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# **SAKE Alcoholic Beverage Production in Japanese Food Industry**

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## **1. Introduction**

*SAKE* brewing is an important sector of the Japanese food industry. It has maintained a strong relation with the culture in areas producing it, as have other alcoholic beverages such as wine, beer, and tequila in other countries. *SAKE* has a history extending back 1000 years into antiquity, and brewers' skills and techniques have been cultivated scientifically for longer than the discipline of chemistry has even existed. Particularly, low-temperature sterilization of *SAKE* was conducted in the 16th century, before Louis Pasteur invented pasteurization. The method is carefully described in old Japanese literature.

The significance of *SAKE* culture and its old techniques of brewing has been investigated using modern scientific analysis and brewing research methods. Furthermore, in *SAKE* brewing, unique techniques have been examined, such as fermenting under low temperature, achieving more than 18% high alcohol concentrations without distillation, open fermentation systems without sterilization, and creation of a fruity aroma in *SAKE*. Furthermore, yeast, mold, and the raw material—rice—have bred to be suitable *SAKE* brewing. Preferences for *SAKE* among young (20–30s) consumers have been elucidated recently, and the potential for new *SAKE* development has been reported. This report describes the history of *SAKE*, propagation methods of *SAKE*, its production materials, and recent research related to it.

## **2. History of SAKE**

Cultivation of rice, the raw material for *SAKE* brewing, originated in China. Seed rice harvested more than 10 millennia ago have been found in Kiangsi province and Hunan province in China. Probably, Japanese rice was introduced from China, where rice was cultivated

in dry fields using dry rice cultivation methods. Introduced from China early, rice was cultivated in dry fields in Japan also. However, the method of rice cultivation in paddy fields boosted yields to higher levels than those achieved in dry fields. The wet method brought social changes: a reliable labor force is necessary for cultivation by planting rice in paddy fields, harvesting it, and maintaining paddy fields, equipment, and irrigation. The labor force resources from families were limited. More labor was required from settlements. The settlements formed communities. Later communities formed ancient Japan. At that time, rice was of particular value: it was a divine food. *SAKE*, made from that divine food of rice, was also revered as blessed. It was used as a sacrifice to the gods. Moreover, people believed in a divine spirit indwelt in rice. Extending that belief, people believed that intoxication by alcohol beverages as *SAKE* made from rice brought gods into the human body. Furthermore, they solidified the community by sharing divine foods as rice and beverage as *SAKE* among members as they cooperated in rice cultivation [1].

Alcoholic beverages can be made from cereals as beer, *SAKE*, or whisky. Saccharification processing is extremely important. Generally, it is an important feature of Asian alcoholic beverage production that mold cultivate in cereal, so-called '*KOJI*', is used for production. However, according to ancient literature, *Osumi-no-Kuni-Fudoki*, which recorded the culture and geography of Kagoshima in ancient times before production of *SAKE* using *KOJI*, alcoholic beverages were made with saliva as a saccharifying agent with a method of chewing rice in the mouth. It was produced by that method until the eighth century [1].

In China, ancient Chinese *KOJI* had been used from ancient times, as described in Chinese ancient texts such as the *Chi-Min-Yao-Shu*. Chinese *KOJI* is made from barley or wheat. It is kneaded cereal flour with water and hardened as a brick or cake. Modern *KOJI* is made from non-heated cereal flour or wheat as material. However, it is described that ancient *KOJI* made from a mixture of heat-treating material with mixed non-heated wheat flour, roasted wheat flour, and steamed wheat flour, and the mixture cultivated *KOJI* mold after kneading with water or extraction without inoculation of seed mold. Kanauchi and co-authors [2, 3] reported their features. Results show that *Aspergillus* spp. was grown on and within steamed cereal cake as the dominant *KOJI* mold, *Rhizopus* spp. was grown on and within a non-heating cereal cake as the dominant *KOJI* mold. Furthermore, both *Aspergillus* spp. and *Rhizopus* spp. as dominant *KOJI* molds were grown on and within a roasted cereal cake and a cereal cake mixed with heat-treating of cereal materials of three kinds [2,3]. Mold strains were dominant selectively in cereal cake because denatured protein was impossible to decompose by *Rhizopus* spp., but *Aspergillus oryzae* was impossible to assimilate non-heated starch in wheat flour [2,3]. In modern China, non-heated cereals such as barley or peas are used, whereby *Rhizopus* spp. or similar physiological features have *Mucor* spp., which can grow on non-cereals, predominant in it. It is difficult to decompose denatured cereal protein to enhance their uptake for nutrition of micro-organisms, *Rhizopus* spp. has a weak protease or peptidase to grow on steamed cereals [4].

It remains unclear whether Chinese type *KOJI* was used for *SAKE* production or not by ancient Japanese. However, steamed rice is used for *SAKE* production where *Aspergillus* spp. has been used since ancient times.

Between the Asuka-Nara Era and Heian Era (5th – 8th century), imperial families and courtiers established huge craft factories of which wide areas were dedicated to crafts. Many technicians and workers were employed in them, monopolizing practical technologies of all areas. Brewery also continued in such factories, and SAKE was brewed using advanced technologies during those eras.

During the Heian period (8th century and thereafter), SAKE rose to importance for use in regional ceremonies or banquets. SAKE brewing by SAKE-NO-TSUKASA was both a SAKE brewery and a supervisory office of the imperial court. Some kinds of SAKE were brewed for emperor, imperial family, and the aristocracy for use at ceremonies or banquets. Brewing methods were described in *ENGISHIKI*, an ancient book of codes and procedures related to national rites and prayers. For example, *GOSYU* was specially brewed for the Emperor using steamed rice, *KOJI*, and mother water. The mash was fermented using wild fermentative yeast for ten days; then the mash was filtrated. The resultant SAKE was used for subsequent brewing as mother water. The SAKE brewed steamed rice, *KOJI*, and strained SAKE brewing were repeated four times to produce SAKE with a very sweet taste [1]. The literature in this period described SAKE of more than two kinds. The minor aristocracy and many people were not able to drink SAKE because it was extremely expensive.

Between the later Heian Era and Muromachi Era via the Kamakura Era (12th–16th century) SAKE was produced and sold at Buddhist temples and private breweries. During that period, it was a popular alcoholic beverage. In the 12th century, the feudal government issued alcohol prohibition laws many times to maintain security. Officers destroyed SAKE containers throughout cities.

At the beginning of the Muromachi Era, according to the '*GOSHU-NO-NIKKI*', SAKE was brewed already using a modern process in which rice-*KOJI* and steamed rice and water were mashed successively step-by-step. Moreover, the techniques applied lactic-acid fermentation, which demonstrates protection of the mash from bacterial contamination and dominant growth of yeast during SAKE production [1, 5].

During the 16th century, the *TAMON-IN* Diary was written for 100 years. *TAMON-IN* were small temples belonging to the *KOFUKUJI* temple in Nara. The diary described heating methods used to kill contaminated germs already in this century. In Europe, Louis Pasteur announced low-temperature pasteurization of wine and milk in 1865. However, Japanese brewers had acquired experimentally pasteurized SAKE during the 16th century [5].

During the Edo Era, the brewing season extended from the autumnal equinox to the vernal. However, results show good tasting SAKE brewing conducted in midwinter using a method called '*KANZUKURI*'. The brewing techniques of those brewers in the Ikeda, Itami, and Nada districts (Osaka City and Hyogo Prefecture) held the leadership in SAKE brewing at that time. During the Genroku period (end of the 17th Century), the total number of breweries was reported as greater than 27,000 [5].

After the Meiji Era, SAKE brewing methods changed drastically based on European science. Many improvements of SAKE brewing were accomplished by applying beer-brewing methods directly. However, many special techniques are used in SAKE production. For example,

mold culture is not required in beer brewing. Japanese brewers built the technology of *SAKE* production which mixed European beer brewing and old Japanese traditional techniques during the Meiji Era [5].

### 3. *SAKE* materials

#### 3.1. Water

Water is an important material used in *SAKE* brewing, accounting for about 80% (v/v) of *SAKE*. It is used not only as the material but also in many other procedures such as washing and steeping of rice, washing of bottles or *SAKE* tanks, and for boiling. Generally, approx. 20–30 kl of water is necessary to process one ton of rice for *SAKE* brewing [5, 6]. The water for *SAKE* brewing must be colorless, tasteless and odorless; it must also be neutral or weakly alkaline, containing only traces of iron, ammonia, nitrate, organic substances, and micro-organisms. In particular, iron ions are injurious to *SAKE*, giving it a color and engendering deterioration [5, 6]. Therefore, iron in brewing water is removed using appropriate treatments such as aeration, successive filtration, adsorption (with activated carbon or ion-exchange resins) and flocculation (with a reagent of alum) [7, 8].

#### 3.2. Rice

The quality of rice, the principal raw material of *SAKE*, strongly affects the *SAKE* taste, but details of its effects are not clearly elucidated. Contrary to the other Asian alcohol production, Japonica short-grain varieties are used for *SAKE* production. In Korea and Taiwan, other short-grain varieties might also be used for alcoholic beverages.

##### 3.2.1. Grain size

Large grains are suitable for *SAKE* production. Figure 1 shows rice grain size. The grain size is generally reported as the weight of 1,000 kernels. A weight of more than 25.0 g has been quoted as a mean value of 101 selected varieties by scholars [5, 9]. The selected varieties have a white spot in the center known as *SHINPAKU*, which contains high levels of starch.

##### 3.2.2. Chemical constituents

Rice contains 70–75% carbohydrates, 7–9% crude protein, 1.3–2.0% crude fat, and 1.0 ash, with 12–15% water. Other components such as proteins or lipids in rice, excepting starch, are unnecessary for *SAKE* production. In fact, *SAKE* produced with rice having excessive proteins or lipids does not have good flavor or taste. Their compounds exist on the endosperm surface, mainly around the aleurone layer. Therefore they are removed by rice polishing. Moreover, the following have close correlations among the weight of 1,000 kernels: crude protein contents, speed of adsorption of water during steeping, and formation of sugars by saccharification of rice with amylases [5, 10].



**Figure 1.** Rice grain size. Left side shows YAMADANISHI for variety of SAKE brewing rice. Right side shows HITOMEBORE for variety of diverting rice.

## 4. Microorganisms

### 4.1. KOJI mold (*Aspergillus oryzae*)

The scientific name of Japanese KOJI mold is *Aspergillus oryzae*. It grows on and within steamed rice grains. The mold accumulates various enzymes for SAKE production. Enzymes of about 50 kinds have been found in KOJI, the most important of which are amylases.  $\alpha$ -Amylase (Endo-  $\alpha$ - amylase, EC.3.2.1.1) and saccharifying amylase (Exo- $\alpha$ -glucosidase; E.C. 3.2.1.20) play important roles in amylolytic action [5, 11]. Furthermore, proteases of some kinds are also important enzymes: acid-proteases and alkaline-proteases are found in KOJI. In SAKE mash, the enzymes decompose protein to form amino acids and peptides (oligo-amino acid) at low pH values such as pH 3–4 [5]. Furthermore, amino acids or peptide-supported yeast grow with food or nutrition. The enzyme acts indirectly, decomposing rice protein while combining to an active site of the  $\alpha$ -amylase [12].

The taxonomy of mold was studied for *Aspergillus oryzae* by Ahlburg and Matsubara (1878) and Cohn (1883). A report by Wehmer (1895) was published, describing KOJI mold class *A. oryzae* in detailed mycological studies as an *A. flavus-oryzae* group. They are slight graded on variations in morphological and physiological properties [5]. Murakami et al. identified and reported that KOJI mold strains used for SAKE brewing belonged to *A. oryzae* and not *A. flavus*. Two species were distinguished based on mycological characteristics of each authentic type culture of the two species [13, 14]. It is noteworthy that no Japanese industrial strain of KOJI mold is capable of aflatoxin production.

In SAKE brewing, conidiospores produced over bran rice, so-called TANE-KOJI, are sprayed and inoculated on steamed rice. KOJI is prepared in an incubation room, a so-called KOJI-MURO.

## 4.2. Yeast

### 4.2.1. Physiology of SAKE yeast

Fermentative multi-budding yeast, *Saccharomyces cerevisiae*, which has been used not only in SAKE brewery, but also in beer brewery, winery and bakery, was discovered in ca. 1830 by J. Meyen; it was named by E.C. Hansen in 1882 [5]. SAKE yeast is classified taxonomically in the *Saccharomyces cerevisiae* group [15]. However, the yeast was distinguished from other strains of *S. cerevisiae* by additional properties such as vitamin requirements [16, 17], acid tolerance, sugar osmophilic character, and adaptability to anaerobic conditions. Additionally, SAKE yeast has advantageous features that enable its growth under high sugar contents and low pH conditions, to produce SAKE under open system fermentation.

SAKE yeast formed a large amount of foam during main mash fermentation. Because one-third of the capacity of the fermentation vessel is occupied by foam during usual main fermentation, preventing foam formation would be greatly advantageous to breweries to save space occupied by the foam and scaling up the amount of MOROMI produced. Some large-molecular-weight compounds that arise from steamed rice grains are also regarded as taking part in foam formation. Recently, foam formation has involved existing proteins, with foam formation on the yeast surface.

Ouchi and Akiyama obtained foam-less mutants that have the same characteristics as the parent yeast except for foam-formation [18, 19]. A foam-less mutant of SAKE yeast, a favorite strain of *Saccharomyces cerevisiae* (The Brewing Society of JAPAN is distributing it as SAKE yeast), has become available for SAKE brewing. Recently, foam protein in SAKE yeast, AWA 1, was cloned. TAKA-AWA foam has been obvious molecular biologically [20].



**Figure 2.** TAKA-AWA foam. (Photograph by Shiraki Tunesuke Co., Ltd.)

### 4.2.2. Aroma production by SAKE yeast

The SAKE aroma is produced by yeast mainly because rice, as a SAKE material, has weaker aroma than materials used for wine or beer. Furthermore, SAKE contains ethanol, higher concentrations of alcohol, and many aroma-producing compounds. Aromatic compounds are an important factor used to characterize SAKE. Recently, a flavor wheel for SAKE was produced similar to existing ones used for wine and beer [21,22]. According to this wheel, the aromas can be categorized as floral aroma, fruit-like nutty, caramel-like, and lipid-like. A

fruit-like flavor is imparted to SAKE from yeast production because many Japanese consumers favor SAKE that has a fruit-like aroma. A yeast mutant producing fruity aromas was isolated for SAKE brewing. Their typical chemical components are ethyl caproate, which gives an apple-like aroma, and iso-amyl acetate or iso-amyl alcohol, which give a banana-like aroma. Before development of methods of breeding yeast to produce aromas, it was not easy for aromatic SAKE to be brewed and supplied stably for customers. Some competent SAKE brewers had controlled temperature severely to adjust enzymes that produced KOJI mold as amylase. Controlling the amounts of sugars as nutrient elements produced by amylase in mash adjusts the metabolisms of yeast growth and production of SAKE aromas as ethyl caproate and iso-amyl acetate. However, it is readily apparent that SAKE aroma synthesis by metabolic pathways or control mechanisms. The yeast producing fruity aroma was bred for use in commercial brewing [1, 5].

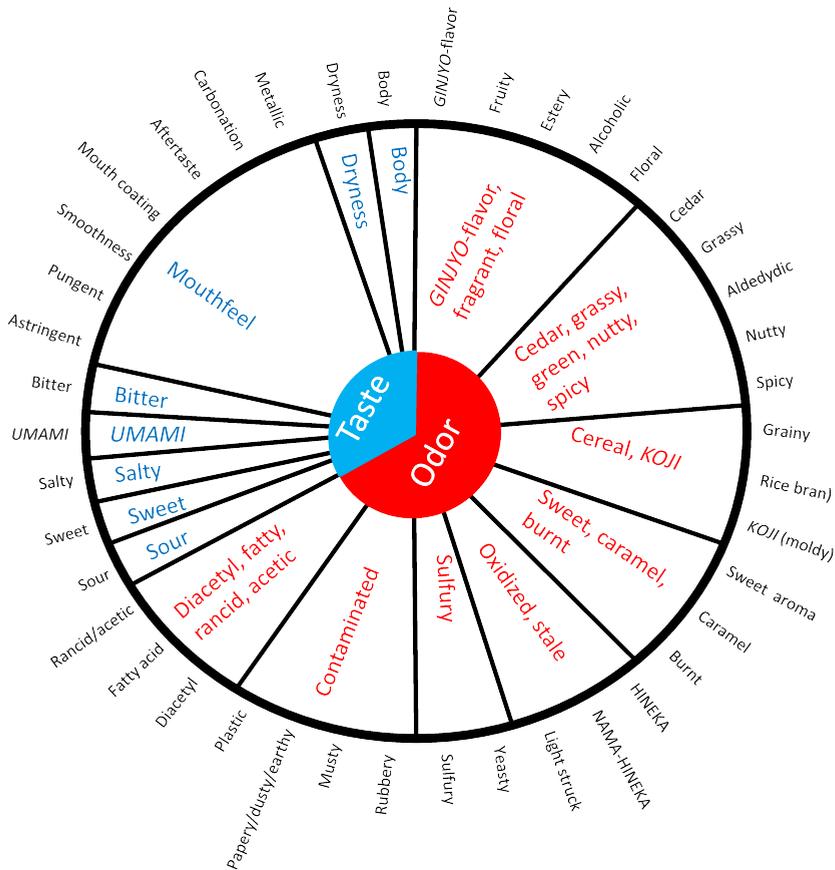


Figure 3. Flavor wheel of SAKE.

Typical yeast metabolic processes producing aromatic compounds are shown in Fig. 3.

- Higher alcohol metabolism pathway [1]

The higher alcohols as aromatic compounds are iso-amyl alcohol and iso-butyl alcohol. The alcohols are produced by two pathways by yeast as shown below.



In these two pathways, 2-oxo acid is produced as a precursor. In the 1 pathway, 2-oxo acid is produced by deamination reaction between Ehrlich pathways. In the 2 pathway presented above, 2-oxo acid is produced between production of amino acid pathway. Oxo acid is produced by decarboxylation reaction and reduction reaction between both pathways, similarly as ethanol is produced from acetaldehyde via pyruvic acid as oxo acid of one kind. For example, lacking amino acids as leucine and valine in *SAKE* mash, the yeast produces leucine and valine in *SAKE* mash. Furthermore, 2-oxo acid was transaminated from other amino acids. It is controlled by the amount of amino-acid-based amino bonds. Therefore, lacking extremely amino acid in mash, 2-oxo acid is converted to higher alcohol as iso-butyl alcohol and iso-amyl alcohol. Sufficing amino acid as leucine and valine in *SAKE* mash, the reaction of the 2 pathway inhibited by native feedback control and uptaken amino acid are converted by the 1 pathway.

- Fatty acid ethyl ester [1]

Ethyl caproate is a favorite flavor providing an apple-like aroma for Japanese consumers. This compound is produced by esterification from caproic acid as a precursor. Caproic acid is synthesized by fatty acid synthase between fatty acid synthesis pathway from acetyl-CoA and malonyl-CoA in *SAKE* yeast. Their synthase composes FAS 1 (Fas1p;  $\beta$ -subunit) and FAS2 (Fas2p; $\alpha$ -subunit), which are hexamer proteins ( $\alpha\beta\beta\beta$  subunit) [23]. Ichikawa reported a yeast breeding method that produces high levels of ethyl caproate that high levels of precursor of ethyl caproate were producing in yeast cells [24]. Cerulenin, an antifungal antibiotic produced by *Cephalosporium caerulens*, inhibits beta-ketoacyl-ACP synthase as *fatty acid* synthetase. A mutant of cerulenin-resistant yeast strain decreases synthesis of long-chain fatty acids by mutating Gly1250 Ser in the gene. The strain can produce high levels of caproic acid [25].

- Ethyl acetate group

Higher alcohol and esterified fatty acid produce a fruity aroma in *SAKE*. Usually, *SAKE* has 0.1 ppm or higher concentrations of ester compounds. That slight amount of ester produces a fruity aroma and intensifies the *SAKE* flavor. Excessive esters destroy the balance of the *SAKE* flavor. Many ester compounds produced mainly by yeast are acetate ester groups that react and which are produced by an alcohol-acetyl transferase reaction that transfers an acetyl bond from acetyl CoA to alcohol. Alcohol acetyl transferase (AATFase; E.C. 23.1.84) catalyzes the following reaction.

Acetyl CoA + Alcohol → Acetyl ester + CoA-SH

This enzyme, a microsomal enzyme, is an endogenous membrane protein dissolving by surfactant. Furthermore, more than 70% of the activity exists in it. AATFase has two isozymes of molecular weight 56 k Da. Isozyme P1 is reacted mainly in the yeast cell. Its activity has ca. 70–80% overall activity. Its optimum temperature is 25°C (Isozyme P2 is 40°C), and the optimum pH is 8.0. The pH range of its reaction is pH 7.5–8.5 (Isozyme P2 is pH 7.0–8.5). Their enzyme inhibited phosphatidylserine and phosphatidylinositol, having interfacial activity, and oleic acid and linoleic acid. Accordingly, this phenomenon showed that this enzyme has a hydrophobic active site in it [26].

### 4.3. Lactic acid bacteria (*Lactobacillus sakei*) [5, 27]

Lactic acid bacteria are the most important bacteria in SAKE brewing. Lactic acid bacteria are defined as listed below.

1. Bacteria ferment glucose and producing more than 50% lactic acid per 1 molar of glucose.
2. Bacteria is Gram positive. Their shapes are cocci or bacci.
3. They are facultative anaerobic bacteria.
4. They have no mobility.
5. They produce no spores.

Their fermentation types are two. One is homo type, 2 molar of lactic acid fermenting from 1 molar of glucose. The other is hetero type, 1 molar of lactic acid, 1 molar of ethanol and 1 molar carbon dioxide from 1 molar of glucose. Typical lactic acid bacteria for food processing are shown as the following: *Leuconostoc* spp. is a hetero-type cocci lactic acid bacteria, and *Pediococcus* spp. is a homo type cocci lactic acid bacteria. *Lactobacillus* spp. belongs to both types of bacci lactic acid bacteria.

In SAKE brewing, lactic acid bacteria are used in traditional seed mash, KIMOTO production for without sterilization safety open fermentation system without sterilization. In traditional seed mash, MOTO, production, it is known that *Leuconostoc mesenteroides* as hetero-lactic acid fermentation grows the MOTO preparation earlier under extremely low temperatures of less than 5°C. *L. sakei* as a hetero-lactic acid fermentation grows in it.

It is rarely that lactic acid bacteria spoil commercial SAKE. The bacteria are called HIOCHI bacteria, and have resistance to ethanol concentrations higher than 18% in SAKE. SAKE-grown HIOCHI bacteria have turbidity and an uncomfortable cheese-like smell from diacetyl [28]. Two types of lactic acid bacteria might be involved: one is *L. homohiochi* (homo lactic acid fermentation type); the other is *L. heterohiochi* (hetero-lactic acid fermentation type). Both bacteria have resistance to ethanol. The coefficient for growth of two bacteria in SAKE is mevalonic acid, which is produced by KOJI mold. Recently, mevalonic acid nonproductive mutants have been bred for SAKE-KOJI production [1, 17, 29].

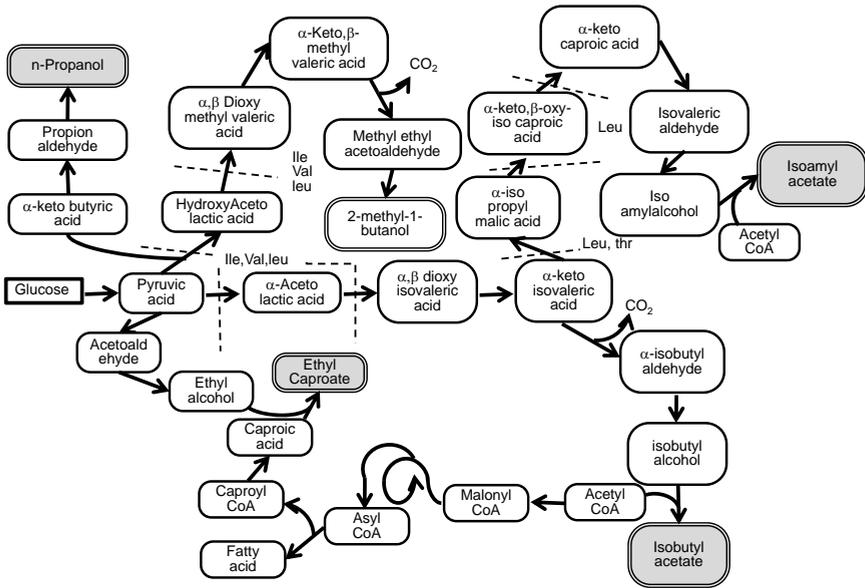


Figure 4. Production of aroma compound mainly by yeast. Broken line shows inhibition of the reaction by amino acid

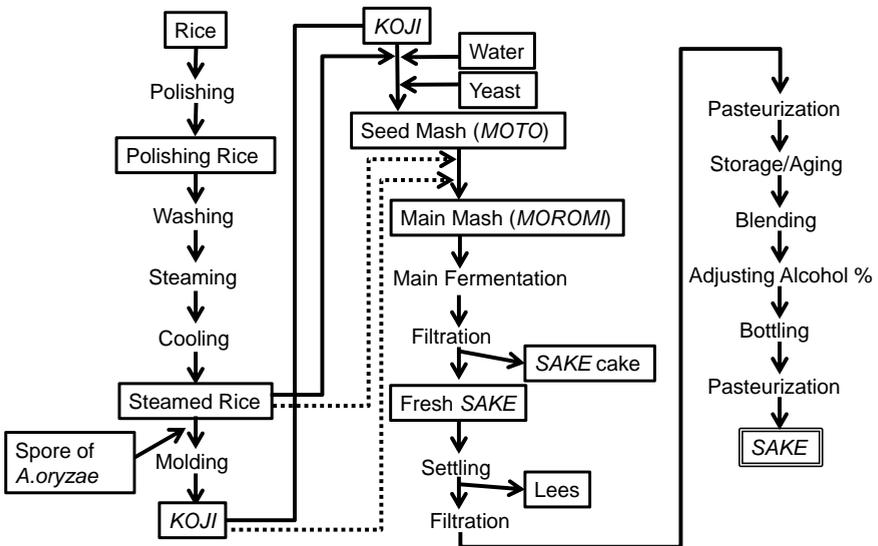


Figure 5. SAKE production.

## 5. SAKE Production

### 5.1. Rice treatment (polishing, washing, and steeping)

In contrast to the use of malt in brewing beer or producing spirits, in *SAKE* brewing, polished rice is used. The main purpose of polishing is to remove unnecessary substances in rice aside from the starch, which are regarded as undesirable in *SAKE* brewing. Polishing removes surface layers of the rice grains, which contain proteins, lipids, and minerals. The ratio of percentages by weight of polished rice to the original brown rice is defined as the polishing ratio. Changes in the amounts of some constituents of the processed grain with various polishing ratios are presented in Table 1 (Research Institute of Brewing, Japan, 1964). Crude fat and ash contents decrease most rapidly, whereas the protein content decreases gradually until the polishing ratio reaches 50%, after which it remains practically constant. In contrast to changes in the crude fat content, the lipid content (by hydrolysis) does not change with increase of the polishing ratio [30].

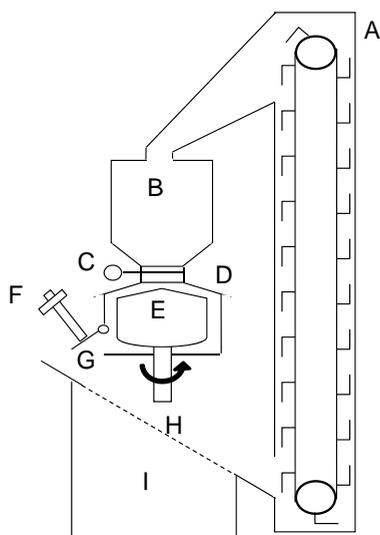
	Polishing ratio (%)			
	100	80	60	50
Moisture	13.5	13.3	11.0	10.5
Crude protein	6.55	5.12	4.06	3.8
Crude fat	2.28	0.11	0.07	0.05
Ash	1.00	0.25	0.20	0.15
Starch	70.9	74.3	76.3	77.6

**Table 1.** Changes in the contents of some rice grain components after polishing [5]

The lowest polishing ratio is strictly regulated under the Liquor Tax Law. In general, polished rice of 75–70% ratio is used for reasonably priced *SAKE* brewing. In contrast, polished rice of a 60% ratio is used for special brewing brands such as GINJYO-SHU, and rice of less than 50% polishing ratio is used for Grand grade *SAKE*, DAIGINJYO-SHU. The latter is a prestige class of *SAKE*. Sometimes, the *SAKE* is brewed using rice of a 30% polishing ratio.

The rice polisher depicted in Fig. 6 is used for *SAKE* brewing. The roller made of carborundum and feldspar rotates around a vertical axis, and scrapes the surface of grains. Rice grains supplied from the hopper are polished and fall to the bottom of the basket conveyer. The grains go through the sieve to remove the rice bran. The rice is carried by the basket conveyer to the hopper. The operation continues until the grains are polished to the required ratio [5].

Generally, with a mill having a roller that is 40 cm in diameter, average times for polishing are 6–8 h for 89%, 7–10 h for 75%, 10–13 h for 70%, and 16–20 h for 60% polishing ratio [5].



**Figure 6.** Diagram of a vertical type rice mill used in SAKE brewing [5]: A, the basket conveyor; B, rice hopper; C, the rice flow adjusting bulb; D, the polishing chamber; E, the roller; F, a resistance; G, exit; H, sieve and I, bran reservoir.

## 5.2. Washing and steeping

Rice is washed and steeped in water before steaming. During washing, the grains are polished further by collision of rice grains in water. During processing, the surface parts of the grains are removed, eliminating approx. 1–3% of the total grain weight [5]. Washed rice grains are passed into a vat and are steeped immediately in water. In washing and steeping procedures, the grains absorb water to about 25–30% of their original weight. The moisture promotes penetration of heat into the grain center during steaming and accelerates gelatinization of starch in the grains. Absorption of water is extremely important for preparing properly steamed rice, and controlling *KOJI* making and fermentation. The water absorption into grains differs according to the variety of rice and the polishing ratio [5, 10, 30]. Generally, rice grains are steeped in water for 1–20 h, and soft rice absorbs water within 1–3 h. Highly polished rice grains absorb water more rapidly. During washing and steeping, potassium ions and sugars are eluted from the grains [1, 31], whereas calcium and iron ions are absorbed onto the grains [5]. After steeping, excess water is drained off from the grains for about 4–8 h before steaming.

## 5.3. Steaming

Starch is changed to the  $\alpha$ -form, and protein is denatured by the steaming process. Moreover, the grains are sterilized by steaming. The grains are usually steamed for 30–60 min, although previous reports show that steaming for as little as 15–20 min is sufficient to modify the starch and protein of rice produced in Japan [1]. During steaming, the grain moisture

is absorbed to the extent of 7–12% of the weight of the starting rice grains, namely total water gain of about 35–40%. Historically, at many breweries, steaming processes usually generated steam from water in a large pot. Today, boilers are often used in many breweries for steaming. A steamer is a shallow and wooden tub in which is bored a hole (1/20 diameter of bottom) at bottom. The steamer is put above the 1.5–2.0 kl caldron, and rice is permeated by large amounts of steam from the caldron. Recently, a modern apparatus for steaming rice as belt conveyor type apparatus is used in automated modern breweries. The steamed rice is cooled to nearly 40°C for *KOJI* production, and the rice used for preparing *MOTO* and *MO-ROMI*-mash is cooled to less than 10°C. Breweries usually use machines to cool the steamed rice with a draft of air as it moves on the screened belt. A pneumatic conveyer system is often used to transfer steamed rice [1].

#### 5.4. *KOJI* preparation [5]

A *KOJI* cultivates the *KOJI* mold, *Aspergillus oryzae* on and in steamed rice grains, and which accumulates various enzymes for *SAKE* production. For the preparation of *KOJI*, seed-molds are used at all breweries. The *Aspergillus oryzae* strains are cultivated in steamed bran rice dredging wood ash at 34–36°C for 5–6 days. This process results in abundant spore formation. Cultivation conditions influence the enzyme production. In general, higher cultivation temperatures (approx. 42°C) develop the activities of amylases. Lower temperatures (approx. 30°C) activate protease activities.

As cultivation times lengthen, more enzymic activities appear in the *KOJI* [32]. Nitrogenous substances and acids are accumulated more in *KOJI* that has been prepared from steamed rice of higher moisture contents [33]. They are regarded as related to the flavors and tastes of *SAKE*. After the steamed rice has been cooled to about 35°C by going through a cooling apparatus, it is transferred into the *KOJI-MURO*, a large incubating room, where temperature (26–28°C) and humidity are controlled at suitable levels to grow *KOJI* mold.

After inoculating or spraying *TANE-KOJI* as seed mold in the proportion of 60–100 g/1,000 kg of rice, then the mixture is heaped in the center of a table for *KOJI* preparation. At this stage, the temperature of the material is 31–32°C. As the spores germinate and mycelia develop, the rice begins to smell moldy like sweet chestnut. After incubation for 10–12 h, the heap of rice grains is mixed to maintain uniformity of growth, temperature, and moisture contents. After another 10–12 h, with growth of the mold, mold mycelia can be observed distinctly as small white spots on the grains. Furthermore, the material temperature has risen to 32–34°C. It is dispensed into wooden boxes, each with 15–45 kg of the grain. To control the rise in temperature and the moisture in the grain mass, the bottom of the box is made of wooden lattice or wire mesh. Temperature and moisture contents are also controlled by the thickness of the heaped grain layer in the box: 8 cm at the beginning, 6 cm at the first mixing, and 4 cm at the second mixing. Thereafter, at intervals of 6–8 h, the material is mixed and heaped again in the box. After incubation for about 40 h, the temperature of the material rises to 40–42°C. The mycelium develops to cover and penetrate the grains which have sufficient enzymes, vitamins and various nutritive substances for mashing and growth of *SAKE* yeast. Then the *KOJI* is taken out of the room and spread on a clean cloth to be cooled

until it is used for mashing.  $\alpha$ -amylase and acid-protease activities increase during *KOJI* making. Carbohydrates are decomposed finally to water and carbon dioxide, which engenders the production of energy for growth of the mold.

## 5.5. SAKE mash fermentation

### 5.5.1. 'MOTO' as yeast starter

In *SAKE* brewing, *MOTO* is important as a yeast starter for the fermentation of *MOROMI*. *MOTO* is necessary to provide a pure and abundant yeast crop, and to supply sufficient lactic acid to prevent contamination of harmful wild yeast or bacteria during *MOTO* production or in the early stages of main fermentation.

In traditional *MOTO* preparation, lactic acid is produced by lactic-acid bacteria in the mash. In the modern method, pure lactic acid is added to the mash at the beginning of *MOTO* preparation. Lately, compression yeast cultivated using a method similar to that for baker's yeast used to ferment main mash safely with this yeast instead of *MOTO*. The amount of rice used for *MOTO* preparation is usually 7% of the total rice used for the entire *SAKE* mash.

- Traditional Seed Mash

*KIMOTO* is a traditional *MOTO*. Actually, *MOTO* has been handed down from early times, and the *MOTO* was modified to be simple and convenience by Kagi et al. [34]. The modified *MOTO* is called *KIMOTO*. The *YAMAHAIMOTO* is based on the same microbiological principle as that of *KIMOTO*, and has practically replaced *KIMOTO* because the related procedure is simpler [5].

Steamed rice (120 kg) is mixed with 60 kg of *KOJI* and 200 L of water in a vessel at an initial temperature of 13–14°C. It is then kept for 3–4 days with intermittent stirring and agitation. During this period, the rice grains are partially degraded and saccharified, and the temperature falls gradually to 7–8°C. The mash is then warmed at a rate of 0.5–1.0°C/day by placing a wooden or metal cask filled with hot water in the mash after warming for an additional 10–15 days, after which the temperature reaches 14–15°C. In *KIMOTO* mash, some micro-organisms grow successively to each other as Fig. 7, and mash brings acid condition to grow *SAKE* yeast easily without contamination [5].

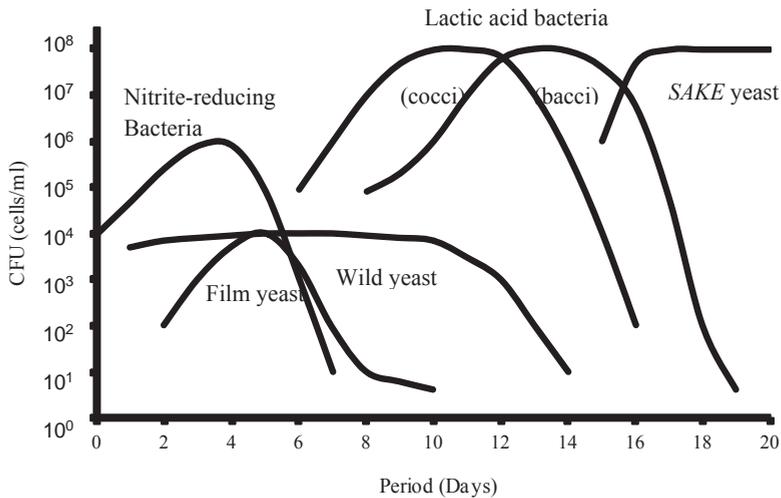
In early stages, contaminating wild yeast or germs disappears within the first two weeks as a result of the toxic effect of nitrite, produced by nitrate-reducing bacteria from nitrate contained in or added to the water. Slight nitrate contained in the mother water is converted to nitrite, which has toxicity for micro-organisms by nitrate-reducing bacteria such as *Achromobacter* spp., *Flavobacterium* spp., *Pseudomonas* spp., and *Micrococcus* spp. (derived from *KOJI* and water). A toxic substance, nitrite, yeast of one kind from *KOJI* as *Pichia angusta* [35] was assimilated after oxidating nitrite during *MOTO* mash. Nitrite is toxic for lactic acid bacteria and fermentative yeast in traditional *MOTO*. Their utility micro-organisms are able to grow under the *MOTO* mash containing nitrite. After removing nitrite, lactic-acid bacteria including *Leuconostoc mesenteroides* and *Lactobacillus sakei* (derived from *KOJI*) can grow in the *MO-*

*TO* mash. These bacteria multiply to reach a maximum count of about  $10^7$ – $10^8$ /g. However, other gram disappear before fermentation by *SAKE* yeast begins because of the accumulation of high concentrations of sugar and because of acidification resulting from the growth of lactic-acid bacteria [5].

The mixing process helps to dissolve the nutrients contained in the *KOJI* and steamed rice, and mash promotes the growth of lactic-acid bacteria in the early stages [36].

- Convenient *MOTO* preparation as *SOKUJYO-MOTO*

Recently, *SOKUJYO-MOTO* is popular for use in *SAKE* brewing. It was devised by Eda [37]. It is based on the principle that addition of pure lactic acid to *MOTO* can prevent contamination by wild microorganisms. It takes a short time (7–15 days) to produce *MOTO* because of the time-saving lactic-acid formation by naturally occurring lactic acid bacteria, and saccharification of the mash proceeds quickly with the high initial mashing temperature (18–22°C). In this production, commercial lactic acid (75%, 650–700 ml/100 L of water) is added to the mash to adjust the pH value to 3.6–3.8. Although pure culture yeast is used as the inoculums, yeast grows more advantageously than do wild yeasts from *KOJI*. Furthermore, the latter eventually predominate during the *MOTO* process [5].



**Figure 7.** Changing numbers of micro-organisms in *KIMOTO* mash.

This predominance might be ascribed to the fact that the high mashing temperature and acidic conditions are close to the optimum for multiplication of both culture and wild yeasts. In addition, as opposed to the behavior in the classical process, no natural selection of wild yeasts by the toxic effect of nitrite occurs because the presence of lactic acid inhibits nitrate-reducing bacteria.

An example of the preparation of *SOKUJYO-MOTO* is the following: *KOJI* (60 kg) is added with 200 L of water and 140 ml of lactic acid (75%). A pure culture of *SAKE* yeast is inoculated to the mash ( $10^5 - 10^6/g$ ). Its temperature is about 12°C. Steamed rice (140 kg) is added to the mixture, cooling it sufficiently to give a temperature of about 18–20°C. After keeping the mash for 1–2 days with intermittent stirring and agitation, it is warmed gradually in the same way as *YAMAHAI-MOTO* by increasing the temperature at a rate of approx. 1.0–1.5°C/day. As the temperature rises to about 15°C, *SAKE* yeast reaches its peak and fermentation begins.

The cultivation period can be shortened further by starting the mashing at 25°C and by keeping the temperature of *MOTO* over 18°C. Moreover, the variety of *SOKUJYO-MOTO* as *KOONTOKA-MOTO* (hot-mashed *MOTO*) is used by Japanese brewers. This mashing method is conducted at 56–60°C during several hours with subsequent inoculation of pure cultured *SAKE* yeast. To prevent excessive accumulation of sugars and the development of a high viscosity, the ratio of water to rice used is raised to 150–160 L/100 kg [5].

### 5.5.2. Main fermentation [5]

*MOROMI*, as main mash, is fermented in a large open vessel with a capacity ranging from 6–20 kl without special sterilization, in an open fermentation system. The weight of polished rice (1.5 t) was used for mashing one lot as standard. However, recently, larger vessels as 3–7 tons or sometimes over 10 tons have been used for mashing one lot. The *MOROMI* mash is brewed steamed rice, *KOJI* and water. Table 2 shows proportions of various raw materials used for a typical *MOROMI* mash. The preparation of stepwise mashing as three steps is one characteristic of *MOROMI* mash production. First, steamed rice, *KOJI* and water are added to the *MOTO*. Consequently, the total acid and yeast population in *MOTO* are diluted to about one-half. The temperature of the first mash is about 12°C, and the yeast propagates gradually. After two days, the yeast grows until  $10^8/g$ , which reaches the same order as that in *MOTO*. As a second addition, the materials are added in an amount that is nearly twice as much as the first addition. The yeast population and total acids are diluted by about half too. The temperature of the second addition is lowered to 9–10°C. In a third addition, materials are added in a larger amount.

	1st addition	2nd addition	3rd addition	4th addition	Total
Total rice (kg)	140	280	890	160	2000
Steamed rice (kg)	95	200	720	160	1580
<i>KOJI</i> rice (kg)	45	80	170		420
Water (liter)	155	250	1260	160	2460

**Table 2.** Proportions of raw materials used in a typical *SAKEMOROMI* [1]

The amount of *MOROMI* bring 14 folds as same as *MOTO* mash. Whereby yeast cells are diluted. This stepwise addition of material plays an important role in suppressing the invasion of wild micro-organisms together with lowering the mashing temperature in each addi-

tion. In *SAKE* brewing, temperature control is also extremely important to balance saccharification and fermentation, both of which occur simultaneously in *MOROMI*. Therefore, we call it 'Parallel Fermentation'. Small quantities of sugars released from steamed rice and *KOJI* are fermented gradually by *SAKE* yeast until the alcohol content reaches nearly 20% (v/v). Accumulated alcohol of 20% v/v in the mash from 40% (w/v) of sugars. If such a high concentration of sugars is supplied at once, then *SAKE* yeast would not ferment alcohol in the mash. Instead, the mash fermentation at a low temperature (below 10–18°C) is also a characteristic of *SAKE* brewing which gives the mash a balanced flavor and taste as well as a high alcohol concentration. After the third addition of materials, the mash is agitated, usually twice a day. The mash density then reaches maximum levels 3–4 days later.

A foam resembles soap suds. Furthermore, it spreads gradually over the surface, and subsequently increases to form a thick layer. A fresh fruit-like aroma at this stage indicates healthy fermentation. The fermentation gradually becomes more vigorous with a rise in mash temperature, and a rather viscous foam rises to form *TAKA-AWA* (a deep layer of foam, shown in Fig. 2), which reaches to the brim of the vessel. In some breweries, it is broken down with a small electric agitator. At this stage, the yeast cell count reaches a maximum of about  $2.5 \times 10^8/\text{g}$  [38]. Because the alcohol concentrations increase, the foam becomes less dense, and is easily dispersed. The fermentation finishes usually during 20–25 days. In some breweries, pure alcohol (30–40%) is added to the mash to adjust the final concentration to about 20–22% (v/v).

Quite often, to sweeten the mash, 7–10% of the total amount of steamed rice is added during the final stage of the *MOROMI* process to produce glucose from starch by the saccharifying action of *KOJI* that accumulates in the mash.

#### 5.5.3. Filtration [1,5]

After alcohol fermentation, the mash is divided into *SAKE* and solids by filtration. The mash is poured into bags of about 5 L capacity made of synthetic fiber, which are laid in a rectangular box. *SAKE* is squeezed out under hydraulic pressure. After complete filtration, the solids pressed in a sheet are stripped out of the bags. Recently several automatic filter presses for filtering *MOROMI* mash have been used. The *SAKE* lees or *SAKE* cake, residue squeezing *SAKE* as cake was called '*SAKE-KASU*', contains starch, protein, yeast cells and various enzymes, *SAKE* lees is used traditionally for making foodstuffs such as pickles and soup. In general, regarding 3 kl of *SAKE* containing 20% ethanol and 200–250 kg of *KASU* are obtained from one ton of polished rice. The slightly turbid *SAKE* is clarified to separate lees by standing in a vessel for 5–10 days at a low temperature.

#### 5.5.4. Storage (aging) and bottling [5]

After settling the clarified *SAKE* for a further 30–40 days, The *SAKE* is pasteurized, killing yeasts, harmful lactic acid bacteria, and enzymes. The *SAKE* is heated to 60–65°C, passing it through a helical tube type heat exchanger for a short time. Recently plate-type heat exchangers with high efficiency of heat transfer have become available.



**Figure 8.** SAKE squeezer (Photograph by Hamada Co. Ltd)

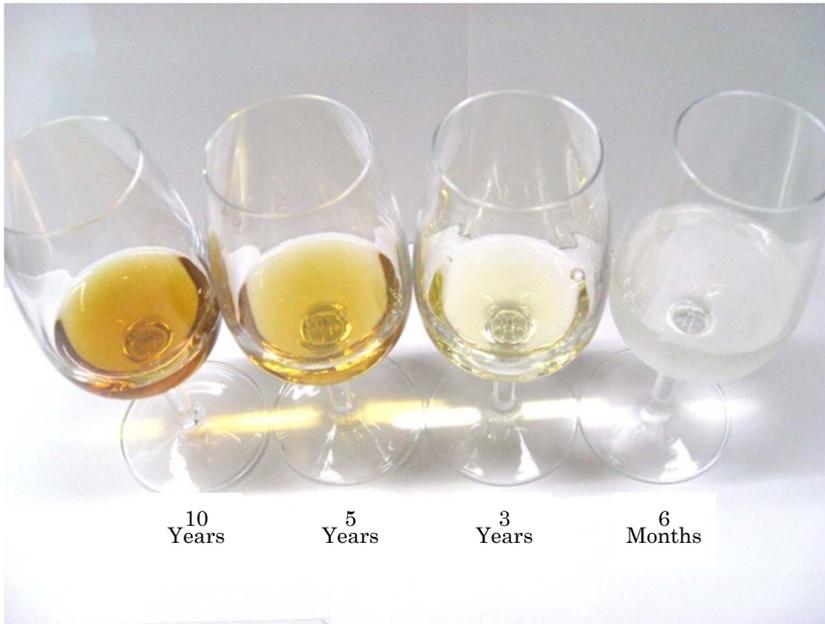
As described in this chapter, the history of *SAKE* pasteurization began in the 16th century, before Pasteur's discoveries. After pasteurization, *SAKE* is transferred to sealed vessels for storage with or without addition of activated carbon. Pasteurization and the high content of alcohol in *SAKE* (usually 20%) prevent microbial infection. The blended *SAKE* is diluted with water to the appropriate alcohol content, usually 15.0–16.5% (v/v), and is filtered through activated carbon to improve the flavor and taste and to adjust the color and clarity. In modern procedures, filtration through activated carbon is followed by filtration through membranes or sheets having numerous pores of micrometer size, thereby removing minute particles including micro-organisms if any are present. This procedure enables the *SAKE* producer to omit pasteurization in the bottling procedure and therefore to prevent deterioration of quality caused by heating *SAKE*. The spoilage of *SAKE* is sometimes encountered, off-flavors and tastes are attributed mainly to the formation of diacetyl and acetic acid by *HIOCHI* bacteria.

*SAKE* is usually sold in a pale blue bottle of 1.8 l capacity, which is pervious to short and medium wavelengths in sunlight, as are beer, wine, and other alcoholic beverages. Coloring is spoilage of *SAKE* by sunlight, deferriferrichrysin precipitates, and tyrosine or tryptophan, kynurenic acid or flavin precipitates as precursors of colorants. Usually *SAKE* is aged and stored for a short time. It does not age for a long time of several years or longer. Vintage wine is aged much longer than *SAKE*. During storage, *SAKE* matures gradually. The maturation process is probably the result of oxidation reactions and physicochemical changes. *SAKE* changes and adopts a smoother taste. The storage temperature should be maintained carefully at 13–18°C, with consideration being devoted to the rate of maturation and the time of bottling.

*SAKE* is browned not only by amino-carbonyl reactions but also by still unknown reactions during aging. Long-aged *SAKE* has a sherry wine-like aroma that is attributable to furfurals

produced in *SAKE* during aging. Furthermore, the *SAKE* taste is smooth and less stimulated by ethanol because of molecules of ethanol and water flocculate in the *SAKE* during aging. However, research of *SAKE* aging has been conducted by many researchers [1].

Recently, aging of *SAKE* to add value has been attempted by some breweries with so-called *KOSYU* as old vintage *SAKE*. *KOSYU-SAKE* has rich and complex flavors and tastes like those of cherry wine and a brown color by amino-carbonyl reaction as shown in Fig. 9. Aged *SAKE* can even have a chocolate color.



**Figure 9.** Changes of color of sake during aging.

## 6. Varieties of *SAKE* [39]

In Japan, *SAKE* production and labeling are regulated strictly by the Liquor Tax Law. According to this law, *SAKE* is made from defined raw materials and methods of production as follows: 1, *SAKE* is an alcoholic beverage produced by fermenting materials such as rice, rice-*KOJI*, and water, with subsequent filtering of the material mixture. 2, *SAKE* is an alcoholic beverage fermenting a material such as rice, water, *SAKE* lees, rice-*KOJI*, and other material as authorized by government ordinance and filtering the material mixture. 3, *SAKE* is an alcoholic beverage filtrate of a mixture of *SAKE* and *SAKE* lees. Moreover, *SAKE* has been categorized as grand, first and second class, by alcohol concentration, and sensory

evaluation by officers until 1992. However, labels and names of *SAKE* have not been regulated by law. For various reasons, many commercial products, *SAKE* which labels producing method or excessive name, was sold in the market and low-quality *SAKE* also was sold. Furthermore, many consumers were confused and purchased it mistakenly. Whereby, they were regulated by law in 1992.

<b>SAKE type</b>	<b>Used material</b>	<b>Used rice Polishing ratio</b>	<b>Requirement</b>
GINJYO -SYU	Rice, rice <i>KOJI</i> and pure distilled alcohol	Less than 60%	Fermentation at low temperature; fruity- flavor; good and clear appearance
DAI-GINJYO- SYU	Rice, rice <i>KOJI</i> and pure distilled alcohol	Less than 50%	Fermentation at low temperature; fruity- flavor; good and clear appearance
JYUNMAI- SYU	Rice, rice <i>KOJI</i>	-	Good flavor; good and clear appearance
JYUNMAI- GINJYO-SYU	Rice, rice <i>KOJI</i>	Less than 60%	Fermentation at low temperature; fruity- flavor; good and clear appearance
JYUNMAI- DAI-GINJYO-SYU	Rice, rice <i>KOJI</i>	Less than 50%	Fermentation at low temperature; fruity- flavor; good and clear appearance
TOKUBETSU- JYUNMAI	Rice, rice <i>KOJI</i>	Less than 60%	Especially good flavor; good and clear appearance
HONJYOZO- SYU	Rice, rice <i>KOJI</i> and pure distilled alcohol	Less than 70%	Good flavor; good and clear appearance
TOKUBETSU- JYUNMAI	Rice, rice <i>KOJI</i> and pure distilled alcohol	Less than 60%	Especially good flavor; good and clear appearance

**Table 3.** Classification of *SAKE* types by law [39]

Instead of *SAKE* grades such as grand grade, fiesta grade and second grade that had been used until 1992, *SAKE* is categorized as *DAIGINJYO-SHU*, *GINJYO-SHU*, *JYUNMAI-SHU*, *JYUNMAI-DAIGINJYOU-SHU*, *JYUNMAI-GINJYOU-SYU*, *TOKUBETSU-JYUNMAI-SYU*, *HONJYOZO-SYU*, and *TOKUBETSU-HONJYOZO-SHU*, and the labeling *SAKE* is regulated by the law as shown in Table 3.

The polishing rice ratio and using *KOJI* ratio regulated by the law to sell their categorized *SAKE*. Then they must be shown on the label. *JYUNMAI* means that *SAKE* is brewed using only rice and rice-*KOJI* and mother water, and *GINJYO* means special brewing. *DAI-GINJYO* means special brewing and prestige class in the *SAKE* brewery. Consequently, *DAI-GINJYO* tends to be expensive, but the price of *SAKE* is decided by the policy of the brewery. Additionally, *KOSYU* as aged vintage *SAKE* or *NAMAZAKE* as non-pasteurized *SAKE* is displayed on the *SAKE* label. It is necessary that some method or public organization manage other *SAKE* label items.

## 7. SAKE tastes

As explained in this chapter, SAKE is a favorite food and beverages and individual favored SAKE and components of SAKE are important factors for purchasing SAKE. Over 500 chemical compounds exist in SAKE, producing a complex flavor and taste in SAKE.

SAKE consumption has decreased since the 1970s, it is 1.7 million kL. Recently, in 2009, SAKE consumption is about one-third that of the 1970s. According to a survey of household spending conducted by the Public Management Ministry in Japan [40], consumers in their 20s spend 1100 yen per month for SAKE, those in their 30s spend 2500 yen per month, and those in their 60s spend 3800 yen per month. Elder consumers spend three times as much as young consumers. To examine favorite tastes of young consumers (20s–30s) play a role to development of new SAKE for them and to increase SAKE consumption in Japan.

	Appearance	Intensity of aroma	Appeal of aroma	Intensity of sourness	Intensity of bitterness	Intensity of sweetness	Appeal (Balance) of tastes	Preference of consumer
Appearance	1.000							
Intensity of aroma	0.450	1.000						
Appeal of aroma	-0.280	0.000	1.000					
Intensity of sourness	-0.590**	-0.300	0.100	1.000				
Intensity of bitterness	-0.200	0.160	-0.430*	0.120	1.000			
Intensity of sweetness	0.030	-0.380**	0.210	0.030	-0.570	1.000		
Appeal (Balance) of tastes	-0.050	-0.190	0.550*	0.070	-0.860**	0.660**	1.000	
Preference of consumer	-0.110	-0.450*	0.650**	0.120	-0.800**	0.670**	0.880**	1.000

(Correlations are significant; \*\*,  $P < 0.01$ , \*,  $P < 0.05$ )

**Table 4.** Correlations for Evaluating SAKE using Sensory Evaluation Methods

Suzuki and co-authors [41] investigated the opinions and preferences of panelists (22 persons, 20s–30s) to conduct a SAKE sensory evaluation for research into favorite tastes and consumer preferences. The correlation of sensory evaluations of SAKE are presented in Table 4. Correla-

tion was found between 'Intensity of aroma' and 'Balance of taste', for which the correlation factor is 0.55, and the relation between 'Appetite of consumers', with a correlation factor of 0.65. However, it showed a negative relation with 'bitterness', and the correlation factor was -0.430. Young consumers hope to buy and drink *SAKE* having a favorite flavor. Furthermore, correlation was found between 'Preferences of consumers', and 'Balance of taste', with a correlation factor is 0.88. 'Preferences of consumers', showed a relation with 'Intensity of bitterness', with close correlation factor of -0.80. Furthermore, a negative and close statistical correlation was found between 'Balance of *SAKE* tastes' and 'Intensity of bitterness', for which the correlation factor was -0.86. These data show that consumers hope to buy or drink *SAKE* with no bitter taste. 'Bitter' is a decreased balance of *SAKE* test and consumer appetite. Although bitter taste has played an important role in giving richness-taste to *SAKE* for a long time, young consumers are sensitive to bitter tastes in *SAKE*. It is therefore considered that the control of bitter taste must be undertaken in brewing processes.

## 8. Conclusions

*SAKE* brewing necessitates the use of high-quality techniques that have been developed experimentally without acquaintance with scientific method. Furthermore, unique techniques have been researched, as fermenting under low temperature, more than 18% of high alcohol concentration without distillation, open fermentation system without sterilization, and having a fruity aroma in *SAKE*. *SAKE* brewing using only rice as a material can yet produce fruity aromas such as those of apple, melon, or banana. Specially brewed *SAKE* for Japanese *SAKE* contests includes 6–7 ppm of ethyl caproate [42], which is a very high amount for alcoholic beverages, which is one reason that producing ethyl caproate yeast has been developed and fostered at public institutes in many Japanese prefectures. However, strong doubts persist that their *SAKE* has been adequately adapted to favor consumers. In questionnaire investigation, young consumers (20–30s) bring up the image that *SAKE* is a beverage for elderly people [41]. This is one reason for their image that *SAKE* is a cheap alcoholic beverage also. It is expected that *SAKE* consumption will decrease because the Japanese population is decreasing as result of the nation's low birthrate and high longevity.

All brewers and researchers of the *SAKE* field must make efforts to brew high-quality *SAKE* and suitable *SAKE* for consumers or for *SAKE* not only in Japan but also in foreign countries exporting it. Furthermore, *SAKE* can be highly appreciated by connoisseurs, just as 'Chateaux' wines, Grand cru, are in European countries.

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