganisms play on aguamiel fermentation, Sánchez-Marroquin et al. restricted the number of cell varieties to be seeded to four groups: *S. cerevisiae*, *L. mesenteroides*, *Lactobacillus* spp.,

and *Z. mobilis*. By using sequential inoculation during the fermentation, further control of the final characteristics of the product can be assured, and even some undesirable properties can be suppressed. For example, by eliminating *L. mesenteroides* from the inoculum, a less viscous pulque is obtained. Reportedly, lower viscosity implies better acceptance in export-markets and among young Mexicans consumers (18,19).

Poor stability was a second major challenge which had to be overcome to commercialize pulque. The end point for pulque production is not the end point of the fermentation process. When the tlaquichero determines that pulque is ready, it still contains 0.2–0.5% (1) fermentable sugars available for further fermentation. In addition, given the high protein concentration of the final product and its richness in mineral and vitamin content, pulque is an excellent medium for proteolytic and acetogenic bacteria. Poor asepsis, normally associated to the traditional method of preparation of pulque, severely increases the probability of microbial contamination. As a result, the shelf life of pulque is very limited if the final product is not processed to stop further microbial activity. Pasteurization is a crucial unit operation in modern pulque production technology.

In summary, modern versions of the process of production of pulque are founded in a better control of some (not all) of the key fermentation conditions such as inoculum age of the substrate (fresh or regular aguamiel), temperature, residence time and commercial quality. The complex ecological diversity and rich microbial variability present in the indigenous process has been sacrificed in modern versions to maximize reproducibility and productivity.

VIII. CRITICAL STEPS IN MANUFACTURE/FERMENTATION

In this section, we discuss some of the steps in pulque production that we consider particularly important. Pulque production is an elaborate process where success depends on multiple factors. From the cultivation of agave to the bottling and/or canning of the final product, several steps are particularly important to assure adequate and reproducible quality.

A good pulque starts with a good or unadulterated aguamiel [Table 1 (5)] and, as a consequence, a good pulque agave plant. A long waiting time (between 8 and 10 years) precedes maturity of the plant. During this time, the agave is subject to diseases, environmental stress, and animal feeding depradation. At the present commercial production level, the supply of agave does not seem to be a problem. However, if demand continues to grow, pulque agave could face the same situation that agave *tequilana* for tequila production is now experiencing.

Aguamiel collection is another important step in pulque manufacturing. Contamination of aguamiel with undesirable microoraganisms could possibly disrupt the fermentation process or at least prolong its lag phase. The transportation conditions from the agave field to the "tinacal" are also considered by local experts to be important. Excessive agitation during transportation may favor growth of aerobic bacteria, again deviating the normal ecological balance during the fermentation start-up.

The nature of the inoculum is also fundamental as a good start-up of the fermentation process. Whereas the ancient traditional process relied on natural flora to start the process, present practices, even for small-scale production, are frequently based on the use of direct inoculation.

The actual fermentation process is still a "black box." No detailed studies have addressed the complex microbial ecology dynamics from inoculation to the fermentation end point. Indeed, the end point is still determined empirically by the tlaquichero once the organoleptic properties of the product are judged satisfactory [Table 1 (1)]. Because the amount of fermented sugars at the end point is still significant and the alcohol content is not high enough to inhibit further microbial growth, pasteurization is then crucial to preserve properties and commercialize a stable product. Nonpasteurized pulque has a very limited shell life (must be consumed 1–3 days after production).

Therefore, a microbial ecology model for minimizing a food-borne hazard and improving its fermented food quality should be capable of describing and controlling four critical factors:

1. Intrinsic factors (physical and chemical composition)
2. Types of adventitious microorganism to initiate the fermentation
3. Extrinsic factors (water activity, time, temperature, and oxygen tension)
4. Implicit factors (specific growth rates, symbiosis or inhibition among flora)

This microbial or nature ecology approach has been adapted in industrial microbial processes, where both the microbiological and process engineering factors are intimately related in technologically successful developments for yeast protein or ethanol fermentations.

IX. MAJOR PROBLEMS IN INDUSTRIALIZATION

The two major commercialization obstacles that pulque has to overcome to reach the market place are quality inconsistency and limited shelf life.

In Section VII, we reviewed the modifications of the traditional process in order to assure less variability in the quality of the final product. They are pasteurization/sterilization of the incoming aguamiel, adjustment of initial pH, and standarization of inoculum. Still, more work has to be done to refine (optimize) the industrial version of the process.

For many years (from 1954 to 1994) the main technical obstacle for pulque commercialization was its short shelf life (6). Unpasteurized pulque must be consumed within 1–3 days after production. Several pasteurization protocols were tested during that period, failing to be effective. This problem prohibited the massive distribution of the product nationally and internationally. At the same time, beer and other mixed drinks gained popularity even in those communities where pulque had been favored by the public.

By the beginning of the 1990s, the idea of modernizing the pulque industry became impractical. It was in 1994 when del Razo succeeded on commercializing a canned and pasteurized pulque. The taste and texture of traditional pulque was not particularly attractive to young Mexicans. It is outside of Mexico were Productos Naturales de Agave has found a potentially promising market, particularly among the Mexican population living in the United States.

Once the pasteurization obstacle has been overcome, the next bottleneck in massive commercialization is the balance between supply and demand. For example, Productos Naturales de Agave is now exporting 50,000 cans/month to the American market. This represents 17 m^3/month or 17,000 L/month. Even at this low scale, aguamiel supply can soon become an issue. Considering the daily average production of aguamiel from a healthy agave plant (during its productive period), at least 190 plants will be needed in order to sustain the actual pulque production. Considering that each plant will produce aguamiel for only 4 months once it is fully mature (after 10 years of development), a plantation of 4500 agave plants will be required to extend production for another 10 years. This is a very demanding proposition, particularly in light of two factors. In an ironic contradiction, two traditions fight for survival. The silky inner membrane of the agave plant from which pulque is produced is being used as a cooking sack that gives a special flavor to a traditional rabbit recipe called "mixiote" (19). On the other hand, agave spp. is succeptibility to plagues and diseases. Bacterial infections are not uncommon among agave plants, mainly in low-altitude and humid regions. For example, *Erwinia carotovora*, the causal agent of stem soft rot is a serious concern among agave *tequilana* growers (23).

If the forecast of the Productos Naturales de Agave is correct, they will be exporting in the short term four times more pulque to North America. At that point, four times more agave plants and a better process control during

fermentation will be required to assure not only a sustained quality but also for demand.

X. MICROBIOLOGY AND BIOCHEMISTRY OF FERMENTATION

The main body of research in microbiology and biochemistry of pulque was generated by Sanchez-Marroquin and his group from 1950 to 1968. Their main contribution was to identify the key organisms in the aguamiel fermentation and recommend variations from the traditional process that will make the scale-up and industrial production processes more feasible and controllable.

Sanchez-Marroquin and collaborators mentioned four microbial groups as the main responsible for the biochemical changes that occurred in going from aguamiel to pulque: *Leuconostoc* spp., *Lactobacillus* spp., yeasts (*S. cereviseae* primarily), and *Zymomonas mobilis* casually. However, no information has been reported on the complex ecology occurring during pulque fermentation, and the interdependence and relative dominance among these groups at different stages of the fermentation is yet to be established experimentally. Several microbiology studies have indicated that aguamiel has a higher bacterial count and a lower yeast count compared to traditional pulque (6): (a) aguamiel had (0.8–1.5) $\times$ 10^6 bacteria/mL and (3–6) $\times$ 10^3 yeasts/mL and (b) pulque contained about (0.1–0.2) $\times$ 10^6 bacteria/mL and (0.25–0.30) $\times$ 10^6 yeasts/mL.

Leuconostoc genus probably contains the most flexible lactic bacteria in terms of conditions for growth in a neutral to slightly acidic media. In pulque fermentation, *Leuconostoc* spp. play three important roles. They increase the acidity of the aguamiel during the first stages of the fermentation, evacuate oxygen by production of CO_2, rendering the media anaerobic, and produce bacterial polysaccharides (dextrans) from dextrose (24) imposing its traditional viscous consistency to pulque. *Leuconostoc mesenteroides* and *L. dextranicum* have been reported in pulque, (Sanchez Marroquin, cited in Ref. 15). The lower limit of the pH growth range for *Leuconostoc* is 5.8–6.0 depending on the species. Once the pH has been reached, it is another lactic group which should take dominance, *Lactobacillus*. The higher growth rate of *Lactobacillus* spp. in the 6.0–4.0 pH range guarantees their dominance over *Leuconostoc* in this second fermentation stage. Several species of *Lactobacillus* have been reported in pulque (among them *L. plantarum* or *L. arabinosus* and *L. brevis*). *Lactobacillus* converts glucose to pyruvate and, subsequently, to lactic acid by the Embden–Meyerhof pathway, increasing the level of acidity in pulque and

inhibiting the growth of most pathogenic bacteria and creating the proper environment for growth of ethanol producing groups, *Z. mobilis* and various yeasts.

When the production of lactic acid is such that the pH has reached a value around 6.0, *Z. mobilis* can start exponential growth (its optimum pH range from 6.5 to 4.5). *Zymomonas mobilis* is considered by some authors to be the main causal microorganism in pulque fermentation (25). *Zymomonas* is a bacterium that lives on the surfaces of plants, including agave species indigenous to Mexico from which pulque is produced. Lindner (3,4) and Gonçalves de Lima et al. (26) discovered another causal organism of the sugary sap to obtain pulque, which they called *Z. mobilis*. This alcohol-producing bacterium has been isolated from fermenting palm juice (palm wine) in India (27) and Nigeria (28), fermenting sugar cane juice (caldo-de-cana-picado) in Brazil, and ripening honey and occasionally from bees (25).

This bacterium may have been the causal organism in the preparation of bread and "solid beers" of the Arabs and the ancient Babylonians. Gonçalves de Lima et al. (27,29) reported the in vitro antagonistic effect of *Z. mobilis,* isolated from pulque and fermented sugarcane juice, against a number of pathogenic bacteria and filamentous fungi. From all usual pulque microoraganisms, it is the only Gram negative that greatly facilitates its identification. *Zymomonas* is catalase-positive, nonsporulating, polar-flagellated, actively motile rod 4–5 μm by 1.4–2.0 μm. It is facultatively anaerobic (producing acetic acid), and under anaerobic conditions, the organism uses the Entner–Douderoff pathway for the catabolism of glucose (Sanchez–Marroquin cited in Ref. 15) displaying a remarkable efficiency in alcohol production from 1.8 g of glucose: 0.81 g of ethanol (45%), 0.79 g of CO_2 (45%), and 0.15 g of lactic acid (8%) and with lower biomass accumulation (~2%) than yeast (27).

During the last stage of fermentation, at a lower level of pH (around 4.5), *S. cerevisiae* and other yeast (*Endomycopsis* sp., *Pichia,* and *Torulopsis)* will assume dominance (24) *Zymomonas mobilis* and yeasts are responsible of the production of ethanol and CO_2.

This ecological structure could be disrupted without apparently loss of the properties profile of pulque. In pilot-plant experiments, Sanchez-Marroquin et al. (7) demonstrated that aguamiel adjusted to pH 5 could be successfuly fermented to pulque in a two-stage process in which the first stage is an aerobic propagation of *S. cerevisiae,* followed by inoculation and anaerobic growth of lactic bacteria and *Z. mobilis*. According to Sanchez-Marroquín et al., the biochemical profile of pulque prepared by this method does not differ significantly from that prepared by traditional methods. Yeast communities found in a natural tequila (Herradura brand) fermentation were gradually reduced in their heterogeneity. *Torulaspora delbrueckii*, *Kluyveromyces marx-*

ianus, and *Hanseniaspora* spp. progressively were replaced by *S. cerevisiae*, *Zygosaccharomyces bailii*, *Candidia milleri*, and *Brettanomyces* spp. It was also concluded that crushing equipment and must holding tanks are the main sources of inocula for the blue agave fermentation and not the adjacent vegetation or *Drosophila* species as internal vectors (30).

Recently, a research group at the Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM) became interested in the ecological dynamics in pulque fermentation (Jimenez et al., personal communication, 2002). Their preliminary data reveal a complex population dynamics during a batch fermentation of sterile aguamiel (agave juice from San Luis Potosi, northeastern Mexico). The batch fermentation presents total counts, in aguamiel agar, in which two serial-growth regimes can be observed. After a short lag phase, the population, mainly dominated by bacteria, reaches a first stationary phase at approximately 12 h after inoculation with 10% (v/v) of pulque. A second exponential growth phase is observed afterward, mainly dominated by yeasts.

If the Sánchez-Marroquin proposition is correct (nobody has proved it inaccurate yet), the logical sequence in dominance in a traditional process would be *Leuconostoc*, *Lactobacillus*, *Zymomonas,* and *Saccharomyces* yeast.

XI. OPTIMUM FERMENTATION CONDITIONS

The optimum conditions for pulque production have not been fully delineated or they are proprietary. Sanchez-Marroquin et al. (7) reported a set of conditions capable of reducing the fermentation time from 10 days (traditional process) to 2–3 days. In a series of pilot-plant studies, they explored a range of temperatures between 28°C and 30°C, a sugar concentration of 8°Brix, and an initial pH adjusted to 5.0. They also tested several inoculation protocols, including simultaneous use of yeast and bacterial pure cultures, or sequential addition starting with yeast, followed by bacterial cultures at different times. A better control on the 12-h fermentation was achieved when a sequential inoculation of starters was implemented (see Secs. VI and XIV). However, no details are offered to compare performances of different versions of the process. In a latter finding, Sanchez-Marroquin et al. (18) recommended using a temperature in the range of 20–25°C and a fermentation time of 24 h for industrial implementation of the process. An additional period of 16–48 h at 15°C is suggested for maturation and "bouquet" development.

At this moment, no other study has presented data on optimization or control of aguamiel fermentation, and the effect of important process variables (such as mechanical agitation, temperature, inoculum/batch ratio) on the quality of pulque remains to be explored more rigorously.

XII. POSSIBLE SPOILAGE MICROORGANISMS

Many alcoholic fermentations containing live lactic acid bacteria along with ethanol can make the food stable and resistant to diarrhea-causingbacteria in nonhygienic rural areas. In principle, the alcoholic content of pulque is high enough to inhibit growth of pathogenic bacteria. However, the peak of flavor of pulque (and the end point of the fermentation) is not conditioned by exhaustion of carbohydrates. The high concentration of reducing sugars (0.5% as glucose) in pulque (see Table 4) and its relatively high protein level (0.2%) makes pulque an excellent culture media for acidophilus spoilage and ethanol-tolerant bacteria and yeast. The continued evolution and metabolic action of the microbial community found in pulque may, indeed, be the most frequent cause of spoilage if pasteurization is not effected immediately after the end point of the fermentation has been reached. Loyola (6) reported the following adulterated and commercial pulques confiscated in Mexico City: sour and putrid spoilage (11%), water dilution (20%), and starch and cactus-gum addition (69%). However, there is no report on the characterization of this industrial spoilage flora.

Alycylobacillus spp. may be one of the few candidates to grow in a pasteurized product. Splittstoesser (31) found that growth of these acidophilic bacteria is obtained over a pH range of 3–6 in agar medium and is only inhibited when the ethanol content exceeds 6%. *Bacillus*, *Clostridium,*

Table 4 Biochemical Changes Occurring in Agave Juice During a Pilot-Plant Fermentation Using Mixed Pure Cultures

Average determination (g/100 mL)	Agave juice pasteurized[a]	Pulque pilot plant[a]
°Brix	11	6
Refractive index (Abbe) at 20°C	—	1.338
Specific gravity	1.042	0.978
Viscosity (cps at 20°C)	—	4.1
pH	7.0	4.6
Total acidity (as lactic acid)	0.018	0.348
Total reducing sugars (as glucose)	10.0	0.48
Direct reducing sugars (as glucose)	2.4	0.06
Gums (as glucose)	0.60	0.33
Sucrose	7.6	0.42
Total solids	15.3	2.9
Total protein	0.17	0.17
Ash	0.31	0.29
Ethanol	—	5.4

[a] Data from Sanchez-Marroquin et al. (7) and Steinkraus (15).

and *Alycylobacillus* are also acidophilic spore-forming, heat-resistant bacteria that may be found in fruit juices and beverages contaminated during harvesting or processing.

XIII. CHEMICAL AND BIOCHEMICAL CHANGES DURING FERMENTATION

Among the most significant biochemical processes and changes occurring in unadulterated agave juice by the mixed flora described in Section X, the following are worth mentioning:

1. Lactic acid fermentation of sugars mainly by *Lactobacillus plantarum*, *L. brevis*, *Leuconostoc mesenteroides* and *L. dextranicum* (24); acetic acid fermentation by *L. brevis*, *L. mesenteroides*, and *Z. mobilis* (25).
2. Alcohol fermentation and flavor production by *Z. mobilis* subsp. *mobilis*, *S. cerevisiae,* and other wild yeasts (3,4,7,18,25)
3. Dextran and levan fermentation of sucrose by *Leuconostoc* spp. and *Z. mobilis* (7,18,27)
4. Pulque nutritional bioimprovement with microbial protein and vitamins due to mixed-flora symbiosis (7,32)

Table 5 Nutritional Composition of Traditional and Industrial Mexican Pulque

Food nutrient (per 100 mL)	Traditional pulque[a]	Pilot-plant pulque[b]	Industrial pulque[c]
Energy (kcal)	43	—	42
Ethanol (g)	4.7	5.4	4.5
Solids (g)	2.2	2.9	1.7
Proteins (g)	0.29	0.17	0.37
Lysine	0.016	0.008	—
Tryptophane	0.003	0.009	—
Ash (g)	—	0.29	0.24
Ascorbic acid (mg)	6.0	—	5.1
B Vitamins (mg)			
Thiamine	0.02	0.02	0.02
Riboflavine	0.02	0.04	0.03
Niacin	0.33	0.38	0.35
Minerals (mg)			
Iron	0.7	—	0.7
Calcium	1	—	11
Phosphorus	8	—	6

Source: [a]Refs. 32, 34, and 35; [b]Refs. 7, 15, and 18; [c]Ref. 14.

5. Food preservation by alcohol, carbon dioxide, and organic acids (lactic and acetic) along with in vitro antagonistic activity of lactococci, lactobacilli, and *Z. mobilis* (26,29,33)

The effect of the processes just described is reflected in Table 4, which compares major biochemical profiles of agave juice and pulque produced with mixed pure cultures. The main effects occurring in pasteurized agave juice during a serial yeast followed by bacterial fermentation with mixed pure cultures are summarized in Table 4: soluble solids (°Brix) decrease from 11 to 6; pH falls from 7.0 to 4.6; total acidity (as lactic acid) increases from 0.018 to 0.348 g/100 mL; sucrose decreases from 7.6% to 0.42%; glucose decreases from 2.4% to 0.06%; ethanol increases from 0% to 5.4% (Sanchez-Marroquin cited in Ref. 15). The agave plant (*A. atrovirens*) can produce at least 150 L of agave juice per month with a total sugar content of 7% (regular juice) to 14% (fresh juice). The agave leaves also have juice with a sugar content of 2–10%.

Sanchez-Marroquin et al. (cited in Ref. 15) compared the chemical analyses of traditional pulques (from Hidalgo, Tlaxcala, and Mexico State) with that produced in a modern pilot plant. The most striking differences are the wider range of ethanol (2.9–6.5%), lactic acidity (0.01–0.96%), and total reducing sugar contents (0.1–2.3%) in the traditional pulques. In contrast,

Table 6 Recommended Dietary Intakes (RDIs) for Pregnant Women and Children for Selected Nutrients, and the Micronutrient Contribution of Mexican Pulque

		Mexican RDI		Percentage of RDI	
Pulque nutrient (per 100 mL/person/day)	Traditional pulque[a]	Woman[b,c,d]	Child[c,d]	Pregnant woman	Child (1–3 years)
Energy (kcal)	43	2385	1300	1.8	3.3
Ethanol (g)	4.7	—	—	1.4	2.5
Solids (g)	2.2	—	—	—	—
Proteins (g)	0.37	75	20	0.5	1.9
Lysine	0.016	0.263	0.163	1.1	1.8
Tryptophane	0.003	0.263	0.163	1.1	1.8
Ascorbic acid (mg)	6	70	40	9	30
B Vitamins (mg)					
Thiamine	0.02	1.5	0.7	1.3	6
Riboflavine	0.02	1.7	0.8	1.2	5
Niacin	0.33	20	9	2	4
Minerals (mg)					
Iron	0.7	24	15	3	9
Calcium	11	800	450	1	2

Source: [a]Refs. 32 and 34; [b]Refs. 16 and 36; [c]NOM-086-SSA1 (Ref. 37); [d]Ref. 38.

the pilot-plant pulque has a narrower range for ethanol (4.1–5.1%), lactic acidity (0.04–1.28%), and sugar contents (0.05–0.15%), primarily contributing to a mildly alcoholic and acidic flavor.

The contents of ethyl acetate and fusel oils were similar and lower, respectively, in the pilot-plant pulque (0.04–22.1 mg% and 10–50 mg%). These components probably add smoothness (bouquet) and aroma to the final pulque. Furthermore, B vitamins and essential amino acids were higher in pilot-plant pulque because the microbial cultures were not filtered in the pulque (Tables 5 and 6).

XIV. STARTER CULTURES

Pulque fermentation can be classified into two different processes: the traditional batch and the modern fed-batch process. Both of these production techniques are in use today and different forms of starter culture application are in place. The traditional method uses samples from a previous batch. The proportion of pulque inocula [~1–3% versus ~25–50% (v/v)] determines the fermentation process (batch versus fed batch) and production method (batch versus semicontinuous).The back-sloping method, as it is often referred to, can be easily applied with minimal cost and technical know-how. However, the limitation using the back-sloping technique is that the cultures tend to lose their fermentative ability as successive uses increase (39). The culture used in this traditional method is usually unknown and not defined (6). Hence, optimization of fermentation conditions and further improvement to the fermentation process are much more difficult to develop. This can also limit improvements in process control and technology development. Another way in which direct inoculation of unknown starter microoraganisms is currently practiced is by using goat excrement as the inocula. It contains different microoraganisms, such as lactic acid bacteria, yeasts, as well as coliforms and pathogens. This adulterated method is certainly not accepted hygienically and the product would not be accepted as pulque by any standard or official code practices (1,5).

The use of defined starter cultures in the modern pulque fermentation in pilot studies was first described by Sanchez-Marroquin et al. (7,18). Initially, the fermentation was done by using mixed cultural strains. In a later development, the fermentations were compared between mixed culture and serial additions of yeast and other bacterial cultures. The fermentation process from serial culture addition consisted of two successive steps followed by aging for 24–48 h prior to bottling and distribution (Fig. 7) as described in Sections VI and X. The first addition was *S. cerevisiae* in agave juice at pH 5, followed by the second-stage fermentation of lactic acid bacteria, *Lactobacillus* sp. and *Leuconostoc mesenteroides* and *Z. mobilis*. Pulque from the serial

fermentation was shown to yield better quality attributes and a shorter fermentation time. The mixed-culture fermentation would be advantageous to lactic acid bacteria due to their faster growth and may suppress the growth of *Z. mobilis* and yeast, both of which are important flavor producers. In this pilot production, pasteurization of commercial agave juice (5) was introduced prior to fermentation as a measure to eliminate the undesirable indigenous microoraganisms. Hence, the final characteristics of the products were the results of starter cultures. The studies analyzed the final products produced using defined starter cultures and the traditional method and showed no significant difference in their biochemical profiles. However, further studies are still needed to show any difference in flavor and sensory quality of products from these starter cultures and the traditional methods. It is possible that the pasteurization would limit the complex interactive contribution of other indigenous microoraganisms resulting in a deviation of products from their usual authenticity. As evidence is the wine fermentation, where the wild strains or natural flora can play an important role in flavor development, even though the fermentation may be dominated with inoculated yeast, *S. cerevisiae* (40).

Thus far, the use of a starter culture has been limited to pilot-scale studies and it was used once in 1967 in three pulque factories making pulque and syrup from the agave stems and leaves (Sanchez-Marroquin, 1977 cited in Ref. 15). The limited development of pulque starter technology in the past may be due to the fact that industry is not sufficiently mature to adopt this technology. Recent development in exporting markets, particularly in the United States, as described earlier would make the adoption of the starter technology more favorable. Hence, a comprehensive research framework to carry out the plan right through to the end users is indispensable to the development and expansion of the pulque industry. The studies as described here focus mainly on the optimization of fermentation conditions for the application of a mixed-starter formula. Further work should focus on the economical production of cell mass for use as starters and starter strain improvement in order to maximize their fermentative ability and pulque quality. The criteria for strain selection in pilot pulque fermentation are not well described. There have been very few in-depth studies on the biochemical changes during the fermentation and on the desirable quality parameters such as flavor, aroma, and nutritional profiles.

XV. EFFECT OF PROCESSING ON NUTRITIVE VALUE

Alcoholic beverages comprise an important part of diets worldwide. In the industrialized countries, alcohol (i.e., beer, cider, wine) often provides 10%

or more of adult energy intakes (41). In developing countries, alcoholic beverages, specially if not distilled or overly processed, can also provide significant amounts of essential micronutrients. This is the case of Mexican pulque.

Rural malnutrition is still a severe national problem in Mexico—a problem which has not improved in the last 20 years according to the National Nutrition Survey in 1996. This survey was conducted not only to analyze energy and nutrient composition but also to show critical deficiencies in the diet, predominantly in pregnant and lactating women coming from low socioeconomic levels living in the rural and indigenous areas of southern Mexico. Mean energy consumption provided only 72% of the Required Daily Allowance (RDA) and the diet comprised 15% protein, 60% carbohydrates, and 25% fat. The percentage of women having intakes lower than 50% of the RDA was 70% for retinol, 75% for pyridoxine, 69% for folic acid, 50% for ascorbic acid, 33% for cyanocobalamin, 33% for calcium, and 22% for iron (42). The Masa Flour and Wheat Milling Industry signed a federal agreement in 1999 to enrich staple flours such as nixtamalized corn for tortillas and wheat flours for bread and flour tortillas (43,44). About 66% of the wheat flour brands are fortified with ferrous iron (3.5 mg%) and folic acid (0.2 mg%), representing about 0.1% of the selling price (Monterrey®, Selecta®, El Diluvio®, and Cantabria®). In addition, all of the masa flour production, which provides at least 30% of the Mexican tortilla consumption, is enriched with reduced iron (3 mg%), zinc (1 mg%), vitamins (niacin: 3.5 mg%; thiamine: 0.5 mg%; Riboflavin: 0.3 mg%; folic acid: 0.05 mg%), adding an extra 0.3% to its flour cost. Furthermore, the fortification cost with defatted soybean flour (6%) and the previous enrichment premix would cost approximately 8% of its marketing price (45–47). This new masa flour (Maseca®), having a 15% protein increase, improved both lysine (4.4 % or 465 mg%) and tryptophane (0.85% or 90 mg%) contents, which, in turn, raised the essential amino acid score (from 55% to 75%) for a 2-year-old child (38). It has been shown that flour fortification (48) is the most cost-effective and simple way to deliver micronutrients to urban and rural populations. At the beginning of any program, this cost may be subsidized by the industry, but in the long term, it will be included in the price to make the program sustainable.

The most severe and widely spread forms of malnutrition in Mexico since 1996 are energy (marasmus) and protein (kwashiorkor) deficiencies,* followed by deficient micronutrient intakes. The areas with the highest pre-

* It is reported that protein malnutrition affects stunting associated with adverse neurobehavioral outcomes and reduction of the brain's DNA (49).

valence are the southern states, which also have a significant indigenous population. Overall malnutrition affects 43% of the children based on the weight/age indicator, 50% are malnourished according to height/age, and 10% are malnourished with respect to weight/height (50). In Otomi Indian communities (from the Hidalgo state located in the central part of México), pulque is a significant bioenrichment source (51), contributing to daily intakes of calories and microbial proteins, ascorbic acid, riboflavin, niacin, thiamine, iron, and calcium (Table 5). In Hidalgo, school children may receive from 2.2% to 12.4% of their calories and from 0.6% to 3.2% of their proteins from daily pulque intake (15). Additionally, pulque can provide low-cost micronutrients (Table 6) for children (1–3 years old), such that 100 mL might supply 3% of the total caloric intake, 2% of high-quality microbial protein (lysine/tryptophane), 30% of ascorbic acid, 9% of iron, 6% of thiamine, 5% of riboflavin, and 4% of niacin (16).

Pulque may also improve the iron status of women in rural central Mexico. A recent prospective cohort study was conducted in the central highlands of Mexico State (52), with the objective of identifying dietary constituents and foods associated with better iron status in nonpregnant women aged 16–44 years old. Iron deficiency and anemia occur in this population sample within a dietary context that is much constrained by economic and environmental conditions that limit food choice. This study reveals that together, maize tortillas (51% provided by ~330 g/day), beans (20% provided by ~130 g/day), pulque (7% supplied by ~200 mL/day) and nopales/quelites (1%) provided nearly 79% of the diet iron. Nearly all of the iron consumed (usually >99%) is nonheme and less bioavailable. Therefore, it is also possible that ferrous lactate and ferrous ascorbate might form after pulque drinking, enhancing the vegetarian diet because the iron in corn tortillas (~1.9%) or in beans, when these are consumed as a single iron source, is poorly absorbed. Chavez et al. (53) had previously reported a negative correlation between a high consumption of corn tortilla and beans during a mother's pregnancy and her infant psychomotor development. In contrast, a moderate pulque consumption during pregnancy correlated positively with child's weight, which depended on the mother's availability of essential amino acids and vitamins during intrauterine development and lactation.

According to Backstrand et al. (16), the median quantity of pulque consumed in the region being studied was 500 mL (24 g alcohol or 168 kcal) which provides about 215 kcal (9% of Mexico's RDA) plus significant quantities of ascorbic acid (45%), iron (15%), niacin (10%), thiamine (7%), riboflavin (6%) and 2.5% of high-quality microbial protein (lysine/tryptophane). Consumption of pulque predicted a lower risk of low-ferritin (iron-deficient) and low-hemoglobin (anemia) values due to a better iron absorption in the presence of ascorbic acid and ethanol (54). Thus, a low-to-

moderate pulque consumption on child growth and mental development should be viewed with caution* although a possible beneficial effect.

In 1977, Sanchez-Marroquin (cited in Ref. 15) provided additional information on the alcohol, B vitamin, and amino acid content of pulque and showed the micronutrients to be highly variable when produced by traditional and self-consumption/barter methods. The Mexican Food Composition tables published by the Instituto Nacional de la Nutrición (34), Cravioto et al. (35), and Massieu et al. (32) are other sources of information.

Industrial (Nectar del Razo®) and pilot-plant pulques (Table 5) contain uniform amounts of ethanol (4.5%), ascorbic acid (5.1 mg%), niacin (0.35% mg%), riboflavin (0.03 mg%), thiamine (0.02 mg%), iron (0.7 mg%), and calcium (11 mg%) and is a good source of lysine (8–16 mg%) and tryptophane (3–9 mg%) mainly derived from bacterial and yeast fermentation. The total solids loss (i.e., 50% on a dry basis) during fermentation appears to increase the total protein in the solids (ie., 15%) along with essential amino acids not present before processing (Table 5).

Contrary to the popular belief, pulque does not appear to contain psychoactive (55) or aphrodisiac substances. It can contain significant quantities of steroidal saponins, many of which are bioactive (56). Saponins (plant sterols) present in agave spp. have shown hypocholesterolemic, anti-inflammatory, and antibiotic activity, but the functional effects of these in pulque remains unexplored (57,58). They may behave as fructans or prebiotics, to selectively foster the growth of probiotic lactic acid bacteria in the gut (Bacteriotherapy using harmless bacteria to displace pathogenic organisms is an alternative and promising way of combating intestinal infections.) Probiotics may have potential to boost disease resistance, treat food allergies, and reduce hypertension, or can be used as vectors for oral vaccines.

XVI. APPLICATION OF NEW BIOTECHNOLOGY AND GENETIC ENGINEERING TO FERMENTATION

In our opinion, there are three obvious areas of application of biotechnology related to pulque production:

1. Development of starter culture technology for pulque fermentation
2. Optimization of the fermentation itself
3. Propagation and cultivation of agave plants

* Maternal alcohol consumption has a strong potential to directly and indirectly compromise child health. Therefore, public dietary interventions are necessary to prevent the heavy consumption of pulque by pregnant women and increase consumption of other green-vegetable foods containing ascorbic acid and high-quality protein.

The starter culture technology is indispensable for the industrialization of pulque fermentation. The use of cultured fermentation should assure both quality consistency and product safety to modern consumers. However, successful and sustainable application of starter culture involves development of starter formula, optimization of the cultured fermentation, economical production of cells, and improvement of strains for their fermentation ability.

Molecular biology techniques have greatly facilitated the selection of appropriate starters. The development of the starter formula usually requires a comprehensive understanding of interactions of different groups of microorganisms during fermentation. Information on the successive growth at different phases of fermentation is important in determining their roles and their importance to fermentation and the final products. Using the molecular approach, individual microorganisms dominant in the microbial population can be identified during fermentation. In the studies of microbial population in nham, a fermented Thai pork sausage, the random amplified polymorphic DNA technique was able to identify dominant groups of microoraganisms during fermentation (59). In the studies of traditional sour cassava starch or a fermented nixtamalized maize dough pozol fermentation, the microbial community of the fermentation was genetically differentiated using denaturing gel gradient electrophoresis and quantitative RNA hybridization (60,61). The molecular techniques could be used to study the population dynamics of pulque fermentation to determine the successive growth and to identify the dominant starter strains during fermentation. Once the dominant strains are identified, the next step would be to optimize the use of selected microbial strains by challenging them for their pulque fermentation ability. The single strain or different combinations of these strains can be used for pulque fermentation in order to determine their suitability for use as starter cultures. The inoculation of different strains of culture at different phases of the fermentation should also be studied. The finished products should then be analyzed for their key attributes (1) such as pH, total acidity, viscosity, brix (total reducing sugars), and ethanol content at the end point of fermentation. Once the combination of starter strains are selected, the fermentation conditions and pulque quality attributes can be further optimized. This technique has been used successfully in the selection of starter formula for fermented Thai pork sausage (62).

An increase in production cost is one of the major handicaps to small and medium enterprises (SMEs), as the cost is inversely proportional to the scale of fermentation. The starter culture cost may constitute less than 1% for a large enterprise or 10% in a small production such as for SMEs. Factors influencing the starter production cost include the number of starter combinations used, the cost for biomass production, and the shelf life of the culture. In many developing and emerging countries where the growth media for

microbial cell production are imported, this is also a significant factor contributing to a high production cost. To minimize production cost, an economical medium using local raw material should be developed, such as the use of yeast from local breweries instead of imported yeast extract. The cell culture can also be suspended in phosphate buffer and distributed to the refrigerated food plants once a week. This model of cell production and distribution requires a minimum capital investment and hence facilitates the adoption of starter cultures to SMEs.

The rapid advance in molecular biology has opened promising opportunities to improve the fermentation ability of starter cultures. The genome sequences of *S. cerevisiae* (63) and lactic acid bacteria (64) are key information which can be developed to gain a better understanding of cell function. The genetic material for desirable traits such as flavor and aroma production can be identified using novel genomic techniques such as DNA microarray and cloned into the starter culture to increase their abilities to make more desirable products. These biotechnological techniques offer promising opportunities for strain improvement using metabolic engineering. *Saccharomyces cerevisiae* has been successfully engineered with two key genes under the control of the promoter in order to increase the sugar utilization by 50%, ethanol formation by 44%, and carbon dioxide production by 35% (65). The use of this yeast in pulque fermentation would reduce its alcohol fermentation time by half.

Extensive laboratory testing has indicated that *Z. mobilis* can ferment glucose to ethanol with a 2–4% higher yield than *S. cerevisiae*. However, after numerous pilot-scale and mill-scale trials, the fuel ethanol industry has not adopted the use of *Zymomonas*. The reasons for this include the following (66): (a) the lack of an established market for spent cells, which decreases the overall revenues, (b) the maximum ethanol concentration tolerated is not as high as yeast, (c) the lack of availability of cells on the open market makes its use difficult for mills that do not have an inoculum laboratory, (d) the use of pH 6 for fermentation results in higher contamination losses than yeast, which run at pH 4, and (e) the smaller cell size makes it more difficult, with existing centrifuges, to settle and separate *Zymomonas* cells the broth than yeast cells.

As mentioned in Section XI, optimum conditions for pulque fermentation have not been completely defined, and questions about the effect of key parameters remain to be answered. Recently, researchers at the Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM, Monterrey, Mexico) started a study on the population dynamics in pulque fermentation. The global aim of this 3-year project is to learn the effects of temperature, inoculum quality and quantity, and agitation rate on the dynamical behavior of the microbial population, the fermentation time, the organoleptic

qualities, and the biochemical profile of the final product. The information derived from this investigation will be made available to small producers in an attempt to help them to optimize their processes at the lowest possible cost.

A third line of application of biotechnology in pulque production is propagation of the agave plant. Agave plants are susceptible to bacterial infections and environmental stresses. The long waiting period from plantation to harvest of aguamiel (8–10 years) suggests the need of assurance of a constant supply of healthy plants with a constant phenotype. In this matter, cloning and propagation techniques may become relevant if massive demand of plants with a balanced phenotypic expression of the selected genotype, which also depends upon environmental factors.

XVII. FORECAST FOR FUTURE FERMENTATION

Many manufacturers are hesitant to change and improve their production practices. However, they will eventually assimilate either an appropriate/emerging technology or a biotechnology transfer if their production cost could be significantly reduced and they could maintain pulque quality, which is of utmost importance for expanding either a modern consumption (US$2.9/L: Nectar Del Razo) or a traditional but declining consumption (US$0.05/L). At present, agave juice cost represents about a 50–60% of the total pulque value (13) with an estimated tax of more than 30–35%.

Sanchez-Marroquin (cited in Ref. 15) studied the effects of mechanically pressing the whole agave plant (*A. atrovirens*), including stems and leaves, to improve the recovery of the sugary juice used for the industrial fermentation. Agave leaves also had a juice with a sugar content of 2–10%. When the juice is filtered and clarified, it needs sterilization and adjustment for optimum sugar concentration and pH prior to a serial yeast and lactic acid bacterial fermentation. Biomass that settles from the fermenters is recovered and it is either added to the pulque, sold for pulque bread, or used in cattle feed. The agave plant residues are combined with urea, phosphate, and biomass to obtain a fodder for ruminants. Mexico is fortunate to harbor 75% of the almost 300 agave species in its territory (19,22). However, other recent uses (67) for agave include both food (syrups for sweeteners and confectionery) and feed (cattle forage). They are apparently increasing both in the central (Tlaxcala) and northeastern (Coahuila and San Luis Potosi) areas (13).

In order to shorten the fermentation and improve the quality of the product, concentrated agave syrups and pure starters can be distributed to manufacturers as previously used for industrial pulque (18). There are a few agave syrup brands (i.e., with 60% total reducing sugar selling at more than

US$7/L) already made in Tlaxcala (Mimex®), Jalisco (Naturel® with GRAS status for the United States) and Coahuila (Nectar de Agave®) states. It is estimated that an agave plant for fructose- or polyfructose-rich syrups (*A. americana* or *A. tequilana*) normally contains from 62% to 63% by weight of core material (>25–45 kilos), from 30% to 32% of pulpy leaf material, from 2% to 3% of stalk material, and from 2% to 6% of root material (68,69).

Therefore, there are immediate opportunities for tailor-made starter cultures for both modern and traditional manufacturers. On the other hand, new syrup brands can be produced from agave leaves and standardized for either modern prebiotic (nutraceutical) or starter cultures (probiotics) needed in modern pulque production for the urban and export market niches.

As mentioned earlier, traditional pulque is losing its battle with conventional alcoholic beverages (beer, distilled spirits, and coolers) among young Mexicans. Still, traditional pulque production will remain alive in rural communities, where, in addition to being a symbol of tradition and culture, pulque is a valuable source of micronutrients, energy, and protein, principally for pregnant and lactating women and children. In Mexico City (one of the most populous cities in the world), pulque culture is deeply rooted as a basic and irreplaceable element of the suburban, traditional, surreptitious personality of a large city. Some old pulquerías with funny and ingenious names will survive the advance of modern times as they have the past three decades, even becoming monuments to the tradition.

Ironically, this Mexican icon, can find redemption and commercial success not only in Mexico but also in international markets. The Latino population is the fastest growing segment in the United States. In particular, Mexicans have become the largest Hispanic group in America, representing an enormous market for Mexican traditional products. Rodolfo del Razo, his family, and Productos Naturales de Agave (his Nectar Del Razo) lead the struggle not only for survival, but internationalization of Mexican pulque, already exporting 20,000 cans monthly. The degree of commercial success of this family business in the years to come may dictate the future of pulque as an industrial product. If demand for canned pulque increases, as Del Razo foresees, a more robust and optimized process will be required (starting with standardized starter cultures). The increase in demand may be the driving force for other producers to go commercial on a larger scale or to franchise from Del Razo's brand to new agroindustrial cooperatives.

DEDICATION

This chapter is in memoriam to A. Sanchez-Marroquin.

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11

Industrialization of Tempe Fermentation

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I. INTRODUCTION

It is important to clarify that tempe is a food native only to the Javanese people who have lived on the island of Java. Tempe originally developed in Central Java a few centuries ago (1). It is not widely accepted throughout Indonesia except among the Javanese.

Traditional tempe is a fermented food in which fungi, particularly *Rizhopus oligosporus*, play an essential role. Fresh tempe could be defined as a compact and sliceable mass of hydrated precooked bean cotyledons bound together by the mold mycellium (2,3). The major desirable aspects of tempe are its attractive flavor, texture, and nutritional properties. In Indonesia, tempe is consumed as a protein-rich meat substitute by all economic groups.

There are many kinds of tempe in Indonesia depending on the raw materials from which tempe is prepared. Tempe made from soybeans is called *tempe kedele*; it is called *tempe koro* if made from sword beans *velvet bean (Mucuna pruriens), tempe benguk* if from *tempe lamtoro* if from *Leucanea leucocephala, tempe gude* if from pigeon peas, and *tempe gembus* if from a waste product of tofu (4). Other kinds of raw material such as sweet narrow-leafed lupin (*Lupinus angustifolius* L) is similar appearance to soybean (5), yellow pea tempe (6), solid waste of mung bean starch (7), wheat tempe (8,9), and tempelike foods produced from broad beans (*Vicia alba*), cowpeas (*Vigna sinensis*), barley (*Hordeum vulgare*), wheat (*Triticum aesticum*), or mixtures thereof (10), and groundnuts (11) have been introduced. In Central Java, people utilize coconut press cake and peanut press cake for production of

tempe, namely *tempe bongkrek* or *dage*. Velvet bean tempe has a higher carbohydrate and fiber content and contains 0.551 mg/L isoflavone aglucone with the higher content of daidzein (12).

A range of tempe-making processes has been described for different localities and countries (13,14). The essential stages in the preparation of tempe include cleaning the beans, hydration/acid fermentation, dehulling, partial cooking, draining, cooling, surface drying, inoculation with starter, incubation in fermentation containers (fermentation), harvesting, and processing. The technology of tempe processing still needs improvement, especially improved equipment. This chapter deals with the production and consumption, historical aspects, traditional fermentation technology and facilities, development of processing, microbiological aspects, nutritive value, and future outlook for fermented tempe.

II. PRODUCTION AND CONSUMPTION

Tempe is consumed practically all over Indonesia, although the level of consumption still varies from one province to another. Total consumption is estimated at 20,000 tons per week (1,040,000 tons tempe/year) with an annual increase of about 3.5–4%. It is believed that Indonesia is the largest producer and consumer of tempe in the world (15). The development offers optimism on the future market of tempe in Indonesia. This development is also supported by the recent government campaign to increase tempe consumption as one of the instruments in improving the nutritional status of the people. However, the existing condition of the tempe industry, particularly the small scale, is still far from meeting international sanitary and hygienic standards (16).

Agranoff and Markham (17), stated that the tempe consumers in Indonesia are mainly Javanese people, whereas other ethnic groups may not like tempe. There is also the matter of cultural perception of tempe as a cheap food among the Javanese. Many other Asian countries have tastes similar to the Javanese. In Thailand, Burma, and Vietnam, tempe has been introduced on an experimental scale with good acceptability. In Malaysia, the racial distinction between local Malays and Chinese segregates tempe along with poorer sectors of society. It is known as a cheap inferior food of Indonesian immigrants and Malays; therefore, it already has a negative connotation. In the United States, Australia, Europe, and most Western societies, tempe is a completely alien food and therefore represents a great and expensive marketing challenge to introduce it into these societies. Holland, which has a large Indonesian community, is already familiar with tempe, as well as vegetarian, vegan, and health food groups. In the United Kingdom,

the British taste is very conservative and the consumers are lead entirely by marketing media. This represents an extremely expensive outlay and the market at present is limited to Indonesians and health food consumers. Introducing tempe into parts of Africa would be of tremendous nutritional benefit, but also would be difficult due to cultural habits. The appearance of fungus on a food is totally unacceptable to most African people. In Zimbabwe, however, cooked tempe (fried) is extremely well liked even though it is unfamiliar food to African tastes.

In industrialized countries, tempe offers significant business opportunities, but only where it has been able to find a good market. The business in the Netherlands is very large and the demand is consistently high. In the United States, it also offers good business opportunities due to the sizeable demand from the health food and ethnic sector. The U.K. market is limited to the health food sector and ethnic sectors because of the unfavorable economy and conservative traditions of the British people: the risk in capital investment is too great (17).

The first commercial tempe shop in North America was fittingly opened by Indonesian, Mary Otten, of Albany, California in 1961. In 1972, the Farm, a large spiritual community from Summertown, Tennessee began the first Caucasian, noncommercial tempe factory. They sold starter culture and tempe-making kits and were responsible for much of the popularization of tempe in the United States. In 1975, Glen Randal began the first commercial Caucasian-run tempe shop in Palmyra, Nebraska. By 1979, in response to many publications about tempe, there were a dozen companies making approximately a combined 10,000 lbs of product a week. The same year, Michael Cohen started the tempe works in Greenfield, Massachusetts. This was the first company to set up shop in a commercial building strictly for the purpose of making tempe (18). By the early 1980s, tempe was the fastest growing soy food in the United States, growing at a rate of about 29% a year. More companies were formed and existing ones diversified their lines to include grain tempe such as soy-rice tempe introduced by White Wave in Boulder, Colorado and Five Grain tempe (soybeans, rice, millet, sunflower seeds, and sesame seeds) introduced by Turtle Island Foods of Forest Grove, Oregon.

In 1981, Pacific Tempe introduced the first tempe burger in United States, which instantly became very popular. The production of tempe in the United States increased from 19,055 lbs to 25,590 lbs/week. By 1984, 53 companies were producing 34,675 lbs of product each week (901 tons/year) at an average retail cost of $2.50/lb or $4.96 million per year on an annual basis (18).

Tempe sales were equal to about 10% of the level of tofu sales. Many "second-generation" products such as tempe burgers, sweet and sour tempe,

mock chicken and tuna salads, tempe lasagna, and various sausage-type products like Soylami and Tempehroni were introduced. Although only 14% of all consumers have heard of tempe, Ben Lee of Quong Hop Company in San Fransisco believes that tempe has a bright future. As consumers look toward soy foods, they want other tastes and texture in addition to tofu. Tempe, barely 40 years old in the U.S. marketplace has come a long way but has even further to go.

An innovative approach occurred when Betty's Tempe entered the regional market in 1987. They made tempe in stainless-steel trays. This tempe form was familiar in the US as "burger" patties and grated like hamburger meat. The production of tempe on a commercial scale required that stainless-steel trays be used. Other trays, metal or fiberglass with special coatings, would not stand up to daily use and the high heat encountered during pasteurization. The quality of "plastic bag" tempe is less than that produced with tray incubation, especially for texture and flavor (19). The varieties of tempe in the United States are shown in Table 1.

The scale of production of tempe in Indonesia can be classified according the amount of soybeans processed, where the lowest is less than 50 kg soybeans/day, the middle class is utilizes between 50 and 100 kg soybeans/day, and the large producers utilizes above 100 kg soybeans/day. There were some large tempe producers (above 500 kg soybeans/day), but the number is limited to less than 100 producers. In all classes, a consistent reduction in terms of cost and profit as size of production got larger was found as shown in Table 2.

Table 1 Varieties of Tempe in the United States May 1997

Variety	No. of brands on the market, May 1997
Tempe burgers	14
Soy tempe	9
Multigrain (mostly five or three grains)	6
Bulk soy tempe	5
Soy-brown rice tempe	4
Sea veggie tempe	3
Wild rice tempe	2
Soy millet tempe	2
Sloppy joe tempe	2
Miscellaneous other styles	8

Source: Ref. 18.

Table 2 Cost Structure of Tempe Production for Different Sizes and Modes of Production

	Individual			Group	
No. Variable	I	II	III	IV	V
1 Fixed cost	94.40	77.60	81.81	81.81	67.68
2 Variable cost	757.20	769.45	763.64	754.55	776.77
3 Marketing cost	—	24.24	60.61	60.61	20.20
4 Total cost	851.60	871.29	906.06	896.97	864.65
Revenue and profit (Rp/day)[a]					
5 Total revenue	660,000	825,000	82,500	82,500	247,500
6 Total profit	97,944	106,178	7,750	8,499	33,449

Note: I = production capacity = 400 kg soybeans/day; marketing activity through direct sale of tempe processed (fried tempe) by a group of people with a simple lorry. II = production capacity = 500 kg soybeans/day. III = production capacity = 50 kg soybeans/day. IV = average production capacity = 50 kg soybeans/day (a group with eight small industries). V = average production capacity = 150 kg/day (a group with 10 small industries).
[a] Rupiahs/kg; 8910 rupiahs = US$1.
Source: Ref. 16.

The scales of production also provided the higher earning power and resulting higher profit that could be invested in improvement of quality in processing. To some extent, marketing of fresh tempe remains suitable for small producers because their of ability to produce varieties of tempe for different purposes. The profile of tempe consumption in Indonesia is shown in Table 3.

In Indonesia, tempe has long served as a key source of low-cost protein in the diets of both the rich and poor. In fact, about 64.1% of all soybeans are made into tempe, as a basic food and the second most popular soy protein is tahu (tofu). Tempe plays its most important role in the diets of those who live in Central Java, especially in the regions of Yogyakarta, East Java, and Lampung, where there are transmigration people from Central Java. In areas outside of Java, most of tempe is made by the people from Java.

Tempe is most widely served at the small street stalls or booths called "warungs," where several popular dishes will generally be offered at very reasonable prices. This food is most popular among the middle and lower classes; while the upper classes use it most widely as a snack in the form of tempe chips.

Tempe factories and their average production in several other countries as shown in Table 4. It demonstrates that tempe is actually an important food in the diet of people worldwide.

Table 3 Tempe Consumption (kg/person/week) in the 13 Provinces of Indonesia

No.	Province	Average (kg/person/week)	City (kg/person/week)	Village (kg/person/week)
1	Yogyakarta	0.240	0.260	0.235
2	Central Java	0.173	0.202	0.166
3	Jakarta	0.137	0.137	—
4	East Java	0.136	0.169	0.128
5	Lampung	0.114	0.142	0.110
6	East Kalimantan	0.077	0.114	0.053
7	West Java	0.065	0.108	0.054
8	Bali	0.059	0.086	0.055
8	Central Kalimantan	0.051	0.067	0.049
10	Irian Jaya	0.052	0.072	0.030
11	South Sumatera	0.050	0.067	0.044
12	North Sumatera	0.037	0.059	0.032
13	Riau	0.034	0.068	0.022

Source: Sayogyo (1981) in Ref. 20.

Table 4 Tempe Factories and Average Production

Factory	Country	Year of production	Average production (kg/week)
1. Marusan-Ai	Japan	1983	6.885
2. Tempe Production Inc.	Netherlands	1969	6.000
3. Quong Hop/Pacific Tempe	United States (California)	1980	3.182
4. White Wave	United States (Colorado)	1979	2.659
5. Soy Foods Unlimited	United States (California)	1981	2.636
6. Torigoe Flour Milling	Japan	1983	2.623
7. The Tempe Works	United States (Massachusetts)	1979	2.500
8. Marukin Foods	Japan	1983	2.100

Source: Ref. 21.

III. HISTORY OF TEMPE

Tempe has been known and produced by Javanese for centuries, as mentioned in the Javanese literature *Serat Centhini* in 1815. It was developed on the island of Java, probably in the central or eastern provinces, and has been used throughout the island for hundreds of years. *Serat Centhini* was written by Rangga Sutrasna in the Javanese language during the reign of the King of Surakarta Pakubuwono V and mentioned that tempe was consumed during the time of King Sultan Agung (1613–1645). Tempe may have originated over 2000 years ago at almost the same time that the Chinese were making a related product soy sauce (22). The rise in popularity of tempe in Java spread to other Indonesian islands and to other countries of the world, probably at the beginning of the 20th century.

Because Indonesia was a Dutch colony in the late 1600s, it was only natural that the first Westerners to study tempe were from Holland. In 1895, H.C. Princen Geerligs, a Dutch scientist, wrote an article entitled "Einige Chinese Voedingsmidden Uit Soyabonen Bereid" (Chinese Food Made with Soybeans) which contained the first known written reference to tempe and included an examination of its nutritional value and methods of production. Princen Geerligs was interested in the identification of the tempe mold, and he was followed by Boorsma in 1900 (see Ref. 23), who analyzed tempe and soybeans to determine the changes that were occurring during the fermentations. Heyne in 1930 (see Ref. 24) published his comprehensive *De Nuttige Planten Van Indonesie* (The Useful Plants of Indonesia), which contained extensive and detailed information about tempe, followed by Jansen in 1924 showing that the thiamine content was reduced in tempe and by Jansen and Donath in 1924 (see Ref. 25), who demonstrated that tempe protein was highly nutritious when fed to animals. The first English-language description of tempe was in Burkill's dictionary of Malayan plant foodstuffs (26). Van Veen and Schaefer (27) studied tempe nutritional value and microbiological changes during fermentation.

During World War II, prisoners of war suffering from dysentery could not tolerate soyabeans but were able to subsist on tempe, providing early indications of increased digestibility (28–30). It was reported from the prisoner of war camp Changi, Vitamin Centre report by A./Sgt. C. Morton as follows: In the later part of 1943, the Japanese, either through shortage of rice or kindness of heart, started to replace part of the rice ration with soybeans. Soybeans are one of the best foods, but the hygiene officer discovered that most of the beans were being passed undigested in the feces. The camp then came to the rescue and showed the prisoners how to make tempe from soybeans. Tempe is a Javanese method of processing the beans and making them easily digestable. The fungus *Rhizopus,* which is found in

hibiscus flowers abounds in Singapore, and this fungus, when grown on the beans softens them and makes them digestible. Before the fungus can grow on the soybeans, the husks have to be removed. This is done by soaking the beans in water and then passing them between two loose rollers. In the tempe factories in Java, the natives substitute their large flat feet or their hands for the rollers and this may be why tempe is not eaten a great deal by the Europeans there. After the husk are removed, the fungal spores are mixed with precooled soaked soybean cotyledons, which are then spread about 1 in thick on trays. After about 36 h, the beans are covered with a whitish fungus and become a solid mass now ready for cooking. It is excellent fried, baked, or boiled. Medical officers were convinced that making tempe from beans was the best way to get the most value from them. Personally, when fried, I thought tempe most tasty—something between mushrooms and pork (31).

In the later years, there was much of research activity and many publications and review articles on tempe, potential micro-organisms, vitamin and bioactive components produced during fermentation, biochemical changes, effects of fermented tempe on digestion, absorption, and diarrhea, and improvement of processing technology.

IV. OUTLINE OF ESSENTIAL STEPS IN FERMENTATION

Fermentation of soybeans into tempe is a traditional technique originating in Indonesia. During fermentation, the micro-organisms, mainly *Rhizopus* spp., induce many changes in nutrient composition. These changes include changes in protein, fat, and carbohydrate composition (32) because of the production of a variety of proteases, lipases, and carbohydrases. During fermentation, there is a reduction of antinutritional factors (33) and synthesis of vitamins (21,34,35) ascribed to the action of the molds and bacteria and their enzymes.

The beans are boiled, dehulled, steamed in a basket over a copper chimney, mixed or inoculated with ragi tempe (mainly *R. oligosporus* mold), wrapped in the banana, teak, or *hibiscus* leaf packages or perforated plastic bags, and left for 36–48 h at ambient temperature to ferment, producing a firm white cake. During fermentation, the nutritional value and digestibility are enhanced, making tempe suitable for use in different applications like dietetics and nutrition improvement (36).

Tempe is made basically in the same way throughout Java, and it is hard work because it requires at least for 2 or 3 days fermentation. Saono et al. (37) presented various methods for preparing commercial tempe in Indonesia, as shown in Table 5.

Table 5 Some Traditional Methods of Preparing Commercial Tempe from Dry Soybeans

	Flowsheets of seven methods						
Step	1	2	3	4	5	6	7
1	Dehull	Boil	Soak	Boil	Boil	Soak	Soak
2	Wash	Soak	Dehull	Soak	Cool	Boil	Boil
3	Boil	Dehull	Wash	Dehull	Dehull	Wash	Cool
4	Drain	Wash	Boil	Wash	Wash	Boil	Dehull
5	Cool	Drain	Drain	Boil	Soak	Cool	Wash
6	Inoculate	Inoculate	Cool	Drain	Boil	Dehull	Soak
7	Wrap	Wrap	Inoculate	Cool	Drain	Wash	Boil
8	Incubate	Incubate	Wrap	Inoculate	Cool	Drain	Drain
9			Incubate	Wrap	Inoculate	Inoculate	Cool
10				Incubate	Wrap	Wrap	Inoculate
11					Incubate	Incubate	Wrap
12							Incubate

Note: Soaking: 10 h to evernight. boiling: if only once, 60–120 min; If twice, first for 30 min, second for 90–120 min. Incubate: 36–48 h at ambient temperature (26–30°C).
Source: Ref. 37.

The essential steps in the manufacture of tempe in Indonesia are the following:

1. **Cleaning the beans.** The beans should be cleaned to remove dirt, weed seeds, stones, damaged and possibly decomposed beans, or other foreign matter. The clean beans are separated from the other matter by use of bean screening or grading of the beans, as shown in Fig. 1. In Fig. 2, the soybeans and dried hulls are shown after cleaning and soybean cotyledon after dry dehulling.
2. **Soaking or hydration.** Soaking soybeans overnight in excess water makes dehulling easier; the beans absorb approximately their dry weight of water (i.e., double their original dry weight) and result in tempe with a desirable soft texture. During the soaking period, bacterial growth takes place and the pH of the beans falls to a range of 4.5–5.3. Acid production does not affect mold growth, but it prevents the growth of undesirable bacteria that can spoil the tempe. Acidification of soybeans occurs naturally during soaking in the tropics. Most of the bacteria are killed or removed during the subsequent procedures of boiling, dehulling, and washing. The soaking water generally is discarded and the beans are washed before the next step. Fresh water is added and the beans are boiled.

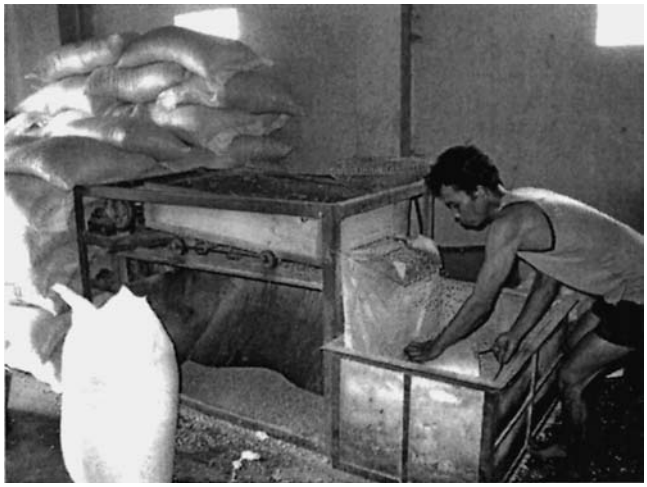

Figure 1 Screening of soybean to remove any foreign matter (From the "tempe murni" industry in Yogyakarta).

There is the possibility that some mold growth inhibitors, soluble in water, are removed by discarding the soaking water. Soaking after boiling and dehulling is mainly for the acidification process or prefermentation for the support of the growth of mold and the prevention of several pathogenic and spoilage bacteria. The other aim of soaking is to support suitable flavor and texture of tempe. The bacterial fermentation during soaking is very important and generally involves one or two soaking periods before and after

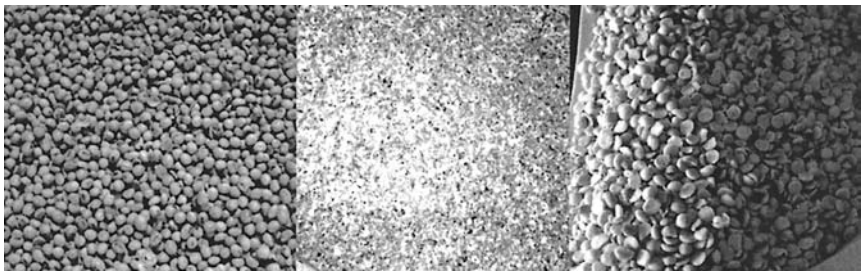

Figure 2 Whole soybean, hulls (center) and dried dehulled bean.

dehulling (6–12 h). Another reason for encouraging acid fermentation or artificially acidifying the bean is that the mold is very proteolytic; deamination following hydrolysis releases ammonia, causing the pH to rise. Above pH 7.0, sufficient free ammonia is released to kill the mold. A lower initial pH requires a longer fermentation time before ammonia is liberated (14).

3. **Boiling.** The aim of boiling the beans is to partially cook the beans, as a result of which the resulting tempe has a good flavor and soft texture. Sutardi and Buckle (38) reported that boiling in excess water for 5 min without pressure produced a satisfactory texture. To obtain a uniformly moist substrate, cooking in excess water is easier than steaming. During boiling, the hulls begin to loosen and can be rubbed off by hand, but many loosened skins are mixed with the beans. Substances that can disturb the fermentation can also be removed during boiling and dehulling. Removal of this material is necessary, as was demonstrated when soybean grits were boiled with soak water until the excess water was evaporated, with no loss of solids. However, in this substrate, the mold showed poor mycelial growth and the product had an unpleasant odor and astringent taste. If the soak water and boil water were discarded, a high-quality product with good mycelial growth was obtained. It was concluded that removal of heat-stable, water-soluble factors is required for the fermentation of soybeans into tempe (39,40).
4. **Dehulling and washing.** There are two different dehulling processes: dry and wet. Dry dehulling is done after the bean is dried or before any hydration and is a desirable and efficient method, provided that suitable mechanical equipment is available. Dry dehulling is carried out by passing preheated and cooled soybeans through a burr mill, properly adjusted to loosen the hulls without cracking the cotyledons (41). The hulls are then removed from the cotyledons by passing the mixture over a gravity separator. The dry-dehulled cotyledons can be stored until they are made into tempe. It is also possible to remove the hulls from dry-dehulled soybeans by placing the beans in water and floating the hulls off during the soaking and boiling steps.

   Wet dehulling is always done after a hydration or soaking procedure and requires no devices other than the hands or feet to rub the hulls from the cotyledons and a supply of water for floating the hulls away. Traditionally, Indonesian people dehulled soybeans after soaking or boiling if the soaking process is done only once. It is done normally by hand, but for large quantities, the beans are

dehulled in a bamboo basket by treading on them at the edge of a stream of water. The seed coats, thus freed from the beans, float away (42). Dehulling can be improved by using a simple roller mill, with the distance between rollers adjusted to somewhat less than the size of the soaked soybeans. In such a mill, the pairs of cotyledons are slightly pressed from each other by rotating cylinders, and at the same time, the hulls are removed by floating away with the water. An abrasive vegetable peeler and properly spaced burr mill are also useful to loosen the hulls from soaked and boiled soybeans.

Wet dehulling is available in almost all of the small tempe factories in Indonesia, whereas dry dehulling is used in the higher-capacity factories processing over 1 ton of soybeans per day and in the Japanese tempe factories, such as Maruzan Ai Co.

5. **Steaming or boiling.** The aims of the steaming or boiling process are mainly to destroy contaminating bacteria that might interfere with subsequent fermentation, to destroy trypsin inhibitors, and to release some of nutrients required for mold growth. This process also enhances the softening of the beans. Sutardi and Buckle (38) reported that a 30-min steaming produced a satisfactory texture for the production of tempe. A temperature of 100°C for 30 min is required to completely destroy mold-growth inhibitors (40). The steaming and boiling processes after dehulling are very important for mold growth and producing the specific flavor of tempe.
6. **Inoculation.** Drained and cooled soybeans are mixed with prepared inoculum, ensuring that the inoculum is well distributed over the surface of the bean. The types of inoculum that are used are leaves (*hibiscus* and teak), powdered tempe, and aqueous spore suspensions. The common inoculum used in Indonesia is powdered "ragi" tempe. The dried powder, about 1–3 g for 1 kg of dehulled beans, is sprinkled over the surface of the cotyledons and thoroughly mixed to distribute the spores over the surface of all of the beans.
7. **Packaging.** After inoculation of the soybeans, it is important to pack them to obtain a final product in which a white mycellium has developed abundantly and has bound the soybeans into a compact cake. An equilibrium should be maintained between keeping the beans moist and allowing sufficient contact of the beans with air. Unrestricted air supply and dehydration of the beans result in poor mycellium growth and excessive sporulation, whereas too little contact with air suppresses mold growth, such as when the soybeans layer is too thick, in which case mycelium growth is poor in the center of the cake. The traditional method of packaging is to wrap small amounts of inoculated soybeans in folded banana, hibiscus, or

teak leaves or bamboo tubes, although the plastic bag is now common packaging throughout the villages in Indonesia.

Martinelli and Hesseltine (43) reported that of all of the types of fermentation container tested, plastic bags and cellophane tubes were the most satisfactory, being inexpensive, easily handled, not requiring sterilization, and producing a very attractive tempe. Steinkraus et al. (44) used covered stainless-steel cake pans (25 cm × 35 cm × 5 cm deep). Each pan could accommodate 100 g of hydrated soybean cotyledons, leaving enough head space so that diffusion of oxygen through the loose edges of the top allowed satisfactory growth of the mold. Later, Steinkraus et al. (41) developed a small-factory process in which dryer trays were used for the fermentation.

8. **Incubation or fermentation.** The last essential step in tempe production is the fermentation that develops in the mass of prepared soybeans under optimum conditions. The incubation takes 36–48 h at a room temperature of about 25–30°C. The conditions for producing tempe are quite flexible as long as the overall growth requirements of the mold, which is the essential micro-organism in the tempe fermentation, are considered. The tempe should be harvested as soon as the bean cotyledons have been completely overgrown and knitted into a compact cake. The cotyledons should feel soft and pasty when pressed and the pH should have risen to about 6.5.

V. INDIGENOUS PROCESSES: RAW MATERIALS USED IN ANCIENT TIMES AND TODAY

The indigenous processes of tempe fermentation are basically the same throughout Java since ancient times, they differ in several minor ways, especially the preparation of the bean and equipment needed.

Tempe traditionally and originally was prepared using soybeans as the raw material. A number of tempe producers mix or substitute soybean with other legumes or cereals in order to promote the utilization of alternative inexpensive legumes and extend low-cost protein sources. The varieties of tempe can be grouped into five basic types according to the primary ingredient used: legumes, grains and soy, grains, press cake residues, and nonlegume seeds. Each variety can also experience changes in its basic nature depending on (1) the type and amount of extenders or adulterants that are mixed with the

basic ingredients, (2) the stage in which the tempe is consumed, and (3) the type of wrapper or packaging in which it is prepared and sold (13).

Legume tempe, especially soybean, is the major tempe manufactured. Soy tempe is the first variety developed and the most popular and important protein food for humans. A number of different towns in Indonesia are well known for the unique varieties of soy tempe, such as Malang (East Java), Bandung (West Java), Yogyakarta, and other places in Central Java. The second most popular legume tempe is velvet bean tempe (*Mucuna pruriens*). Velvet beans are boiled before fermentation, the thick, hard seed coats removed, and the cotyledons then soaked for 22 h in water that is flowing or changed several times to remove the cyanogenic content of the bean. This kind of tempe is popular in the Yogyakarta region, as shown in Fig. 3.

Wing bean tempe is a source of low-cost, high-quality protein in the tropics. The wing bean (*Psophocarpus gonolobus*) has a protein content equal to that soybean and the seed contains 20–40% more essential amino acid lysine than soy. The hulls are somewhat harder to remove than soybeans, and being relatively heavy, they do not float off easily. If boiled in water containing a small amount of baking soda, the beans will soften well and removal of the hulls is facilitated. This kind of tempe is found in the dry land area. The bean and tempe product are shown in Fig. 4.

Leucaena tempe or "tempe lamtoro" is made from leucaena seed prepared by the people in the Wonosari area or Yogyakarta region. This kind of tempe is not popular because of the content of a toxin called mimosine, which may cause loss of hair. During World War II, this kind of tempe was used as food in many areas of Java, especially Central Java.

Figure 3 Velvet beans and its tempe product (tempe benguk).

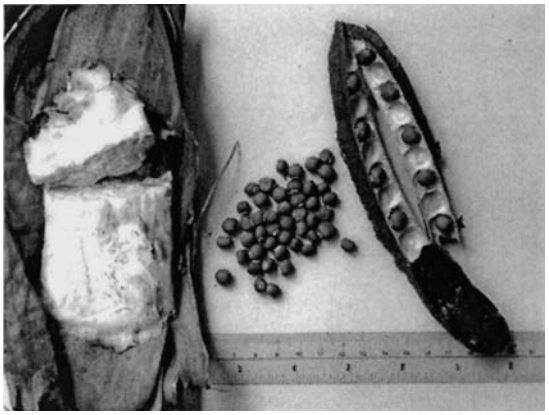

Figure 4 Wing beans and its tempe product (tempe kecipir).

Mung bean tempe or "tempe kacang hijau" is made from the seeds of *Vigna radiata*. This kind of tempe is only found in the local area around Yogyakarta, and not other areas of Java.

Lima bean tempe, local name "tempe koro," is made from the seeds of *Phaseolus lunatus*. This kind of tempe is also popular in the dry land area, where the bean is easily grown. The bean and tempe product are shown in Fig. 5.

Press cake tempe is made from tofu waste, peanut oil, and coconut oil. There are three kinds of tempe; Tempe gembus is made from soy curd/tofu solid waste, as shown in Fig. 6; Tempe bungkil is made from peanut press cake mixed with copra press cake; and tempe bongkrek is made from coconut press cake, usually the fresh residue from coconut milk production. These kinds of tempe are popular among the people of Central Java, and are shown in Fig. 7.

The unique process of preparing traditional soy tempe is the dehulling process, where the bean, after boiling, is placed into woven bamboo baskets and the people step barefoot into the basket, tread the beans underfoot for 3–5 min, and the loosened hulls are floated away. Currently, this process is not used. Now, they use simple hand dehulling machines. Using a metal plate or small bucket, one-half of the dehulled beans are scoop into a colander and immersed in a the pot full of water and the hulls floated off by lifting up a corner of the colander and stirring or fanning the floating hulls toward the far edge. This process is repeated four or five times until most of the hulls have been removed. The beans are then moved to a metal or clay pot and soaked overnight. This process is still used for tempe making in the villages. Most use

Figure 5 *Phaseolus lunatus* and its tempe product (tempe koro).

a simple dehulling machine. Only a few developed factories use dry dehulling; most use wet dehulling, as shown in Fig. 8.

After boiling for 30 min the next day, the cotelydons are cooled and inoculated with "usar" inoculum—covered hibiscus leaves. The usar starter culture is shown in Fig. 9. Two hibiscus leaves ("usar") inoculate about 2 kg of beans. Since ancient times, especially in Central and East Java, tempe has

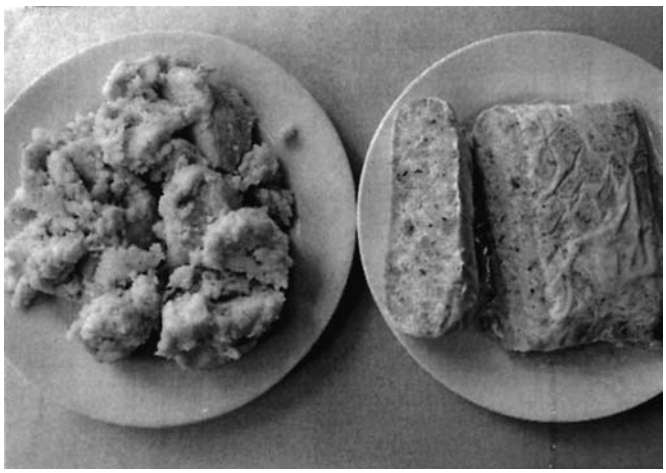

Figure 6 Soy curd (tofu) solid waste and its tempe product (tempe gembus).

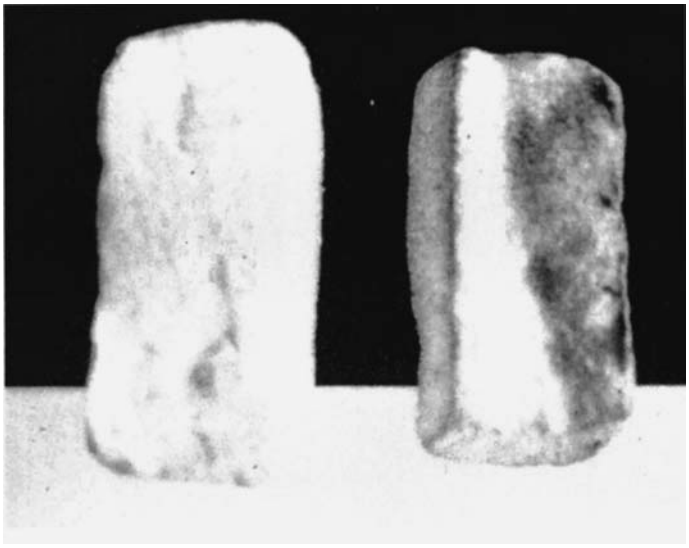

Figure 7 Tempe bongkrek from Purwokerto.

Figure 8 Hand dehuller machine.

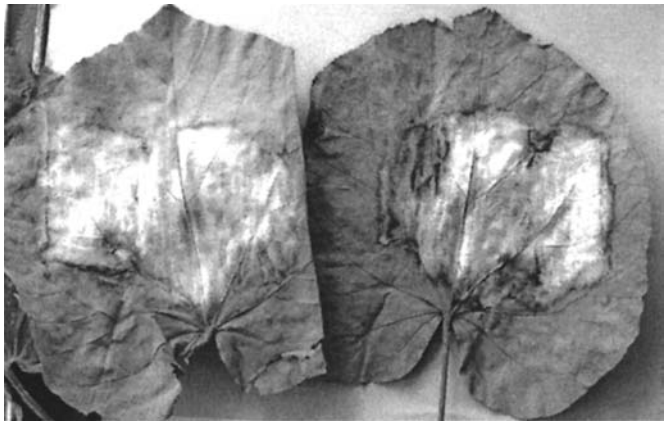

Figure 9 Usar starter culture for tempe.

been incubated and wrapped in banana leaves in various sizes and shapes, such as a square or triangle, as shown in Fig. 10.

The most popular way of making tempe inoculum since ancient times involves the use of hibiscus leaves (*Hibiscus tiliaceus* Linn), commonly called waru in Java. This leaf is used because the underside of each leaf is covered with downy hairs (trichome) to which the mold mycelium and spore can

Figure 10 Tempe wrapped in banana leaves.

adhere. In East Java, they used teak (*Tectona grandis*) leaf instead of hibiscus because of the same properties. The method for preparing hibiscus inoculum leaves is as follows: Place the hibiscus leaf on the bottom of the tray with the underside of the leaf facing up. Sprinkle 30–40 inoculated soybean halves or whole beans over the surface of the leaf. Then place a second leaf of about the same size underside down on top of the first leaf to form a sandwich of two leaves with inoculated soybeans between them. Proceed to make 50–60 such sandwiches until the tray is full, up to four to six layers. Fold the side ends of the plastic over the top of the leaves and place the tray on a double thickness of gunny sacking (jute bags) on a clean section of floor or on a sturdy bench or shelf. The incubation of this tray with a single layer is about 48 h or until the mold has sporulated. The downy hair of the leaf should be covered with a black or dark gray mycelium, and spores are dried under sunlight. The dried leaves are then ready to use for inocula.

In 1935, the Dutchman Burkill (26) wrote that to make the inoculum, a portion of the older preparation of tempe is wrapped in a rather young teak leaf freely punctured with holes. This preparation is allowed to dry for 2 days, during which the fungus spreads to the leaf. In East Java, some producers use teak leaf wrapper as the inoculum.

The wrapping leaves depend on the variety of tempe substrates. For example, tempe made from soy curd/tofu solid waste, or tempe gembus, is wrapped with bamboo leaves. Tempe benguk is wrapped with hibiscus leaves or teak leaves. Banana leaves play an important role in the incubation techniques. Other leaves are used for certain varieties of tempe.

VI. MODERN INDUSTRIAL AND COMMERCIAL PROCESSING METHODS

The development and establishment of the modern tempe industry is about bioprocessing and equipment development in the food industry. The traditional tempe industry can be converted into a modern tempe industry by utilization bioprocessing innovation to improve and assure quality and to introduce new tempe products (45).

The bioprocessing development activities are defined as attempts to create new bioprocesses and equipment rather than to achieve improvements in an existing traditional tempe industry. The use of biotechnology in the manufacture of *Rhizopus* sp. inoculum has been practiced for many years.

The hygienic design factors in the modern tempe industry must be considered in terms of site selection, housing, and layout of the processing equipment. The construction and installation of new plants for tempe production

must follow a good hygienic design to avoid tempe contamination. Such a modern tempe industry can produce a competitive quality of tempe which can be exported to other countries. The various types of equipment in the modern tempe industry can be divided into the following:

1. Equipment (nonbioprocess) such as dehuller, separator, and aspirator
2. Equipment (bioprocess) production of starter culture
3. Equipment for incubation, especially controlled incubator temperatures (30–37°C) and humidity control
4. Equipment for utilities, including a steam boiler, electrical power, and compressed air
5. Equipment for piping and the belt conveyor

Soybeans are transported by a belt conveyor into a hopper. The hopper containing soybeans is transported by an electric hoist and dipped in hot water (80°C) for 30 min in stainless-steel tanks. Soybean handling equipment is completed with an electric hoist to carry the hopper. The hopper can act as a storage container for intermediate products. A dehulling machine for soaked soybeans will separate the hulls and the cotyledons. A dipping tank containing a water and lactic acid solution is used to dip dehulled soybeans and skin for 3 h. An electric hoist can bring this hopper containing soybean skin and dehulled soybean to a flotation tank in order to separate the soybean skin and dehulled bean; it is then transported by an electric hoist to the retort. The sterilization in the retort is held for about 10 min. This retort consists of double jacks that can accommodate the hopper of dehulled soybeans. The sterilized soybeans are then transported by an in lot conveyor, which can act as a cooling system for the mixers. The inoculation system is used spore solution into the sterilized soybean. This material is then transported to the table distributor and transferred to the stainless-steel tray; Steinkraus et al. (44) used covered stainless-steel cake pans (25 cm × 35 cm × 5 cm deep). Each pan accommodates about 100 g of sterilized soybean cotyledons, leaving enough head space so that diffusion of oxygen through the loose edges of the top allowed satisfactory growth of the mold. The development of the fermentation container in a small factory process is done in the dryer trays (35 × 81 × 1.3 cm) (41). The trays are lined with waxed paper to maintain moisture in the beans and prevent excessive aeration, which leads to rapid sporulation and darkening of mycelium as the mold grows. The developed process of tempe factory is shown in the flowsheet in Fig. 13 (the large-scale tempe process at the Farm, Summertown, Tennesse). In the Maruzan Ai factory, the fermentation containers were perforated plastic trays covered by perforated plastic, and the flowsheet of tempe processing is shown in the Fig. 12.

Steinkraus (14) stated that any container can be used for fermentation as long as it permits access of sufficient oxygen for growth but not for sporulation, controls the temperature, and retains the moisture content without excess free water in the bean so that the fermenting tempe remains clean and wholesome.

The incubation temperature mainly in Indonesia is ambient or room temperature, whereas the normal temperature of 25–37°C is suitable for the growth of *R. oligosporus*. Tempe should be harvested as soon as the bean cotyledons have been completely overgrown and knitted into a compact cake.

VII. CHANGES FROM INDIGENOUS TO MODERN PROCESSING METHODS

The traditional tempe industry has been known and disseminated by the Javanese for several hundred of years as a cottage industry on a household scale. The technology applied to making tempe can be easily replicated and used in other similar conditions. Tempe making has spread throughout Indonesia because of the simple technology involved. It is likely that tempe will be developed throughout the world in a similar manner. Efforts to patent tempe processing is futile because the technology belongs to everybody and the patent holder would have difficulty protecting his property rights. On the other hand, biotechnology can be used to improve the inoculum which makes a tempe variant possible that may be protected by an international patent (15).

The common problems faced by such a production scale usually are that the economic efficiency is low, there is a lack of quality standardization, sanitation and hygiene during processing are often poorly managed, and the concern for waste and environment management is minimal. The intervention for improvement is done through promoting cooperative activities among the processors. The support from the government has been in the form of securing the availability of good quality raw material, developing good cooperative organization and management, improving processing technology, and introducing the importance of waste management. The Indonesian government policy for a small production business should be securely participated in the production system, while the production monopoly by a large enterprises has to be avoided. The intervention of the government for improvement is done through promoting cooperative activity among the processors.

The changes from indigenous to modern processing methods is mainly by improving equipment needed, maintaining a sanitary and hygienic environment, inoculum by selected micro-organisms, or the method of pack-

aging. The changes in the method from traditional to modern processes are shown in the flowsheets in Figs. 11–14, and 30.

The main changes in the tempe industry from the indigenous to modernized processes are as follows.

A. Treatment of Raw Material

In the traditional method, soybeans are soaked, dehulled, and then boiled. Soaking in the traditional way before boiling is shown in Fig. 15, and the

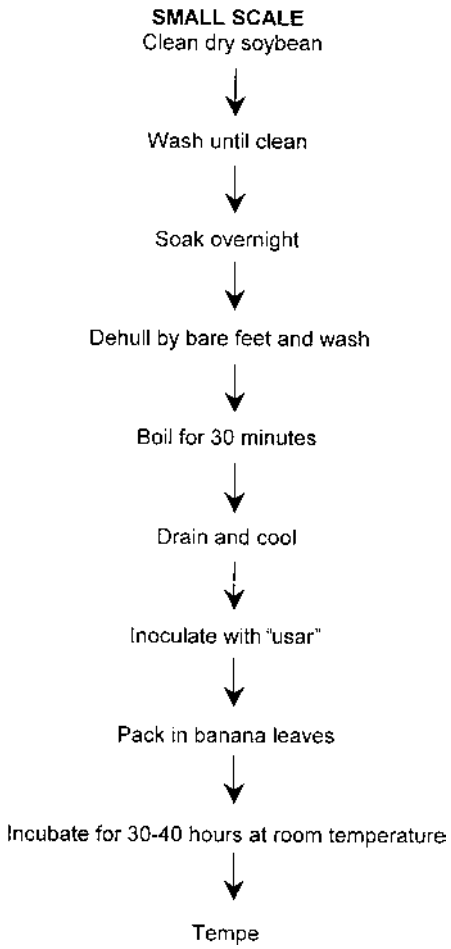

Figure 11 Flow sheet: traditional tempe production since ancient times.

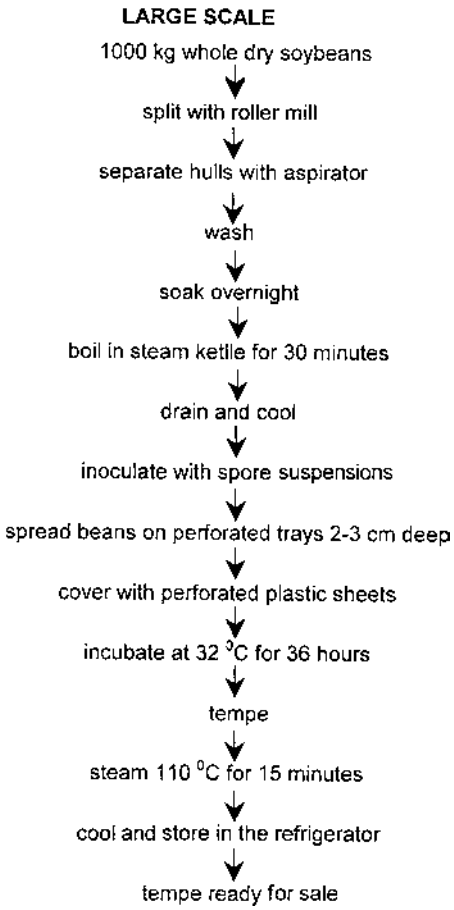

Figure 12 Flowsheet: tempe process (Maruzan Ai Company, Japan).

improved process after dry dehulling and soaking overnight is shown in Fig. 16. Soaking overnight or about 12 h or more before dehulling is to make the process easier. The dehulling process, where the beans after soaking are placed into woven bamboo baskets and then the people step barefooted into the basket, as they did in a flowing river. Around that time to make tempe only once for soaking and once for boiling. They used local soybeans, which were a size smaller than imported beans. The boiling process is not controlled, usually using kerosine or firewood for fuel, and takes approximately 30 min. Use of firewood leads to product contamination in open cooking vessels. Control of cooking temperature is difficult with open fires and produces an

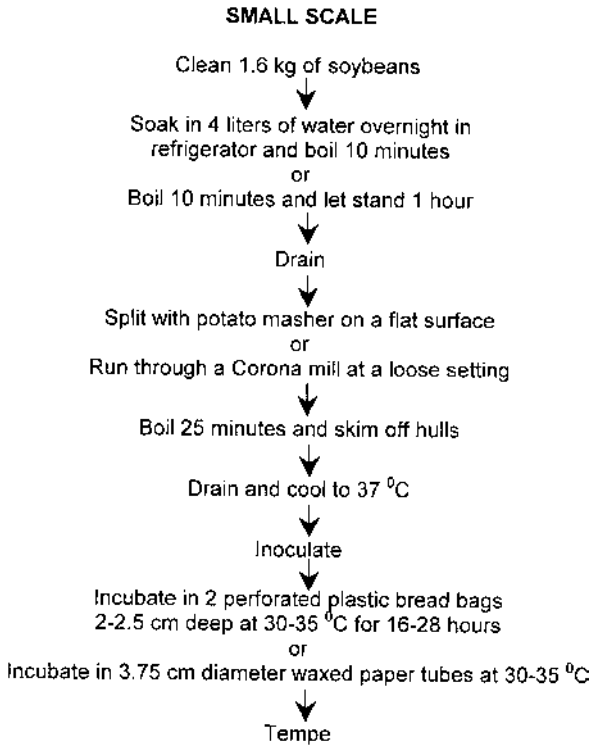

Figure 13 Flowsheet: tempe production (The Farm, Summertown, Tennessee). (From Bates et al., 1977, reprinted in Ref. 14.)

uncomfortable work environment because of smoke and heat. Since 1970, many researchers helped improve tempe processing. Therefore, the dehulling process was improved by using a hand peeler; furthermore, they improved an electrical dehuller, and some factories used dry dehulling. The time of soaking and boiling also differed according the variety of raw materials, and the tempe industry generally prefers soaking twice and boiling twice to achieve more softening of the beans.

In the Tempe Murni factory, they do the first soaking before the first boiling, followed by the second soaking before the second boiling or after the first boiling. The first and second boiling of the cotyledons is shown in Figs. 17 and 18. For easier transportation, they use a conveyor to transfer the boiled beans to the drain and cooling tray, as shown in Fig. 19, and the drain and cooling tray is shown in Fig. 20.

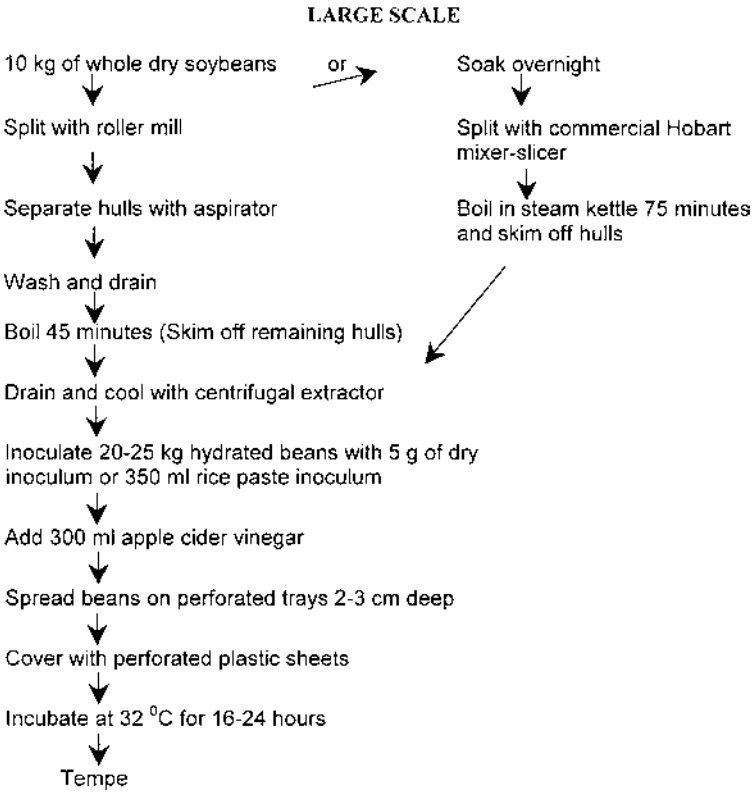

Figure 14 Flowsheet: tempe process (The Farm, Summertown, Tennessee). (From Bates et al., 1977, reprinted in Ref. 14.)

B. Starter Culture

Traditionally, the people prepare the starter culture in the hibiscus leaf (viz, usar) or by collecting small pieces of a previously fermented tempe or wrapper of former tempe. The collected pieces of tempe are air-dried or sun-dried, ground to a smooth powder, and used as inoculum. In some cases, the surface of a previously fermented tempe cake, where most of the mycelium is found, is sliced, sun-dried and ground. At present, Indonesia has a ragi tempe factory that produces starter culture containing the improved strain of *R. oligosporus* and also some important cultures for tempe production. The common ragi tempe in Indonesia is the Raprima inoculum produced by The Institute of Science at Bandung, as shown in Fig. 21. Inoculation was done after the

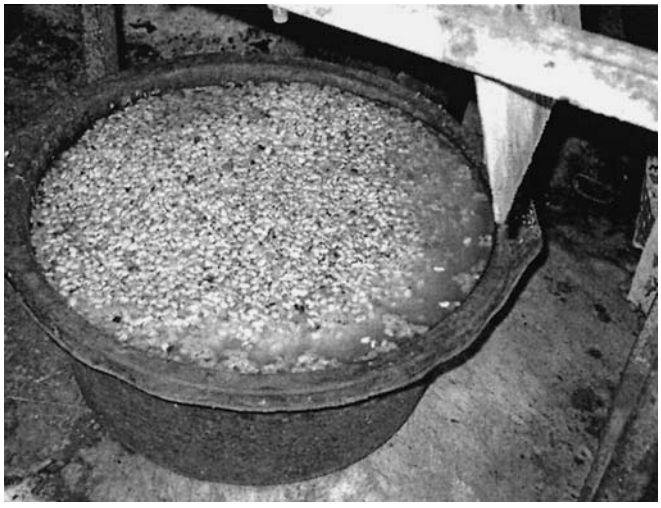

Figure 15 Soaking whole soybeans before boiling in the traditional way.

Figure 16 Soaking soybean cotyledons after dry dehulling.

Figure 17 The first boiling of cotyledons, after the first soaking.

boiled beans cooled and the surface dried, as shown in Fig. 22. tempe factory in Japan uses spore suspension of *R. oligosporus* as the inoculum, as shown in Fig. 23.

C. Packaging and Incubation

The packaging of tempe since ancient times is banana leaves, as shown in Fig. 24, or other leaves such as hibiscus, teak, and bamboo. At present, the

Figure 18 The second boiling of cotyledons after the second soaking.

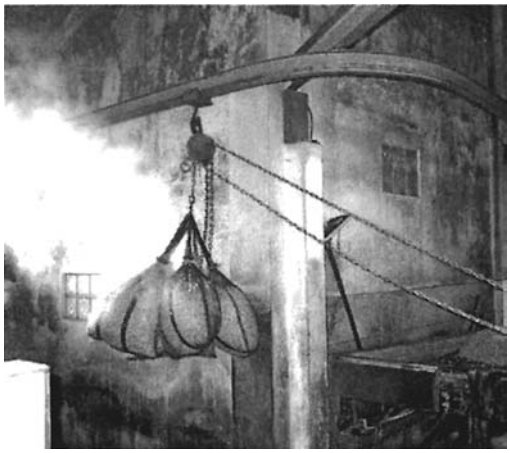

Figure 19 Transferring the boiled cotyledons.

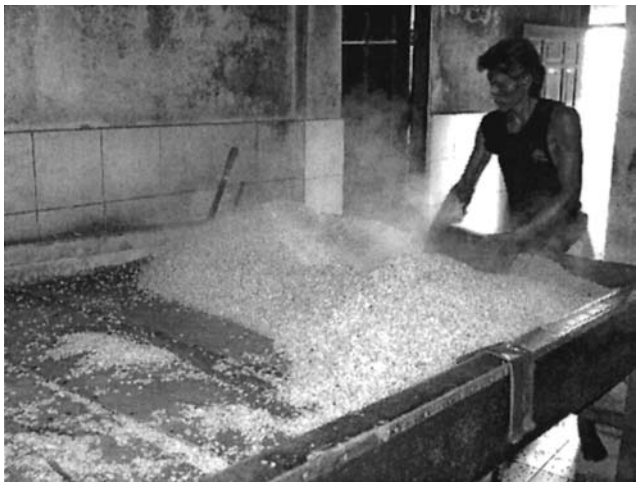

Figure 20 Draining and cooling tray.

Figure 21 Raprima inoculum from Bandung, Indonesia.

industry uses perforated flexible plastic bags, as shown in Fig. 25, or perforated plastic tubes, wooden trays covered with plastic sheeting, and plastic trays covered with perforated plastic (Japan), as shown in Fig. 26, and stainless-steel cake pans with sliding covers (14). However, several small industries in the village still use many kind of leaves, especially banana leaves.

Figure 22 Inoculation of boiled beans with "ragi tempe."

Figure 23 Inoculation with spore solution.

The tempe wrapped with banana leaves was incubated in a bamboo basket, as shown in Fig. 27, whereas plastic bags were incubated in a bamboo rack, as shown in Fig. 28.

The final tempe in a plastic bag is sliced is shown in Fig. 29. The figure shows that the mycelia of *R. oligosporus* knits the cotyledons together to yield a compact cake. During fermentation, the mycelium penetrates several layers of cells into the soybean cotyledon. Infiltration occurs to a depth of 2 mm in 40 h for soybean tempe (46).

D. Sanitation and Waste Management

The immediate environment of tempe factories is not maintained in a way that will promote sanitation in the processing plant. Most of the traditional

Figure 24 Wrapping the cotyledons in banana leaves at village level.

Figure 25 Packing in a perforated plastic bag.

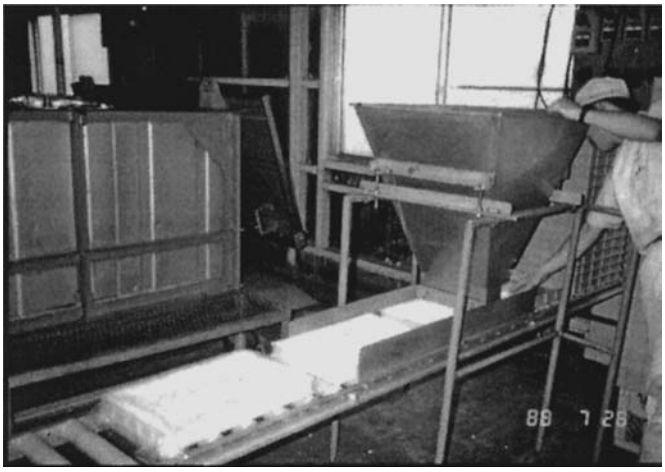

Figure 26 Packing in plastic trays covered with perforated plastic.

Figure 27 Incubating tempe in bamboo baskets.

producers are not aware of solid and liquid waste management. Recently, the government of Indonesia organized several small-scale factories into one area so that they can manage their waste treatment. The quality of tempe depends on the sanitation of the factory.

Among the tempe processes in Indonesia, the "Tempe Murni" Company in Yogyakarta is the most modernized compared to the other tempe companies. The capacity is about 5 tons of soybean a day with 30 workers and the flowsheet of its tempe processing is shown in Fig. 30.

The strategy for modernizing the existing tempe industry basically consists of three important aspects; increasing the scale of production, improving processing technology, and introducing suitable management. These factors can be considered as the source of improving efficiency and quality of tempe production.

The scale of production is an important factor in modernization. The producer gains efficiency at a lower cost from larger-scale production.

Technology is an important factor in improving efficiency and quality. The improvement of technology is aimed at reducing the time and cost of boiling and dehulling. The constraint of improvement in the boiling and dehulling process is partly due to the high cost of processing tools, especially when applied to small industry. The Indonesian government has supported a prototype tempe factory at Cibitung, near Jakarta. This factory was designed as an ideal example of a modern tempe factory.

The other factor for modernization of the tempe factory is the human workers. Most of the producers learned the technology from parents or rela-

Figure 28 Incubating tempe on bamboo racks.

tives. Formal training and learning process should be part of modernization. The training will provide basic knowledge of the technology and quality concerns to new participants in the industry.

Nout and Kiears (47) reported that in Belgium, an elegant semicontinuous process line is commercially used for production of 600 kg of tempe per day. Dry-dehulled beans are soaked overnight in the hopper and transported by belt conveyor through a boiling water bath in a period of 20 min. Next, they are drained, dried, and cooled under a fan; if needed, the bean temperature can be adjusted using a heated section. Inoculation takes place using a mechanical dispenser, and the inoculated beans are filled into perforated

Figure 29 Tempe wrapped in plastic bags.

5-cm-diameter polyethylene tubing. The incubation of these packets take place at 32°C for 24 h in a carefully designed room with forced ventilation. The tempe products are smoked tempe with a sausage taste, various salads, burgers, and meatloafs. In order to obtain the reddish color of bacon, a mixed culture is used consisting of *R. oligosporus* and *Neurospora intermedia* or *N. sitophila*.

VIII. MAJOR PROBLEMS IN INDUSTRIALIZATION

Tempe has been produced traditionally in a small-scale unit, with the range of capacity of less than 20 kg of soybeans as a raw material per day to the largest capacity 5 tons of soybeans a day. It is the government's policy that the small tempe production business should be securely protected in the production system. A production monopoly by a large enterprise has to be avoided. On the other hand, we consider that with the cultural and economic characteristic of tempe as described earlier, it would be unproductive or even self-defeating to change the processing and marketing of tempe into a modern, sophisticated, profit-oriented, centralized institution.

The number of tempe producers has increased in all large cities of Java and the production is mostly small scale such that the management of production especially influences the quality and hygienic standard of the

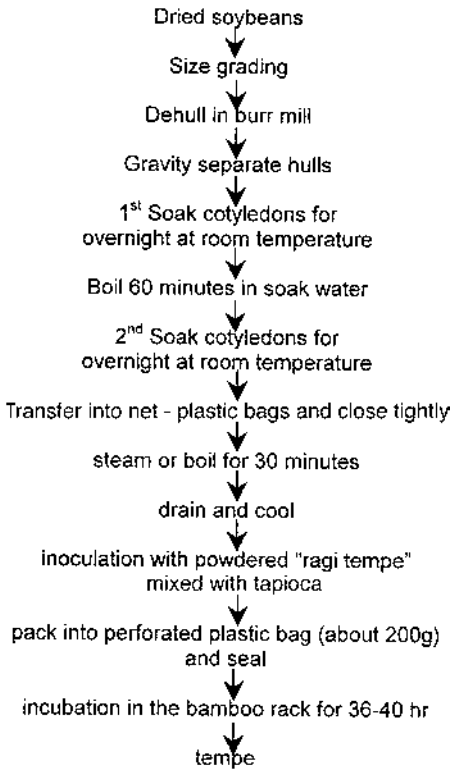

Figure 30 Flowsheet: tempe processing in Tempe Murni, Yogyakarta (data from October 2002).

industry. The immediate vicinity of enterprises is not maintained in a way that will promote sanitation in the processing plant.

Historically, the small-scale tempe industry has developed into, and stabilized at, subsistence level. This industry serves the domestic market. The consumer accepts the product in the present form, despite the many deficiencies that can be readily seen in the processing of the product and its quality. No competing products of better quality are on the market. Hence, the producer does not feel any pressure from the consumer to bring about change voluntarily.

The major problems in the development of a small-scale tempe industry are upgrading processing equipment and technology. The strategies are to improve the present conditions of plant sanitation and hygiene, to improve currently used processing equipment and methods, and to introduce improved product quality and prolonged shelf life of the product.

IX. MICROBIOLOGY AND BIOCHEMISTRY OF FERMENTATION

Tempe is made by fungal solid-state fermentation, mainly of cooked soybeans. The microbiological composition of tempe includes high numbers of bacteria and yeasts in additional to the molds. Microbial interactions are clearly important during tempe fermentation. The activity of lactic acid bacteria during the initial soaking of soybeans can have a direct impact on the proliferation and possible toxin formation of potential pathogens and toxigenic bacteria in tempe. Soaking of soybeans can be considered part of overall fermentation process because the micro-organisms not only produce organic acids that lower the pH but also utilize some of the nutrients and other growth factors influencing subsequent growth of micro-organisms during solid-state fermentation (14).

The microbiological aspects of the tempe process can be divided into two parts: the bacterial interactions of relevance to public health and the environmental parameters that influence the fungal growth during the fermentation (47). A Netherlands Food Inspection team analyzed 11 samples of commercial tempe of Netherlands origin, and found that *Staphylococcus aureus* and *Bacillus cerreus* occurred at 10^5 cells/g in 13% and 11% of all samples, respectively (48).

Mulyowidarso et al. (49) reported that micro-organisms reached level of 10^7 to 10^9 cells/mL during soaking. The pH falls to 4.5. *Lactobacillus casei, Streptococcus faecium*, and *S. epidermis* were responsible for most of the pH drop. *Streptococcus dysgalactiae*, *Klebsiella pneumoniae*, *K. azonenae*, *Enterobacter cloacae*, *E. agglomerans*, *Citrobacter diversus*, and *Bacilus brevis* were also present, along with several yeast types—*Pichia burtonii*, *Candida diddensiae*, and *Rhodotorula rubra*.

Tuncel et al. (50) reported that the acid fermentation by lactic acid bacteria during the soaking of soybeans contributed to the microbiological safety and acceptability of tempe. Recently, many tempe factories are doing twice as much soaking to improve the acceptability of tempe. The bacteria growing during soaking are very important to the production of a good quality tempe. Nout et al. (51) developed a simple technique to enrich a lactic acid bacteria starter in soyabean soak water or accelerated acidification by a "back-slopping" process. This process was done by inoculation of soak water with 5% of soak water from a previous production.

During the soaking and cooking of soybeans, a considerable amount of dry matter is lost (52). Although some solids are lost during fermentation due to microbial metabolism, the solubility of solids also increases in the tempe. The absorbability of tempe is slightly increased, and this condition indicates possible better digestibility after fermentation.

Earlier studies on tempe before World War II and some reports around 1960 revealed that *Rhizopus oryzae* was the dominant isolate from highly preferred tempe samples, such as tempe Malang and tempe Purwokerto.

Dr. Keith H. Steinkraus isolated *R. oligosporus* NRRL strain 2710 (now *Rhizopus microsporus* var. oligosporus) from the dried Indonesian tempe brought to Cornell University from Indonesia by Ms. Yap Bwee Hwa and identified by Dr. C.W. Hesseltine at Northern Regional Research Laboratory (NRRL) of the United States Department of Agriculture (14). It has unique characteristics such as the ability to grow rapidly at temperatures from 30°C to 42°C, high proteolytic and lipolytic activity, production of strong antioxidants, ability to produce the typical tempe cake with its characteristic aroma and flavor, and growth on wheat and other starch cereal substrates without producing noticeable amounts of organic acids that would cause a sour product (14).

This species was then used by many Indonesian microbiologists for their studies on tempe, especially in the studies of nutritive value. Wang et al. (53) and Hesseltine et al. (54) conducted studies on the mass production of fungal spores. At present, an inoculum for tempe, composed of *R. oryzae* and *R. oligosporus* has been developed by the National Chemistry Institute–Indonesian National Institute of Science (LKN–LIPI) and distributed to tempe producers to prevent failure of their products (55).

Rhizopus molds are primitive coenocytic micro-organisms which grow fast on any substrate, are often the first invaders on natural organic substrates, and are very easily isolated. The bundles of sporangiospores which bear the sporangia are opposite the rhizoids, which can be varied from very simple (*R. arrhizus*) to well developed (*R. stolonifer* and *R. oryzae*) or rosette forming (*R. microsporus* group). The sporangiospores vary from subglobose, globose, to hexagonal with a smooth or striated surface. Most studies on species of this genus were carried out by Boedijin (1958), Inui (1965), Zycha and Siepmann (1969), Scholer (1971), Zheng (1995), Schipper and Stalpers (1984), Ganjar (1995–present), and Santoso (1996–present).

The benefit of the traditional tempe process is a microbial ecological process where the molds work in succession. It starts with *R. oryzae,* a strong amylase, protease, and pectinase producer, followed by *R. arrhizus*, which is also very active like *R. oryzae*. During fermentation, the temperature increases which is suitable for *R. oligosporus*, to complete the tempe fermentation. Arbianto (56) mentioned the exchange of genetic information occurring through the heterocaryotic hyphae of mold and he confirmed these results by experiments on the recombination of the protoplast fusion between *R. oryzae* and *R. oligosporus*. He concluded that the preparation of traditional inoculum through hibiscus leaves, as it is usually done in the villages, is a valuable effort to preserve the biodiversity of the genus *Rhizopus*.

Several researchers found that tempe inocula contain molds other than *R. oligosporus*.

Gandjar (57) isolated molds from tempe Malang, and tempe Purwokerto and found mainly *R. oryzae* and *R. arrhizus* as the dominant molds. The tempe products were well known among the Javanese people because of their delicious taste. However, recently, the dominant mold in the same areas was found to be *R. microsporus* var. *oligosporus*. Fortunately, in places outside Java, specifically in Aceh and North Sulawesi, other specieas of *Rhizopus* were found, namely *R. microsporus* var. *chinensis* and *R. microsporus* var. *rhizopodiformis*, respecively (58). These species are already fully adapted to that environment and should not be replaced by the commercial inoculum.

Kiers et al. (59) found that several species of the genera *Mucor* and *Rhizopus* are capable of making tempe, although the *Rhizopus* spp. are considered to be most suitable. In the soybean substrate, the most suitable for making tempe is *R. microsporus,* followed by *R. oligosporus*. During fermentation, some solids are lost due to the metabolic activities, and the solubility is increased in the tempe. The absorbability of tempe is slightly increased, indicating a possible better digestibility after fermentation.

Primarily, *Rhizopus oligosporus* is used in tempe fermentation because of a rapid and successful fungal growth, germination of sporangiospores, and mycelial growth. Nout and Kiers (47) illustrated the influence of acidity, temperature, and the composition of the gas atmosphere on germination and growth of *R. oligosporus*. Resting sporangiospores germinate best in media of slightly acidic pH. Whereas acetic acid inhibits germ tube extension, lactic acid favors mycelial growth and allows a rapid start for fermentation. When the lactic acid fermentation is stimulated using "back-slopping, heterofermenters produce also some acetic acid and this results in a much longer lag phase of the fermentation.

X. OPTIMUM FERMENTATION CONDITIONS

The essential fermentation conditions for supporting the mold growth include the preparation of raw materials, culture conditions, fermentation containers, and the environment during fermentation.

1. Preparation of raw material for suitable growth of the mold. Sufficient hydration, dehulling, and cooking for softening of the beans, acidification to a suitable pH (4.5–5.3), and surface drying to inhibit bacterial growth are needed. The most important of this preparation is acidification of the beans during soaking. The growth of lactic acid bacteria inhibits pathogenic and toxigenic bacteria

such as *Salmonella*, *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas cocovenenans*, and *Clostridium botulinum*. Boiling and partial cooking are used for killing the contaminant micro-organisms, especially pathogenic ones, softening of beans and, support of sufficient flavor of the tempe or reduction of the beany flavor. The optimum condition for the preparation of softening of the bean is soaking overnight and boiling or partly cooking. The time of treatment affects the final product, especially texture and flavor.

2. Culture condition. A number of mold starter cultures have been selected for tempe fermentation. The availability of an appropriate starter culture is essential for producing a good quality tempe. Selecting potential micro-organisms and improving strains which produce flavor and vitamins, and increases the functional properties of tempe should be done. The utilization of a mixed culture could be done for better functionality of tempe.
3. Fermentation containers. There are several types of containers such as leaves, perforated plastic bags or tubes, plastic or stainless-steel trays can be used. Banana leaves support specific flavor development of tempe; therefore, the Javanese people prefer to consume tempe wrapped in those leaves. The optimum condition for the containers is sufficient aeration for the growth of the mold.
4. Environmental condition of fermentation. The temperature and relative humidity of the room are important. A suitable temperature for *R. oligosporus* is 25–37°C with sufficient but not excess moisture and oxygen. The rate of fermentation depends on the temperature. Water quality is an important consideration for safe life of the product. The main concern with direct use of groundwater is microbiological quality; therefore, the factory should upgrade plant sanitation as a first priority and concentrate on water quality as a second priority.

XI. BIOCHEMICAL CHANGES DURING FERMENTATION

During fermentation, the microorganisms, mainly *Rhizopus* spp., induce many changes in nutrient composition. This changes include protein, fat, and carbohydrate composition due to the production of a variety of proteases, lipases, and carbohydrases. A reduction in antinutritional factors and production of vitamins also occurred (33). Thus, during the fermentation, total transformation of the soybeans is brought about and, simultaneously,

nutritional value and digestibility are enhanced, making tempe suitable for use in different applications like dietetics and nutrition improvement (60).

Because of the process, different components are hydrolyzed into simple compounds; therefore, tempe processing has many advantages, such as easy digestibility. Regarding nutritive value, the tempe is rich in unsaturated fatty acids and vitamins and contains less antinutritive substances. The chemical changes occurring during tempe fermentation are shown in Table 6.

Biochemical changes as the result of high lipolytic and proteolytic activities of the proliferating mycellium within the soybeans characterize the fermentation into tempe. Fatty acids are liberated during fermentation, resulting in hydrolysis of over 30% neutral lipid, with a preferential utilization of α-linolenic acid (62). Nutritionally significant consequences of this enzymic action are an increased digestibility of the product and an accompanying elevated level of free fatty acids and soluble protein (41).

Table 6 Biochemical Analysis of Tempe

Nutrient value	Soybean (dried)	Tempe (dried)	Tempe (fresh)
Calorie content	392	—	157
Global composition (%)			
Protein	48.2	50.5	19.5
Lipid	23.6	19.3	7.5
Carbohydrate	28.5	30.2	9.9
Fiber	3.7	7.2	3.2
Ash	6.1	3.6	1.6
Soluble solid	14.0	34.0	28.0
Soluble Nitrogen	6.5	39.0	—
Vitamins (in 100 g)			
Thiamine (B_{-1})	0.05 mg	0.15 mg	0.28 mg
Riboflavin (B_{-2})	0.15 mg	0.85 mg	0.65 mg
Niacin (B_{-3})	0.67 mg	4.35 mg	2.52 mg
Pantothenic acid	0.46 mg	1.0 mg	0.52 mg
Pyridoxine (B_{-6})	0.08 mg	0.47 mg	0.83 mg
Biotin	34 μg	71.0 μg	53.0 μg
Folacine	25–30 μg	140–170 μg	0.1 μg
Cyanocobalamin (B_{-12})	0.15 μg	5.0 μg	3.9 μg
Vitamin A	—	—	42 IU
Minerals (in 100 g)			
Calcium (Ca)	254 mg[a]	347 mg[a]	142 mg
Phosporus (P)	781 mg[a]	724 mg[a]	240 mg
Iron (Fe)	11 mg[a]	9 mg[a]	5 mg

[a] Data from Ref. 61.

During the fermentation, the principal changes in carbohydrates are rapid removal of hexoses and the slow hydrolysis of stachyose. The mold used glucose, fructose, or galactose as a source of energy but does not utilize sucrose or raffinose under the same conditions (Sorensen and Hesseltine, 1996, in Ref. 14.).

In addition to nutrient constituents, tempe is also rich in active substances produced during tempe fermentation by micro-organisms. The active substances in general constitute a secondary metabolite which potentially have a specific influence on metabolism and a potential benefit for health. Currently, several active substances in tempe and its potencial for medical or health use are identified in Table 7.

In the traditional method of tempe preparation, there are many micro-organisms in addition to *R. oligosporus* such as other fungi, yeasts, and bacteria, which also contribute to the final fermentation products. The characterization of the micro-organisms followed by selection of those micro-organisms considered as critical play an important role in the final product. Furthermore, the selected micro-organisms should be formulated into tempe

Table 7 Active Substances Identified in Tempe

Active subtances	Potency/function	Ref.
Isoflavones: daidzein, glycitien, genistein and Factor-2	Antioxidant, antihemolysis, antifungi and anticancer	63–66
Unsaturated fatty acids: oleic acid, linoleic acid, and linolenic acid	Antioxidant, hypocholesteremic	67, 68
Fat-soluble vitamins: vitamin E (mixed with α-tocopherol) and β-carotene (pro-vitamin A)	Antioxidant, antihemolysis, protection,	69
Antibacterial compounds	Inhibits the growth of several bacteria	70
Ergosterol	Hypocholesteremic, pro-vitamin D	69
Vitamin B complex: thiamine, riboflavin, niacin, panthothenic acid, cyanocobaltamin, folacin	Metabolism (coenzyme), pernicious anemia	69,71,72
Enzyme: protease, lipase, amylase, glycosidase, superoxide dismutase	Metabolism/hydrolysis	73,74

Source: Ref. 20.

Table 8 Potential Strains Selected for Improvement of Active Substances in Tempe fermentation

Micro-organism Isolates of	Potency	Ref.
Micrococcus luteus, Microbacterium arborescens, and *Brevibacterium epidermis*	Transformation of daidzein/glycitein to Factor II	Barz et al. (1993)
R. oligosporus MS5 7 Pur	Vitamin B_{-2} (riboflavine) and vitamin B_{-6} (pyridoxine)	Keuth and Bisping (1993)
Klebsiella pneumoniae and *Citrobacter freundii*	Vitamin B_{-12} (cyanokobalamine)	Denter and Bisping (1994); Liemfetd (1977)
R. oligosporus MS5	β-Carotene (pro-vitamin A)	Bisping et al. (1993)
R. oligosporus MS1, MS5 and *R. arrhizus* EN	Ergosterol (pro-vitamin D)	Bisping et al. (1993)
R. oligosporus IK and *R. arrhizus* EN	γ-Linolenic acid	Hering et al. (1993)

Source: Ref. 20.

inoculum. A single culture or multiculture of micro-organisms inocula can be designed as needed and depend on the final products desired. The selected strains for improving the content of active substances have been identified by different authors as shown in Table 8.

XII. STARTER CULTURE

The starter culture for inoculation tempe can be obtained from the following sources:

1. A previous batch of sporulated tempe; from previous leaf wrappers or bamboo trays.
2. The mold grown and air-dried on the *Hibiscus tiliaceus* (waru leaf), namely usar. This is still used in the villages.
3. Ragi tempe: dried powder containing tempe mold and a variety of micro-organisms produced by an industry in Indonesia, and currently very common in the tempe industry. They improved the properties of the *R. oligosporus* strain, which produced more active substances.

4. Sun-dried or freeze-dried pulverized sound tempe; not common in Indonesia.
5. Ragi tempe inocula-flattened dry round rice cakes (2.5 cm in diameter) containing tempe mold and a variety of micro-organisms, common in Malaysia.
6. *Rhizopus oligosporus* strain NRRL 2710, available as a slant or a freeze-dried culture from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, IL (14).
7. *Rhizopus oligosporus* CBS 338.62, available from the Centralbureau voor Schimmelcultures Baarn, Holland.
8. Spores of *R. oligosporus* (dried); Used for inoculation by making a suspension in which the concentration of spores is 10^8/mL. This type of inoculum is used by the tempe industry in Japan.

XIII. EFFECT OF PROCESSING ON NUTRITIVE VALUE

The benefit of tempe to humans can be divided into two categories: sources of nutrients and sources of active substances, which are potentially useful for health. Because of these advantages, tempe is prospectively used as a functional food (20). The nutritive values of tempe are easy digestibility, rich in unsaturated fatty acids and vitamins, and containing less antinutritive substance, as shown in Table 6.

During fermentation, *R. oligosporus* MS5 has the potential to produce riboflavin (vitamin B_{-2}), pyridoxin (vitamin B_{-6}), β-carotene (pro-vitamin A), and ergosterol (pro-vitamin D). *Klebsiella pneumoniae* and *Citrobacter fruendii* as contaminating micro-organisms in tempe are sources vitamin B_{-12} (cyanocobalamine), whereas *Micrococcus luteus*, *Microbacterium arborescens*, and *Brevibacterium epidermis* are sources for the transformation of daidzein and glycitein to Factor II, as shown in Table 8.

Tempe is beneficial for those suffering from nutrition deficiency because of its high content of soluble nitrogen and many important vitamins; it is also beneficial for those suffering overnutrition because of the high content of dietary fiber. In addition, tempe is useful in treating some diseases such as diarrhea and has the potential to protect against heart disease, hypercholesterol, cancer, and high blood pressure. Astuti (56) found an increase in the bioavailability of iron in tempe, which is, of course, very important for people with anemia, especially for pregnant women, toddlers, and low-income people who are not able to serve meat dishes in their daily menu.

Tempe is well known for its antioxidative constituents. The research on the antioxidant was initiated by Gyorgy et al. Murata (63), after they had isolated a new isoflavone (viz., 6,7,4′-trihydroxyisoflavone) from tempe.

Furthermore, Jha et al. (65) have investigated the antioxidative properties of the isoflavonoids as critical constituents of tempe. Kolvenbach (1994) in Jha et al. (65) found derivates of isoflavones in tempe; the isoflavanone and isoflavan of Factor II were also important for antioxidative activity.

Siregar and Suyanto (75) (1996) found that the addition of *Brevibacterium epidermidis* and *Micrococcus luteus* to tempe inocula was able to increase the amount of isoflavone formation during tempe fermentation. Astuti (76) showed that tempe contains the antioxidant enzyme superoxide dismutase (SOD). Therefore, soybean tempe provides a defense mechanism against lipid oxidation. This fact could be applied in preventing health problems related to oxidation. Copper as a constituent of tempe influenced the activity of SOD to prevent lipid peroxidation in the body. The activity of catalase was not influenced by the copper in the diet. Based in Astuti's study, tempe can be recognized as a functional food for the prevention of degenerative diseases.

Fermentation of soybeans, either with *Rhizopus* spp. or with *Bacillus* spp., leads to a major degradation of polymeric macronutrients, resulting in increased nutrient availability, and tempe allowed a higher absorption of nutrients during intestinal perfusion as well (31). Tempe has been shown to inhibit *E. coli* infection in rabbits (60,77) and was reported to be beneficial when it supplemented the diet of malnourished children (78).The absorbability after fermentation is slightly increased, also indicating possibly better digestibility. It had been shown earlier that fermentation decreases flatulence in consumers (79).

In conclusion, tempe fermentation improves the nutritional quality and functional properties of soybeans, protein digestibility is improved, it removes antinutritive compounds, and decreases the flatulency of soybeans. It improves the availability of minerals, increases vitamin B content, especially B_{12}, and produces antibacterial substances and antioxidants.

XIV. FORECAST FOR FUTURE FERMENTATION

Tempe is a traditional fermented food that is rich in nutrients and active substances. The active substances, which consist of isoflavones, unsaturated fatty acids, ergosterol, and vitamins, are important for health. Improvement of micro-organisms and further inocula formulation will play an important role in the successful development of functional foods based on tempe fermentation.

People in the developed countries have a keen awareness of health because they have sufficient quantities of foods, but, at the same time, because of their consumption of foods they suffer from geriatric diseases, such as

obesity, cardiovascular disease, and cancer. Soybeans are highly nutritious, are an excellent source of proteins, oils, and minerals, and have a variety of physiological functionalities. Recently, consumption of tempe has been increasing rapidly, not only in Indonesia but also in the United States and European countries, especially the Netherlands and Germany. The physiological functionality of tempe has attracted the attention of many people.

The aspect of functionality of tempe must be mentioned from previous experiments in which the digestibility of soybeans was shown to be influenced by the tempe process (80) and antioxidant substances were shown to be formed by the transformation of flavonoids (81). In the future, the consumption of tempe will probably increase significantly.

In order to improve the quality of tempe in Indonesia, the tempe industry must be modernized.

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12
Tempe Production in Japan

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I. CIRCUMSTANCES AND BACKGROUND OF TEMPE

Scientific exchange between Japan and southeastern Asian countries has been carried out actively since around 1975, and in 1985, the Society of Tempe Research was establish (1). Since then, multilateral research, including academic and industrial studies, have continued. In 1983, several companies, such as Torigoe Foods, Marusan Ai, and General Federation of Natto Manufacturers' Cooperatives, started the industrial production of tempe, responding to public demand. The total annual production at that time was nearly 500 tons.

Prior to the movement toward tempe production, the methods of production and involved microorganisms were already seen in publications in Japan in 1950 (2). In 1965, Ohta (3) reported in detail the historical background, major microorganisms involved in the fermentation, culturing, and the appearance and disappearance of components in the production of tempe. In the 1960s, studies on the production methods and the selection of useful microorganisms were actively carried out at the research level in universities and companies. In particular, the establishment of the Society of Tempeh Research in 1985 and the several sessions of the International Non-salted Fermented Soybean Food Society in Tsukuba, Akita and other cities spurred the progress of tempe research in Japan.

In Japan, however, there are many types of traditional processed soybean food, such as natto, tofu, yuba, soybean milk, and cooked soybeans, and the annual consumption of natto alone is reported to be 250,000 tons. Therefore, there was an unavoidable competitive relationship between these soy-

bean products and tempe. Because tempe has a characteristic smell and bland flavor and requires further processing, it has less impact. As a result, the industrial production of tempe decreased. At present, only about 30 tons of tempe is produced industrially in Seto-cho in Okayama Prefecture and Shiroishi-cho in Saga Prefecture. Most of the other factories have been closed (4).

II. STARTERS FOR TEMPE PRODUCTION

Although tempe production in Japan declined significantly in the past 10 years, all of the starters presently used in Okayama and Saga prefectures are five strains used solely for tempe production selected by the True Fungi Functional Development Institute (Akita Konno Co. Ltd.) in Kariwano, Akita Prefecture (5).

The representative strain is selected from several strains of *Rhizopus oligosporus* isolated from tempe produced in Indonesia, strains isolated by Teruo Ohta, and strains of the culture collection of the Tokyo University of Agriculture (6). The sporangium and chlamydospore of these strains have a characteristic sporangiospore, and the chlamydospore is not as thick (Figs. 1 and 2).

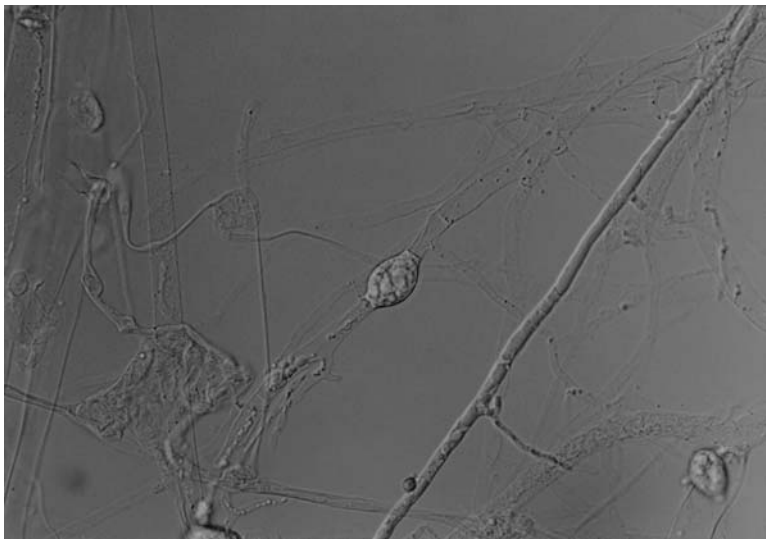

Figure 1 Chlamydospore of *Rhizopus* (tempe making).

Figure 2 Sporangium of *Rhizopus oryzae*.

This strain has excellent features, in that it grows at a relatively high temperature (35–42°C), which is suitable for tempe production, spore production is sparse, the tempe produced using this strain has little musty smell and has a rich flavor, a large quantity of strain can be cultured because the oxygen demand is less than that of general molds, and it grows well on steamed soybeans.

The selected strains distributed by Akita Konno Co. Ltd. are five to six strains based on the NRRL 2710 strain, which is said to have the above characteristics, and one of them is known as *Rhizopus oryzae*. According to Professor I. Gandjar of Indonesia University in the second International Non-salted Fermented Soybean Food Society, the microorganisms involved in the fermentation of tempe is not only *R. oligosporus*, as reported by Dr. Ko Swan Djien, but several *Rhizopus* sp., such as *R. oryzae* and *R. stolonifer*, isolated from a usar of hibiscus. Akita Konno also combines the *Rhizopus* genus without emphasizing the NRRL 2710 strain, aseptically culturing them, and supplying them as the starter for better tempe production.

III. TEMPE *RHIZOPUS* IS DERIVED FROM HIBISCUS

Mold is the main microorganism involved in the production of most traditional eastern Asian foods, such as miso, soy sauce, and cereal wine, the mold involved in many Japanese fermented foods is *Aspergillus oryzae*, and its origin is known to be rice straw (7).

Mold spores or ragi for tempe production in Java are generally used as the starter for tempe production today. The leaves of the hibiscus plant, known as usar, were used until several years ago. When cooked, soybeans are sandwiched between hibiscus leaves, *Rhizopus* sp. grows around soybeans, and this is used as the starter. However, the origin of the *Rhizopus* sp. was unknown.

In order to determine the origin of the *Rhizopus* sp., Tsubaki et al. studied the appearance of fungi from usar that had been cold-stored (4°C) for 2 years and obtained *Rhizopus oligosporus* at a very high frequency (90%) (8). Because the sporangiospores of *R. oligosporus* have little resistance to drying, they cannot be considered to be present on the surface of usar kept dry for 2 years, but they are present inside the leaves as resistant organisms. Because a large number of sporangiospores of *Rhizopus* were observed in frozen sections, it was concluded that *Rhizopus* is an endogenous fungus.

Therefore, molds and their frequencies from fresh usar, green leaves of the hibiscus plant, which are the substrate of usar, and wild hibiscus on the seashore of Bali Island, were investigated. Table 1 shows the results.

Rhizopus was observed in all the samples of usar used as a starter. *Rhizopus* also appeared from fresh leaves of hibiscus growing in the garden of a tempe factory and from green leaves of wild hibiscus on the seashore at frequencies as high 100% and 95%, respectively. From the above results, *Rhizopus* can be considered to be a mold coexisting with hibiscus (8).

Table 1 Frequency (%) of Mold Genera Isolated from Fresh Usar and Wild Hibiscus

Origin and place of collection	Genus	Frequency (%)
Usar of Bali Island (1997)	*Rhizopus*	100
	Aspergillus	60
	Cladosporium	20
	Fusarium	15
	Curvularia	10
	Penicillium	5
Green leaves of tempe factory (1997)	*Rhizopus*	100
	Aspergillus	15
	Penicillium	15
	Curvularia	5
Wild green leaves of seashore in Bali (1997)	*Rhizopus*	95
	Cladosporium	60
	Aspergillus	10
	Chaetomium	5

Table 2 Mold Genera Isolated from Green Leaves of *Hibiscus hamabo* of Japan, and Their Frequencies

Origin and place of collection	Genus	Frequency (%)
Shimoda City (green leaves)	*Rhizopus*	100
	Cladosporium	95
	Alternaria	55
	Fusarium	15
	Curvularia	5
	Aureobasidium	5
Hachijo Island (young leaves)	*Rhizopus*	45
	Phoma	90
	Fusarium	10
	Penicillium	20
	Alternaria	5
	Cladosporium	5
Hachijo Island (matured leaves)	*Rhizopus*	30
	Phoma	65
	Cladosporium	50
	Penicillium	15
	Fusarium	5

Furthermore, Tsubaki et al. are studying whether *Rhizopus* can be isolated from hibiscus growing in places other than Bali Island at high frequencies using *Hibiscus hamabo* in Japan as an example.

As a result, it is known, as Table 2 shows, that the *Rhizopus* genus is present at high frequencies even if the kind of hibiscus differs. To summarize these results, the *Rhizopus* genus used as the starter for tempe production can be considered to be a mold always inhabiting several hibiscus species, and hibiscus is obviously the origin of tempe fungus *Rhizopus*.

IV. PRODUCTION OF TEMPE

The flow sheet of tempe production in Japan exemplified by the method used in Okayama Prefecture is shown in Fig. 3 (9).

Cleaned soybeans are soaked in water for several hours, or boiled with water for about 10 min, and after draining, they are dehulled and soaked in 1% lactic acid solution or 0.2% acetic acid solution. However, in the case of mass production in factories, previously dehulled soybeans are washed well and soaked in a lactic acid solution. The pH of the lactic acid solution is about 3.0, which prevents contamination by harmful bacteria and facilitates the

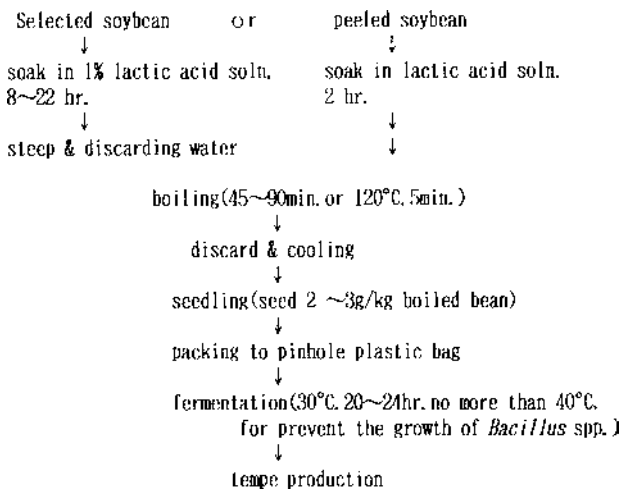

Figure 3 Production of tempe.

multiplication of tempe fungi. In conventional tempe production in Java, soybeans are immersed in water overnight, and undesirable bacterial contamination is prevented by natural fermentation by lactic acid bacteria. However, at present, lactic acid is added.

By pressure-cooking under 1 kg/cm^2 for 5 min, or under 0.7 kg/cm^2 for 7 min, soybeans are cooked but remain hard. When the temperature of cooked soybeans has decreased to 40°C or below, 2–3 g of the starter is sprinkled on 1 kg of the cooked soybeans and mixed well. Although usar or ragi for tempe is used as the starter in Java, a pure mold spore starter is used in Japan. After mixing, the soybeans are packed in perforated polyethylene bags 2–3 cm thick. The bags are placed in a constant-temperature chamber or vessel maintained at 30°C to allow them to ferment for 20–24 hours. When the tempe is completely with mold hyphae the fermentation is complete.

The preferred product is entirely covered with white mold hyphae and has a sweet fermentation odor and moderate firmness. If the fermentation temperature is too high, the fermentation time is too long, black spores are produced, resulting in an astringent taste and poor flavor and occasionally free ammonia odor. Tempe products must be stored in a cold place (4–5 days) or frozen.

In Shiroishi-cho in Saga Prefecture, an agricultural product-processing group (eight females and one male) produces tempe at a farmhouse level. Therefore, they do not use pressure-cooking, but they immerse soybeans in

water, cook them under normal pressure for 60 min, dehull them, and cook them again. After draining and cooling, the starter is sprinkled onto the cooked soybeans; the mixture is packed in bags and placed in a constant-temperature oven for 24 h for fermentation (10).

Because tempe is not consumed as it is, but used as foodstuff, many cooking methods have been studied. Production of tempe not only from soybeans but also from green peas or adlay is studied.

V. NUTRITION AND SECONDARY PROCESSING OF TEMPE

The general food composition of tempe made in Okayama is shown in Table 3. When adlay tempe is compared with soybean tempe, the water content is almost the same. Soybean tempe has higher contents of components other than carbohydrates.

Tempe contains high-quality vegetable protein and is rich in unsaturated fatty acids and vegetable fiber, minerals, and lecithin. Digestion and absorption are further enhanced with fermentation, and the vitamin B group is also increased. It is also known that antioxidation power and SOD-like activity are stronger than those of the starting soybeans. From these facts, tempe is said to prevent mutagenicity and obesity, lower the cholesterol level, as well as prevent chronic diarrhea. Tempe protects against lifestyle-related diseases.

Because tempe has a bland taste, it lacks impact as a food. Therefore, the value of tempe as a food is elevated by secondary processing. Examples include tempe miso produced in Okayama.

Ebine et al. (11) studied the production of miso and soy sauce using *Rhizopus* sp. and Nozaki et al. (12) succeeded in the production of salty miso and sweet miso using tempe as the substrate. The formulation of salty miso and sweet miso is shown in Table 4. The salty miso is produced by replacing half of the cooked soybeans with tempe, adding rice koji, and fermenting and

Table 3 Food Composition of Tempe in Japan

Name of tempe	Water (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Soybean tempe	60.5	16.6	9.5	12.4	1.0
Adlay tempe	60.1	6.4	0.4	32.9	0.2

Table 4 Formulation of Tempe Miso

Material (kg)	Salty tempe miso	Sweet tempe miso
Cooked soybeans	50	—
Tempe	50	50
Rice koji	25	60
Salt	17	7

aging at 30°C for about 45–50 days. The product is rich in flavor and taste and has a dark color. Sweet white miso is produced from tempe, to which 1.2 times rice koji and a rather low content of about 6% of salt are added. This is sweet tasty miso, and in addition to cooking, it is also utilized in producing pickles.

Tempe can be an ingredient in many processed foods, including tempe croquettes, tempe sausages, tempe cookies, as well as tempe miso.

VI. FUTURE OF TEMPE IN JAPAN

Tempe production at an industrial level began in Japan in the 1980s; however, due to diversified soybean processing in Japan, tempe production decreased gradually, and a total annual production of about 30 tons is now continued at an agricultural community level in Okayama and Saga.

However, tempe production tended to increase again through efforts to develop tempe miso, various kinds of tempe such as adlay tempe and green pea tempe, followed by the processing of tempe such as for cookies and bread, and studies of cooking such as hamburger steaks, dumplings, and fried rice utilizing the ease of secondary processing, a feature of tempe. Although there is a problem of competition with natto, tempe will continue to occupy a position in processed foods.

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13

Industrialization of Thai Fish Sauce (Nam Pla)

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I. INTRODUCTION

Fish sauce is a clear, amber to reddish brown liquid with a predominantly salty taste and characteristic aroma. It is obtained from the hydrolysis of fish with salt through natural fermentation for 6–12 months. Fish sauce is well recognized as a food flavoring by the people in Southeast Asia for many centuries. Hundred of millions of liters of fish sauce are produced and consumed by at least 300 million people annually (1). In Thailand, fish sauce or nam pla is the major and the most common condiment in the household. Nam pla is used as a flavoring sauce in almost every Thai dish, as well as in various types of dipping sauce. Budu is another fish sauce product similar to nam pla. It is produced and consumed only in the provinces in the south close to Malaysia. Thus, it is believed to be of Malaysian origin and mostly used by the Muslim people (2). Not only Thailand, in Asia, but particularly Southeast Asian countries such as Burma, (Myanmar), Laos, Kampuchea, Malaysia, Indonesia, Philippines, and Vietnam with a similar tropical climate and high humidity also produce fish sauce under different names (Table 1). At present, Thailand is the largest producer of fish sauce in the region.

Hydrolysis of fish protein results in small-molecule peptides, nitrogen compounds, and amino acids. Fish sauce contains about 20 g/L of total nitrogen, of which 80% is in the form of amino acids. Therefore, it can provide

Table 1 Local Names of Fish Sauce in Different Countries

Countries	Local names
Burma	Ngapi
Indonesia	Ketjab-ikan
Japan	Uwo-shoyu or qunaga, kaomi, ounago, ika-shoyu, shottsuru
Kampuchea	Tuuk-prahoc, nuoc-mam-gau-ca
Khmer Republic	Nuoc-mam, nuoc-mam-gau-ca
Laos	Nam pla
Malaysia	Budu
Philippines	Patis
Thailand	Nam pla
Vietnam	Nuoc-mam

Source: Ref. 3

up to 7.5% of daily nitrogen intake (4). In addition to the protein and amino acids, fish sauce is also a good source of minerals and vitamins (5). Vitamin B_{12} is found in a significant amount in fish sauce (6). Thearawibul (6) tested 108 nam pla samples and found 0.30–5.82 μg/100 g vitamin B_{12} with an average of 1.91 ± 1.24 μg/100 g nam pla. According to Areekul et al. (5), consumption of 25 mL of fish sauce would result in 0.08–1.46 μg of vitamin B_{12} intake, which is sufficient to prevent megaloblastic anemia.

II. HISTORY

Thai people have consumed fish sauce or nam pla as a flavoring agent since ancient times. "Always fish in the water and rice in the field" is the common phrase known by all Thai. This means fish is abundant and rice can be grown anywhere in the field. The way of life is attached to water, and native Thai tend to live near canals and rivers. Thus, it is not surprising that fish has become the main source of animal meat and that many traditional fish products have been developed over the years. Drying, salting, and fermenting seem to be the most typical and simple ways to preserve the fish. Fermented fish products, apart from being a valuable source of protein, have gained acceptance due to their characteristic flavor and aroma suitable for diet. Fish sauce or nam pla became the most important and the major condiment in the Thai diet. Asian countries like Japan, Korea, Hong Kong, and India also produce fish sauces,

but the sauces are not as popular as in Southeast Asian countries. Other kinds of sauces such as soy sauce is perhaps more common than fish sauce.

III. PRODUCTION AND CONSUMPTION

Fish sauce factories are mainly located along the coastal provinces near raw material sources or harbors. The number of fish sauce factories (nam pla and nam budu) and quantity disposition of marine catch for their production are shown in Tables 2 and 3. Most nam pla factories are located in Coastal zones 1 and 2, which are in the east and central parts of the country, whereas budu is produced only from two provinces of Coastal Zone 4 in the south. Production of fish sauce increased dramatically during the last decade although the numbers of producers are relatively unchanged. Introduction of modern technology and equipment brought about the expansion of production capacity to serve the increasing demand. Utilization of marine fish for different types of product is shown in Table 4. In general, 1 ton of fish yields about 500 L of first-grade nam pla and 1000 L of lower grade nam pla (9).

Fish sauce has long played an important role in the Thai diet as a staple condiment. In the northeastern part of the country, the consumption is at an average of four bottles (750 mL) per family per month. Average consumption of fish sauce for Thai people is 20 mL/person/day. Approximately 365 million liters are produced annually with the value of 5000 million bahts or over US$100 million (10). At present, about 20 popular brands of Nam pla from the 10–15 largest factories produce 80% of the total market share (10). Small industries have a tendency to quit the business due to the increase of capital investment, labor cost, and price of petroleum for transportation.

Table 2 Number of Factories and Quantity of Fish Used in Nam Pla and Budu

	No. of factories		Quantity of fish (ton)	
Year	Nam pla	Budu	Nam pla	Budu
1995	102	54	50,745	318.4
1996	102	57	65,090	364.0
1997	97	70	58,514	409.8
1998	88	70	99,546	647.1
1999	89	105	96,329	165.3

Source: Ref. 7.

Table 3 Number of Fish Sauce Factories and Quantity of Fish Used in Nam Pla and Budu by Province, 1999

Province	No. of factories		Quantity (tons)		Total (tons)
	Nam pla	Budu	Nam pla	Budu	
Coastal Zone 1					
Trat	4	—	20,490	—	20,490
Chantaburi	3	—	79	—	79
Rayong	24	—	17,615	—	17,615
Coastal Zone 2					
Chon Buri	5	—	8,308	—	8,308
Samut Prakan	9	—	16,098	—	16,098
Samut Sakhon	9	—	1,357	—	1,357
Samut Songkhram	21	—	30,659	—	30,159
Petchaburi	2	—	84	—	84
Coastal Zone 3					
Prachuab Khiri Khan	5	—	900	—	900
Chumphon	4	—	645	—	695
Surat thani	1	—	—	—	—
Coastal Zone 4					
Nakhon Si Thammarat	1	—	42	—	42
Pattani	—	43	—	301	301
Narathiwat	—	62	—	364.3	364.3
Coastal Zone 5					
Phuket	1	—	2	—	2
Total	89	105	96,329[a]	665.3	96,994.3

[a] Quantity from anchovies is 96,187 tons or 99.85 % of total.
Source: Ref. 8.

In the past, Thailand produced nam pla solely for domestic consumption. Nam pla has been exported in the last few decades and the amount of export increased considerably. About 75% of total production is for local consumption and 25% for export. Most major factories export about 20–25% of their products. Export of nam pla has constantly expanded since 1997. Between 1985 and 1990, nam pla was considered to be one of the main exports of fishery products. The amount increased from 9100 tons in 1985 to 16,000 tons in 1990, or about a 76% increase (11).

At present, Thailand is the largest exporter of fish sauce in the Southeast Asian region, and nam pla is well recognized in the international market, not only in Asian countries such as Japan, Hong Kong, Philippines, and Indonesia where people are familiar with fish sauce but also to the Western markets, especially the United States and the European Union. The quantity

Table 4 Disposition of Marine Catch by Disposition Channel (%)

Year	Fresh consumption	Fresh, chill and frozen for export	Canning	Steamed or smoked	Salted/ dried	Fish meal and animal stuff	Fermenting		Others	Total (%)
							Fish sauce	Fish paste		
1993	14.5	26.1	14.8	0.3	5.0	36.1	2.3	0.2	0.7	100
1998	19.7	26.5	18.3	0.3	4.6	26.6	3.2	0.3	0.5	100
1999	21.0	24.8	18.5	0.3	5.8	26.0	3.1[a]	0.3	0.2	100

[a] Total amount of 96,329 tons were used for nam pla and 665 tons for budu from a total marine catch of 3,434,456 tons.
Source: Ref. 7.

Table 5 Export of Fish Sauce

Year	Quantity (1000 tons)	Value (million bahts)
1988	12.0	202.9
1989	13.2	233.8
1990	16.0	288.4
1991	17.1	308.7
1992	19.0	346.0
1993	18.3	342.9
1998	31.7	637.6
1999	38.2	637.8
2000	28.8	702.5
2001	30.9	835.9
Jan–July 2002	17.7	450.4

Source: Compiled from Refs. 8 and 12–16.

of export increased from 12,000 tons in 1988 to 30,900 tons in 2001, with a value of 835.9 million bahts or about US$20 million (Table 5). The quantity and value of fish sauce distributed to Asian countries and major importing countries during 2000–2002 are shown in Table 6. Countries that import over 1 million tons annually are the United States, Hong Kong, Japan, France, Australia, and Canada. Major competitors for that market are Vietnam and Philippines, which share about 30% of total export to the United States, Canada, Japan, and Hong Kong (10).

Migration of Asian people to the Western countries and Australia and the expansion of Thai restaurants all over the world apparently help promote the expansion of fish sauce. Improvement of product quality through modern processing development and compliance with international standard (viz, HACCP, and ISO 9002) also guarantee the quality and safety of the product in the world market (Fig. 1).

IV. RAW MATERIALS

Fish and salt are the main raw materials used for making nam pla. Fish can be freshwater and marine fish, but 97% are marine. Most factories of the industrial-scale level use marine fish, which are an abundant and readily available raw material. The highest-quality nam pla is believed to be made from anchovies (*Stolephorus* spp.). Other species include *Clupeoides* sp., *Sardinella* sp., *Rastrelliger* sp., *Dussumieria* sp., and *Nemipterus* sp. (17). Anchovies (pla-

Table 6 Export of Fish Sauce

Country	2000		2001		Jan–July 2002	
	Quantity (1000 tons)	Value (1000 bahts)	Quantity (1000 tons)	Value (1000 bahts)	Quantity (1000 tons)	Value (1000 bahts)
Australia	1,814	54,310	1,663	60,646	994	26,698
Belgium	176	3,606	88	3,093	25	752
Brunei Darussalam	52	1,245	110	2,176	55	791
Cambodia	258	3,677	349	5,305	135	1,975
Canada	1,327	32,189	1,486	38,614	576	13,860
France	1,647	42,906	2,037	52,790	647	14,123
Germany	410	10,846	373	11,539	175	6,294
Hong Kong	4,736	73,200	5,913	113,703	3,573	72,255
Indonesia	278	4,554	308	5,376	226	4,073
Israel	114	2,874	166	5,006	84	2,488
Japan	2,077	71,062	2,523	124,915	1,516	64,717
Korea, Republic	14	408	195	2,506	951	11,329
Lao	783	13,761	911	15,124	595	9,724
Malaysia	219	3,767	478	7,047	607	9,354
Mauritius	77	1,585	138	2,892	41	927
Myanmar	461	7,457	152	2,352	17	289
The Netherlands	890	23,750	961	25,120	394	11,477
New Zealand	164	4,284	197	5,673	95	2,772
Philippines	473	5,053	222	1,995	153	1,612
Saudi Arabia	383	6,897	390	6,656	280	4,824
Singapore	748	13,535	704	11,937	412	7,642
Sweden	122	4,232	241	8,464	93	3,396
Switzerland	110	3,767	158	5,507	64	2,102
Taiwan	757	17,743	928	19,256	334	7,142
United Kingdom	681	26,483	744	24,046	384	13,427
United States	9,390	253,654	8,775	253,456	4,912	140,596
Vietnam	39	562	31	1,201	26	3,930
Others	558	15,099	706	19,499	348	8,843
Total	28,758	702,507	30,948	835,894	17,713	450,412

Source: Adapted from Ref. 16.

Figure 1 Export brands. (Courtesy of Pichai Fish Sauce Co., Ltd.)

ka-tak), which are abundant in the catching area of the east coast, account for about 90.82% of total fish; other types of fish used in relatively small amounts include the following: sardinellas (pla-lang-keow) in the southern part, 0.17%; Indo-pacific mackerel (pla-tu), 7.0%; other food fish, 2.01% (2). In 1999, the quantity of anchovies for fish sauce increased to 99.85% (96,187 tons) compared to 0.06% and 0.09% of Indo-pacific mackerel and other food fish, respectively (7). Freshwater fish such as *Cirrhinus* sp., *Puntius* sp., and *Rasbora* sp. are also used, especially in the central part of Thailand (Ayuttaya and Saraburi provinces), but in limited quantity. The quality in terms of flavor and aroma of nam pla from freshwater fish is lower (18). Moreover, freshwater fish for making nam pla is becoming less available and the number of nam pla producers from freshwater fish has been reduced markedly.

Fine-grain salt is used for preparing nam pla. Crude sea salt or solar salt is added in the range of 20–50%. Rock salt is not used. Solar salt contains about 85% sodium chloride and 11% moisture (19). The purity of salt is also important for processing because highly contaminated salt retards the rate of diffusion of salt into the fish. The impurity on salt, apart from dirt and sand,

includes magnesium sulfate, calcium chloride, magnesium chloride, sodium sulfate, calcium sulfate, and heavy metal (20). A high concentration of salt reduces the water activity (A_w) of the mixture and provides a bacteriostatic effect against Gram-negative putrefactive bacteria and other spoilage microorganisms. Normally, bacteria spoil fish when the salt concentration is lower than 7%. In addition, salt is essential for the growth and enzymatic action of halophilic and halotolerant bacteria responsible for fish sauce fermentation. However, a too high salt concentration can slow down the fermentation rate. According to Saipin (21), the analysis of salt content in 120 nam pla samples from various factories were in the range 27–31% (w/v).

V. INDIGENOUS PROCESS

The traditional process of fish sauce making is very simple. Whole fresh fish are mixed with salt at the ratio 2–5 : 1 (w/w), fish : salt. The mixture is placed in a wooden barrel or large earthen jar (Fig. 2) or concrete tank with a hole at the bottom to drain off the liquid from the fermentation (Fig. 3). A layer of salt is

Figure 2 Traditional method of fish sauce fermentation using earthen jars. Corrugated fiberglass is used as cover over the nylon net to protect from rain, dust, and insects while allowing the sunlight to go through.

Figure 3 Fish and salt are mixed (3 : 1 ratio, w/w) and put in concrete tanks (2.5-ton capacity) covered with a nylon net and fiberglass lid for fermentation. After 12–15 months, fish sauce is filtered a charcoal bed packed at the bottom of the tank and the clear sauce is collected from the pipe at the opening channel.

normally spreaded at the bottom of the tank and also about 2–3 cm thick on the top over the fish. During fermentation, it is necessary to submerge fish under liquid. This was done by weighing down with heavy stones or pressing down with a bamboo mat. The mixture is normally left outdoors for autolysis and natural fermentation. The fermentation period can vary from 6 to 18 months depending on the size of fish, the amount of salt, and other environmental conditions. The end of the fermentation period is determined from the color, aroma, flavor, and clarity of the nam pla, which solely depends on the judgment and experience of the producers. Then, supernatant liquid with a strong fishy odor is drained off and sun-ripened for 1–3 months to improve the color and aroma of the sauce (Fig. 4). It is then filtered and distributed as first-grade nam pla. The residue is further extracted with saturated salt solution and fermented for 3–4 months. The extraction process can be repeated two to four times to produce second and lower grades of nam pla. In some areas, home/cottage-scale producers use fish waste such as heads, guts, and other leftovers to make low-grade nam pla for distribution locally.

In other cases, the producers mix a portion of the first grade with the lower grades to achieve the specification and standard quality of fish sauce.

Figure 4 After the fermentation period, fish sauce is transferred to a nearby concrete storage tank to age before bottling. This practice is for small- to medium-scale production.

The whole process is not controlled, resulting in an inconsistency of the product quality. Production is at the home/village level and distribution is limited to the communities and nearby areas.

VI. MODERN INDUSTRIAL PROCESSING

Due to an increasing demand for fish sauce over the years. The processing method of most medium-scale factories has been improved and new technol-

ogy introduced to increase the productivity and consistency of product quality. The ratio of fish to salt still varies in the range of two to four parts to one part (fish to salt). Concrete tanks of various sizes are mainly used in place of the wooden barrel and jar (Figs. 5–7). Normally, the capacity of the tank is between 2000 and 3000 L, but a very large size can accommodate up to 15 tons. Some examples of the dimension of the outdoor tank are $2 \times 2 \times 2.5$ m or $2.2 \times 2.3 \times 2.4$ m (width $\times$ length $\times$ height), and the indoor tank can be $2.5 \times 2.5 \times 2.75$ m. Small- and medium-scale processors have less than 30 and 30–150 tanks, respectively, whereas the large producers may have over 150 tanks (22). The outdoor tanks must be covered with tiles (Fig. 6) to prevent contamination from dust, rain, insects, and animals. Indoor fermentation tanks under a roof are also used in some areas. For the latter, the tank is not covered.

The fermentation time also varies from 6 to 18 months until the desired characteristics are reached. The modern fish sauce process is shown in Fig. 8.

At present, most fish sauce producers still use traditional methods during the fermentation step. However, the quality of initial raw material is intensively monitored and inspected to ensure the freshness and wholesomeness. Starter culture or the addition of enzymes still has not been commonly practiced in commercial production, although much work has been carried out in the attempt to reduce fermentation time. This is probably due to the loss of the characteristic flavor and aroma, which is the most significance requirement of the consumers. For other production steps, the application of modern technology and machinery for mixing, filtering, blending, bottling, and packing (Figs. 9–16) have been used extensively. Laboratory testing for chemical, physical, microbiological, and organoleptic evaluation at different stages of fermentation until the finished product is also carried out to achieve quality standards and uniformity of the products.

Some manufacturers mix BX or meiki, a byproduct from the precipitation of monosodium glutamate, with brine for making fish sauce. Grade 1 nam pla is usually added to get the desired flavor and aroma of nam pla. Meiki is a black concentrated liquid with substantial amounts of nitrogen and amino acid. According to the Thai Food and Drug Administration (FDA) definition, this type of nam pla cannot classify as grade 1 because it is not naturally processed. At present, there is no regulation concerning the use of BX in fish sauce.

New types of modern packaging (Figs. 17 and 18) suitable for different purposes have been introduced. In the past, fish sauce was packed in glass bottles or earthen jars with tightly sealed cork caps for distribution in the markets. Since the past decade, plastic containers have gained much acceptance and popularity for fish sauce due to its light weight, unbreakablity, and reduced transportation cost. Major kinds of plastic used are polyvinyl chlo-

Figure 5 Outdoor concrete pools for fish sauce production in a large factory. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Figure 6 Fermentation pools with a corrugated tile roof. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Figure 7 Fermentation pool model showing the mixture of fish and salt in the pool. The top layer is salt. (Courtesy of Pichai Fish Sauce Co., Ltd.)

ride (PVC), polyethylene terephthalates (PET), and high-density polyethylene (HDPE). PET is used mainly for 300-, 670-, 750-, and 1500-mL-sized bottles, whereas PVC is for a gallon of 4500 mL and HDPE is used for the 30-L and 200-L drums. Some manufacturers use bag-in-box (Cubitainer) and 1000-L spacekraft for industrial purposes and large demand. Also, a small-sized package of a 5-mL individual serving in a laminated aluminum foil sachet is available for carryout food, airline kitchens, and fast-food establishments. Somboonyarithi and Suwansakornkul (23) studied the quality of 44 fish sauce samples packed in glass and different types of plastic packaging and concluded that there was no difference in pH, salt, total nitrogen, and histamine contents.

A. Critical Steps in Processing

The crucial steps in the production of fish sauce are as follows:

1. The quality of raw materials. Total volatile base (TVB) or histamine contents, which can be used as indicator for freshness of the fish, should be determined. Visual inspection also helps in judging the

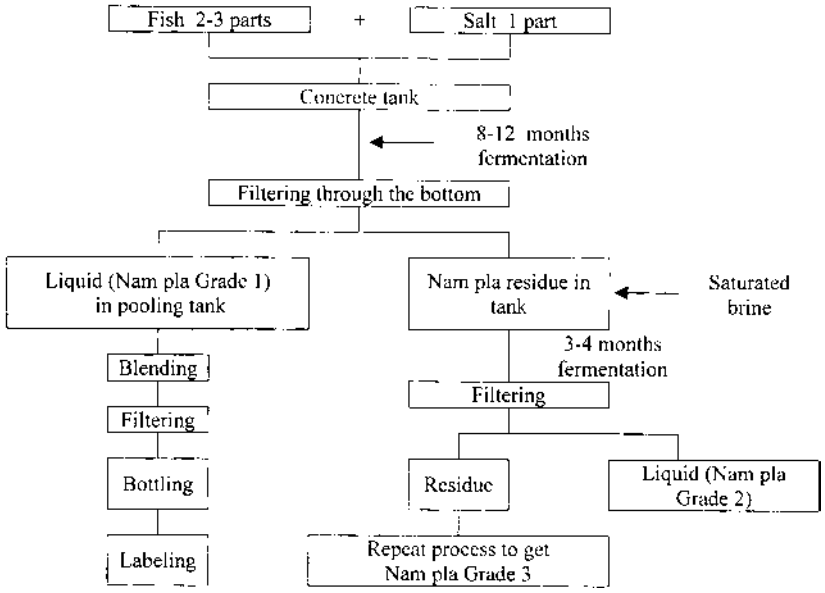

Figure 8 Production of nam pla. (Adapted from Ref. 31.)

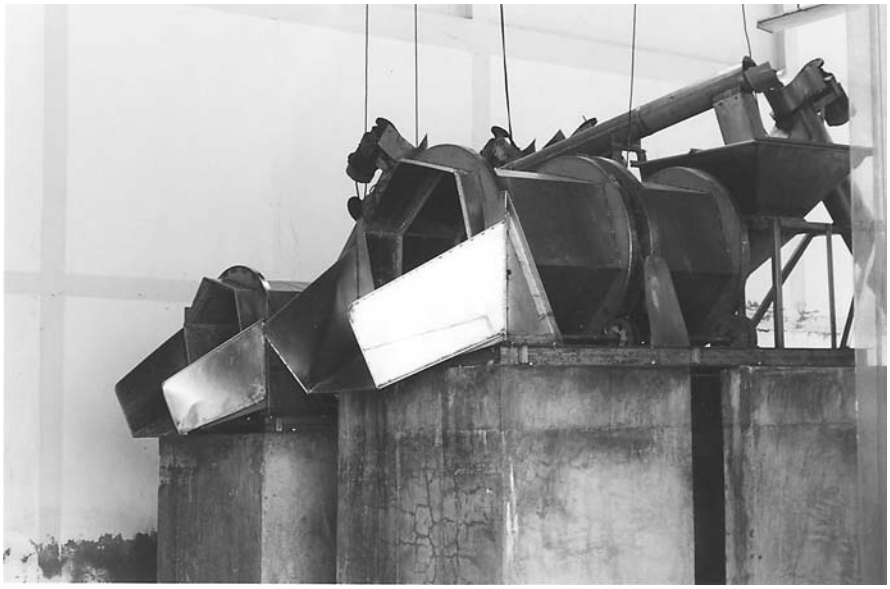

Figure 9 Mixer for fast and thorough mixing of fish and salt. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Figure 10 Fiberglass storage vats. (Courtesy of Pichai Fish Sauce Co., Ltd.)

freshness. Good quality anchovies are transparent, less slimy, and have bright black eyes and firm texture in contrast with the opaque appearance, slimy, soft texture, red color, and apparent sign of decomposition (24). Virulhakul et al. (24) studied the quality of fresh fish maintained at a low temperature in ice for up to 60 h after harvesting and those under normal handling practice. They reported the higher amount of TVB and histamine contents in the latter (i.e., 40.11–72.09 mg/100 g for TVB and 63.21–168.61 mg/100 g for histamine in comparison with 19.45–36.87 mg/100 g TVB and 4.4–26.97 mg/100 g histamine in the former). Thus, the handling of

Figure 11 Bottle cleaning machine. (Courtesy of Pichai Fish Sauce Co., Ltd.)

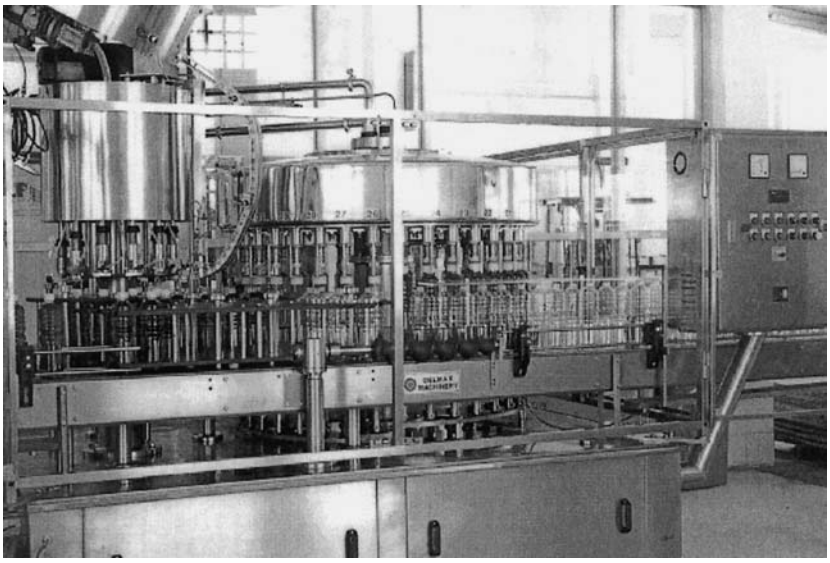

Figure 12 High-capacity filling machine. (Courtesy of Pichai Fish Sauce Co., Ltd.)

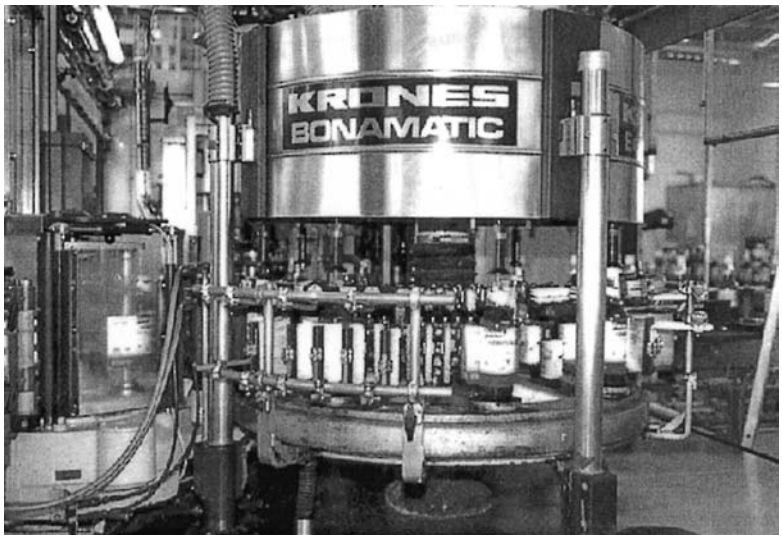

Figure 13 Capping and labeling machine. (Courtesy of Pichai Fish Sauce Co., Ltd.)

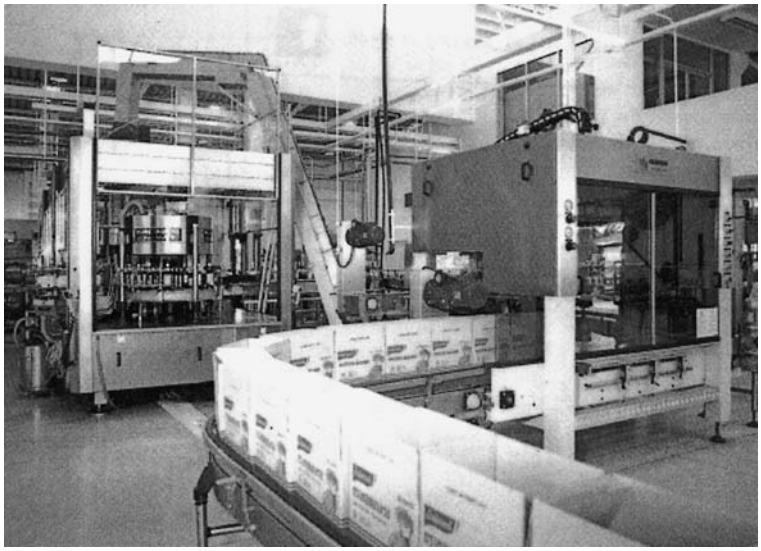

Figure 14 Carton packing. (Courtesy of Pichai Fish Sauce Co., Ltd.)

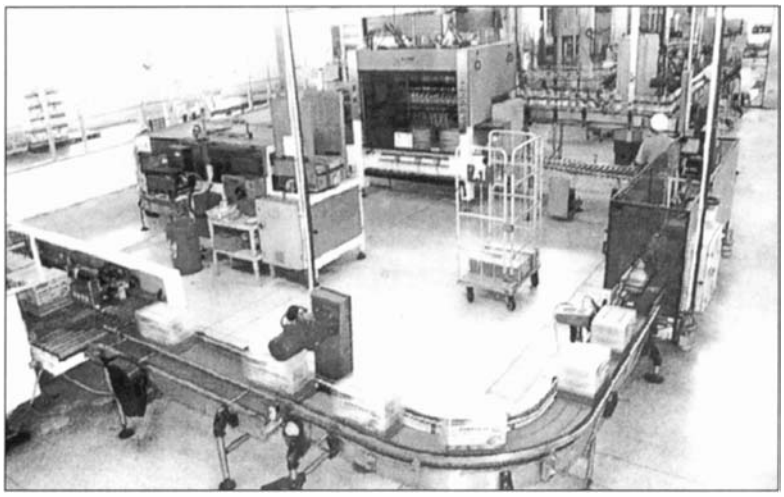

Figure 15 Packing line. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Figure 16 Products storage. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Figure 17 Different sizes of fish sauce on the market. (Courtesy of Pichai Fish Sauce Co., Ltd.)

the fish after harvest should be well controlled to maintain the freshness, which will result in good quality fish sauce with a low level of histamine.

2. The mixing step of fish and salt. Rapid and thorough mixing (Fig. 9) give better quality product because the fish still retains its freshness and does not undergo incipient spoilage. Some producers use a specially designed machine that has the capability to mix 1 ton of fish with salt within 10 min to quicken the mixing process. (Pichai Fish Sauce, personal communication, 2002.).
3. Fermentation time and temperature. Temperature in the fermentation tanks is not controlled, depending on the climate. The outdoor tank temperature can rise to 37–40°C during fermentation. Thus, the period of fermentation varies according to the rate of hydrolysis of fish protein. The acceleration of the process by the addition of enzymes of a starter culture is sometimes introduced.
4. Equipment and mechanization. Equipment and utensils for fish sauce must be resistant to corrosion from the high concentration of salt. According to HACCP Generic Model for Fish Sauce, potential hazards are identified as bacterial spoilage of fish due to mishandling and temperature and time abuses as well as incorrect ratio of fish to salt and uneven mixing. Other hazards include the growth of bacteria on the membrane filter and contamination of the lubricant

Figure 18 Different types of packaging material. (Courtesy of Pichai Fish Sauce Co., Ltd.)

from the pooling tank and bottling steps. Hence, equipment must be maintained in good condition and food-grade lubricant must be used (25). Modern technology and mechanization of the process such as mixing, pumping, filtering, filling, and packing should be performed. Some factories filter the sauce using crossflow microfiltration with a membrane as fine as 0.8 μm to ensure the clarity of the product. An efficient bottle cleaning process and an automatic

bottling system can minimize the risk of contamination and exposure to air that can alter the product quality.

5. Product evaluation (Fig. 19). Strict control of the raw materials and subsequent analysis of the parameters required by the regulating standard during the fermentation period should be done to ensure the quality of the finished product. During fermentation, samples should be drawn for analysis of total nitrogen, sodium chloride, pH, color, specific gravity, histamine, and total viable counts at 2–3-month intervals until the quality standard is reached.

B. Major Problems for Industrialization

There are constraints in the processing and industrialization of fish sauce, which can be summarized as follows.

1. Shortage of raw materials. The major problems in using marine resources for fishery products are the shortage and increasing cost of raw materials. This is clearly seen from the marked increase in demand for anchovies for making fish sauce from 50,745 tons in 1995 to 68,409 tons in 1997 and 96,329 tons in 1999 (Table 2).

Figure 19 Quality control laboratory. (Courtesy of Pichai Fish Sauce Co., Ltd.)

Seasonal change affects the amount of marine catch as well as quality and quantity of the visceral and muscular enzymes of the fish (26). Salt should be clean, white, and fine grained so that it can easily attach to the fish when mixed. Production of solar salt is sometimes not sufficient and has to be imported from neighboring countries such as India (Pichai Fish Sauce, personal communication, 2002.). The total catch of trash fish is about 800,000 tons annually during 1997–1999 (7) and can be considered as a substantially inexpensive aquatic resource. At present, most of by-catch is used for animal feed and fishmeal production. The development of using by-catch fish as a raw material for making nam pla seems feasible, but there are still problems of flavor and color, which might lead to consumer resistance.

2. Quality of the raw materials. Resulting from the shortage of raw material near the coast, fishing vessels have to go further for the fish, thus, it may take over 24–48 h before coming to the production site. If the handling practice after harvesting the fish is not monitored, either by application of ice or mixing with an appropriate amount of salt during transportation to the shore, incipient spoilage can occur and result in a low quality of incoming raw material.
3. Wholesomeness and hygiene. Fish sauce is mainly produced by small- and medium-scale enterprises (SMEs). For manufacturers with a high-production-capacity supply for both domestic and export markets, quality standards such as GMP, HACCP, and ISO 9002 are achieved and certified by authorized bodies. The investment for an automatic processing line are incompatible and troublesome for small-scale factories with more limited production capacity and marketability. This is primarily in the case of producers of grade 2 or lower-grade fish sauce with a limited area of product market. Hygienic and sanitation practices are sometimes neglected or overlooked. This has a great effect on the wholesomeness and quality of the products.
4. Histamine level. In Thailand, a survey was conducted by the Fisheries Inspection and Quality Control Division, Department of Fisheries to determine histamine levels of nam pla samples submitted for export certification and also the relationship of histamine to total nitrogen content (27). A total of 549 samples were drawn for inspection randomly from 10 fish sauce processors during the period from May 1998 to May 1999. The histamine-level examination revealed that <200 ppm was found in 26% of the samples tested and the rest contained >200 ppm. The majority of the samples (85%) contained between 100 and 600 ppm, few at 100

ppm, few above 600 ppm, and scarcely any above 1000 ppm. These samples, although from exported product, also represent the values of histamine for local fish sauce because there are no differences in the process and the quality for both markets. Srisomboon et al. (28) reported the average level of histamine in 26 samples of fish sauce to be 29.28 mg/100 g. The range of histamine content in five fish sauce samples were 4.57 ± 0.13 to 102.99 ± 1.16 mg/100 g as determined by the colorimetric method (29).

For consumer protection from histamine poisoning, the Food and Agricultural Organization (FAO) and World Health Organization (WHO) set the limit of histamine in fisheries product at 200 ppm. Many countries established the maximum level of histamine at 10–20 mg/100g (30). The United States and Canada monitor the maximum content of histamine at 20 mg/100 g as the safety level for tuna products and fish sauce (32). Many countries use a maximum limit of histamine at 100 ppm for canned tuna (27).

Brillantes (27) noted that the amount of histamine obtained from 56 g of tuna (recommended serving size for the United States) with 100 ppm of histamine is 5.6 mg/meal. If Thai people consume the average amount of 20 mL/person/day or 6.7 mL/meal of fish sauce, the amount obtained from fish sauce containing 200 ppm histamine will be 1.34 mg/meal. If we calculate using the fish sauce with the more common level of histamine at 600 ppm, the amount consumed will be 4.02 mg/meal, which is still lower than that from tuna. If a level of 5.6 mg histamine, based on one serving of tuna, is an acceptable safe level, a maximum limit of 200 ppm histamine in fish sauce may be too conservative. A figure around 600–800 ppm seems more realistic and still ensures the safety of consumers (27). Virulhakul et al. (24) recommended the safe level of histamine to be 40 mg/100g (400 ppm).

Nevertheless, it is apparent that the control of the histamine level in nam pla is still a major concern for the producers, especially for the export market. Many studies are carried out to control the histamine content of raw material as well as to regulate the fermentation process in order to keep the histamine content within the acceptable limit.

VII. MICROBIOLOGY

There is still contradictory data about the role of specific microflora responsible for fermentation of fish sauce, although a large number of organisms

have been isolated and reported. Moreover, micro-organisms reported are often in a wide range, depending on which stage of fermentation they have been isolated as well as contaminating organisms. Research to study the microflora of nam pla has been carried out by many investigators. The micro-organisms isolated from wide range of samples in different areas, both in the finished product and during fermentation, have been reported (Table 7). Due to the high salt content of fish sauce, most microflora found are halophilic and halotolerant or moderate halophilic bacteria. The halotolerant group may not require salt for growth but can grow in the presence of up to 10% or more salt (48). This group includes sporeformers, particularly *Clostridium* spp., Micrococci, and Staphylococci. Halophilic bacteria can be divided into two groups: moderate halophilic and extremely halophilic. The first group, which include *Pseudomonas, Achromobacter, Micrococcus, Vibrio, Pediococcus, Coryneform,* and *Bacillus,* require 3–25% salt for growth (49). The second group is extremely halophilic bacteria in the family Halobacteriaceae, which require higher that 2 M or about 12% of sodium chloride for growth (50). The most common are *Halobacterium*, the Gram-negative rod and *Halococcus*, a coccoid highly resistant to osmotic damage that can grow only in the presence of 2.5 M or higher salt contents. Smittasiri (51) reported that isolates of extreme halophiles from nam pla, namely *Pseudomonas, Achromobacter, Vibrio, Micrococcus, Pediococcus, Bacillus, Halobacterium,* and *Halococcus,* could grow in higher than 20% salt. He also isolated *Bacillus* sp., *Micrococcus* sp., *Sarcina* sp., and *Halobacterium* sp. from sea salt. Salt is also the source of microflora that can contribute to the flavor and aroma of fish sauce.

In an early study, a nonpathogenic *Clostridium* was reported to be responsible for the flavor of nuoc-mam, a Vietnamese fish sauce (43). Saisithi et al. (32) pointed out that *Pediococcus halophilus* produced the flavor typical for nam pla. *Bacillus* spp, *Coryneform* spp., *Streptococci* spp, *Staphylococcus* spp., and *Micrococcus* spp. were also isolated (32). They also found 2.7×10^4 colony-forming units (cfu)/g of bacteria in solar salt used and thus concluded that some could be derived from the salt. This supported the work of Bain et al. (52), who reported a count of 10^5–10^6 bacteria/g of solar salt. Among them, 83 % were *Bacillus* and the rest were *Micrococcus* and *Sarcina* spp. Kasemsarn (53) reported that a high-salt-tolerant strain of *Staphylococcus* sp. is responsible for the fermentation of nam pla. Halophilic bacteria isolated from nam pla on media containing 20% NaCl were identified as the genera *Pseudomanas, Bacillus, Staphylococcus,* and lactic acid *Streptococcus* (33). Crisan and Sands (40) believed that *Bacillus* spp. found in nam pla and patis (Philippines fish sauce) were the predominating organism. They identified *B. cereus* and *B. licheniformis* as the dominant bacteria in the early stage; after 7 months of fermentation, other strains of *B. licheniformis*, *B. megaterium,* and *B. subtilis* were the dominant species. *Micrococcus copoyenes, M. varians, B. pumilus,*

Table 7 Microflora Isolated from Fish Sauce

Genera	Species	Ref.
Bacillus	*Bacillus* sp.	Saisithi et al. (32) Saisithi (33) Suntinanalerts (34) Saono et al. (35) Choorit and Prasertsan (36) Bulan et al. (37) Wongkhalaung (38) Padongkeittiwong (39)
	B. cereus	Crisan and Sands (40)
	B. licheniformis	Crisan and Sands (40)
	B. megaterium	Crisan and Sands (40)
	B. subtilis	Crisan and Sands (40)
	B. pumilus	Crisan and Sands (40)
	B. pantothenticus	Klomklang (41)
	B. firmus	Aroonpiroj (42)
	B. pasteurii	Aroonpiroj (42)
Candida	*C. clausenii*	Crisan and Sands (40)
Clostridium	*Clostridium* sp.	Boez and Guillerm (43)
Coryneform	*Coryneform* sp.	Saisithi et al. (32) Suntinanalerts (34) Liptasiri (44)
Corynebacterium	*Corynebacterium* sp.	Aroonpiroj (42)
Halobacterium	*Halobacterium* sp.	Suntinanalerts (34) Saono et al. (35) Wongkhalaung (38) Padongkeittiwong (39) Aroonpiroj (42) Thongthai and Siriwongpairat (45)
	H. salinarium	Klomklang (41) Aroonpiroj (42)
Halococcus	*Halococcus* sp.	Suntinanalerts (34) Saono et al. (35) Padongkeittiwong (39)
Lactobacillus	*Lactobacillus*	Saono et al. (35) Liptasiri (44)
Micrococcus	*Micrococcus* sp.	Saisithi et al. (32) Suntinanalerts (34) Saono et al. (35) Wongkhalaung (38) Padongkeittiwong (39) Bulan et al. (37) Liptasiri (44)

Table 7 Continued

Genera	Species	Ref.
	M. varians	Crisan and Sands (40) Aroonpiroj (42) Itoh et al. (46)
	M. sedentarius	Aroonpiroj (42)
	M. copoyenes	Crisan and Sands (40)
Paracoccus	*P. halodenitrificans*	Itoh et al. (46)
Pediococcus	*P. halophilus*	Saisithi et al. (32) Wongkhalaung (38) Itoh et al. (46)
	Pediococcus sp.	Saisithi (33) Saono et al. (35) Bulan et al. (37) Wongkhalaung (38) Padongkeittiwong (39)
Pseudomonas	*Pseudomonas* sp.	Saisithi (33) Liptasiri (44)
Sarcina	*Sarcina* sp.	Saono et al. (35) Bulan et al.(37) Liptasiri (44)
Staphylococcus	*Staphylococcus* sp.	Saisithi et al. (32) Saisithi (33) Suntinanalerts (34) Saono et al. (35) Wongkhalaung (38) Padongkeittiwong (39)
	S. saprophyticus	Klomklang (41) Itoh et al. (46)
Streptococcus	*Streptococcus* sp.	Saisithi et al. (32) Suntinanalerts (34) Saono et al. (35) Bulan et al. (37) Liptasiri (44)
Tetragenococcus	*Tetragenococcus* sp.	Thongsanit (47)
	T. halophilus	Thongsanit (47)
	T. muriaticus	Thongsanit (47)

and *Candida clausenii* are reported to be other halotolerant organisms isolated from fish sauce. Several studies have indicated the presence of *Pediococcus halophilus, Staphylococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Halobacterium* spp., and *Halococcus* spp. (Table 7).

Itoh et al. (46) identified acid-producing bacteria in Nam pla and were reported to be *P. halophilus, S. saprophyticus, M. varians,* and *Paracoccus halodenitrificans*. Bacterial isolates possessing high protease activity from 12 fish sauce samples were identified as *B. firmus, M. varians, Corynebacterium* spp., *M. sedentarius, B. pasteuii, H. salinarium,* and *Halobacterium* spp. (42).

Padongkeittiwong (39) studied the change of total viable count of halophilic bacteria during the fermentation period in three fermentation tanks (2.19 × 2.33 × 2.41 m, width × length × height) in relation to pH and salt content (Table 8). Three groups of halophiles according to the color profiles i.e. orange/red, white/cream and yellow were isolated during the 12 month-fermentation. Most of the isolates with high protease activity were identified as *Halobacterium* spp. A Halobacterium medium containing 25% sodium chloride was used to compare the number of bacteria from these 3 fermentation tanks. During the early stage, the halophilic count was hardly detected. From the second month on, an increase in the total number was observed in two fermentation tanks. The counts were highest from month 6 to

Table 8 Change of pH, % NaCl, and Total Viable Count of Bacteria in Three Fermentation Tanks

Time (months)	pH			% NaCl			Total viable count (cfu/mL)		
	1	2	3	1	2	3	1	2	3
0	6.45	6.41	6.24	22	25	22	2.5×10^4	0	0
1	6.32	6.39	6.04	25	28	24	0	0	0
2	6.12	6.04	6.12	28	30	27	3	10	1.2×10^3
3	5.97	6.02	6.02	30	27	30	1.1×10	4×10^2	2.89×10^3
4	5.95	5.97	5.95	27	29	34	1.6×10	1.3×10^3	4.12×10^3
5	5.73	5.95	5.82	31	30	33	1.9×10	4.35×10^3	6.25×10^3
6	5.84	5.46	5.67	34	34	32	3.2×10^3	5.4×10^4	3.39×10^4
7	5.78	5.23	5.53	34	32	30	1.2×10^3	5.63×10^4	6.27×10^4
8	5.52	5.27	5.58	28	28	30	1.5×10^3	7.42×10^4	6.89×10^4
9	5.67	5.20	5.43	26	27	28	7.3×10^3	6.73×10^4	2.31×10^4
10	5.84	5.02	5.38	27	26	26	9.2×10^3	9.2×10^3	8.14×10^3
11	5.92	5.34	5.31	26	25	25	1.5×10^4	3.5×10^3	6.35×10^3
12	5.95	5.69	5.45	25	24	26	2.2×10^3	2.2×10^3	3.25×10^2

Source: Ref. 39.

Table 9 Fermented Fish: Total Viable Counts (Bacteria/mL)

Fermentation time (months)	Brain–heart infusion (Difco)		Nutrient agar (Difco)	
	0.5% NaCl	10% NaCl	10% NaCl	20% NaCl
0.5			2.8×10^3	1.1×10^3
1	3.0×10^3	1.4×10^5	9.6×10^4	3.0×10^3
3	2.0×10^3	4.0×10^4	5.5×10^2	1.7×10^2
6	1.1×10^2	3.8×10^2	7.1×10^3	5.5×10^2
9	7.0×10^1	2.4×10^3	2.4×10^1	2.1×10^1
12	4.0×10^1	3.4×10^2	6.2×10^2	2.7×10^2

Source: Ref. 32.

month 9 and gradually decreased. Salt concentrations were also at a maximum during the middle of fermentation and decreased toward the end. This was also true for patis, Philippine fish sauce, studied by Hamm and Clague (54). Immediately after salting, a bacteria count of 2.9×10^6 was obtained and after 1, 2, and 3 weeks, the counts decreased to 4.6×10^4, 5.4×10^3, and 3.2×10^2, respectively. Halophiles are believed to contribute mostly to the flavor of fish sauce, whereas anaerobic bacteria have not yet been demonstrated to be the responsible organism (55). The total viable count during the fermentation period for fish sauce studied by Saisithi et al. (32) showed a marked decrease in bacteria per milliliter grown on brain–heart infusion with 0.5% and 10% NaCl as well as nutrient agar with 10% and 20 % NaCl (Table 9).

VIII. CHEMICAL AND BIOCHEMICAL CHANGES

After death, fish decomposition occurs very rapidly. Salt penetrates the fish tissues by osmotic pressure, causing a protein-rich liquid to develop through autolysis. Biochemical changes are the result of both bacteria and intrinsic enzyme activities from fish. Fish tissue degradation to lower molecular compounds by these proteolytic and lipolytic enzymes contributes to the aroma and flavor of fish sauce during fermentation. According to Han-Ching (1), muscle and visceral enzyme activities hydrolyze proteins and lipids. He pointed out that various fish muscle protease are present, but only cathepsins (acidic proteases) are significantly involved in fermentation. Orejana and Liston (56) reported that the main enzyme involved was a trypsinlike enzyme. Other proteases such as cathepsin B increases the soluble protein content of the liquid during the first 2 months of fermentation and gradually decreases.

They indicated that new polypeptide formation occurs during the last fermentation stage as the level of free amino acids increase. Ricke and Keeton (57) noted that pepsin in viscera is the major gastric protease; trypsin, chymotrypsin, and carboxypepsidases are of pancreatic origin. Cathepsins in muscle tissue of herring could be inhibited by salt concentrations over 15% (58). High salt concentrations have less effect on fish digestive enzymes, thus fish fermentations using high-salt content are never eviscerated (1). Aroonpiroj (42) reported that at the beginning of fermentation, the total protease activities of fish visceral, muscular, and indigenous bacterial origins were 48.23%, 19.43%, and 32.34%, respectively. Higher pyrolic caeca in fish intestines and stomach can accelerate the digestion of fish to amino acids and peptides.

IX. TOTAL NITROGEN AND AMINO ACIDS

Hydrolysis of protein of the fish tissues results in small-molecule peptides, nitrogen compounds, and amino acids. The total amino acids of nam pla is much the same quantity as in fish tissue, as shown in Table 10. However, Saisithi (59) pointed out that taurine, a nonprotein component found in nam pla but not in fish flesh could be derived from cysteine or cystine, and citrulline is derived from arginine. Moreover, these acids and others such as tyrosine, tryptophan, proline, OH-proline and γ-amino butyric acid are metabolized by the bacteria (32,60). Itoh et al. (46) observed a high glutamic acid concentration (486–1179 mg/100 g) and a low content of trimethylamine (0–7.5 mg/100 g) in commercial fish sauces from Japan, Thailand, and Singapore.

Amino acids not only contribute to the nutritive value of nam pla but also to its flavor. Free amino acids such as glutamic acid, proline, leucine, serine, lysine, arginine, glycine, and alanine were reported to be the taste-active substances in capelin fish sauce (62). A prolonged fermentation period resulted in the breakdown of amino acid and loss of nitrogen as ammonia (63). The increase in the pH after 15 months might be due to ammonia and amines such as dimethylamine or trimethylamine (56). Ammoniacal nitrogen in fish sauce was found to increase during the first 6 months and remained relatively unchanged (33). Orejana and Liston (56) indicated that amino–N in fish sauce are in the form of peptides more than amino acids. The molecular weight of the peptides was in the range 700–1500 Da during the first 40 days. The enzymes involved are endopeptidase and trypsin. From 70 to 140 days, smaller-molecule peptides were present, resulting from the action of exopeptidase and cathepsin. First-grade nam pla contains high levels of glutamic acid, lysine, alanine, and valine. The amino acid compositions of nam pla from five producers are presented in Table 11. Histidine gives rise to the flavor

Table 10 Amino Acid Composition of Nam Pla (Undigested and Digested) and Fish Flesh

	Quantity (mg/100 mL)		
Amino acid	Undigested	Digested	Fish flesh
Taurine	167	211	—
Aspartic acid	1601	2009	1800
Threonine	819	873	834
Serine	683	861	924
Glutamic acid	1448	3280	2704
Glycine	552	1041	816
Alanine	1194	1272	1136
Valine	469	974	928
Methionine	331	335	432
Isoleucine	372	379	800
Leucine	434	431	1472
Tyrosine	99	63	656
Phenylalanine	519	541	752
Histidine	707	649	576
Lysine	1767	1982	1692
Arginine	4	34	1120
Citrulline	1300	894	—

Source: Ref. 59.

of nam pla made from fatty fish species, whereas taurine may play the role in the Maillard reaction. A comparison of amino acid contents between Vietnamese fish sauce and Thai nam pla is shown in Table 12.

A. Organic Acids

Lactic acid and acetic acid are found in large quantity in nam pla as a result of lactic acid fermentation. Other acids are pyruvic, formic, malic, propionic, α-ketoglutaric, and levolinic acids (64). They also reported that isovaleric and butyric acids are found after prolonged fermentation. The total amount of organic acids increased as the fermentation time increased, with the highest amount in the middle period, and tended to decrease toward the end of fermentation. The organic acid contents in nam pla is shown in Table 13. Acetic, pyroglutamic, and lactic acids are present in large quantities, and are thus believed to contribute to the flavor and aroma of nam pla.

Table 11 Amino Acid Composition of Nam Pla from Five Processors

	Quantity (mg/g N)					
Amino acid	1	2	3	4	5	Suggested essential amino acid level
Aspartic acid	383	359	288	192	274	
Threonine	257	240	205	146	188	
Serine	123	163	116	66	126	
Glutamic acid	853	831	792	2210	1088	
Glycine	187	176	151	122	132	
Alanine	365	362	318	290	268	
Methionine	127	133	143	138	226	220
Cystine	95	88	90	180	0	
Valine	345	316	306	308	378	310
Isoleucine	236	185	274	226	362	250
Leucine	306	229	431	324	638	440
Tyrosine	39	41	46	0	68	380
Phenylalanine	195	178	184	266	246	
Lysine	500	477	414	386	362	340
Histidine	208	205	163	180	180	
Arginine	0	0	0	0	0	
Proline	0	79	0	0	0	

Source: Ref. 31.

B. Flavor and Aroma

The flavor and aroma are the most important characteristics for acceptability of nam pla. They are very complex and cannot be attributed solely to specific volatile fatty acids or amino acids derived from fermentation. The aroma of nam pla as investigated by Dougan and Howard (65) was classified into three major groups: (a) the ammoniacal group from ammonia and trimethylamine; (b) a "cheesy" aroma due to the low-molecular-weight fatty acids (volatile fatty acids, VFAs) with ethanoic and *n*-butanoic acids predominating; and (c) a "meaty" aroma due to a large number of volatile compounds. The ketones, ketoacids, and amino acids, especially glutamic acid, seem to be important and contribute to part of the aroma.

Volatile acids in nam pla by many investigators as compiled by Saisithi (59) is shown in Table 14. As the fermentation time increased, the amount of total volatile acids (TVAs) also increased and reached its maximum value after about 9 months (33). These acids include formic, acetic, propionic, and isobutyric acids and are believed to contribute to the flavor and aroma of nam pla. The rate of formation of the volatile acids, volatile bases, and ketones

Table 12 Comparison of Amino Acid Contents Between Vietnamese Fish Sauce and Thai Nam Pla

Amino acid (g/L)	Vietnamese nuoc mam	Thai nam pla
Alanine	4.20	6.49
Arginine	2.00	5.71
Aspartic acid	2.40	6.38
Cystine	0.25	0
Glutamic acid	4.00	3.59
Glycine	2.40	2.95
Histidine	0.30	5.16
Isoleucine	4.00	3.36
Leucine	4.00	3.84
Lysine	4.00	11.20
Methionine	0.80	2.65
Phenylalanine	1.50	3.68
Proline	0.50	2.18
Serine	0.80	2.95
Threonine	2.00	3.64
Tyrosine	0.80	0.78
Tryptophan	0.50	3.84
Valine	0.30	5.17

Source: Ref. 51.

Table 13 Organic Acid Contents in Nam Pla (mg%)

Organic acid	Sample 1	Sample 2
Unknown	Trace	Trace
Pyroglutamic	384	489
Lactic	397	602
Acetic	536	453
Levalinic	—	—
Pyruvic	24	62
Formic	14	Trace
Malic	Trace	Trace
Propionic	30	31
Citric	9	16
Succinic	78	126
Butyric	32	11
Isovaleric	24	194

Source: Ref. 64.

Table 14 Volatile Acids Composition of Fish Sauce

Volatile acids		Total amount (mEq/L)	Ref.
Formic, acetic, propionic, and isobutyric acids		87	33
n-Butyric/acetic,	1/1–1/20	175	66
Acetic,	210–530 mg/100 mL	78	67
propionic,	11–47 mg/100 mL		
isobutyric,	1 mg/100 mL		
n-butyric,	6–13 mg/100 mL		
isovaleric,	3–30 mg/100 mL		

Source: Data from Ref. 59.

were studied by Saisithi et al. (33). They reported an increase of these compounds during the fermentation period. An attempt had been made to analyze the aroma of the fish sauce by extracting with an ether–alcohol mixture and separating the amino acids from the VFAs by ion exchange. It was found that the typical fish sauce aroma did not derive from the amino-group fraction. Trimethylamine and a fraction similar to that obtained from the Strecker degradation of phenylalanine were also eluted. The investigators indicated that the fraction is fairly typical of the fish sauce aroma. Trying to reproduce aroma constituents did not give satisfactory results except that glutamic acid did produce a meaty aroma, typical for fish sauce. Further investigation revealed that by an ether–alcohol extract, six amino acids as well as glucosamine and histamine were present. Comparison of volatile acids in fish sauces from Thailand, Vietnam, and Malaysia are presented in Table 15.

Table 15 Comparison of Volatile Acids Content in Fish Sauces from Different Countries

	Quantity (mg/mL)		
Volatile acids	Nam pla	Nuoc-mam	Budu
Ethanoic	0.25–1.40	0.7–1.4	2.1
Propanoic	0.05–0.67	NA[a]	0.12
Isobutanoic	0.06–0.12	NA	0
n-butanoic	0.06–0.42	0.35–0.7	0.23
Isopentanoic	0.03–0.31	NA	0.07
Isovaleric	NA	NA	NA

[a] Not available.
Source: Data from Ref. 21.

Virulhakul (31) reported that major aromatic compounds in nam pla (viz. formic, acetic, propionic, and isobutyric acids) are produced by *Pediococcus halophilus* during fermentation. These acids result from amino acid catabolism through bacterial oxidative deamination in the presence of catalase enzyme. Recently, a profile of the flavor compounds of Nam pla has been analyzed and found to be acetic acid, propanoic, 2-methyl propanoic, butanoic, benzene acetaldehyde; 2-methyl 1-propanal, 3-methylthio pentanoic acid; 2-methyl 1-propanol, 3-methylthio pentanoic acid; phenyl ethyl alcohol; Phenol; indole, 3-methylthio 2-furan-methanol; penta decanoic acid; ethyl ester; tetradecanoic ethylester butanoic acid (ethylester); and 2,5 dimethyl 1-4-hydroxy-s (2*H*)-furanone (31).

Worapong (68) analyzed 8 samples of nam pla at different ages of fermentation and reported that 213 detectable volatile compounds were separated from nine-month samples. However, only part of these compounds had been identified as 12 acids, 2 carbonyls, 4 hydrocarbons, 10 alcohols, 2 nitrogen compounds, 6 esters, 1 phenol, and 1 dioxane. Most samples were found to contain *n*-butanoic acid, phenylacetic acid, propionic acid, isopentanoc acid, *n*-pentanoic acid, benzoic acid, and indole. These compounds are believed to contribute to the agreeable aroma in nam pla, as has been reported by other investigators (21,31,58). High-boiling-point volatile components (viz. heptane, benzyl alcohol, and phenol) are also present in all specimens but probably share less aroma profile for the sauce. Quantitative analysis indicated that the separated volatile compounds increased with the fermentation period, from 150 to 228 after 3 days and 4 months, respectively. A slight decrease was observed after 9 months, but markedly decreased if kept in cold storage at 4°C. The result (68) also demonstrated that long-term storage of the 9-month fermented fish sauce at 4°C caused the loss of some low-carbon volatile compounds such as acetic acid, propionic acid, isobutanoic acid, pivalic acid, *n*-heptanoic acid, *n*-octanoic acid, ethyl acetate and bis-(2-ethyl)hexyl phthalate.

Quantities of volatile acids, alcohols, and aldehydes from 10 commercial brands of nam pla on the Thai market are presented in Table 16. Pengsopar and Chaiyanan (69) reported that acetic acid was commonly found in 30–48% of all samples, whereas propionic acid and *n*-butyric acid were detected in an average of 5%. Other volatile acids were found in small amounts. For alcoholic substances, ethanol and propanol were found at 10.68% and 17.57% of samples, respectively. Only acetaldehyde was found in the aldehyde group. The study of volatile substances produced from *P. halophilus* isolated from nam pla was carried out using fishmeal as the substrate (69). It was found that similar volatile compounds were observed, although the quantities were different to some extent (Table 17). However, propionic acid was not detected. Isovaleric acid was found in higher amounts (19%) compared to that of 1.5%

Table 16 Quantities of Volatile Acids, Alcohols, and Aldehydes from 10 Brands of Fish Sauce

Fish sauce	Volatile acids[a] (%)						Alcohols (%)			Aldehyde (%) (Acetaledhyde)
	A	B	C	D	E	F	Ethanol	Propanol	Butanol	
Brand 1	36.98	6.37	—	4.18	2.46	—	14.70	18.02	1.89	1.11
Brand 2	36.74	2.54	—	10.72	—	—	8.33	—	1.09	1.35
Brand 3	42.26	4.05	—	3.68	—	—	—	22.53	0.84	1.63
Brand 4	30.17	5.76	0.33	9.52	1.69	2.29	27.02	11.01	2.08	0.08
Brand 5	48.00	0.42	0.61	0.05	0.09	—	8.44	—	1.42	1.45
Brand 6	33.00	11.29	0.13	50.9	0.48	—	3.87	11.28	—	2.84
Brand 7	36.58	6.44	1.19	4.26	0.07	1.39	8.86	17.82	1.82	1.58
Brand 8	36.65	3.60	—	4.77	4.72	—	10.90	17.34	2.10	2.02
Brand 9	43.28	3.62	0.05	2.61	0.43	—	8.74	18.35	2.31	1.23
Brand 10	35.26	6.45	0.31	5.58	1.26	1.13	5.29	24.21	—	2.71
Maximum	48.00	11.29	1.19	10.72	4.72	2.29	27.02	24.21	2.31	2.84
Minimum	30.17	0.42	0.05	0.05	0.07	1.13	3.87	11.01	0.84	0.08
Average	37.89	5.05	0.44	5.05	1.50	1.60	10.68	17.57	1.69	1.60

[a] A = acetic acid, B = propionic acid, C = isobutyric acid, D = *n*-butyric acid, E = isovaleric acid, F = *n*-valeric acid.
Source: Ref. 69.

Table 17 Quantities of Volatile Substances from the Activity of Bacteria, *P. halophilus*

Samples	Volatile acid[a] (%)						Alcohol (%)			Aldehyde (%) (Acetaledhyde)
	A	B	C	D	E	F	Ethanol	Propanol	Butanol	
1	56.06	—	4.92	8.83	15.75	0.24	0.84	—	11.66	1.06
2	51.73	—	5.06	6.29	22.27	0.23	0.61	0.14	12.34	0.80
Average	53.89	—	4.99	7.56	19.01	0.24	0.73	0.14	12.00	0.93

[a] A = acetic acid, B = propionic acid, C = isobutyric acid, D = *n*-butyric acid, E = isovaleric acid, F = *n*-valeric acid.
Source: Ref. 69.

in the commercial sample. Isobutyric acid was also 10 times higher. Butanol was the major alcoholic substance detected, which is about six times higher, whereas ethanol and propanol were less than 1%. A similar study was done by Chaiyanan and Chaiyanan (70). Nine isolates of bacteria from fish sauce (i.e., *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., and *Pediococcus* spp.) cultivated in fishmeal hydrolysate produced methanol, ethanol, propanol, isobutyl alcohol, and acetaldehyde. Only *Pediococcus* spp. could produce every kind of VFA found in fish sauce, indicating that they probably contribute to the aroma development in fish sauce.

X. COLOR

The color of first-grade nam pla is amber or reddish brown, which is desirable. Grade 2 or a lower grade normally has a lighter or pale color that can be regulated by addition of caramel. Carbonyl compounds resulting from the reaction of amino and aldehyde groups in phospholipid and lipoprotein in the presence of sugar and oxygen can give rise to the browning of fish sauce. The color of fish sauce is the result of the nonenzymatic browning reaction between the amino acids in the fish sauce and ribose (33).

Orejana (58) reported that total volatile bases, ammonia, and free fatty acids increased during patis fermentation. Thus, the amino acid fraction (and some polypeptides) was probably involved in the browning reaction upon storage.

XI. NUTRITIONAL QUALITY AND SAFETY

Because the official standard for fish sauce requires a minimum concentration of 230 g/L of salt, the spoilage of fish sauce from micro-organisms is unlikely

Table 18 Proximate Composition of Nam Pla

		(b)	
Percent	(a)	Grade 1	Ordinary
Moisture	70.6–76.7	73.0	75.8
Protein	1.8–2.2	11.6	2.0
Fat	0.7–4.7	Trace	Trace
Carbohydrate		2.4	12.7
Ash	25.6–29.4	36.4	30.8

Sources: (a) Data from Ref. 37; (b) data from Ref. 71.

to occur. The product quality for different grades of nam pla has been set up primarily based on nitrogen content, amino acid nitrogen, and ratio of glutamic acid and total nitrogen. Moreover, export products must comply with an international standard as well as the regulation and requirement of the buyers.

Proximate composition and some mineral contents of fish sauce are presented in Tables 18 and 19. Grade 1 fish sauce contains up to 11.6 % protein. Chemical analysis of nam pla during a 12-month fermentation showed that the total nitrogen markedly increased after 9 months (Table 20). Ammonia–N was increased two times after 6 months, as supported by Uyenco et al. (63) that prolonged fermentation caused the loss of nitrogen in the form of ammonia. The chemical composition of fish sauce from many investigations are shown in Table 21. The salt content is high in the range 26–31%, which is comparable to the amount found in fish sauce from other countries (Table 22). Normally, the fish sauce producer adds a small amount of sugar to reduce the explicit salty taste and improve the flavor, monosodium glutamate (MSG) is sometimes added to increase glutamic acid content and also enhance

Table 19 Some Mineral Contents in Nam Pla

	(a) mg/100 g	(b)	(c) mg/L
Phosphorus		0.266–5.66 g/L	200
Calcium	39–59	0.439–0.541 g/L	700
Magnesium		2208–2310 mg/L	
Organic S		0.54–1.16 g/L	
Iron	2.5–29	10–22 mg/L	6
Sodium	9,294–10,032		
Iodine			154–790

Sources: (a) Adapted from Ref. 59; (b) data from Ref. 71; (c) data from Ref. 21.

Table 20 Chemical Analysis of Thai Fish Sauce

Fermentation period (months)	pH	NaCl (%)	Mmoles/100 ml				Volatile base (mEq/100 mL)
			Total N	Ammonia-N	Titratable acid—lactic	Volatile acid—lactic	
1	6.4	30.1	49	8	6.8	4.3	4.01
3	6.2	30.3	52	7	8.0	3.3	6.61
6	6.6	30.2	56	14	5.2	8.7	10.21
9	6.2	30.2	130	15	5.9	4.3	14.71
12	6.4	27.9	140	15	15.8	6.3	3.01

Source: Ref. 32.

the flavor. A byproduct (Meiki or BX) from the MSG industry is used by some producers to mix with pure fish sauce. The total nitrogen and ratio of glutamic to total nitrogen of different grades of nam pla and commercial samples collected from 1979 to 2000 are shown in Table 23. The composition of BX and blended fish sauce/BX at different proportions are presented in Tables 24 and 25. The proportion of mixing is adjusted to meet the legal requirement for protein and nitrogen. Another report revealed that blended fish sauce with BX contains 12.1 g/L of total nitrogen, 7.2 g/L of amino nitrogen, and 247 g/L of sodium chloride (73).

Table 21 Chemical Composition of Thai Nam Pla from Different Studies

Composition	1	2	3	4	
				High grade	Low grade
NaCl, %	26.06	26–31	26.8	28.15	28.9
Total N (TN) (%)	2.30	0.06–4.41	1.86	1.92	0.92
Formaldehyde-N (FN) (%)	1.74		1.05		
FN/TN	74.4		56.5		
Organic N (%)				1.64	0.62
Ammonia-N (%)		10–30[a]		0.28	0.28
Amino-N (%)		40–60[a]		0.85	0.55
Volatile base-N (mg%)			338		
Trimethylamine-N (mg%)			3.7	20	20
Glutamic acid (mg%)			1179		
Total solid, (% without salt)	5.3	5.4–6.6	5.58		
Specific gravity	1.24	1.19–1.22			

[a] Percent of total N.
Source: Ref. 3.

Table 22 Chemical Composition of Fish Sauce from Different Countries

Composition	Malaysia budu[a]	Indonesia ketjab-ikan	Japan uwo-shoyu	Philippines extra patis	Singapore fish sauce	Vietnam nuoc-mam	
						First quality	Ordinary
NaCl (%)		27.0–28.2	28.2	28.21	26.8	27.5	28.0
Total N (TN) (%)	1.77	1.6–1.93	1.08	2.20	0.38	2.2	1.1
Formaldehyde-N (FN) (%)	1.85		0.369	2.20	0.199	1.6	0.8
FN/TN			34.2		52.4		
Organic N (%)		1.10–1.17		2.20		1.5	0.75
Ammonia-N (%)				0.18		0.7	0.35
Amino-N (%)	1.17	0.5–0.87					
Volatile base-N (mg%)	120		24.0		23.0		
Trimethylamine-N (mg%)			7.5		0		
Glutamic acid (mg%)			642		1151		
Total solid (% without salt)		5.4–6.6	6.2	5.3	10.7		
Specific gravity		1.19–1.22	5.77	1.24	4.67		

[a] Data from Ref. 21.
Source: Ref. 3.

Table 23 Total Nitrogen and Glutamic/Nitrogen Ratio of Different Grades of Fish Sauce

Fish sauce	Price (bahts) (725-mL bottle)	Total N (g/L)	G/N ratio
Grade 1 (5)[a]	7.00–22.00	8.7–32.0	0.4–0.9
Grade 2 (8)	3.00–5.00	2.7–1.9	0.2–1.5
Grade 3 (6)	2.00–2.50	0.3–3.4	0.1–1.2
Commercial nam pla in			
1979 (11)		18.9–40.0	0.2–1.8
1982 (18)		16.9–28.0	0.4–1.6
1983 (16)		16.2–33.3	0.2–1.9
1985 (15)		16.7–28.6	0.5–1.2
1987 (21)		19.1–29.3	0.5–0.6
1990 (9)		16.3–34.2	0.5–0.7
1991 (15)		18.4–34.5	0.4–0.9
1992 (10)		14.4–37.2	0.4–0.9
1993 (13)		19.1–36.6	0.4–0.7
1995 (7)		26.5–33.7	0.4–0.7
1996 (9)		16.3–34.2	0.5–0.7
1997 (5)		13.1–27.9	0.7–0.8
1999 (11)		9.1–33.3	0.1–0.9
2000 (11)		2.9–26.4	0.7–1.2

[a] Figure in parentheses is the number of samples.
Source: Ref. 72.

Table 24 Different Types of Fish Sauce on the Thai Market

Item	Total N (g/L)	Amino-N	Glutamic acid	G/N ratio
Fish sauce (100)[a]	0.2–27.0	0.1–47.6	0.2–19.2	0.6–5.6
Fish sauce blended with BX	2.5–10.6	4.0–26.9	4.9–10.4	1.0–1.9
Fish sauce from BX	1.7	1.6	4.2	2.4
Fish sauce from BX + MSG	1.3	0.1	7.3	5.6
BX	10.1	7.6	24.5	2.4

[a] Total of 100 commercial samples sold in the country.
Source: Adapted from Ref. 72.

Table 25 Fish Sauce Blended with BX at Different Ratios

Fish sauce	Total N (g/L)	Amino-N (g/L)	G/N ratio
Fish sauce	17.0	29.6	0.5
BX	10.1	7.6	2.4
Fish sauce + 25% BX	15.6	24.8	0.8
Fish sauce + 50% BX	14.2	20.6	1.2
Fish sauce + 75% BX	12.7	16.2	1.8

Source: Ref. 72.

XII. CLASSIFICATION AND STANDARD OF FISH SAUCE

In Thailand, the grade or quality of fish sauce is classified by the total nitrogen content in conjunction with its aroma and flavor. There are two standard levels for nam pla: the compulsory standard from the Thai FDA, Ministry of Public Health (74) and the industrial standard from the Thai Industrial Standards Institute (TISI), Ministry of Industry. The minimum legal requirement is set by the Thai FDA for three types of nam pla, as shown in Table 26. The minimum content of total nitrogen of the product to be labeled as nam pla must not be less than 4 g/L and the salt content must not be less than 200 g/L.

Table 26 Thai FDA Standard for Nam Pla

Requirement	Natural or pure fish sauce	Fish sauce from other animals[a]	Blended fish sauce[b]
Sodium chloride (g/L)	NLT[c] 200	NLT 200	NLT 200
Total nitrogen (TN) (g/L)	NLT 9	NLT 9	NLT 4
Amino acid-N (%) [(AA-N/TN) × 100	40–60	40–60	—
Glutamic acid/total N (G/N)	0.4–0.6	0.4–0.6	0.4–1.3
Clarity	Clear, no precipitation unless naturally occurring and must be <0.1 g/L		
Sweetening agent	Not allowed, except sugar		
Color	Not allowed, except from caramel		

[a] Includes the sauce fermented by the same process from animals other than fish and also the mixture of those with natural fish sauce.
[b] Includes the sauce from the first two categories that are flavored, diluted, or concentrated.
[c] NLT = not less than.
Source: Ref. 74.

Table 27 TISI Standard of Nam Pla in Thailand

Requirement	Grade 1	Grade 2
Sodium chloride (g/L)	NLT[a] 230	NLT 230
Total nitrogen (g/L)	NLT 20	NLT 15
Glutamic acid/total N (G/N)	0.4–0.6	0.4–0.6
Amino acid-N (g/L)	10	7.5
pH	5.0–6.0	5.0–6.0
Relative density at /27°C	1.2	1.2
No preservative allowed		
No color allowed except from caramel		

[a] NLT = Not less than.
Source: Ref. 75.

The TISI, Ministry of Industry has established an industrial standard for local fish sauce known as TIS 3-1983 (75) to upgrade the fish sauce industry in the complying country. Those fish sauces complying with the standard can use the mark of TISI on the container and are recognized as good quality products. In view of industry, the TISI classifies fish sauce in to two grades, as shown in Table 27. Those with total nitrogen below 15 g/L cannot be labeled as pure or natural fish sauce but must be labeled as diluted, blended, or mixed fish sauce. An analysis of 549 fish sauce samples for export during 1998–1999 showed that 31% fell into grade 1, 33% into grade 2, and 36% into lower grades (27).

XIII. IODINE SUPPLEMENTATION

The iodine nutritional requirement is recommended to be 150 μg/person/day (76). Iodine deficiency results not only in the development of goiter but also malfunction of the brain; thus, the term iodine deficiency disorders (IDDs) is suggested. In Thailand, concern for IDDs is emphasized in children and pregnant and lactating women, especially in the north and northeastern parts of the country. Iodine deficiency anemia in elementary school children in Thailand was reported to be 16.78% in 1992 (77). An iodine level in urine less than 50 μg/g of creatine indicates iodine deficiency (78). According to the Department of Health, the recommended daily intake of iodine is 50–70 μg/day for adults, 90–150 μg/day for children up to 12 years, and 170 μg/day for pregnant women and 200 μg/day for lactating women.

A survey of iodine content in fish sauces revealed that pure and blended fish sauces contained up to 134.6 and 92.4 mg/L, respectively (78). The average content was 19.5–21.0 mg/L, thus, consumption of 20 mL fish sauce per day is usually considered sufficient to prevent IDDs. However, lower grades of fish sauce, which are normally consumed by the low-income group, contained only 0.75–1.59 mg iodine/L (79). To supply the iodine needs of the risk group, iodine fortification in the form of potassium iodate at 8 mg/L fish sauce has been recommended by the Ministry of Public Health. Moreover, the stability of iodine in fish sauce is essential to ensure the availability of the amount required. A study of fish sauce fortified with 8 mg/L potassium iodate revealed that 2.7–5.1 mg iodine/L were detected after storage for 6 months. No physical change in terms of color and precipitation was observed. Light exposure has no effect on the iodine content in fish sauce upon storage (80).

XIV. HISTAMINE FORMATION

Red-fleshed pelagic fish such as sardines, mackerels, and anchovies, which are used for nam pla making, usually contain a generous quantity of free histidine in their white muscle (27). High free histidine in the flesh of anchovies serves as a substrate for histamine production by the action of histidine decarboxylase enzyme from bacteria. Due to the depletion of natural resources, fishing vessels have to go at a greater distance to catch fishes; thus, it may take as long as 24–48 h before the boats are back to shore. If insufficient ice or salt is incorporated, it is likely that the fish will be in an incipient stage of spoilage when they reach the processing sites. At temperatures around 0°C and below, histamine formation is restricted. The level of histamine in the fish is thus a control measure for decomposition and temperature abuse (27).

Bacteria containing histidine decarboxylase are capable of histamine production. Okuzumi et al. (81) isolated histamine-producing bacteria and identified them as psychrophilic and halophilic, which are the groups normally involved in nam pla fermentation. Many types of bacteria, including *Hafnia* sp., *Klebsiella* spp., and *Proteus* spp., found on fish skin are capable of producing histidine carboxylase (82).

Cousin and Noyer (60) first identified histamine in nam pla in 1944 and Saisithi et al. (32) found tyramine and tryptamine to be present in addition to histamine. Beddows (61) identified putrescine, cadaverine, and histamine in both budu and nam pla but concluded that the levels found were relatively low and may not contribute to the aroma. A high level of histamine over 100 mg% could give rise to Scombroid poisoning (83).

Consumption of a high concentration of histamine can cause histamine poisoning. Although it is considered a rather short, mild illness, it could be a

major impairment or even fatal to people with high susceptibility to histamine. The symptoms include gastrointestinal (nausea, vomiting, diarrhea, abdominal cramps) and neurological (facial flushing, itching, urticaria, localize inflammation, palpitation, hypertension, and headache) effects (84). People with histamine poisoning may experience only a few of these symptoms, depending on the level of histamine intake and susceptibility of each individual (27). The effect is very rapid, from immediately to a couple of hours after ingestion. It normally takes 2–3 days for recovery. It is estimated that a man of 70 kg body weight can ingest up to 6 mg histamine with no apparent symptom; from 6 to 70 mg histamine results in mild forms of poisoning; from 70 mg to 1 g histamine, disorders are stronger and more serious; above 1 g histamine can result in major injury (27).

The wide range of histamine levels in nam pla, grades 1 and 2, showed that histamine content does not limit the production of total nitrogen (TN) (27). Nam pla samples with the same TN content may have histamine at 100 or 1000 ppm. From this investigation, it seems to be apparent that the processors can control the amount of TN according to quality categories but can hardly control the level of histamine. Nonthaphala (85) studied histamine contents of nam pla grades 1 and 2 from five producers, as presented in Table 28.

There are no reports of illness associated with histamine poisoning from consumption of fish sauce.

Table 28 Histamine Contents in Nam Pla Grades 1 and 2 from Five Producers

Nam pla	Histamine[a] (ppm)	Standard deviation
Grade 1		
1	258.25	±6.87
2	194.39	±8.75
3	176.13	±10.27
4	236.27	±7.75
5	225.57	±10.22
Grade 2		
1	208.92	±8.02
2	210.68	±7.54
3	194.68	±9.90
4	210.71	±8.04
5	189.73	±11.83

[a] Mean of five samples taken at various times of the year.
Source: Adapted from Ref. 85.

XV. RESEARCH AND DEVELOPMENT

The increase in demand and legal standards requirements of fish sauce led to substantial studies to improve the quality and the fermentation process. Due to the long period of natural fermentation, which can reach 18 months, acceleration of fish sauce processing has gained much attention and were carried out by many means. Research and development are also focused on many other areas, such as quality improvement of raw material, application of starter cultures, and standardization of products and processing operations to increase productivity and international standard quality.

A. Methods Used for Acceleration the Fermentation Process of Fish Sauce

The traditional process through natural fermentation is still practiced and the period required to obtain desirable quality and characteristic aroma takes 8–12 months. Attempts have been made over the years to accelerate the fermentation process without altering the typical quality. It is well recognized that the hydrolysis of the fish protein is due to the actions of enzymes from the viscera and muscle of the fish as well as from the bacteria (32). Thus, the addition of proteolytic enzymes in various forms have been studied. Other acceleration methods have also been used, such as additing acids, raising the temperature, lowering the salt content, and using amino acid, as shown in Table 29. Although these processes were reported to be accelerated, more of these methods has proved to be able to replace the traditional method commercially or to produce fish sauce with the same typical quality. At present, all fish sauce producers still rely on the traditional fermentation process, which normally takes 8–12 months to ensure adequate development of flavor and aroma of the product. The major development of the fish sauce manufacturer has been aimed toward the postfermentation processing control in terms of the application of modern technology and equipment for uniformity and wholesomeness of the product.

Chaveesuk et al. (93) studied the effect of the addition of 0.3% (w/w) trypsin and chymotrypsin at various proportions to accelerate fish sauce fermentation from herring. Whole uneviscerated herring was minced, mixed with salt (1 : 1.5 parts of 25% salt solution), enzymes added, and fermented at 37°C for 42 days. They reported the increase of protein hydrolysis in terms of significantly higher total nitrogen, soluble protein, free and total amino acid contents in fish sauces with 50 : 50 and 0 : 100 trypsin : chymotrypsin supplementation when compared with fish sauce with no added enzyme. Chemical and microbiological qualities were similar to a commercial first-grade nam pla from Thailand. The color was lighter than nam pla, which was

Table 29 Methods Used for Acceleration of the Fermentation Process of Fish Sauce

Acceleration methods	Material used	Ref.
Enzymes/source of enzymes	Enzymes from Bacillus subtilis at 50 C for 3–5 hr	Amano (4)
	Fresh pineapple juice	Guillerm (86)
	Papain	Santos et al. (87)
		Ooshira et al. (88)
	Bromelain	Beddows et al. (89)
		Ooshira et al. (88)
	Plant protease	Murayama et al. (90)
		Beddows and Ardeshir (91)
	Proteolytic enzymes	Raksakulthai et al. (92)
	Trypsin	Ooshira et al. (88)
	Trypsin and Chymotrypsin	Chaveesuk et al. (93)
Addition of oxygen	Aeration	Chuapoehuk et al. (94)
Raising the temperature	To 45 C and reduce salt	Hamm and Clague (54)
		Amano (4)
		Orejana et al. (95)
Addition of acid	At low pH	Beddows and Ardeshir (96)
Addition of acid and lowering the salt		Gilberg et al. (97)
Use of amino acid	Histidine	Sanceda et al. (98)
Use of antibacterial instead of salt		Amano (4)
Recycling system with microbilal inoculation		Chaiyanan et al. (99)
Packed-bed column		Chaiyanan (100)
Continuous process		Pucharoen (101)

preferred by the panelists, but no significant difference in aroma and flavor preference was reported.

The addition of oxygen to the fish–salt mixture (3 : 1) during the fermentation of fish sauce from anchovies was studied (94). Aeration at 50 rpm, 8 h/day, 5 days/week, was applied to 175-l fiberglass containers containing about 100 kg of the fish–salt mixture. The propellant also provided uniform temperature distribution in the fermentation tank and improved enzymatic reactions of the fish visceral enzymes. The aerated sample had higher total and amino nitrogens than the control during 2–40week fermentations. Total nitrogen content reached 20.14 g/L (grade 1 fish sauce) compared to 15.64 g/L of the control after 20 weeks. However, the filtration was difficult with lower yield and the color was found to be darker. Sensory

evaluation of the 6-month sample was judged as not significantly different from the control and commercial samples ($p < 0.05$), whereas the 12-month sample was not unacceptable due to the much darker color and oxidized flavor from the fish fat.

Sanceda et al. (98) reported that the addition of histidine shortened the fermentation process of fish sauce manufacturing to 4 months compared to the normal 6–12 months in the traditional process. The liquefaction rate was faster than the control without added histidine and the degree of hydrolysis was much greater, resulting in higher nitrogen and amino acid content (Tables 30 and 31). The conversion of insoluble protein to soluble protein was increased in all histidine-added samples except for the 0.1%. Analysis of amino acids revealed that histidine-fortified samples were in the same pattern with slightly higher values than those in the control, except for histidine content, which was about five times higher. This indicated that histidine was not degraded during fermentation. The amount of histidine in 4-month fish

Table 30 Nitrogen in Fish Sauces

	g N/100 mL[a] Incubation	
Samples	2 months	4 months
A. Histidine-added sauces		
Control	0.97 ± 0.01[c]	1.80 ± 0.01[c]
0.1%	1.10 ± 0.01	1.82 ± 0.03
0.2%	1.91 ± 0.02	2.22 ± 0.02
0.5%	1.97 ± 0.03	2.64 ± 0.02
1.0%	2.15 ± 0.02	2.95 ± 0.02
2.0%	2.91 ± 0.02	3.70 ± 0.02
B. Commercial fish sauces[b]		
Patis (Philippines)	0.80 ± 0.07	
Nam pla (Thailand)	0.71 ± 0.06	
Shottsuru (Japan)	1.60 ± 0.07	
Salted anchovy sauce (Korea)	0.77 ± 0.08	
Nam pla (Japan)	0.61 ± 0.05	
Anaerobically fermented (Japan)	2.01 ± 0.06	

[a] Values are average of three replicates by the Kjeldahl method.
[b] Commercial fish sauces with traditional names of the products were fermented in the country of origin.
[c] Values of all the histidine-added samples are significantly different from the control ($p < 0.001$) in both the 2- and 4-month fermentation.
Source: Ref. 98.

Table 31 Volatile Acids in Fish Sauces Incubated for 4 Months[a]

Acids	Control (mean ± SE 1%)	Histidine-added (Mean ± SE 1%)
Acetic	12.32 ± 0.22	6.11 ± 0.02
Propionic	27.58 ± 0.26	23.42 ± 0.20
Isobutyric	0.40 ± 0.01	Trace
n-Butyric	13.06 ± 0.12	11.16 ± 0.02
Isovaleric	1.05 ± 0.05	0.74 ± 0.02
n-Valeric	1.07 ± 0.03	0.81 ± 0.02
Isohexanoic	Trace	Trace
n-Hexanoic	Trace	Trace
n-Heptanoic	Trace	ND
Isononanoic	Trace	Trace
n-Octanoic	Trace	ND
n-Nonanoic	Trace	Trace
n-Decanoic	Trace	Trace

[a] Values are the average of three replicates
[b] Significantly different from the control at $p < 0.001$. Significance test was done using Student's *t*-test.
[c] Significantly different from the control at $p < 0.01$.
[d] Trace-values are less than 0.01%; ND = not detected.
Source: Ref. 98.

sauce was 21.02 ± 0.37 mg/mL compared to 4.44 ± 0.05 and 4.25 ± 0.56 mg/mL in the control and reference fish sauce (patis), respectively. The nutritional impact of high histidine is not known because the recommended dietary allowance of histidine is set at 28 mg/kg body weight/day for infants (3–4 months), with no specific levels for children or adults.

The profile of volatile acids, which has been reported to contribute for the flavor and aroma of fish sauce (65), was similar to the control and histidine-added fish sauce. Nevertheless, a quantity difference was noted. Acetic, propionic, *n*-butyric, isovaleric, and *n*-valeric acids were higher in the control than in histidine-added samples, as shown in Table 31. Histamine in the resulting fish sauces with added histidine and the control was hardly detected (Table 32). This suggested that the addition of histidine did not result in histamine formation during fermentation. The sauces were also tasted and no symptoms of histamine poisoning were observed.

Fermentation by recycling system was tested in industrial fish sauce production (99). Using a recycling system and microbial inoculation can

Table 32 Histamine Contents in Fish Sauces

Samples	Mean ± SD[a] (mg/mL) Incubation period	
	2 months	4 months
A. Histidine-added sauces[b]		
Control	0.11 ± 0.03	0.12 ± 0.02
0.1%	0.05 ± 0.02	0.07 ± 0.02[c]
0.2%	0.04 ± 0.02[d]	0.05 ± 0.01[c]
0.5%	0.07 ± 0.01	0.08 ± 0.03
1.0%	0.15 ± 0.03	0.15 ± 0.01
2.0%	0.12 ± 0.04	0.15 ± 0.01
B. Commercial fish sauces		
Patis (Philippines)	0.04 ± 0.01	
Nam pla (Thailand)	0.43 ± 0.02	
Shottsuru (Japan)	0.37 ± 0.01	
Salted anchovy sauce (Korea)	1.38 ± 0.02	
Nam pla (Japan)	ND	
Anaerobically fermented (Japan)	ND	

[a] Values are average of two replicates.
[b] Histidine added to fish mixture before incubation.
[c] Significantly different from the control ($p < 0.05$) from the control in the 4-month fermentation.
[d] Significantly different from the control ($p < 0.05$) from the control in the 2-month fermentation.
Source: Ref. 98.

reduce the fermentation time. The efficiency of the system was improved by the addition of proteolytic enzymes, neutrase, papain, bromelain, and tuna visceral enzyme. The most preferable product was obtained from the one with 0.3% neutrase. Sensory evaluation showed that the acceptability was the same as for the commercial product with no significant difference ($p > 0.05$). Chaiyanan (100) used a packed-bed column in fish sauce production to reduce fermentation time. Fish and salt in a ratio of 3 : 1 was packed alternately in the column. Drainage collected in the container at the bottom of the column was slowly recycled back through the column two to three times. The amounts of protein, pH, salt content, color, odor, and taste of the fish sauce after 7 days and 1 month were determined as presented in Table 33. The result indicated that the quality of the resulting fish sauce after 1 month was comparable to the fish sauce produced in the traditional method in a closed container for 8 months. The addition of natural or commercial proteolytic enzymes (i.e.,

Table 33 Comparison of Fish Sauce Produced by Packed-Bed Column and Traditional Process

Fish sauce	Aroma		Color		pH		% Salt	Protein (g/L) (1 month)
	7 days	1 month	7 days	1 month	7 days	1 month		
1. Recycle through column 2 times	+ + +	+ + + +	Light brown	Reddish brown	5.85	6.01	17.8	50.4
2. Recycle through column 3 times	+ + + +	+ + + +	Light brown	Reddish brown	6.13	6.13	26.6	75.6
3. Traditional method in closed container	–	+ + +	Light pink	Light brown	5.42	5.58	26.8	55.7

Source: Ref. 100.

neutrase, papain, bromelain, and tuna visceral enzyme) improved the efficiency of the fermentation system. By sensory evaluation, fish sauce with 0.3% neutrase was the most preferred product, with no significant difference in quality from commercial fish sauce. Pucharoen (101) developed an accelerated continuous process for fish sauce by using rotary fermentation. Fish processing waste (head and bone of big-eye scad) and salt in the ratio 4 : 1 (w/w) was fermented at 35°C, 40°C, and 45°C. Pineapple peel (10%) was added as a source of bromalain and *Pediococcus halophilus* was inoculated for flavor development during ripening. The fish sauce at 45°C ripened faster than the others. After 21 days, the resulting liquid contained total nitrogen and amino nitrogen at 18.02 and 6.97 g/L, respectively. However, the flavor of the sauce was very mild and the total viable count, acid-producing bacteria, and proteolytic bacteria decreased during fermentation.

B. Starter Cultures

Although many studies in the application of starter cultures to nam pla fermentation have been carried out, none has been used commercially as a routine practice. Most studies for using starter cultures to accelerate the fermentation process are performed at laboratory- or semipilot-scale levels. Chaiyanan et al. (99) applied bacterial strains isolated during the fermentation process and subsequently inoculated back in a controlled fermentation. They reported the reduction of fish sauce production time from 1 year to a few months. Klomklang (41) studied the fermentation of fish sauce by using halophilic bacteria with koji. Koji was prepared by growing *Aspergillus oryzae* on wheat bran 1 in.-thick and 65% moisture. The temperature was 28–30°C and enzymes activities (i.e., proteases and amylases) were highest after 50 h.

The koji was used in combination with different strains of halophilic bacteria isolated from fish sauce. Results indicated using 12.5% koji at 25% salt and a starter of *Halobacterium salinarium* yielded fish sauce with 7.42 mg/mL of soluble protein in 42 days compared to 3.96 mg/mL and 11 months, respectively, in the control. A comparison of flavor and aroma of the resulting fish sauce and commercial product was not reported. The addition of koji from fermented soybeans and wheat in a ratio of 3 : 1 : 1, fish : salt : koji, was also studied by Togano et al. (102). Korean fish sauce produced from sandfish, soybean koji, and salt was also reported (103).

Other research and development included study of processing fish sauce tablets (104). Fish sauce was dehydrated by a spray-dryer using three different temperatures of 200°C, 220°C, and 240°C before being made into tablets. Iodine in the form of potassium iodate was added. The appropriate drying temperature was 200°C. A shelf-life study of aluminum-laminated bags for 2 months indicated no change of total nitrogen content. Acceptability of reconstituted fish sauce varied from neither like nor dislike and like slightly.

Fish sauce production from freshwater fish as an alternate raw material for the anchovy has been carried out using small-size *Tilapia nil.otica* Linnaeus (105). Tilapia is one of the most cultivated freshwater fish in many parts of Thailand. They are fast growing and easy to raise. About 9–10-cm-long (3-month-old) fish were mixed with salt at 2 : 1, 3 : 1, and 4 : 1 (w/w), fish : salt. The ratio of fish to salt at 4 : 1 gave the product quality of grade 1 nam pla after 20 months. The specific gravity, pH, NaCl, total nitrogen, and amino acid nitrogen were 1.19, 6.6, 259.33 g/L, 20.13 g/L, 0.4 g/L, and 10.82 g/L, respectively. Sensory evaluation showed no difference in total score from the commercial sample. The odor received a higher score but the taste and color received a slightly lower score. However, the total fermentation time was much too long compared to that from marine fish; thus, further development is required.

XVI. FUTURE TRENDS

To promote the fish sauce industry in terms of quality standard and capability to compete in the international market, the National Center for Biotechnology and Genetic Engineering (BIOTEC) set up a series of intensive research studies (106) to cover different topics of fish sauce processing as follows:

1. Raw materials
    - Research and development to replace anchovies with other types of fish without damaging the typical fish sauce characteristics and quality

- Utilization of appropriate industrial byproducts for making fish sauce

2. Postharvest technology
   - Monitor the histamine content within an acceptable level by control of the histamine level in the fish from harvesting to fermentation
   - Storage procedure to retain the freshness of raw material and study the mechanisms of histamine formation before and during fermentation
3. Processing
   - Reduction of fermentation time while maintaining the typical quality
   - Effect of salt on histamine level during fermentation
   - Flavor profile of fish sauce
   - Application of research outcome to industry
4. Packaging: development of new packaging for value-added product
5. Product development: innovative development (e.g., low-salt and high-amino-acids fish sauce and concentrated fish sauce)
6. Study of total quality assurance system in fish sauce processing as well as certain quality criteria of fish sauce such as glutamic acid content for further regulating of current national standards

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14

Production of Thai Fermented Fish: Plara, Pla-som, Som-fak

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I. PLARA

A. Historical Perspective

Plara (fermented fish) is the rural staple food in the north, northeast, and central regions of Thailand. Traditionally, almost every household, especially in northeastern Thailand, make their own fermented fish for domestic consumption. Thai local names of this fermented fish are plara or pla ha (north), pla-daek (northeast) and ra (south) (1,2).

During the last decade, plara has been produced for sale and some consumers who once would have made the fermented fish paste themselves have increasingly bought if from the market. The producers have also developed a new formula to meet market demand, so the fermented fish production has become a promising new small-scale industry. Production factories are usually located where there are plenty of freshwater fish. The Thai Farmers Research Center reported in 2000 that from just a household production to small-scale industry, the yearly production volume of this fermented fish has now reached 40,000 tons, worth about 800 million bhat (Bt) with estimated export values of Bt20 million bhat each year (3), whereas the production capacity of plara in the whole country was 20,000–40,000 tons per year. The average of plara consumption for Thailand was 15–40 gr/day/person (4). Currently, trade and marketing of plara has progressively expanded through an e-commerce system set up by one Thai entrepreneur (5).

Plara is one of the Thai export products. In 1998, the value was US$292,050 US$(FOB) from the export of 86 tons of plara to the European Union; 800–900 tons per years of Plara was exported to Taiwan (6). Other main markets for plara are the United States, Australia, Singapore, Brunei, and Middle East countries such as Saudi Arabia, Iraq and Kuwait. The shelf life of plara is approximately 6 months to 3 years, depending on the fermentation period and good handling, such as tight packaging and occasionally mixing to exchange the upper and lower portions in the jar.

Plara composition varies depending on the type of fish, the ratio of fish to salt, and the fermentation period (1).

B. Raw Material Needed

The main raw materials or ingredients consist of the following:

1. Freshwater fish. There are several kinds of freshwater fish normally used: *Channa striatus* or *Ophicepharus striatus* (serpent-head fish; pla chorn); *Puntius gonionotus* (silver barb, pla ta-pian); *Trichogaster trichopterus* (gouramy, pla kra-dee); *Cirrhina jullieni* (Jullien's mud crap, pla soi); *Cyclocheilichthys* sp. (Pla ta-kok) and *Nile tilapia* (tilapia; nile)
2. Salt. Good quality sea salt is recommended. It serves as an environmental controller for suitable fermentation, which selects and encourages lactic acid bacteria to grow.
3. Rice bran. Rice bran is a natural nitrogen source for microorganisms. Also, it provides good color and good aroma for plara (7). Normally, bran is roasted before use in order to obtain a good flavor profile. It is used for producing a kind of plara called rice bran plara or plara-rum (rum is a Thai word that means bran).
4. Roasted rice. Rice and sticky rice are popular for use as roasted rice preparation. Sometimes, dried cooked rice is preferred. In the case of rice or sticky rice, it is soaked in tap water for 8 h and then drained until only a small amount of water is retained. Then, it is roasted until the color of the rice turns to a dark yellow or brown. Roasted rice is then ground prior to use so that good and specific flavor of the product is obtained. Roasted rice is used to produce a kind of plara called roasted plara or plara-kua (kua is a Thai word meaning roasted).

C. Production

There are two kinds of plara: freshfish plara and rotten plara, depending on the process used in fermentation. Freshfish plara is manufactured in two

phases. First, fresh fish is mixed with salt and then roasted rice or rice bran is added which introduces a sweet taste and aroma. Second, the sour taste is developed by lactic acid-producing bacteria.

Rather than fresh fish, rotten fish plara is commonly produced and the method of manufacture remains unchanged except the addition of roasted rice or rice bran may differ.

The plara preparation methods vary from simple to complicated processes by using salt and raw material in appropriate ratios.

Generally, freshwater fish are descaled, deheaded, eviscerated, and washed with tap water and then rapidly dried. The prepared fish is mixed with salt in a fish to salt ratio of 3–5:1 by weight and then left at ambient temperature for 12–24 h before packing in an earthenware jar and letting it ferment for 1 month. Then, salted fish is added with roasted rice or rice bran in a salted fish to roasted rice or rice bran ratio of 4–5:1 by weight. It is put in earthenware jars and held at least 6 months (8). Figure 1 shows a local plara product after 6 months fermentation, when it is ready to sell on the market.

The fermentation process depends on the enzyme from the fish gut and micro-organisms. Both roasted rice and rice bran or roasted rice bran are used as carbon and nitrogen sources for lactic acid bacteria (LAB). In addition, various enzymes, including the proteolytic enzymes amylase and lipases, produced from micro-organism cause chemical changes in the product.

Figure 1 Plara in a local market. (Courtesy of W. Krusong, KMITL, Bangkok.)

Various kinds of processed plara product are developed for market. Plara cube and plara powder are two main products in the market. The processing steps are as follows (6). Plara is processed by high heat treatment for softening of fish scales and then dried and ground as plara powder or dried and formed as cubes for plara cubes. Plara powder is packed in glass bottles or plastic bags, whereas plara cubes are wrapped individually and pack in laminated bags as shown in Fig. 2. Processed plara can be used as one of the ingredients for many recipes. In addition, there is product development to add more value to Plara powder and Plara cubes by supplementing them with herbal ingredients such as ginger or roasted rice (9). Iodated plara is one being studied to enhance the nutritional value of the product (10).

D. Product Process Flow

The production process flowsheet for plara is shown in Fig. 3.

Figure 2 Sample of processed plara in a Thai Market. (Courtesy of W. Krusong, KMITL, Bangkok.)

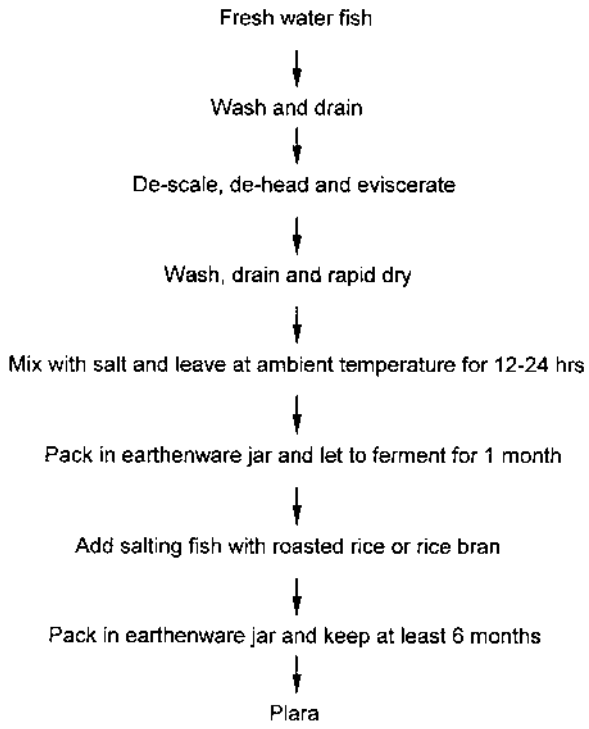

Figure 3 Process flowsheet for plara. (From Ref. 8.)

E. How to Consume

Plara is consumed either raw or cooked. In the northeast, it is generally eaten raw, but this has the out hazard of liver parasites, especially when it was fermented for a short time. It is recommended that plara be cooked before eating (11). The types of dish made with raw plara includes mixing with chopped shallots, lemongrass, chili, and so forth to use as a condiment with vegetables and rice. The cooking method can be frying or boiling with spices to make a soup or it can be wrapped with banana leaves and baked over a fire (1). Processed plara can be used as a seasoning to make various kinds of recipe.

II. PLA-SOM AND SOM-FAK

A. Historical Perspective

The local names of this kind of Thai fermented fish are pla-som, pla-khao-suk, pla-prieo. Two main low-salt products which have in a sour taste due

to the fermentation of LAB are of pla-som, which is made from whole fish or fillet, and som-fak, which is made from minced fish, as shown in Fig. 4. Both are commercialized. To make pla-som, cooked rice is added during preparation, and it can be called as pla-khao-suk (Fig. 5). Som-fak is sometimes called as nham-pla, a Thai fish nham.

Pla-som is produced mainly in the northern part of Thailand; 1–1.5 tons a year is produced yielding Bt60 million (12). Som-fak is valued at Bt8–10 million per year (13).

B. Raw Material Needed

The main raw materials or ingredients consist of the following:

1. Freshwater fish. There are several kinds of freshwater fish are normally used to produce pla-som and som-fak. They are *Puntius gonionotus* (silver barb; pla tapien khao), *Pungasius sutehi* (striped catfish; pla sa-waai), *Pangasius larnaudi* (black ear catfish; pla tay-poh), *Cirrhina jullieni* (Jullien's mud carp; pla soy), *C. microlepsis* (small-scale mud carp; pla nuanchan), *Notopterus chitala* (spotted featherback; pla krai), and *Labeo rohita* (rohu; pla yesok).
2. Salt. As previously described for plara.
3. Cooked rice or cooked sticky rice. Rice or sticky rice is recommended. It is used as the main carbon source for lactic acid bacteria (LAB). It was reported that a slight extra decrease in pH occurred when the percentage of rice in som-fak was increased from 5% to 20% (14). In addition, Owens and Mendoza (15) mentioned that the main role of rice was to reduce the high buffering capacity of the fish in order to obtain a rapid decrease in pH.
4. Garlic. Garlic is a spice usually used as an ingredient in various fermented foods and produces the good smell of these products. Reports have already been published on several effects of garlic on the growth of LAB and their lactic acid production in various kinds of fermented product (16,17). However, it is believed that garlic affects the microbial flora in two ways: (1) by stimulating the growth of LAB (18) and (2) by acting as an antimicrobial agent, in particular against Gram-negative bacteria due to allicin (19,20).

C. Production

In case of pla-som, freshwater fish is cleaned, descaled either by hand or by a descaling machine depending on the factory size; it is then eviscerated, washed, drained, and mixed with salt is a ratio of fish to salt of 8 : 1 by weight

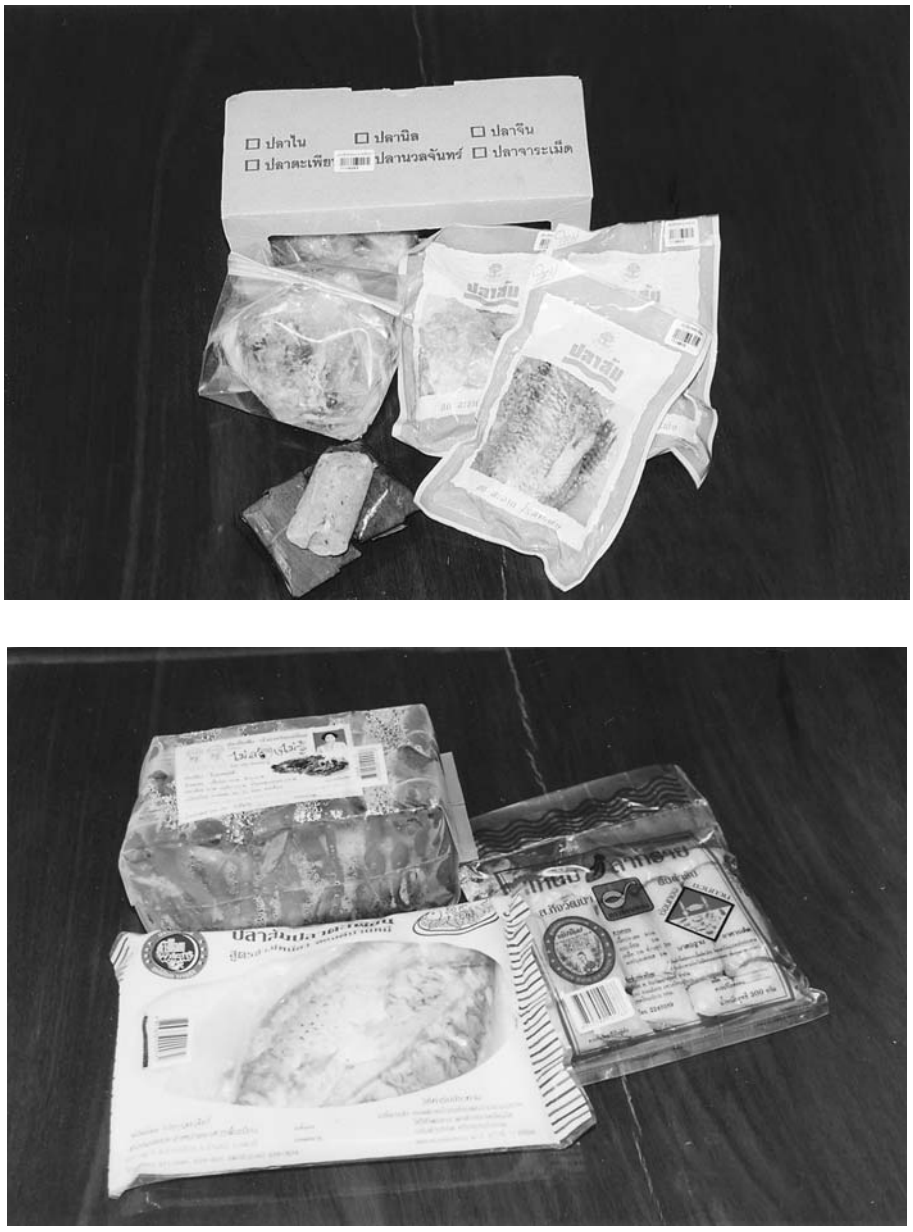

Figure 4 Various brands of pla-som and som-fak. (Courtesy of W. Krusong, KMITL, Bangkok.)

Figure 5 Pla-khao-suk, a type of Thai pla-som. (Courtesy of W. Krusong, KMITL, Bangkok.)

(1) and held overnight. Then, the cooked sticky rice or cooked rice and minced garlic are added to the mixture of fish and salt in the ratio of mixture to rice to garlic of 20:4:1 by weight (1), packed into a ceramic jar or plastic bag, and left to ferment for 2–5 days at ambient temperature of at the factory.

For som-fak preparation, freshwater fish are cleaned, filleted, and washed to remove the skin and blood. The flesh is collected and the water is pressed out in a cloth bag; it is then minced and mixed with ingredients consisting of (ratio of fish to each ingredient by weight) cooked rice (6:1), garlic (20:1), and salt (20:1) (1). The mixture is kneaded until the appearance becomes gel-like and forms a sticky, elastic paste. The paste is divided into small portions and then packed and tightly wrapped in banana leaves or plastic sheeting. The raw som-fak is elastic and firm and left to ferment at ambient temperature for 3–5 days. For garnish, whole bird peppers or whole chilis are added to yield the typical som-fak product at each factory.

D. Production Process Flow

The production process flowsheet for pla-som is shown in Fig. 6 and that of som-fak is shown in Fig. 7.

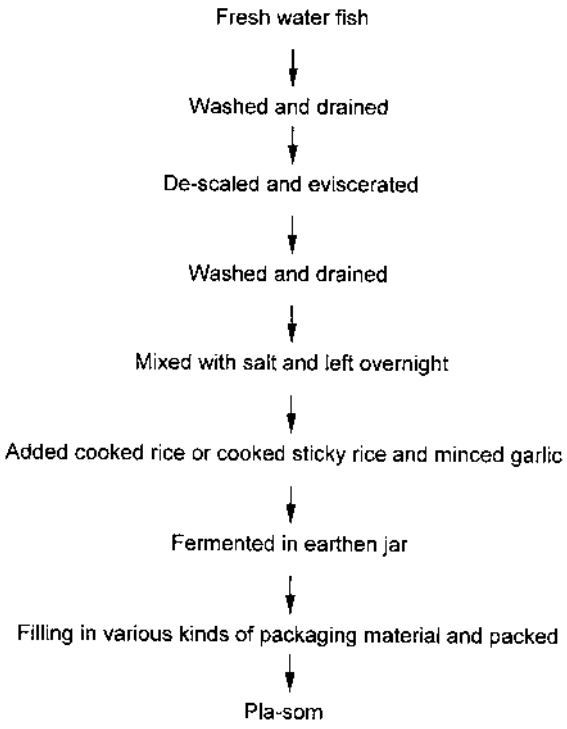

Figure 6 Process flowsheet for pla som. (From Ref. 1 and (1) W. Krusong, personal communication, 2002.)

III. QUALITY OF THAI FERMENTED FISH PRODUCTS: PLARA, PLA-SOM, AND SOM-FAK

The quality aspects of Thai fermented products are color, odor, texture, and flavor. Species, saltiness, and quality of fish are major influences (9). Freshwater fish are most popular. However, there are many investigations using marine fish to substitute for freshwater fish, but, commercially, they are not successful on the market.

Good quality som-fak was obtained when the lactic acid content was above 2%, the pH was below 4.5, and the salt content was between 2.9% and 4.4% (wt/water phase) (21). It had the solid texture of fish flesh, was slightly pinkish in color, and had a slightly sour and salty taste with a garlic flavor. In the case of pla-khao-suk, cooked sticky rice was used and closely attached to the fish flesh (12). Som-fak is characterized as slightly sour and salty with a

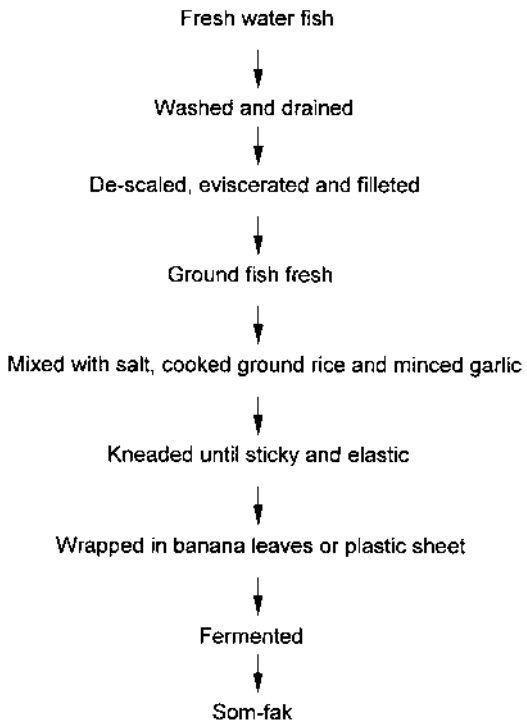

Figure 7 Process flowsheet for som-fak. (From Refs. 1 and 21.)

firm, elastic texture (1,22). The color of som-fak depends on the kinds of fish used, which vary from white to brown, but sometimes it depends on the sanitariness of the process during removal of blood from the fish flesh.

Both pla-som and som-fak are foods enriched with protein, fat, vitamins, and minerals, which depend on the kinds of freshwater fish used. However, due to the activity of lactic acid bacteria in products, some of the protein and fat are degraded to form amino acids, amines, keto acids, ammonia, carbon dioxide, fatty acids, and glycerol in the products (23,24).

IV. MICROBIAL ASPECTS OF THAI FERMENTED FISH PRODUCTS: PLARA, PLA-SOM, AND SOM-FAK

The initial levels of LAB ranged from 10^4 colony-forming units (CFU)/g to 4×10^6 cfu/g and reached 10^8 cfu/g within 1 day (21,25). LAB isolated from

Table 1 Nutrient Composition of Thai Fermented Fish: Plara, Pla-som, and Som-fak

Food description	Nutrient composition per 100 g edible portion							
	Energy (Kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Dietary fiber (g)	Ash (g)	Calcium (mg)
Plara	147	52.5	15.3	8.0	3.4	— (0.5)	20.3	22
Plara powder	273	4.8	28.0	13.9	8.9	— (0.4)	44.0	2392
Pla-som, small fish	127	64.6	16.5	5.3	3.3	— (0.1)	10.2	—
Pla-som, large fish	106	69.4	19.4	0.8	5.4	— (0.1)	4.9	55
Som-fak	96	74.4	18.0	1.4	2.8	— (0.1)	3.3	14

Food description	Phosphorus (mg)	Iron (mg)	Retinol (μg)	β-Carotene (μg)	Total vitamin A (RE)	Vitamin E (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)
Plara	20	3.4	—	—	—	—	0.02	0.16	0.8	—
Plara powder	1312	15.2	—	—	—	—	0.12	0.19	1.8	—
Pla-som, small fish	509	3.2	0	—	0	—	0.03	—	1.3	—
Pla-som, large fish	157	1.6	43	—	43	—	0.05	—	2.5	—
Som-fak	110	Trace	0	0	0	—	0.05	0.06	4.6	1

Source: Ref. 28

processed som-fak were reported to be *L. lactis* subsp. *lactis* and *Leuconostoc citreum* specifically associated with fish fillet and minced fish, *L. paracasei* subsp. *paracasei* associated with cooked rice, and *Weisella confusa* associated with garlic and banana leaves. Various kinds of LAB was found in Thai fermented fish. *Pediococcus pentasaceus, Lactobacillus pentosus, L. plantarum, L. fermentum*, and *L. sake* were found in pla-som and som-fak whereas *L. farciminis, L. acidipiscis* and *Weissella thailandensis* were found in pla-ra (26). Many kinds of micro-organism were found in Thai fermented fish products. The case of plara, there were *Bacillus subtilis, B. licheniformis, Micrococus* sp., *Pediococcus* sp., *P. halophilus, Staphylococus* sp., and *Staph. epidermidis* whereas *Lactobacillus plantarum, P. halophilus, Bacillus* sp., *Micrococcus* sp., *Staph. epidermidis, Streptococcus faecalis, Saccharomyces* sp., *Candida* sp., and *Pichia* sp. were found in pla-som (1). In addition, *Candida* sp., *L. brevis, L. plantarum, L. fermentum, P. pentasaceus*, and *Strep. faecalis* were found in som-fak. However, Hong-tongdaeng (8) investigated micro-organisms in plara sold on the market and classified them into five groups as follows: (1) *Pediococcus halophilus*, which is found mainly in plara fermented for 3–5 months and provides a typical flavor to plara, (2) *Pediococcus* sp., which is found in small numbers during fermentation of plara also adds flavor to plara, (3) *Staphylococcus* sp. and *Staph. epidermidis*, which are found from the start of fermentation until 3–5 months and may participate in protein degradation, (4) *Micrococcus* sp., which acts in protein degradation, and (5) *B. subtilis* and *B. licheniformis*, which also act in protein degradation. In addition, there is a report which concludes that extremely halophilic bacteria are found in plara (27).

V. NUTRIENT COMPOSITIONS OF THAI FERMENTED FISH PRODUCTS: PLARA, PLA-SOM, AND SOM-FAK

The nutrient compositions of Thai fermented products including plara as plara powder, pla-som and som-fak are shown in Table 1.

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15

Industrialization of Thai Nham: Fermented Pork or Beef

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I. HISTORICAL PERSPECTIVE

Thai Nham is a lactic-fermented pork or beef semidry sausage with a our and slightly salty flavor. It is one of the most popular traditional Thai fermented meat products. It is widely produced by natural fermentation for local consumption at the homemade scale. After 3–4 days of spontaneous fermentation, it is considered ready to eat without cooking. Because of the natural fermentation, the most common problems found are quality deviation from batch to batch and the contamination by undesirable micro-organism.

Nham is produced from fresh meat and there is no heat treatment step. There is a high risk of *Salmonella* contamination in nham (1). However, it was found that lactic acid produced during nham fermentation can inhibit the growth of *Salmonella* (2). The study of the bacteriostatic effects of garlic (one of the ingredients in nham production) on meat lactic acid starter cultures and pathogens in nham was investigated (3). The results show that the pathogens found primarily in nham, *Salmonella anatum* and *Staphylococcus aureus* can be inhibited by the presence of garlic; however, lactic acid bacteria (LAB) could grow. Garlic extract can also enhance the growth of LAB and lactic acid production (3). In order to improve the quality of nham and eliminate the contamination, the following researches have been conducted. The potential for use of isolated bacteriocin-producing *Pediococcus pentasaceus* TISTR 536 from nham can control the growth of *Salmonella anatum* (1). The development of the nham formulation and controlled fermentation can be made with mixed bacterial starter cultures (4). The effect of mixed starter

cultures on the reduction of *S. typhimurium* and *S. anatum* in nham fermentation was reported (5). The effect of mixed starter cultures in nham fermentation on the reduction of *Staph. aureus* (6) and the improvement of starter cultures for nham fermentation (7) were studied.

Currently, nham is not only widely consumed domestically, but it has also been exported to other countries such as Japan, Hong Kong, Vietnam, Singapore, Taiwan, New Zealand.

II. DESCRIPTION

Nham, is a fermented pork or beef semidry sausage. It is red with a sour and slightly salty taste (Figs. 1 and 2). Nham is one of the lactic acid-fermented foods. The intentional encouragement of a LAB growth is useful as a meat preservative as well as producing the typical tangy flavor (8). The nutritional values of nham per 100 g are as follows: energy, 185 kcal; protein, 20.2 g; fat, 9.9 g; carbohydrate, 3.6 g (9) In addition, vitamins B1 and B2, ferric iron and phosphoros were found in nham (10).

Figure 1 Nham produced on an industrial scale. (Courtesy of Payon Factory, Chiangmai.)

Figure 2 Nham presented in a fresh market. (Courtesy of W. Krusong, KMITL, Bangkok.)

The German official recommendations for the assessment of the safety of fermented sausages that might be applied to nham are as follows (11): A sausage is fermented (and thus no longer subjected to regulations on minced meat) if the following hold:

- Its pH is below 5.6 and D-lactic acid content above 0.2%.
- Its color is heat stable (curing color).
- Its texture is no longer crumbly.
- Its aroma is typical.
- Lactic acid bacteria predominate.
- Enterobacteriaceae counts are low.

III. PRODUCTION

The essential steps in the fermentation of nham include the following:

1. Ingredient preparation
2. Mixing and kneading
3. Inoculation
4. Filling or wrapping

5. Fermentation
6. Preservation

A. Ingredient Preparation

The main ingredients are fresh pork or beef, pork rind, salt, garlic, cooked rice and sticky rice, potassium nitrate, and seasonings. Lean pork is suitable for producing nham because it contains less fat. All fibrous parts are removed and then the meat is minced. It will provide the proper texture to the end product. In the preparation of pork rind (Fig. 3), the hair and excess fat are removed. Then, the skin is boiled and shredded into thin slices about 2–4 cm long. In some recipes, pork ears (Fig. 4) are used to replace the rind so that a chewy quality can be obtained. Pork ears are prepared using the same method: boiling and then shredding. The percentage of pork ear or rind in the formulation is approximately 20–30% by weight.

Washing the fresh meat before mincing is avoided because the water can cause product spoilage. The water activity of the mixture must be per-

Figure 3 Boiled and sliced pork rind. (Courtesy of Payon Factory, Chiangmai.)

Figure 4 Boiled pork ear. (Courtesy of Payon Factory, Chiangmai.)

fectly controlled. The general formulation of nham is as follows: cooked rice and glutinous rice [7–10% (w/w) of mince meat], peeled garlic (3–10%), salt (2–3%), pepper (0–0.5%), and potassium nitrite (0–200 ppm). The quality of nham depends significantly on the quality of ingredients. Most nham is consumed without cooking. The freshness and cleanliness of meat is very important in order to minimize contamination by pathogens and spoilage micro-organisms.

Rice or sticky rice is cooked separately prior to mixing with other ingredients. It could be a carbon source for LAB.

Garlic is peeled and minced. Garlic serves as a seasoning agent and also contaminating a micro-organism inhibitor. The role of garlic in the growth and lactic acid production of the starter was determined (12). It was found that garlic could enhance the growth of LAB and their lactic acid formation, which results in rapid decreasing of pH. A further study was made on the effect of garlic extract on the growth of LAB (*Lactobacillus curvatus*, *L. sake*, and *Pediococcus acidilactici*) and commonly found pathogens (*S. anatum* and *Staph. aureus*) in nham (12). Garlic extract was prepared by peeling the cloves of garlic, blending with distilled water at a ratio of 1 g peeled garlic to 2 mL distilled water, filtering , centrifuging at

5000 rpm for 20 min, and sterilizing. The results showed that all mentioned LAB cultures were resistant to the antimicrobial activity of garlic is (known as allicin). All tested strains could grow in MRS broth with 3% garlic extract and survive in MRS broth with 5% garlic extract, whereas the pathogens were inhibited by the presence of 1% garlic extract in trypticase soy broth (TSB). From the study, *S. anatum* was more sensitive than *S. aureus* to allicin in garlic extract.

Salt is added to enhance the growth of LAB (10) and provide a better flavor. It also acts as an inhibitor of undesirable micro-organisms. Normally, salt is used at a level of 2–3%.

Nitrate or nitrite is used to develop the color of the products. It also plays a role in enhancing LAB and inhibiting the undesirable micro-organism by controlling the fermentation, maintaining an anaerobic condition (10).

Bird chili is added to improve the flavor. It is also reported (4) that 1% minced bird chili can have significant effects on the production of lactic acid (13). The spices could increase the efficiency of carbohydrate use by LAB.

B. Mixing and Kneading

Minced red meat and sliced pork rind are mixed well; then, the cooked rice and other ingredients are added and mixed as shown in Fig. 5.

Figure 5 Mixing and kneading. (Courtesy of Payon Factory, Chiangmai.)

C. Inoculation

Nham production by household scale normally uses the method of natural fermentation without inoculation. However, some producers have been using the fermented nham from a former batch to mix with the next batch. The fermented nham can help to shorten the fermentation period (10). It has been known that starter cultures are widely used for producing various kind of fermented meat products to shorten the ripening period, ensure the color and texture development, improve the flavor, and assure the safety of the product (4,14,15).

Some important properties of LAB involved in the fermentation are shown in Table 1.

In Thailand, a systematic research regarding the use of starter cultures in nham was made. The development of nham formulation and controlled fermentation made with a mixed bacterial starter was investigated (4). LAB used in this study were *Lactobacillus plantarum* and *Pediococcus cerevisiae*. By this basic technology, both cultures acted simultaneously. Nham produced using a mixed starter provided better quality. There is a report showing that the effect of mixed starter cultures on the reduction of *Salmonella typhimurium* and *S. anatum* in nham fermentation. Pure cultures of *L. plantarum* and *P. cerevisiae* were used to reduce the number of *Salmonella* in MRS broth and nham fermentation. In conclusion, adding pure or mixed starter cultures can reduce the Salmonella contamination problem in nham (5). In addition, the improvement of the starter culture for nham fermentation was also reported (7). *Lactobacilli*, including *L. plantarum*, *L. sake*, *L. curvatus* and *L. zeae*, were used as starters. Nham fermented by *L. plantarum* BTEC 109 had a strong unusual smell and was unacceptably sour. Nham fermented with *L. curvatus* at 10^4 cfu/g received a higher score for red color and overall performance, flavor, and sourness than nham fermented by other strains. The product had a pH of 4.47 and total acidity of 1.0% at 72 h. When *P. acidilactici* and *P. pentosaceus* were studied, nham fermented by *P. acidilactici* received higher scores *P. pentosaceous* for overall preference and the most acceptable scores for flavor, sourness, and texture.

The selection of Salmonella-inhibiting LAB and preparation of powder inoculum of nham were reported (16). The strains of *Lactobacillus* and *Pediococcus* that perform high efficiency in acid producing and inhibiting *Salmonella* spp. were selected. *Lactobacillus* spp L 23 and *Pediococcus* spp P42 were selected. In vitro studies revealed that the selected strains were resistant to 15% bile salt. The growth was good when the medium contained 3–5% NaCl. These strains could grow in the medium containing antifungal substances such as 0.3% propionic acid and 0.1% potassium sorbate.

Preparation of powder inoculum could be conducted by modified solid substrate cultivation and the methods developed (16). Solid substrate culti-

Table 1 Some Important Properties of LAB Involved in Sausage Fermentation

Property	*L. curvatus*	*L. sake*	*L. plantarum* and *L. pentosus*[a]	*P. pento saceus*	*P. acidi lactifi*
Cell shape	Rod	Rod	Rod	Cocci in tetrads	Cocci in tetrads
Growth at a_w–0.93	–(+)[b]	+(–)	+(–)	V	+
Growth at 4°C	+	+	–	–	–
Growth at 50°C	–	–	–	–	+
Peptidoglycan type	Lys-D-Asp	Lys-D-Asp	Meso-DAP	Lys-D-Asp	Lys-D-Asp
Lactic acid enantiomer	DL(L)[c]	DL	DL	DL	DL
Nitrate reductase	–	–	V	–	–
Nitrite reductase					
+ heme	–	–	V	V	–
– heme	–	V	V	–	–
Catalase					
+ heme	–	+	V	–	+
– heme	–	V	V	+	–
Ammonia from arginine	–(+)	+	–	+	+
Fermentation[d] of					
Maltose	V	V	+(–)	+	–
Sucrose	+(–)	+	+(–)	V	V
Lactose	V	V	+(–)	V	V
Melibiose	–(+)	+	+(–)	V	V
Mannitol	–	–	+	–	–
Gluconate	–(+)	+	+(–)	V	–
Minimal pH in MRS broth	~4.0	~4.0	~3.7	~3.8	~3.8

Note: L. = *Lactobacillus*; P. = *Pediococcus*

[a] *L. pentosus* is similar to *L. plantarum* but capable of fermenting D-xylose.

[b] +(–), positive with exception; –(+), negative with exception; V, variable (strain dependent).

[c] Strains forming L-lactate only have been formerly classified as *L. bavaricus*.

[d] All strains ferment glucose and ribose.

Source: Ref. 11.

vation involved the following steps: Put 10 g rice flour in a plastic bag, sterilize at 121°C for 30 min, cool and add 5 mL yeast extract–peptone broth, inoculate *Pedicoccus* or *Lactobacillus* at 10^3–10^4 cell/g, and incubate at 37°C for 24 h. Then, mix with 56 g sterilized rice flour (sterilized at 110°C for 48 h and cooled). The moisture content of mixture is about 13%. Finally, propionic acid is added at 2000 mg/kg, Another method developed involved

the following: Cells were grown in yeast extract–peptone broth con-taining 1% glucose. The cultures of *Lactobacillus* spp. L23 and *Pediococcus* spp. P42 were incubated for 16 h and 20 h, respectively. After harvesting and washing in 100 mL saline, the suspensions were mixed with sterilized rice flour. The ratio was 1 mL cell suspension /20 g rice flour. Aseptic technique was needed thorough all of the steps. The powder starters obtained from both methods could be kept in the refrigerator for 8 weeks with a reduction of less than 1 log cycle of viable cells. The comparison of shelf life between *Pedicoccus* spp. and *Lactobacillus* spp. powdered inoculum prepared by both methods are shown in Tables 2 and 3.

D. Filling and Wrapping

The mixture (Fig. 6) is separated into small portions. On a household scale, the mixture is wrapped in plastic film or plastic bags and then covered with green banana leaves. Industrial-scale equipment is shown in Fig. 7. Cylindrical plastic bags are normally used. The wrapping step is very important for the quality of the products. By tightly wrapping anaerobic fermentation is maintained. This condition is suitable for LAB.

E. Fermentation

At the beginning of nham fermentation, the pH of mixture is around 6.05 (17). LAB are responsible for the souring and ripening of nham. Changes of pH and acid formation during nham fermentation were investigated (10). As shown in Table 4, it was noted that fermentation of nham was rapid during the first 4 days. The pH was decreased dramatically from 5.65 to 4.45. At the early fermentation stage, *Pediococcus* sp., *P. cerevisiae*, and heterofermentative *Lactobacilli* predominated. After 3 days before the later stage, *L. plantarum* and *L. brevis* became more prominent. The report concluded that LAB and related bacteria found in nham are *L. pentosus*, *L. plantarum*, *L. sake, Lactobacillus* spp., *Leuconostoc* spp., *P. acidilactici*, and *P. pentosaceus* (18). At the third or fourth day of fermentation, the product was considered to have attained optimum flavor and texture, the pH value was about 4.5. The pathway of lactic acid fermentation is shown in Fig. 8.

F. Preservation Method

Radiation is the method that is currently used on an industrial scale. The effect of radiation on *Salmonella* spp. Was reported; the optimum condition to eliminate the *Salmonella* contamination and to provide unchanged sen-

Table 2 Comparison of Shelf Life Between *Pediococcus* spp. P42 Powdered Inoculum Prepared by Modified Solid Substrate Method and Broth Cultivation at Moisture Content of 13% and 19%

	13% MC powdered inoculum		19% MC powdered inoculum	
	Modified solid substrate	Broth cultivation	Modified solid substrate	Broth cultivation
Type of medium	YP broth and rice flour	GYP broth	YP broth and rice flour	GYP broth
Cell washing	No	Twice with saline	No	Twice with saline
No. of initial cells	9.183×10^3 cell/g	5×10^7 cell/mL	9.75×10^3 cell/g	1.23×10^7 cell/mL
No. of fresh inocula (incubate at 37°C for 24 hrs)	2.17×10^8 cell/g	—	3.75×10^8 cell/g	—
Ratio of powdered inoculum to rice flour	1 : 3.5	1 : 20	1 : 2	1 : 10
Moisture content of powdered inoculum (%)	12.84	13.21	19.10	18.96
pH of powdered inoculum	5.9	6.05	5.9	6.05
No. of initial powdered inocula (cell/gr)	6.23×10^7	3.85×10^7	2.22×10^8	1.07×10^7
No. of powdered inocula (cell/g)				
After 2 weeks				
• Refrigerated temp.	3.62×10^7	1.09×10^7	2.11×10^8	6.11×10^6
• 35°C	1.01×10^7	4.33×10^4	4.97×10^5	1.63×10^5
After 4 weeks				
• Refrigerated temp.	3.50×10^7	1.52×10^7	1.74×10^8	5.81×10^6
• 35°C	2.22×10^5	2.30×10^2	1.15×10^3	2.30×10^4
After 6 weeks				
• Refrigerated temp.	3.36×10^7	1.37×10^7	1.60×10^8	5.88×10^6
• 35°C	1.85×10^3	9.61×10	1.30×10	5.54×10^2
After 8 weeks				
• Refrigerated temp.	2.805×10^7	1.29×10^7	1.60×10^8	5.88×10^6
• 35°C	1.66×10^2	ND	ND	ND

Source: Ref. 16.

Table 3 Comparison of Shelf Life Between *Lactobacillus* spp. L23 Powdered Inoculum Prepared by Modified Solid Substrate Method and Broth Cultivation at Moisture Content of 13% and 19%

	13% MC powdered inoculum		19% MC powdered inoculum	
	Modified solid substrate	Broth cultivation	Modified solid substrate	Broth cultivation
Type of medium	YP broth and rice flour	GYP broth	YP broth and rice flour	GYP broth
Cell washing	No	Twice with saline	No	Twice with saline
No. of Initial cells	3.82×10^4 cell/g	9×10^7 cell/mL	3.14×10^4 cell/g	2.16×10^8 cell/mL
No. of fresh inocula (incubate at 37°C 24 hrs)	1.03×10^8 cell/g	—	1.84×10^8 cell/g	—
Ratio of powdered inoculum to rice flour	1 : 3.5	1 : 20	1 : 2	1 : 10
Moisture content of powdered inoculum (%)	12.65	13.35	19.17	19.05
pH of powdered inoculum	5.9	6.10	6.0	6.05
No. of initial powdered inocula (cell/gr)	1.85×10^7	7.36×10^7	3.93×10^7	1.24×10^8
No. of powdered inocula (cell/g)				
After 2 weeks				
• Refrigerated temp.	1.06×10^7	2.05×10^7	6.20×10^7	9.90×10^7
• 35°C	2.18×10^4	2.20×10^4	1.67×10^3	6.86×10^5
After 4 weeks				
• Refrigerated temp.	2.13×10^6	1.90×10^7	4.93×10^7	9.90×10^7
• 35°C	5.67×10^3	1.60×10^2	ND	7.32×10^2
After 6 weeks				
• Refrigerated temp.	2.10×10^6	1.32×10^7	3.59×10^7	9.90×10^7
• 5°C	ND	ND	ND	ND
After 8 weeks				
• Refrigerated temp.	2.10×10^6	1.21×10^7	3.45×10^7	9.90×10^7
• 35 °C	ND	ND	ND	ND

Abbreviations: See Table 2.
Source: Ref. 16.

Figure 6 The mixture that is ready to fill. (Courtesy of Payon Factory, Chiangmai.)

Figure 7 Filling machine. (Courtesy of Payon Factory, Chiangmai.)

Table 4 Changes of pH and Acid During Nham Fermentation with 3% Sodium Chloride

Fermentation time (h)	pH	% Acid (as lactic acid)	Fermentation time (d)	pH	% Acid (as lactic acid)
0	5.65	0.22	4	4.45	0.50
12	5.16	0.22	5	4.35	0.62
24	5.27	0.23	6	4.35	0.68
36	5.01	0.26	7	4.32	0.85
48	4.77	0.32	14	4.27	0.97
60	4.64	0.35	21	4.15	1.25
72	4.63	0.40	28	4.13	1.38

Source: Ref. 10.

sory evaluation was found to be 0.2 μrad. The comparison reduction rate between radiation and pasteurization, at 0.2 μrad, provided 4–7D (decimal reduction time) (19).

IV. PRODUCTION PROCESS FLOW

The production process flowsheet is shown in Fig. 9.

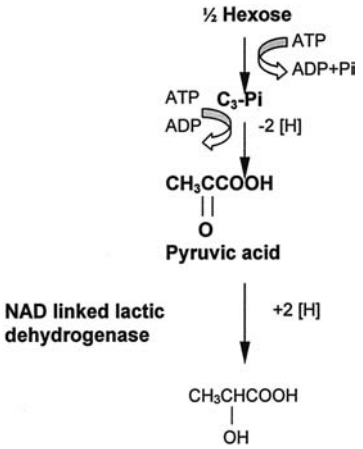

Figure 8 Lactic acid fermentation. (From Ref. 13.)

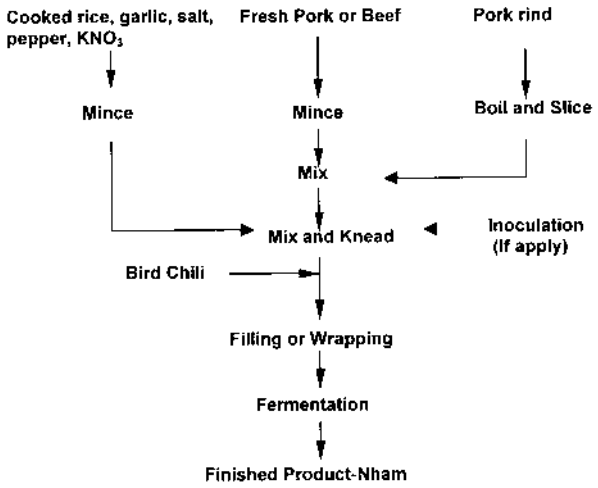

Figure 9 Flowsheet of the nham manufacturing process. (Modified from Ref. 20.)

V. HOW TO CONSUME

Nham is considered to be ready to eat. It is normally eaten as a side dish with fresh ginger, green leaf vegetables, roasted peanuts, and bird chili. For people who worry about the risk of pathogens and parasites, nham must be cooked before eating. The favorite dishes are fried rice with fried or grilled nham.

VI. FOOD SAFETY ASPECTS

Normally, nham is consumed as raw food because heat treatment may destroy its typical flavor. There is evidence showing the harmful effects of consumption of nham without cooking. The causes of contamination are from raw materials especially fresh meat (21–24) and food handlers (1,6). Nham is occasionally contaminated with some parasites such as *Trichinella spiralis* and *Taenea solium* and some enteropathogenic bacteria (e.g., *Salmonella* and coliform bacteria) (17). Samples from the market were obtained from nine nham producers. Fifty-six samples out of 450 samples (12.44%) contaminated with *Salmonella*. The isolated *Salmonella* found were *S. agona*, *S. anatum*, *S. bovis-morbificans*, *S. derby*, *S. heidelberg*, *S. java*, *S. krefeld*, *S. lexington*, *S. london*, *S. senftenberg*, *S. typhimurium*, *S. virchow*, and *S. weltevreden*. *Salmonella* was not the only contaminant found in

nham. *Staph. aureus* and *Escherichia coli* were also detected (17). The cause of contamination is either poor quality of ingredients or improper hygienic control by food handlers (6). However, the use of selected strains of LAB as mixed starter cultures could help reduce the problem as reported (1,5,6,16).

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Industrialization of Myanmar Fish Paste and Sauce Fermentation

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I. INTRODUCTION

Fermented fish paste and sauce are generally known in Myanmar as ngapi and nganpyaye respectively. Literally, ngapi means "pressed fish," named by in its traditional processing technique. According to the type of raw materials used (whether fish or shrimp*), it could be classified into two groups: nga-ngapi (for fish) and hmyin- (or seinsa) ngapi (for shrimp). Fermented fish and shrimp products are indigenous food greatly relished by Myanmar people and consumed daily along with their staple meal (i.e., rice). No lunch or dinner is complete without a dish of boiled nagpi liquor.

Previously, Ngapi was viewed with disfavor by some people as a decayed fish product. However, recent research invalidated this assertion and proved that ngapi is a protein-rich, nutritious food containing high percentages of calcium, phosphorus, and several essential amino acids; its caloric content is comparable to beef, pork, and mutton. Because of its adoration by Myanmar people and its attractive food value, ngapi has rightly attained its respectable status as national food (2).

* Shrimp (crustaceans) are included in the term "fish" as defined in 21 CFR 123.3(d)(1).

II. PRODUCTION AND CONSUMPTION

Ngapi and nganpyaye are basic foods for the Myanmar diet and is produced by a single fermentation process. According to the manufacturer's choice, either ngapi could be a main product for which nganpyaye is a by-product or nganpyaye could be a sole product without producing ngapi at all. In Myanmar, ngapi is normally produced as a main product and nganpyaye is obtained as a by-product. Depending on the manufacturing practice and required product quality, the volume of nganpyaye produced in Myanmar is normally 25–35% that of ngapi (both for nga and hmyin). The annual production of hmyin-ngapi is generally about three times higher than nga-ngapi. All of the ngapi and nganpyaye produced are consumed locally and there is no export of this commodity. The annual volumes of production of ngapi products are presented in Table 1.

Table 1 Annual Volume of Production of Ngapi in Myanmar (in Thousands)

	Nga-Ngapi		Hmyin-Ngapi	
Year	Viss[a]	Metric ton	Viss	Metric ton
1980	—	—	43,931	71.7
1984	14,761	24.1	46,796	76.4
1985	15,788	25.8	49,237	80.4
1986	16,138	26.4	51,560	84.2
1987	16,928	27.6	53,453	87.3
1988	16,133	26.3	48,994	80.0
1989	17,329	28.3	50,626	82.7
1990	17,414	28.4	53,272	87.0
1991	17,635	28.8	50,677	82.8
1992	17,615	28.8	50,453	82.4
1993	19,083	31.2	53,395	87.2
1994	—	—	53,323	87.1
1995	—	—	1,147	1.9
1996	—	—	1,700	2.8
1997	—	—	1,467	2.4
1998	—	—	771	1.3
1999	—	—	765	1.5
2000	—	—	624	1.0

[a] 1 viss = 1.6329 kg.
[b] From 1994 onward, the data from the FGMTC factories were probably not included.
Source: Ref. 3 (1993, 2000, 2001).

III. HISTORY

The earliest record depicting the importance of ngapi in the Myanmar diet appeared on the Mon stone inscription dating the 1st century AD (4). More evidence could be found in the list of occupations of the ancient Myanmar inscribed on the stone conscription of the 12th century and marble monument of 15th century where ngapi manufacturers and salt manufacturers had been registered (5,6).

The trading pattern of nagpi was already established by the Mons of lower Myanmar by about the 15th century. The Mons sold their products (viz. ngapi, salt, and rice) to the dry zone of upper Myanmar and brought back rubies, benzoin, and musk (7). The selling price of ngapi fixed by Damazedi (AD 1460–1492), King of the Mons, during this period was about 5 kyats per 100 viss* (8). The trade of Ngapi from lower to upper Myanmar has increased steadily since then. As mentioned in one of the poems composed in the 17th century, Ngapi was transported by large sailing boats called pain-gaws carrying more than 10,000 viss worth of Ngapi per shipment (9). In the Muttama Sittan 45 (Census of Martaban 32 townships in AD 1783), ngapi was found to be included in the list of the royal presents submitted annually to the Myanmar king, Bodawphaya (1782–1819). Also in this Sittan 45, the royal tax for fishing in the lakes and rivers was collected in terms of ngapi (10).

From 1 November 1854 to 1 November 1855, the total amount of ngapi traded from lower to upper Myanmar was about 13,500 tons. This annual trade figure for ngapi almost doubled after 10 years (11).

During the years from 1861 to 1869, the annual consumption of ngapi (and dried fish) in the whole Myanmar country was found to be about 32,000 tons (12).

Yaw Atwin Wun U Pho Hlaing, a Myanmar scientist of the Konebaung period, stated in his treatise "Utubawzana Thingaha Kyan" in 1880 that ngapi (salted fish) could digest phlegm and bile and enhance diffusion, indicating the medical significance of ngapi (13).

In 1880, an interesting episode occurred in Nyaungdon, the largest production center of ngapi and nganpyaye in the delta region of Myanmar, in conjunction with an outbreak of cholera. The Deputy Commission of Nyaungdon, an English officer, announced that the outbreak of cholera was due to the unwholesomeness of ngapi and nganpyaye and ordered its production and sale closed down. Because these delicacies were the ones most relished in Myanmar, the whole town boiled up and a riot was inevitable. The towns-

* 1 viss = 1.6329 kg.

people marched to the Deputy Commissioner's office and claimed that ngapi and nganpyaye had nothing to do with the cholera outbreak and demanded that he rescind his order. The Deputy Commissioner had to consent; the production and sale of ngapi and nganpyaye were then resumed. Within a few days, luckily, the cholera also died down, proving the wholesomeness of ngapi and nganpyaye (14).

Not only Bhama (Burman) and Mons but also among most of the other Myanmar nationals [i.e., Rakhine (Arakanese), Shan, and Palaung] are enormously fond of Ngapi. A Portugese missionary named Sebastein Manrique had mentioned in his account of travels in Rakhine (Arakan) in 1634–1635 that the relish, sidol (the Rakhine equivalent of ngapi), is included into every dish of a Rakhine meal—even in the feasts during the coronation ceremony of the Rakhine king Thirithudhama (15).

Malcom also described in his book published in 1839 that the Shans bought ngapi, salt, and rice from Ava (upper Myanmar) and from Moulmein (lower Myanmar) (16). In the story of the Myanmar Crown Jewel Nga Mauk (the fabulous ruby), kept by successive kings of Myanmar since King Bodawpaya, another interesting episode is that this ruby, formerly owned by a Palaung woman, was used in a bargain, giving it to a trader named Nga Mauk in exchange for some ngapi. Nga Mauk dared not keep such a valuable gem with him and hence presented it to King Bodawpaya, who named it Nga Mauk, after the name of its last owner (17).

The importance of ngapi as an army ration for ancient Myanmar was stated by an Italian chaplain, Sangermano, who lived in Myanmar from 1783 to 1808; every Myanmar soldier carried ngapi and rice with him when he went out for war (18). The political significance of ngapi could also be seen in Myanmar history. In 1881, the British, who ruled the lower Myanmar at that time, attempted to use the method of halting or restricting the shipment of ngapi to upper Myanmar to counteract the trade monopoly of the Myanmar king Thebaw (1878–1885) (19).

Although this historical review indicated that ngapi is an important element of commerce and is also a socioeconomically and politically significant food of Myanmar for a long time, the method of processing it is still primitive (20). Only recently, some improvements are being made in ngapi processing by incorporating modern and appropriate technology (21–23).

IV. OUTLINE OF ESSENTIAL STEPS IN NGAPI FERMENTATION

Ngapi is generally prepared by pounding or grinding fish (or shrimp) with 20–25% salt and partially drying in the sun for 3–4 days. The product is then

stored in earthen jars or concrete ponds for 3–6 months to mature. This process is usually called fermentation of fish or shrimp (2).

Fermentation of fish (or shrimp) could be defined as controlled microbial conversion of highly perishable constituents of fresh fish (or shrimp) into nutritious, edible, and preserved products to be consumed at a later date. The microbial conversion is usually administered by common salt.

Edible portions of the fish or shrimp generally contain water, protein, lipid (oil and fats), minerals, and so forth. All of these constituents are important factors contributing to each and every step of fermentation. Essentially, there are three main steps or periods in fish or shrimp fermentation and are discussed in the following subheadings (21,23,24).

A. Enzymatic and Microbial Degradations Period

In this period, proteins and lipids are decomposed into simpler products (polypeptides, amino acids, and volatile compounds) by proteolytic hydrolysis, autolysis, and bacteria degradation; water and minerals enhance the decomposition. Salt added at this stage assists in the selection of beneficial bacteria and their population through dehydration of the the tissue by osmosis and diffusion. The highest volume of exuded liquid (i.e., nganpyaye) is usually collected. The main job of this step is to produce an initial amount of nutritive and taste factors. Incipient aroma or flavor initiates only in the later part of this period. The duration of the enzymatic and microbial degradation (EMD) period is about 30 days from the start of fermentation (i.e., during the first month of fermentation).

B. Buffer Period

In this period, most of the enzymes previously very active are beginning to deactivate because of the salt and lack of moisture, whereas halophilic bacteria become more active. Hence, the hydrolysis of proteins by proteolytic enzymes (i.e., autolysis) gradually slows down so that hydrolysis does not proceed further than the amino acid-producing stage. Few amino acids continue to be produced in this buffer period. Slight lipolysis of lipid also continues, imparting the aroma through volatile fatty acids. The total volatile basic nitrogen (TVN) content becomes more pronounced contributing to the good flavor and aroma of ngapi. The formation of ammonia is also noticed, showing that microbial degradation still continues; it is possible that halophilic bacteria are responsible for this. The aroma and flavor are significantly developed in this period. It is relevant to designate this buffer period as a preripening or a prematuring step of ngapi fermentation. This buffer period exists generally between 30 and 60

days from the start of fermentation (i.e., during the second month of fermentation).

C. Stabilizing Period

All necessary reactions (i.e., autolysis, lipolysis, microbial degradation, etc.) required for transforming fish or shrimp into ngapi are more or less achieved in the EMD period and these active reactions are hampered and not allowed to proceed further than the amino acid-producing stage throughout the buffer period. After the buffer period, most of the reactions are stabilized and a steady-state condition is attained. The stabilizing stage is controlled by salt, only allowing further ripening or a maturing process. This stabilizing period generally commences about 60 days after the start of fermentation and may continue from 90 to 180 days (i.e., during the third month of fermentation up to the sixth month).

V. INDIGENOUS PROCESSES

According to the type of raw materials used and the processing technique employed, several types of ngapi available in Myanmar could be generally classified into into two groups: (a) nga ngapi and (b) seinsa ngapi or hmyin ngapi (2,21,25).

Nga ngapi is prepared from fish and consists of three types: ngapi gaung, yegyo ngapi, and damin ngapi. Ngapi gaung is made from whole fish of fairly large size. After scaling, cleaning, and removing entrails, fins, and tails, the fish are thoroughly rubbed with salt, tightly packed in bamboo baskets, and well weighted and pressed down. After 12–24 h, by which time the superfluous juices, nganpyaye (fish sauce), would have gradually oozed out, the weight is removed and the fish are gently taken out, rubbed with salt again, and dried in the sun for a day. On the next day, they are neatly packed, layer by layer, in a large container with plenty of salt in between and on the uppermost surface. Normally, the fish are ready for consumption after about 2–3 three months and will keep for more than a year.

Yegyo ngapi is prepared from fish of medium and small size. The heads, fins, tails, and entrails are removed and the fish cleaned. After mixing with some salt, the fish are spread evenly on bamboo mats and left to dry under the sun for a day. In the evening, the fish are partially pounded and spread on slightly inclined bamboo platforms and left overnight. This procedure is repeated three times. On the fourth day, they are firmly pressed down into huge containers and covered with a thick layer of salt and then by bamboo mats over which heavy weights are placed. The fermentation takes 4–6 months

during which a reddish brown liquid, nganpyaye, is occasionally drawn out from the outlet at the bottom of the containers.

Damin ngapi is a fermented paste made from various kinds of small fish (<5 cm) caught by a bamboo trap (damin). The fish are washed, mixed with some salt, and spread out on bamboo mats in the sun for 3–4 days until the mass becomes dry. The mass is then thoroughly pounded or ground for 3 h,

Figure 1 Process flowsheet for ngapi and nganpyaye.

adding some salt and occasionally water. The paste obtained is dried for a day. The pounding and sun-drying operations are repeated four to five times until a homogeneous and smooth paste is obtained. It is tightly pressed into large containers, the surface is covered with a thick layer of salt, and it is kept for fermentation for 4–6 months; during this period, nganpyaye that oozes out from the fermented mass is collected from time to time.

Seinsa ngapi or hmyin ngapi is a pink to reddish colored shrimp paste made in a manner similar to damin ngapi using small planktonic shrimps (*Acetes* spp. and *Mysidacea*) from Tanintharyi or Ayeyarwady delta areas. That made from Tanintharyi shrimp is called seinsa ngapi and the one made from Ayeyarwady Delta shrimp is called hmyin ngapi. Actually, these shrimps are in different small pelagic larval stages of a shrimp called mysis caught from brackish water (26).

The process flowsheet of the above products is shown in Fig. 1.

VI. COMMERCIAL PROCESSING METHODS

The commercial manufacturing practice involves two steps. Small-scale cottage factories produce the semifinished ngapi and sell it to the nearest depots owned by large-scale manufacturers. The semifinished products are then sent to the main (large) factories and reprocessed.

The semifinished product, say, hmyin ngapi, is usually made from freshly caught shrimp and then cleaned by washing and removal of foreign matter as well as fish larger than 5 cm. The shrimp are mixed with salt and sun-dried for a day. Then, they are pounded (or ground) and sun-dried. This is repeated twice, and the semifinished ngapi is obtained.

In large factories, semifinished ngapi is sorted and blended according to the quality requirement and then repeatedly ground. The final ground paste is packed in plastic woven bags and pressed tightly for at least 3–4 months. Nganpyaye squeezed out during the process is collected during this period.

VII. MAJOR PROBLEMS IN INDUSTRIALIZATION

The major problems in the industrialization of ngapi are mechanization of the processing technique and upgrading of the quality. Although the Myanmar ngapi industry is designated as a factory, there is hardly any machine in it except the grinders. The processing and handling are done manually. Hence, there is plenty of room for mechanization of the Ngapi industry. Finding the right kind of machinery is rather difficult. The food machinery manufactured

by foreign firms are not appropriate for ngapi processing. Therefore, research is being carried out to design and fabricate the ngapi and nganpyaye processing machinery locally.

Another difficulty is the lack of appropriate laboratory equipment for testing moisture, salt (NaCl), color, protein, sand, and fish bone at the depot so that results are obtained quickly and accurately. Equipment for quick testing of moisture and salt is being developed, in order that performance trials can be made at the buying depots.

Washing, salting, and pounding (grinding) are the main machineries used in operation; grinding is one of the most important parts of the process for both small-scale and large-scale factories. Manual pounding of ngapi using wooden mortar and pestle was replaced by a mechanical grinder some years ago. The present ngapi grinder is essentially a scaled-up model of a household meat grinder with some modifications using a 5–10-HP motor. Two sizes of grinder are normally used: one with a 30-cm (12-in.)-diameter bore has the capacity to grind 1000 viss of ngapi per hour, and one with a of 25-cm-diameter bore has the capacity to grind about 750 viss per hour. For coarse grinding, ngapi is pushed through the screen with large openings, about 6 mm ($\frac{1}{4}$; in.) by screw action. For fine grinding, a screen with smaller holes, about 1.6 mm ($\frac{1}{16}$ in.) is used. In both cases, there is a rotating blade on the entering side of the screen which masticates, homogenizes, and pushes the ngapi through various holes of the screen. The fine grinder provides good quality ngapi with a very smooth texture.

As ngapi is a very important article of commerce and an important source of protein, proposals (2) were made for upgrading the quality of ngapi and nganpyaye.

Market research on ngapi was conducted in various towns (e.g., Yangon, Mandalay, Pyi, Shwebo, etc.) starting from 1978 and consumer responses on quality were analyzed. The state-owned old ngapi processing factories were renovated; new, modern ngapi processing factories are also being constructed. A central quality control laboratory for ngapi was established in 1979 and it is functioning satisfactorily. At every buying depot for semifinished ngapi, quality control laboratories were set up in order to help in buying quality ngapi according to the specified standards. Quick testing methods and laboratory equipment have been invented for use in the depots where large amounts of ngapi samples have to be analyzed within a very short time. The training for quality control procedures and short orientation courses were organized to improve the knowledge of quality control procedures not only in the processing of ngapi but also in assessing its quality at the buying depot.

The above activities are performed under the guidance of the working group of scientists and engineers form various institutes (Chemistry Depart-

ment, Arts and Science University, Yangon; Chemical Engineering Department Yangon Institute of Technology; Food Division, Central Research Organisation, Yangon; Research and Development Division, Foodstuffs Industries Corporation, Yangon; and National Health Laboratory, Yangon), organized by the Foodstuffs and General Merchandise Trade Corporation (FGMTC), which controled the state-owned ngapi processing factories and various buying depots until 1994 (21,23,24,27). The quality improvement scheme shown in Fig. 2 and adopted by the FGMTC has been successfully since 1980 (2).

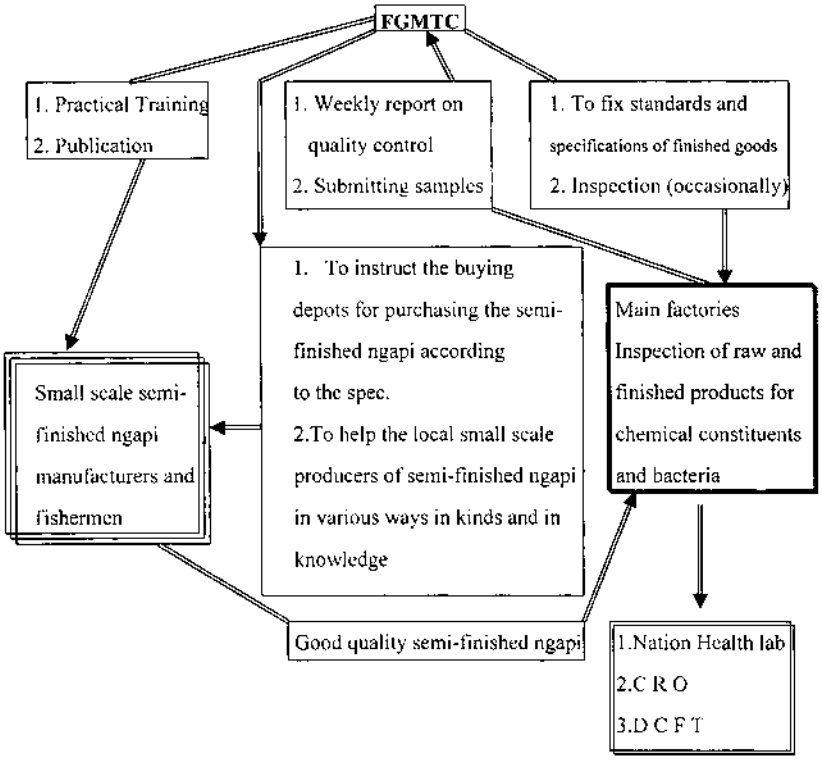
Because of these endeavors in organizing integrated utilization of emerging modern technologies for improvement of the traditional ngapi industry, the new commercially viable quality products in attractive packages such as bleached hmyin nganpyaye and powdered nagpi have been successfully developed; the quality and packaging system of the existing ngapi products are also being effectively improved.

A. Bleached Nganpyaye

Nganpyaye is the exudate or extract obtained as a by-product of the fermentation of fish or shrimp during the manufacture of ngapi. Usually, it has a dark brown color with a strong characteristic smell and is not attractive for table use. Although there is export potential, its color and strong smell are usually offensive to foreign customers. Therefore, in 1979 a preliminary study on the pilot-scale production of blanched nganpyaye was conducted (28). A pilot plant (shown in Fig. 3) was designed on the basis of appropriate technology developed by the Research and Development Division of Foodstuffs Industries Corporation. Most of the materials used for the equipment of this plant are of wood. Activated carbon is used for bleaching—the color of the nganpyaye changed from dark brown to the golden yellow of whiskey. Part of the smell is also removed simultaneously. Due to the overwhelming response in test marketing, bleached nganpyaye was promoted as a commercial product since the end of 1979. Bleached nganpyaye under the tradename Myint-Hmo-Hmew has now become a favorite commodity among the various culinary preparations of Myanmar.

B. Powdered Hmyin Ngapi

The traditional ngapi products are usually manufactured in the form of either whole fish (ngapi gaung) or paste. A new type of ngapi was introduced in 1983: dried and powdered hmyin ngapi. The strips of hmyin ngapi [0.6 cm ($\frac{1}{4}$ in.) in diameter] are obtained by extruding the first-grade hmyin ngapi



FGMTC = Foodstuffs and General Merchandize Trade Corporation
C R O = Central Research Organization
D C F T = Development Centre for Food Technology

Figure 2 Quality improvement scheme for hmyin ngapi and nganpyaye of the FGMTC.

paste of smooth texture with 40–50% moisture through the extruding machine resembling the coarse grinder. The strips are dried either in the sun or in the dryer until the moisture is reduced to about 10%. Dried strips are then ground to a very fine powder in a hammer mill and packed in laminated plastic bags for retail distribution. This new product is popular and has a great potential for a wider market (29). The analysis of the dried ngapi powder is given in Table 2.

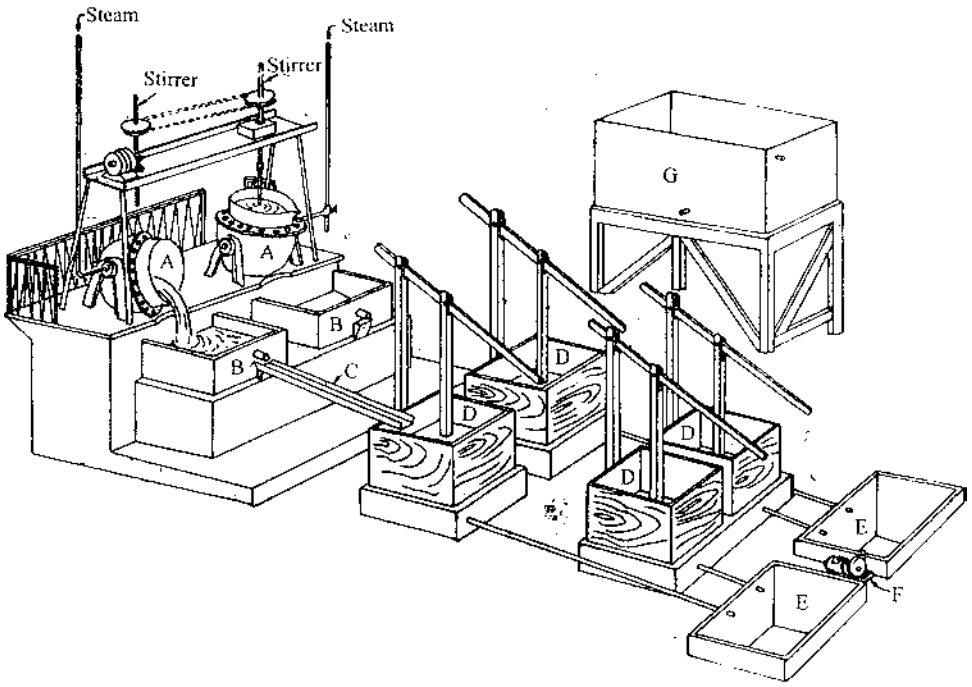


Figure 3 Bleaching process for hmyin nganpyaye. A = Steam-jacketed stainless-steel pan; B = receiving ponds for boiled nganpyaye; C = trough for collecting nganpyaye; D = wooden fitter presses; E = receiving ponds for filtered nganpyaye; F = nganpyaye pump; G = nganpyaye storage overhead tank.

Table 2 Chemical Analysis of Ngapi Powder, Myint-Hmo-Hmew Superior Brand

Component	Percent
Moisture	7.6
Protein	31.9
Fat	8.4
Ash	34.8
Sand	0.5
pH (5% w/v)	6.65

Source: Development Centre for Food Technology, FIC, Yangon, 1983.

VIII. MICROBIOLOGY AND BIOCHEMISTRY OF FERMENTATION

During fermentation of fish or shrimp, microbiological and physiochemical changes of edible parts take place. The initial composition of freshly caught fish and shrimp is generally found to be within 70–85% water, 15–25% crude protein, 1–10% fat (or lipid), and 1–1.5% minerals. The trend in the change of this composition is very important to the understanding of the mode of fermentation. Some information is available (24,27) on the physio-chemical and microbiological changes, including those of amino acids, during the fermentation period up to 90 days and are summerized in Tables 3–5.

The test samples were made by villagers at Kyonkan Creek in Pyapone District, Ayeyarwady Division, using traditional method. The data for finished product of Hmyin Ngapi from other private manufacture and from the FGMTC factory are also presented for comparison. The overall trend of changes of pH, water, salt, total nitrogen, protein, lipid, TVN, and ammonia in Table 3, free amino acids in Table 4, and bacteria genera and standard plate count (SPC) in Table 5 clearly reveal that there exist generally three periods of changes (viz, EMD period, buffer period, and Stabilizing period) during the course of fermentation, as mentioned earlier.

All of the reactions of the enzymes (i.e. hydrolysis, autolysis and lipolysis) and bacteria seem to be very active during the EMD period in comparison with the buffer or stabilizing periods, as indicated by the rapid fall of protein and lipid content and the rapid rise in TVN and NH contents. Only the indigenous enzymes and bacteria originally present in the fish or shrimp are responsible for these reactions. At present, no external enzymes are incorporated into the fish or shrimp fermentation process in Myanmar, although some attempts are being made eleswhere (31).

A. Degradation of Proteins and Lipids

Proteins and lipids are major components of the muscle and tissue of fish (and shrimp) responsible for the nutritive (taste) and aroma (flavor) factors of ngapi, respectively.

As soon as a fish or shrimp dies, the muscle and tissue start to decompose due to the actions of enzymes and bacteria present in the fish or shrimp. The two processes that occur simultaneously are usually known as autolysis, responsible for enzymes, and microbial degradation, responsible for that of bacteria. The decomposition will continue to the putrefaction stage if there is no control. Salt is used to control the autolysis to a required stage (i.e., up to the amino acid-producing stage) and then stabilized. This is essentially the main course for ngapi fermentation.

Table 3 Physio-chemical Properties of Hmyin Ngapi During 90-day Fermentation Period

Fermentation time (day)	pH	Water (%)	Salt (%)	Total N (%)	Protein (%)	Lipid (%)	TVN (%)	NH_3 (%)	Period
Fresh shrimp	7.1	80.03	1.17	—	17.69	0.42	0.084	0.064	
0	8.02	47.13	20.75	3.97	23.57	1.25	0.199	0.151	EMD
10	8.09	46.92	20.67	4.00	23.33	1.21	0.281	0.244	
20	8.14	46.54	21.26	4.12	23.09	1.20	0.423	0.333	
30	8.16	46.18	21.96	4.13	22.87	1.18	0.471	0.430	
40	8.17	45.91	22.80	4.17	22.85	1.11	0.513	0.493	Buffer
50	8.19	45.54	23.13	4.20	22.85	1.10	0.543	0.510	
60	8.21	45.36	23.33	4.23	22.80	1.10	0.581	0.525	
70	8.22	45.27	23.40	4.23	22.79	1.11	0.592	0.545	Stabilizing
80	8.23	45.21	23.43	4.23	22.68	1.13	0.601	0.551	
90	8.23	45.18	23.45	4.23	22.69	1.14	0.604	0.554	
Private[a]	8.15	36.34	21.55	3.73–4.29	29.01	2.02	0.690	0.597	
FGMT[a]	8.26	45.42	20.05	3.39–4.74	27.56	2.25	0.991	0.912	
Literature (Ref. 30)	7.1–8.3	37–64	10–29	2.99–4.65	18–32	2–8	—	0.4–1.4	

[a] Hmyin ngapi from private corporations and FGMTC are from Ref. 27.
Source: Data from Refs. 24 and 27.

The proteins of fish and shrimp are highly complex compounds and can be classified into three types according to their solubility: water-soluble, salt-soluble, and insoluble proteins. The contributions of salt-soluble protein (myofibrillar), water-soluble protein (myogen), and insoluble protein in fish or shrimp muscle are about 65–75%, 20–30%, and 2–3%, respectively. The highly complex proteins of the tissue are hydrolyzed into simpler soluble proteins, polypeptides, with the help of proteolytic enzymes, and this process of self-digestion, generally known as autolysis, continues in collaboration with the action of other beneficial bacteria and micro-organisms to form amino acids (24). If contamination by outside bacteria occurs during the process, the amino acids are further decomposed into amines or ammonia and carbon dioxide, spoiling the products. Salting prevents the entry of undesired external bacteria by removing water from the tissue cells. Because the main constituents that contribute the nutritive, aroma, and taste factors of ngapi are the amino acids, it is important to control the fermentation steps as much as possible up to the amino acid-producing stage. The trend of decrease in protein and that of increase in amino acid during fermentation, as shown in Tables 3 and 4, respectively, confirms that the combined effects of autolysis by enzymes (more pronounceded in the EMD and buffer periods) and that bacterial degradation (more pronounced in the stabilizing period) have occurred.

Table 4 Amino Acid Contents During the Fermentation of Hmyin Ngapi

			Content (mg/g)				
			Sample 1			Sample 2	Commercial
No.	Amino acid		0 day	30 days	90 days	90 days	>90 days
1	Alanine	Ala	4.59	4.62	7.22	8.37	10.31
2	Arginine	Arg	3.65	3.68	0.83	10.63	0.71
3	Asparagine	Asn	0.12	0.16	0.24	0.45	0.09
4	Aspartic acid	Asp	2.19	1.74	3.01	3.37	2.54
5	Cystine	Cys	0.02	0.00	0.08	0.05	0.06
6	Glutamic acid	Glu	5.35	4.40	7.56	7.72	9.88
7	Glycine	Gly	3.40	3.27	5.16	5.34	5.36
8	Histidine	His	0.33	0.22	0.68	1.05	0.67
9	Leucine[a]	Leu	4.29	4.32	4.65	9.24	7.88
10	Isoleucine[a]	Ile	2.09	2.14	2.94	4.99	4.52
11	Lysine[a]	Lys	4.32	3.28	6.05	10.40	8.51
12	Methionine[a]	Met	1.03	1.04	1.73	2.52	2.59
13	Phenylalanine[a]	Phe	1.93	1.95	2.88	4.06	3.02
14	Proline	Pro	1.29	1.27	1.92	2.22	1.95
15	Serine	Ser	0.90	0.92	0.64	2.14	1.88
16	Threonine[a]	Thr	1.19	1.02	1.13	3.20	3.17
17	Tryptophan[a]	Typ	2.63	2.86	12.03	2.94	3.62
18	Tyrosine	Tyr	1.25	1.36	2.09	3.69	3.43
19	Valine[a]	Val	2.92	2.87	4.38	5.61	6.04
Total			43.47	41.12	65.23	87.99	76.25

Note: Sample 1 used analar salt and sample 2 used solar salt. The commercial sample was from private manufacturers.

[a] Essential amino acid.

Source: Ref. 24.

The total nitrogen, containing both protein nitrogen and nonprotein nitrogen such as TVN and amino acids, sharply increases in the EMD period, slows down in the buffer period, and then stabilizes in the stabilizing period. This trend of increase in total nitrogen content could be attributed to the extent of formation of volatile bases and amino acids. However, it can be seen from Table 4 that the amino acid content does not increase notably in the EMD period, but it increases significiently in the later buffer and stabilizing periods of fermentation. Therefore, it is envisaged that in the EMD period, the hydrolysis of complex proteins into simpler polypeptides is the major reaction of enzymes. The autolysis reaction producing amino acids normally accelerates from the buffer period and then proceeds throughout the stabilizing period.

Table 5 Bacteria Genera Isolated from Shrimp (*Acetes* spp. and *Mysids*) and Shrimp Paste Fermentation as a Percentage of Total Microflora for Various Genera Groups

	Bacteria genera			
Sample	Gram(−)	Gram(+)	Standard plate count	Period
Shrimp (*Acetes* spp.)	*Ac* (8%), *Ae* (10%), *En* (10%), *Fl* (8%), *Ps* (15%), *Vi* (2%)	*Ba* (15%), *Mi* (17%), *St* (5%)	1.11×10^5–2.06×10^6	
Shrimp paste: fermentation time (days)				
0	*Ps* (0.17%)	*Ba* (99.69%), *St* (0.04%), *Mi* (0.10%)	2.40×10^4	EMD
10	*Ps* (0.12%)	*Ba* (99.76%), *St* (0.03%), *Mi* (0.09%)	4.15×10^3	EMD
20	*Ps* (0.07)	*Ba* (99.82%), *St* (0.02%), *Mi* (0.09%)	7.50×10^3	EMD
30	*Ps* (0.03)	*Ba* (99.87%), *St* (0.02%), *Mi* (0.08%)	6.92×10^3	EMD
40	*Ps* (0.01)	*Ba* (99.91%), *St* (0.01%), *Mi* (0.07%)	6.19×10^3	Buffer
50	—	*Ba* (99.96%), *Mi* (0.04)	5.90×10^2	Buffer
60	—	*Ba* (10%)	5.65×10^2	Buffer
70	—	*Ba* (100%)	4.70×10^2	Stabilized
80	—	*Ba* (100%)	4.65×10^2	Stabilized
90	—	*Ba* (100%)	2.70×10^2	Stabilized
Private (Ref. 27)	*Ps* (0.02%)	*Ba*, *St*, *Mi*	2.85×10^3–0.55×10^4	
FGMTC (Ref. 27)	*Eu*, *Kl*, *Ps*	*Ba*, *St*, *Mi*	5.32×10^4–0.18×10^5	
Literature	*Ae*, *Al*, *Ps*	*Co*, *Ba*, *Se*, *La*, *St*, *Ku*	9.5×10^4–4.0×10^6	

Note: *Ac* = *Acinetobacter*; *Ae* = *Aeromonus*; *Al* = *Alcaligenes*; *Ba* = *Bacillus*; *Co* = *Corynebacterium*; *En* = *Enterobacter*; *Fl* = *Flavobacterium*; *Kl* = *Klebsiella*; *Ku* = *Kurthia*; *La* = *Lactobacillus*; *Mi* = *Micrococcus*; *Ps* = *Pseudomonas*; *Se* = *Serriata*; *St* = *Staphylococcus*; *Vi* = *Vibro*.
Source: Data from Refs. 23, 24, and 27.

The other factor which determines the aroma of ngapi is the ratio of ammonia to total nitrogen content. From Table 3, this ratio is found to be rapidly increased in the EMD period and then slows down and stabilizes in the remainder of the fermentation period. This fact also explains that Myanmar ngapi has higher pH values and has a distinct ammoniacal aroma.

Lipid degradation also occurs by lipolysis to form some fatty acids, giving a cheesy aroma, and also some unsaturated fatty acids. The fatty acids thus formed are gradually neutralized by the nitrogen compounds present in hmyin ngapi in the later stabilizing period, thus maintaining the pH of ngapi at about 8.2. Due to the strong basicity of the hmyin ngapi fermentation process, considerable volatile organic compounds are developed; among them, 2-ethyl 5-methylpyrazine is the most abundent compound, contributing the characteristic aroma of Myanmar hmyin nagpi (24).

According to these trends of protein and lipids degradation, it could be envisaged that proteolysis and lipolysis are essential processes in the fermentation of Myanmar hmyin ngapi, rendering its characteric delightful taste and flavor and important nutritive value.

B. Salt-to-Water Ratio

The notable decrease and increase in water and salt content, respectively, denote that osmosis and diffusion mechanisms are also very active in the EMD period. The water contained in the shrimp tissue oozed out by osmosis and salt from outside also enter into the tissue cells by diffusion. The solubilized proteins of autolysis products then dissolves in the incoming salt and residual water with the tissue cells forming a colloidal solution, sol. This colloidal protein solution is held within the tissue cells and then gradually converts to a pasty consistency. The above opposite movement of salt and water in and out of tissue cells infers that a balance of salt and water exists which could be employed as a controlling parameter in ngapi fermentation. From Table 3, the ratio of salt to water increases fairly rapidly in the EMD period and then smooths out in the buffer and stabilizing periods. It is recommended that the salt-to-water ratio should be kept at about 0.500 throughout the fermentation period to obtain a quality-grade ngapi (24). Below the value of 0.500, the growth of harmful micro-organisms is enhanced due to the lack of salt content and ngapi could undergo spoilage or have a shorter shelf life.

C. Halophilic Bacteria

For ngapi processing in Myanmar, solar salt is usually used. Because solar salt is produced by evaporation of seawater under solar heat and winds, the

chance of contamination by micro-organisms from the atmosphere is high in addition to the original contamination from seawater. It was reported that the bacterial content (SPC) in the solar salt showed a maxmium value of 3×10^5 cells/g at 20°C and 1.4×10^3 cells/g at 37°C. In freshly caught shrimp, the highest count of 2.06×10^6 cells/g was found. However, the count decreases to 2.4×10^4 cells/g in the freshly prepared shrimp paste sample, as shown in Table 5. In the course of fermentation, the bacterial count decreases sharply in the early days during the EMD period, as the salt inhibits the growth of micro-organisms, sparing only halophilic bacteria. This rate of decrease then starts to decrease within in the buffer period; when the salt content reaches about 23%, it becomes stabilized. The cause of the decrease is possibly due to the exhaustion of halophobic bacteria, leaving only halophilic ones to survive throughout the later stabilizing period. This finding indicates that shrimp paste does not seem to be a good growth medium for bacteria.

From Table 5, one can observe that the Gram-negative bacteria genera gradually decrease from the shrimp paste flora and the Gram-positive bacteria are found to be the most predominant during the late period of fermentation. Among the Gram-positive bactera, *Bacillus* spp. were the most abundant, mingling only with some trace of *Micrococcus* spp. The *Bacillius* spp. are resistant both to heat and to high salt concentration and are the sole bacteria genera dominating in the buffer and stabilizing periods of ngapi fermentation. Therefore, the increase of amino acid content of the shrimp paste in these periods as shown in Table 4 can be regarded as a contribution by the halophiles, *Bacillus* spp. This is probably due to the strong proteolytic action of *Bacillus* spp., enabling them to participate in hydrolysis of protein into amino acids in the buffer and stabilizing periods. Moreover, it has been reported (31) that the halophilic micro-organisms were found in Myanmar hmyin nganpyaye (shrimp sauce) and the aroma of the sauce was caused by these halophiles. It is also relevant here to say that the characteristic aroma and the quality of Myanmar hmyin ngapi may be mainly attributed to the *Bacillus* species.

IX. HARMFUL AND SPOILAGE MICROORGANISMS

Myanmar Public Health Law (1972) set the limits of SPC for foods as 1×10^6 in 1 g of sample for the minimum limit and 1×10^7 in 1 g of sample for the maximum and samples must be free of *Escherichia coli* and *Salmonella* and must not exceed 5×10^2 to 5×10^4 cells/g of *Staphylococcus aureus* (32).

Analysis of 70 samples in 1967 and 1969 (2,33) indicated the presence of 23 types of bacteria (of which the majority, 15 types, were *Bacillus* spp. in

Myanmar hmyin ngapi. The SPC of these samples ranged between 9.5×10^4 and 4.0×10^6 cells/g. In recent studies (22–24,27,31), 61 samples were analyzed and 5 more additional *Bacillus* species were identified, altogether totaling 20 *Bacillus* species found in Myanmar shrimp paste to date. The SPC of all these samples were also less than the allowed limit of 10^6 cells/g. Therefore, it supports the conclusion that ngapi of Myanmar is fit for consumption.

Recently, the presence of food-poisoning bacteria such as total coliforms, *E. coli*, *Salmonella*, *Vibrio*, and *Staph. aureus* in the shrimp paste were tested based on the most probable number (MPN) method (24). In the raw material, shrimp, total coliforms of 43 MPN/g were found, of which *E. coli* and *Staph. aureus* were 4.7 MPN/g and 4 MPN/g, respectively, and no *Salmonella* and *Vibrio* were detected. At the begining of fermentation, the MPN of total coliform, *E. coli*, and *Staph. aureus* were 11, 3, and 4, respectively, which reduced to ≤ 3 in the later period and also in the finished product. The other food-poisoning bacteria *Vibrio* and *Salmonella* were not found in the finished product.

In the samples from the private enterprise and FGMTC, the total coliforms were found to be <3–11 MPN/g and 3–23 MPN/g, respectively. In the former samples, *E. coli* and *Staph. aureus* were found to be < 3–5 MPN/g and 7–11 MPN/g, respectively. whereas the latter are <3–8 MPN/g and 7–14 MPN/g, respectively. No *Salmonella* or *Vibrio* were found in these samples. Although these poisonous pathogens are destroyed by proper cooking at high temperature, they can cause spoilage to the product throughout the fermentation period. The other major contaminants were dead flies belonging to *Drosophilidae*, *Borboridae*, and *Calliphoridae* species. Traditional methods of preventing the contamination of ngapi from fly larvae by covering with the leaves called lauk-thay-ywet (*Desmodium triquetrum*) was effecient and had a lethal effect on the flies (50).

It is nessary to thoroughly clean the raw material and also to take measures to prevent contamination during processing and fermentation because most of the contamination was caused by negligence of elementry rules of hygiene.

X. ESSENTIAL MICROORGANISMS FOR FERMENTATION

Generally, the majority of the Gram-negative bacteria that predominate in the raw material were significantly diminished during the course of fermentation, leaving only a trace of *Pseudomonas*, whereas the majority of Gram-positive bacteria *Bacillus* (>99.9%), *Micrococcus* (<0.7%), and *Staph. aureus* (<0.01%) survived, as shown in Table 5. From these observations, it could be deduced that during the EMD period, the micro-organisms responsible for

the initial fermentation are enzymes presented initially in the shrimp and the extracellular enzymes produced by *Pseudomonas* spp. In the buffer and stabilizing periods, the genus *Bacillus* was the most predominant, with few *Microcoecus* spp. and *Staph. aureus*. Due to their strong proteolytic action, these halophilic micro-organisms are responsible for the later periods of shrimp paste fermentation.

XI. STANDARDS OF NGAPI AND NGANPYAYE

From the nutritional, chemical, and microbiological characteristics, it is evident that ngapi and nganpyaye are important sources of amino acids, some minerals, and vitamins which are indispensable for maintaining good health. Therefore, nutritionists, including those from developed countries, have realized that ngapi ingestion could augment the intake of protein, especially for those from the southeast Asian countries.

Because ngapi and nganpyaye are nutritionally important traditional foods relished by a majority of people, it is desirable to fix standards and safeguard them from adulteration. U Chit Thoung proposed fixed standards for Myanmar ngapi and published data which could serve this purpose (34). A considerable amount of data is now available for fixing the required standards (2,21).

In 1972, the standards for nga ngapi (fish paste), hmyin ngapi (shrimp paste), and hmyin nganpyaye were included in the Union of Burma Food Standards and specifications. The proposed standards are outlined in the Appendix.

XII. CONCLUSION

A comparison of the nutritional status of people from different regions of Myanmar in 1947 (35) revealed that those from Paung township, mostly farmers and fishermen, could obtain 3355 cal from their daily diet containing hmyin ngapi. Ever since ngapi came to be regarded as a protein-rich, nutritious, traditional food, efforts have been made by scientists and industrialists to upgrade the quality and status of ngapi and nganpyaye.

Many research projects on ngapi are being carried out in the universities and in the learned institutes, and some valuable theses and papers are being published (22,24–26,36–47,49).

Although some initial success has been achieved, much remains to be done, particularly integrated utilization with available modern technologies such as biotechnology and genetic engineering.

APPENDIX: STANDARDS FOR NGAPI AND NGANPYAYE

A. Ngapi (Fish Paste)

Ngapi (fish paste) as sold, offered, or exposed for sale or manufactured for sale shall conform to the following standards:

(a) Quality: The fish paste shall be a product made by the process of salting, drying, fermentation, and grinding from sound, clean, fresh fish. No foreign matter shall be present. Only permitted dyes may be used.
(b) Inspection: The analysis of the fish paste shall conform to the specifications given in Table 6.
(c) Bacteria: The bacteria count should not be more than one million per gram of Ngapi incubated at 37°C. The following bacteria should not be present in ngapi: coagulase-positive *Staphylococci*, *Salmonellae*, and *Coliform bacilli*.

B. Nga Nganpyaye (Fish Sauce)

Nga nganpyaye (fish sauce) as sold, offered, or exposed for sale or manufactured for sale shall conform to the following standards:

(a) Quality: The fish sauce shall be a product extracted by the salting, drying, and fermentation process from sound, clean, and fresh fishes. Adding of dyes and chemicals is prohibited.
(b) Inspection: The fish sauce analysis shall conform to the specifications given in Table 7.
(c) Bacteria: The bacteria count should not be more than one million per gram of ngapi incubated at 37°C. The following bacteria

Table 6 Fish Paste Specifications

	Percentage	
Component	Maximum	Minimum
Water	40	—
NaCl	25	—
Protein	—	18
Fat	—	1.5
SiO_2	0.5	—
Mineral salts (other than NaCl)	8	—
Free ammonia	1	—

Table 7 Fish Sauce Specifications

Component	Percentage	
	Maximum	Minimum
Water	55	—
NaCl	25	—
Protein	—	18
Fat	—	1
Copper content[a] (per 100 g of ash)	—	0.0007
pH	7.5	6.0

[a] Copper content determined according to Ref. 47.

should not be present in ngapi: coagulase-positive *Staphylococci, Salmonellae*, and *Coliform bacilli.*

C. Hmyin (Seinsa) Ngapi (Shrimp Paste)

Standards and specifications for shrimp paste (hmyin or seinsa ngapi) as sold offered, or exposed for sale or manufactured for sale must conform to the following standards:

(a) Quality: The shrimp paste shall be a product made by the salting, drying, fermentation, and grinding process from sound, clean, and fresh shrimps. The addition of starch or bran is prohibited. Only permitted dyes may be used.

Table 8 Shrimp Paste Specifications

Component	Percentage	
	Maximum	Minimum
Water	40	—
NaCl	25	—
Protein	—	18.5
Fat	—	1.5
SiO_2	0.5	—
Mineral salts other than NaCl	8	—
Free ammonia	1.0	—
Fish[a]	15	—

[a] Fish is determined according to Ref. 48.

Table 9 Shrimp Sauce Specifications

Component	Percentage	
	Maximum	Minimum
Water	55	—
NaCl	25	—
Protein	—	18
Fat	—	1
Copper content[a] (per 100 g of ash)	—	0.01
pH	7.5	6.0

[a] Copper content determined according to Ref. 47.

(b) Inspection: The analysis of shrimp paste shall conform to the specifications given in Table 8.
(c) Bacteria: The bacteria count shold not be more than one million per gram of nganpyaye incubated at 37°C. The following bacteria should not be present in Nganpyaye: coagulase positive *Staphylococci, Salmonellae* and *Coliform bacilli.*

D. Hmyin Nganpyaye (Shrimp Sauce)

Hmyin nganpyaye (shrimp sauce) as sold, offered, or exposed for sale or manufactured for sale shall conform to the following standards:

(a) Quality: The shrimp shall be a product extracted by the salting, drying, and fermentation process from sound, clean, and fresh shrimps. Adding of dyes and chemicals is prohibited.
(b) Inspection: The shrimp sauce analysis shall conform to the specifications given in Table 9.
(c) Bacteria: The bacteria count should not be more than one million per gram of nganpyaye incubated at 37C. The following bacteria should not be present in Nganpyaye: coagulase-positive *Staphylococci, Salmonellae,* and *Coliform bacilli.*

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Industrialization of Indigenous Fermented Food Processes: Biotechnological Aspects

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I. INTRODUCTION

The use of microorganisms in the preparation of foods and beverages is ancient. Microorganisms have been consumed for thousands of years in fermented dairy foods, fermented beverages, and traditional Oriental foods and sauces (1–4). Preservation of foods by fermentation has been widely practiced in many communities throughout the world (5,6).

Some of these fermentation processes have been developed very successfully to commercial scale (e.g., cheese-making and yogurt-making, soy sauce and miso production, brewing and wine-making), whereas others, such as tempe and indigenous fermented beverages, offer the opportunity for extending village-level technology to larger-scale processes. The advantages of this scale-up lies in more economic and standardized fermentations and in making the indigenous foods more commercially attractive and freely available to urban dwellers in developing countries and to a growing market in the West. It is not envisaged, however, that such process developments will supplant the village- and household-level fermentations, which will clearly continue among low-income families.

In assessing the opportunities which new developments in biotechnology can offer in the scaling-up of indigenous fermentation processes, it is worthwhile to subdivide them into two broad categories: (a) submerged culture fermentations and (b) solid-substrate fermentations (SSF). In the

former case, microbial activity occurs at a relatively low biomass concentration in the liquid phase, whereas in the latter case, microbial growth and product formation occur on the surfaces of, or within, solid substrates. Some examples of traditional fermented beverages/foods in each category are given in Table 1 (7–9) with a recent extensive review of a wide range of indigenous fermented foods by Beuchat (4).

One of the major characteristics which distinguishes SSF from submerged cultures is that SSF processes usually occur at low-moisture content, conditions under which filamentous fungi are the preferred microorganisms. However, for many indigenous fermentations, the microbial interactions are complex and mixed fungal/bacterial, fungal/yeast, or yeast/bacterial cultures occur. These interactions play an important role in the organoleptic and nutritional characteristics of the final product.

A comparison of the main features of the two types of fermentation processes is given in Table 2.

The practice of submerged culture fermentation is much more developed technically with large-scale industrial processes now existing for a wide range of products (e.g., single-cell protein, yeasts, alcohols and organic acids, amino acids, enzymes, antibiotics, vaccines, human therapeutics using recombinant bacteria, yeast, and mammalian cells, etc.) using single-micro-organism cultures. Microbial growth and product formation occur in a two-phase (gas–liquid) system.

By comparison, SSF processes are more complex and involve three-phase interactions (gas–liquid–solid) as well as mixed microbial cultures in many instances. Interestingly, as outlined in recent reviews by Pandey et al. (10) and Mitchell et al. (11), there have been significant developments over the past decade in the understanding of SSF processes and in their range of products. These include SSF processes for environmental applications (detoxification and biodegradation of hazardous wastes), biotransformation of

Table 1 Some Examples of Fermentation Practice for Indigenous Foods and Beverages

Submerged culture	Solid-substrate fermentation
Palm and rice wines	Tempeh
Kaffir beer	Oncom
Pulque	Ragi
Soy sauce	Natto
Fish sauce	Miso

Table 2 Comparison of Indigenous Fermentations in Submerged Culture with Those in Solid-Substrate Processes

Submerged culture
1. Relatively rapid growth and product formation can be achieved by yeasts and bacteria.
2. Mixed or pure cultures of filamentous fungi, yeast, and/or bacteria at relatively low cell concentrations.
3. Aeration and agitation requirements can be high for aerobic cultures.
4. Process scaled-up readily to large fermenter volumes.
5. Process control (pH, temperature, dissolved oxygen) relatively easy to achieve. Processes suited to computer control.
6. Product recovery (e.g., cell separation, distillation) can be an important part of overall process.
Solid-substrate fermentation
1. Slower fermentation due to slow growth of fungi on solid substrate.
2. Mixed fungal cultures predominate; relatively high biomass and enzyme concentrations.
3. Relatively low levels of aeration and agitation, the latter due to the shear sensitivity of filamentous fungi.
4. Scale-up problems due to limitations of heat and mass transfer and process control.
5. Process control more difficult due to three-phase (gas–liquid–solid), heterogeneous fermentation.
6. Final product obtained from SSF process.
7. Possible problems due to contamination and mycotoxin production.

crops and agricultural residues for value-added products and nutritional enhancement, and production of enzymes and secondary metabolites as well as organic acids, biopesticides, biosurfactants, and biofuels. However, despite the potential for the larger-scale development of indigenous food fermentation, there is little discussion of these opportunities in these reviews.

II. FERMENTATION KINETICS

In evaluating the differences between growth in submerged culture and in solid-substrate fermentation, it is worthwhile to consider the various kinetic models that have been proposed. These are discussed in detail by Pirt (12), who confirmed that bacteria and yeast growing in submerged culture on a single limiting substrate followed an exponential growth law in which the growth rate of the culture was dependent on the biomass concentration. By

comparison, fungal cultures followed a growth kinetic pattern in which the growth rate was dependent on the biomass concentration to the one-third power. This was due to the very different morphology and nature of growth of the fungal mycelia by comparison to bacterial and yeast growth. In the latter case, the equation used to incorporate the effect of single substrate limitation was based on the Monod model. In this model, there is a linear relationship between the specific growth rate and substrate at low concentrations and a "saturation effect" with increasing substrate concentrations having little or no effect on specific growth rate. Additional effects such as product inhibition and substrate inhibition (at a high substrate levels) can be included in a model to provide a more complete picture of cell growth, substrate uptake, and product formation.

The application of Monod kinetics to SSF is much more difficult, primarily because a complex substrate is involved, mass and heat transfer effects can influence the growth kinetics, and mixed culture interactions are involved in many cases. An early approach was made to characterize SSF growth kinetics by analyzing fungal growth on a cellulose-based substrate (13), with the rate of biomass production being affected by such factors as the rates of fungal penetration of the solid substrate, production of cellulase/hemicellulase enzymes, cellulose degradation, and uptake rate of simple sugars.

More recent and extensive modeling work by Mitchell and his group is well reviewed in Ref. 11 in which the SSF system is analyzed in relation to the following:

1. Microscale phenomena, which influence cell growth and death kinetics, as well as the growth of the microorganisms at the particle level. Issues such as local mass transfer by diffusion, heat transfer, and the effect of particle size of these transfer rates are critical at this microscale level.
2. Macroscale phenomena in which the main influences are the degree and nature of the agitation (which affect the degree of shear on the fungal mycelia) and the degree of aeration and oxygen availability in the SSF bioreactor.

Based on an understanding of these phenomena, a more rational approach can be made to optimize SSF bioreactor design and scale-up.

III. BIOREACTOR DESIGN

Critical issues in the design of SSF bioreactors are outlined in Table 3. For such bioreactors, the overriding issues of how to provide adequate heat and

Table 3 Factors in Bioreactor Operation and Design for Submerged Fermentation Process

1.	Microbiological characteristics of the selected microorganisms
2.	Kinetics of cell growth and product formation
3.	Genetic stability of selected strains
4.	Hydrodynamic characteristics of bioreactor
5.	Aseptic or sanitary equipment design
6.	Control of bioreactor environment (e.g., temperature, pH, dissolved oxygen, humidity control, etc.)
7.	Effect of bioreactor design and operation on downstream processing and product recovery
8.	Mass and heat transfer characteristics of the bioreactor, high rates of oxygen transfer and significant heat removal required in large-scale aerobic processes
9.	Potential for bioreactor scale-up to commercial-scale operation
10.	The fixed and operating costs of bioreactor operation

mass transfer dominate their design and operation. The availability of moisture and oxygen and the removal of heat and carbon dioxide are critical to the success of the fermentation. Various means have been used to improve the mass and heat transfer characteristics of SSF bioreactors, as outlined originally in the review by Mudgett (14):

1. The particle size of the solid substrate is a key variable that can be controlled initially to provide optimal surface area for fungal growth, heat transfer, and gas exchange. However, once the SSF process commences, it is likely that a fungal matte will form over the particle surfaces, possibly also causing aggregation, and this will significantly influence critical heat and mass transfer rates during the fermentation
2. Pretreatment of solid substrates can be used to make their chemical constituents more accessible and their physical structure more open to mycelial penetration. Mild thermal processing may be employed to pasteurize the solids and expose the substrate structure. This is done in traditional SSF fermentations by preparative steaming of the solids at 100°C for relatively short times.
3. The moisture content of the solid-substrate process is critical to a successful SSF fermentation, with a balance between having sufficient moisture for fungal growth but not an excess amount that might promote contaminant yeast or bacterial growth. The effect of water activity on the growth of various fungal cultures has been

studied in our laboratories (15), with the growth of the yeastlike *Aureobasidium pullulans* and the filamentous fungi *Aspergillus niger*, *A. oryzae,* and *Rhizopus oligosporus* being examined. The graphical plot of the maximum rate of colony expansion against the water activity for each culture demonstrates the relationship between their growth rates and the water activity (Fig. 1). The results indicate that *A. niger* and *A. oryzae* are capable of good growth over a relatively wide range of water activities, whereas *R. oligosporus* (used in tempe production) is very sensitive to the water activity and is unable to germinate at water activities below 0.92.

4. The conditions of agitation and aeration are of critical importance. Not only is it essential to maintain aerobic conditions in order to maximize growth and minimize the formation of by-products, it is also necessary to ventilate the CO_2 produced during growth, as CO_2

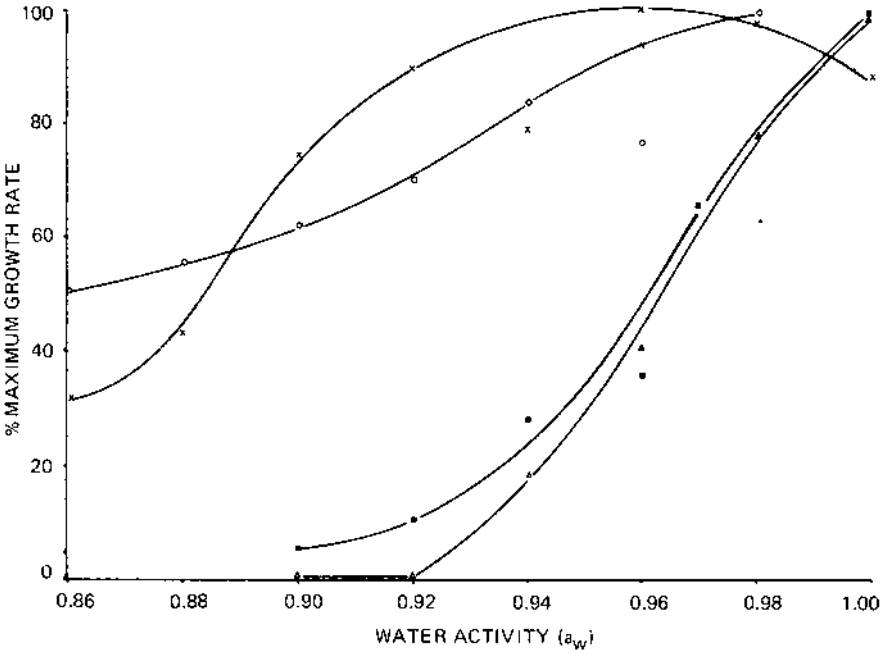

Figure 1 Effect of water activity on the growth rate of selected fungi: (×) *Aspergillus niger*; (○) *A. oryzae*; (▲) *Rhizopus oligosporus* (■) *Aureobasideum pullans*.

accumulation can inhibit further growth (9,16). Aeration and agitation have an impact on the onset of sporulation of the fungal culture, on the removal of heat from the fermenting mass, and on the actual growth of the mycelium itself if it is shear sensitive.

From experience it is evident that optimal design and operating conditions need to be established independently for each SSF process. If the growth and metabolism of the fungal culture are highly sensitive to shear stress, particularly in the early stages of fermentation, then this will require very careful attention to bioreactor design and to process control (including the nature and degree of agitation) at this critical stage. As pointed out by Mudgett (14), this shear sensitivity may be attributed to disruption of contact between the mycelia and the solid substrate, with this being particularly true for fungi with mycelial-bound enzymes required for the hydrolysis of solid-phase nutrients.

Many of the difficulties associated with SSF processes center on the problems of heat accumulation, overcoming fungal growth limitations due to inadequate mass transfer, in addition to the need for effective process control and scale-up (17). The larger-scale processes developed by the Japanese for traditional food products, enzymes, and organic acids have been based on stationary or rotary-tray methods in which temperature- and humidity-controlled air is circulated through stacked beds of fermenting solids (8). Other configurations such as the rotary drum or scraped tubular reactor offer more potential for process control but are more difficult to scale-up (13).

Mitchell et al (11) provides a comprehensive review of available SSF designs. These include the following:

1. Tray bioreactors: unmixed beds without forced aeration
2. Packed beds: unmixed beds without forced aeration
3. Continuously rotating drums: continuous agitation without forced aeration
4. Intermittently stirred beds: intermittent agitation with forced aeration
5. Bioreactors with both continuous mixing and forced aeration

As indicative of the increasing interest in larger-scale SSF processes, two promising bioreactor designs have emerged over the past decade. The first is the Zymotis design of ORSTROM (Montpelier, France) for an SSF process in which the substrate bed must remain static during the fermentation. To achieve this and to facilitate effective heat transfer, closely spaced internal heat transfer plates are constructed within a packed-bed bioreactor (18). For the second design for an SSF bioreactor, in which mixing can be tolerated, a stirred bioreactor has been designed by INRA (Dijon, France) and success-

fully evaluated at production capacities of between 1 and 25 tons of raw material (19).

IV. PROCESS CONTROL

In terms of achieving adequate process control in an SSF bioreactor, the following key variables are critical:

1. Temperature. This may be measured at different depths below the bed surface in a static fermentation by thermistors or thermocouple probes. Heat generation within the bed and the low level of agitation are likely to result in thermal gradients within the solid substrate. A humidified airstream may be used to either supply or remove heat from the SSF bioreactor via control of the airstream temperature.
2. pH. Miniaturized pH probes may be used at different positions in the SSF bioreactor. However, the liquid-phase conditions may not reflect the localized pH changes or the pH changes likely to be occurring within the interstices of the particles due to mycelial penetration and growth
3. Moisture content. Some control can be achieved via air temperature and humidity
4. Composition of input gas (e.g., the % CO_2 and % O_2). This may influence growth of biomass and product formation as shown in studies reported by Bajracharya and Mudgett (16) for the production of amylase by *A. oryzae* on rice. Oxygen partial pressures above 0.21 atm stimulated amylase production while having little effect on growth, whereas CO_2 partial pressures above 0.01 atm severely inhibited amylase productivity but stimulated biomass production. Again, more studies are needed for the different SSF processes to further characterize this phenomenon.

The problem of direct estimation of biomass has necessitated the use of indirect methods. In previous SSF studies, biomass production has been correlated with such by-products and enzymes as ergosterol, glycosamine, and laccase (20) and soluble protein (21). For rapid on-line estimation of the rate of fungal growth, Ryoo et al. (22) have used the rate of carbon dioxide evolution during fermentation as the basis for process control and optimization. Given the heterogeneous nature of most SSF processes and the problems with the direct and rapid measurement of other process variables, it may well be that on-line gas estimations will be the best way to develop any large-scale processes. Both the rates of oxygen uptake and carbon dioxide evolution can

be readily measured via exit gas analysis from the SSF bioreactor. These could then provide the basis for an effective process control strategy, particularly if they can be related to fungal growth and metabolism. At a more sophisticated level, on-line mass spectrometry can be used for exit gas analysis (23) and this could provide additional information on the production of various volatile products (such as alcohols, esters, volatile fatty acids) which could also be related to the status of an SSF process.

V. SCALE-UP

For most submerged culture processes, scale-up is concerned with providing adequate agitation and aeration to larger-scale fermentations as well as ensuring effective heat removal. Scale-up is often based on maintaining geometric similarity with the smaller-scale bioreactor, although in some cases where the fermentation has very high oxygen requirements, the scale-up will be based on trying to maintain similar oxygen transfer coefficients (24). For anaerobic processes such as occur with traditional beverage fermentations, scaling-up will be less of a problem. However, in this latter case, the maintenance of temperature control may be difficult, particularly if the process is being carried out in high-temperature/high-humidity environments. Under such conditions, it may be worthwhile to screen for microorganisms which can ferment at the higher temperatures (e.g., 40–45C) in order to minimize the problems of maintaining adequate temperature control in the large-scale fermentations.

From an economic viewpoint, it is worth noting that the relationship between the cost and size of the various items of fermentation equipment for submerged culture fermentations is as follows:

$$\frac{\text{Cost}_2}{\text{Cost}_1} \text{ is proportional to } \left(\frac{\text{Size}_2}{\text{Size}_1}\right)^n$$

where size_1 and cost_1 refer to the smaller process, size_2 and cost_2, refer to the larger process, and n is the exponent or scale factor.

As discussed by Stanbury and Whitaker (25), scale factors of 0.6 have been estimated for the brewing industry and 0.7–0.8 for single-cell-protein production. The relationship establishes clearly the economies of scale likely to be achieved with larger-capacity equipment. However, the advantages of these improved economies of scale need to be balanced against the additional risks of having a large enough market to meet the additional production.

With SSF processes, the same economies of scale are likely to apply, although the scale factors will depend on the particular type of SSF bioreactor. In some cases where the fungal culture is highly sensitive to shear forces,

scale-up usually involves the repetition of individual units (e.g., additional shallow trays in the koji process). The Zymotis process discussed previously (see Ref. 18) has been designed for scale-up of an SSF fermentation where the substrate bed must remain essentially static. On the other hand, where the fungal culture or the fungal–substrate interaction is not shear sensitive, a stirred SSF bioreactor has been scaled-up to process up to 25 tons of raw material in one operation (19).

VI. PROSPECTS FOR PROCESS IMPROVEMENT

In developing a larger-scale process for traditional fermented foods or beverages, there are a number of improvements likely to flow from an associated research and development program. Significant advances are likely to come from inoculum development (either pure or mixed cultures) and from the isolation of strains that have more desirable characteristics in an SSF process. These may come from screening natural isolates, or from screening and selection following a mutation program, or be produced from a range of recombinant DNA techniques that may be applied to fungal cultures.

In addition, a better understanding of bioreactor design, process control, and scale-up will lead to enhanced SSF processes.

A. Inoculum Development and Germination

In commercial-scale SSF processes, the use of inocula comprising spores that germinate quickly will be a major contribution to the production of a standardized fermented product (26,27). The potential for process improvement by giving careful attention to inoculum development and concentration is well illustrated in the production of spores of *R. oligosporus* (28); also rice-grown inocula produced more spores, had a longer shelf life, and improved the quality of the tempe produced (29). Germination of spore inocula in tempe fermentation may be optimized also by manipulation of conditions such as acidity, temperature, and aeration (30,31).

B. Strain Improvement by Mutation

The qualities of the final fermented foodstuff may be influenced by the characteristics of the fermenting organisms. Desirable properties of fungal cultures may include the following:

1. An improved amino acid balance with enhancement of the sulfur amino acids (cysteine and methionine) and possibly also lysine. The fungal strains used for single-cell protein for supplementation of the

human diet are often deficient in the sulfur amino acids by comparison with the FAO/WHO standard.
2. Improvement of storage properties of the fermented food achieved by the use of asporogenous mutants of conidial fungi. Such mutants would also be useful in extending the fermentation time, thereby allowing more biomass and increased protein production.
3. The inability to produce secondary metabolites such as pigments or toxins.
4. An enhanced ability to produce flavour components and other desirable qualities (e.g., texture, aroma) in the fermented food.
5. The ability to utilize a broader substrate range to allow flexibility in the development of indigenous foods for a wider market (32).
6. Thermotolerance or thermophilic characteristics giving the fermenting organism a selective advantage during the SSF process.
7. Osmotolerance providing a selective advantage to desirable organisms as well as allowing modification of the indigenous food during fermentation.

Strains of filamentous fungi may be developed with a range of characteristics designed to improve the operation of an SSF process. For example, fungal mutants which have improved amino acid profiles (particularly with respect to lysine) and are both asporogenous would have application for an SSF process designed to produce enriched animal feed supplements from various starch sources and cereals (e.g., cassava, sorghum, low-grade wheat, etc.).

1. Development of Asporogenous Mutants

In most solid-substrate fermentations, the limiting factor for biomass production by conidial fungi is the onset of sporulation (13,33). Sporulation may be undesirable in an SSF process for a number of reasons. First, additional growth and substrate utilization can be greatly reduced once sporulation begins. The onset of sporulation can decrease the yield of enzymes (such as proteinases, amylases, cellulases, etc.) and, therefore, may decrease the extent of substrate hydrolysis (9). Second, spores can present a health hazard to fermentation workers or to those who handle the end product. Finally, spore formation during fermentation can result in the formation of undesirable flavors or odors in the product (1,4). To minimize some of these problems, the extent of sporulation may be reduced by alteration of the mechanical shear on the mycelium in the SSF process or possibly by maceration of the fungal culture (21), although both of these destructive techniques damage the mycelium and could affect fungal growth and biomass yield.

On the other hand, spores are the most efficient form of inocula for filamentous fungi (34). To facilitate this, a significant development in the use

of filamentous fungi in solid-substrate fermentation would be the development of stable asporogenous strains which are unable to sporulate at a higher temperatures than those normally used for growth. This would allow the preparation of a conidial inoculum at a permissive temperature, but would result in mycelia with no propensity to sporulate at a higher, nonpermissive temperature. As reported by Glenn (15), such temperature-sensitive asporogenous mutants of *A. niger* have been developed by our group (see Figs. 2 and 3) and it was found that these strains gave improved biomass yields by extending the fermentation time past the limitation otherwise imposed by the onset of sporulation. This resulted in an increase in the protein content of the final cereal-based product. In addition, the final fermented product would have more satisfactory organoleptic characteristics due to the absence of fungal spores. At the same time, the efficiency of the overall SSF process could be maintained through the preparation of a spore inoculum.

2. Amino Acid Enhancement of Temperature-Sensitive Asporogenous Mutant

An L-lysine enhanced, analog-resistant derivative of the temperature-sensitive aconidial mutant of *A. niger* (40-1) has been isolated that produced twice the level of free lysine of the wild type while retaining the asporogenous char-

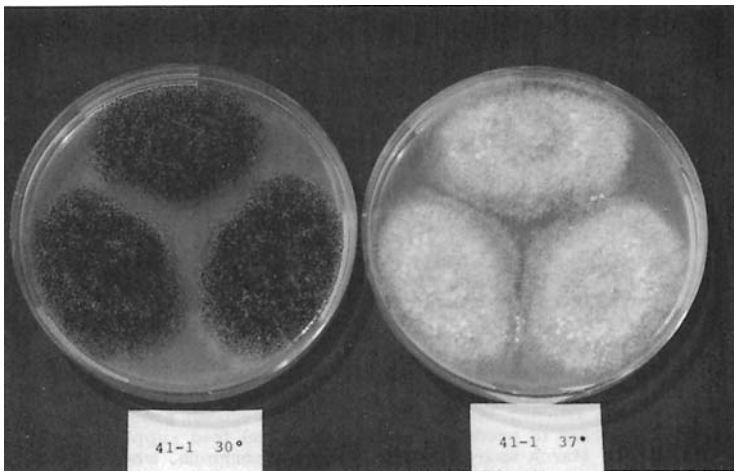

Figure 2 Temperature-sensitive aconidial isolate of *A. niger* at permissive and nonpermissive temperatures. The minimal medium agar plates were incubated for 3–4 days at the designated temperatures.

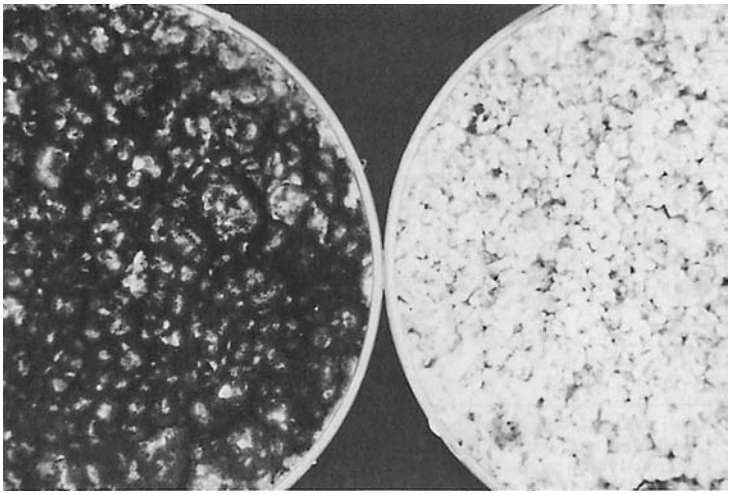

Figure 3 Growth of analog-resistant, temperature-sensitive asporogenous derivative of *A. niger* at the nonpermissive temperature on a solid substrate (cracked sorghum, 1–2-mm-diameter particles). Growth of mutant strain is shown on right.

acteristic of the parent; see Table 4 (15). This is significant, as many cereal crops (and starch sources) used in animal feeding are deficient in L-lysine. Fungal strains with a combination of such improvements have the potential for use in the production of enhanced fermented foods/animal feed, which are free of fungal conidia but enriched in a higher-quality protein with improved nutritional characteristics. Such a strategy of strain improvement and SSF processing could be extended to other raw materials to enhance the quality, safety, and flavor characteristics (through enhanced production of nucleotides, for example) of a range of indigenous fermented foods.

C. Strain Improvement Through Genetic Manipulation

Genetic and physiological analyses of filamentous fungi are increasingly revealing the effectors of important cellular functions relevant to production of foods by SSF. Such cellular functions include conidiation and hyphae production, amino acid synthesis, enzyme and other metabolites (such as polyunsaturated fatty acids) production, and protein secretion (35–38). Gene manipulation tools, such as plasmid-based vectors (39,40), transposon-based gene disruption (41), parasexual recombination (42), and protoplast fusion (43) have been applied to filamentous fungi, extending the application of conventional technologies of protoplast fusion (44) and mutant generation (45). In

Table 4 Lysine Overproduction by Lysine Hydroxamate-Resistant Mutants of *A. niger*

	Total free lysine (mg/g biomass)	
Strain	48 h	72 h
Wild type	3.9	2.9
40-1	3.2	8.2
41-4	1.2	4.7
42-10	4.1	3.4
42-11	4.1	3.4
42-13	ND	3.4
44-11	5.25	3.0

Note: The free lysine included both intracellular and extracellular. The estimated internal lysine content was combined with excreted lysine to give a total free lysine. ND = not determined.
Source: Ref. 15.

combination with the increasing understanding of fungal genetics, the tools of genetic manipulation can be used to generate targeted changes in fungi, without the loss of vigor or other advantageous characteristics of the parent strain. These methods could play an important future role in the industrialization of indigenous fermented foods through strain enhancement or the addition of desirable attributes, such as production of hydrolytic enzymes, or elimination of undesirable attributes, such as toxin production.

D. SSF Process Development

The production of the fermented soybean product tempe provides an interesting example of aspects of process development and potential larger-scale operation. In a review on the developments in tempe research, Nout and Rombouts (46) anticipated increasing consumption and a growing interest in the availability of this low-cost source of protein. They identified the following key challenges:

- The safety and health of the product are key priority areas, with it being imperative to understand and control this nonsterile process in such a manner that food infections and toxin production by contaminant microorganisms are entirely ruled out. In a mixed-culture

fermentation, where many yeast and bacterial species play an important secondary role (including *Lactobacillus* and *Lactococcus* sp.), as well as the primary fungal culture *Rhizopus oligosporus*, (47–50), safe and reproducible process control strategies will need to be developed for larger-scale processes.

- The process engineering aspects of SSF and tempe, in particular, were identified as lagging behind those concerned with the microbial ecology and the nutritional aspects of the fermented product. To fully understand the process and provide the basis for future scale-up and industrialization, more research is needed into mathematical modeling of fungal growth kinetics, the heat and mass transfer limitations of the various bioreactor designs, the effects of shear on fungal growth and metabolism, the development of effective material balances for the process (to minimize dry-matter loss reductions), as well as analysis of the energy requirements of the process.

Early work by Steinkraus et al. (51,52) characterized the fermentation and outlined the design of a pilot-scale process for the larger-scale production of the dehydrated tempe.

In further work, Wang et al. (53) reported on techniques for the mass production of spores of *R. oligosporus* for the process. Subsequently, Steinkraus (2) and Steinkraus et al. (54) proposed an essentially semidry process with reduced cooking periods, thereby resulting in higher tempe yields and less wastes. Studies by Berghofer and Werzer (55) emphasized that an important factor in determining the proper harvest time was the achievement of an adequate cohesive strength in the fresh tempe, which must subsequently withstand slicing, handling, and transport. These authors were also able to monitor pH changes and achieve optimal temperature in their process. Later work by Nout and Rombouts (46) focused on the mass and energy balances, with the authors noting that "reduction of dry matter losses and required process energy is a challenge to food technologists."

More recently, De Reu et al. (56) reported on improved temperature control in an SSF process through discontinuous rotation of a rotating-drum reactor (RDR). This reactor was applied subsequently to the fermentation of soya beans by *R. oligosporus*, with the aim of developing a process for the controlled fermentation of soya beans to produce a microbiologically safe and protein-rich product (57). Automatic control of the process was achieved, with the most important process parameters being rotation speed, substrate temperature, rotation frequency, and relative humidity. By using discontinous RDR operation, the fungal activity was maintained for up to 70 h, whereas in the nonagitated systems, fungal activity decreased after 36 h incubation (De Reu, 1995).

This brief review of the developments of the tempe fermentation process over the past decades is illustrative of the potential which exists for the larger-scale production of indigenous foods—a progression which hopefully will not supplant any traditional village-level production but will make a traditional nutritionally attractive food more available to an increasingly urbanized society.

VII. CONCLUSION

New developments in biotechnology are likely to have an important impact on our ability to scale-up processes for indigenous foods. These include techniques for the production of standardized inocula and for fungal strain enhancement through the use of mutation/selection and recombinant DNA techniques. There is a paucity of data available on strain improvement of filamentous fungi, particularly those used for traditional food fermentations, and this should be a fertile area for future research. Likely benefits of such strain improvements will be a more standardized product with enhanced nutritional, organoleptic and storage characteristics. Over the past decade, increased attention has been paid to a more detailed understanding and modeling of the SSF process itself and this has resulted in improved bioreactor design and operation. As a result, larger-scale systems have been designed both for processes requiring static SSF (Zymotis process) and for cultures where the mycelia or the mycelial–substrate interactions are not shear sensitive (INRA process scaled to 1–25 tons capacity). Such advances in SSF technology now provide a much stronger basis for their direct application to the scale-up and industrialization of a range of indigenous food fermentations.

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