The Story of Sake

NRIB
(National Research Institute of Brewing)
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2

Featured article

Sake(Seishu) 2
(The Integration of Science and Technology)

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Ginjo-shu has a fine fruity aroma and exquisite mouthfeel.*Jummai-shu* has moderate sweetness and great palatefulness.These two types of sake significantly changed its stereotyped image.The market share on shipment basis of *ginjo-shu* and *jummai-ginjo-shu* has been increasing year by year. Japanese term *Ginjo* literally means carefully selected and brewed. So *ginjo-shu* is the culmination of sake brewing technology, embodying the craftsmanlike spirit of sake brewers throughout the brewing processes of sake. Sophisticated brewing technology through the development of *ginjo* making has contributed to the quality improvement of the whole sake industry.The integration of the traditional skills of the sake brewers (called *toji* and *kurabito*) and cutting-edge sake research by scientists has led to the production of not only *ginjo-shu* but also various types and kinds of deep flavored and high quality sake.
This booklet The Story of Sake 2 introduces a more detailed and deeper discussion of how sake is made, including the integration of science and technology.
This booklet also introduces the processes that were not included in The Story of Sake 1, from the step of pressing the fermentation mash to shipping sake as a finished product. It also provides information about the types and storage management of sake.

We hope you will enjoy sake more, after learning about the features of each type of sake, including the different designations derived from respective ingredients and brewing methods.

The beginnings of the science of sake brewing

Sake manufacturing industry is one of the major time-honored fermentation industries in Japan.Toward 1887, the modern sake manufacturing industry established. Simultaneously, the mass production by private sake companies began in *Nada* in the Hyogo Prefecture and *Fushimi* in the Kyoto Prefecture.

At that time, however, sake brewing relied on the experience and intuition of the sake brewers, and therefore the production process was unstable, and frequently lead to spoilage of sake.Research and development, e.g. the isolation of indispensable microorganisms for sake brewing, *koji* mold and yeast were just beginning. However, it was still a long time before we attained a full scientific elucidation of sake brewing.

Besides, by 1899 the liquor tax had become the biggest source of revenue for the Japanese government, exceeding even the land tax. When sake spoiled, the prepaid tax had to be returned, meaning revenue derived from liquor tax was not stable.

The elucidation and countermeasure against sake spoilage came to an urgent matter. Academic research and investigations into how to improve sake brewing technology began as a national project essential to supporting the basis of national revenue.

With this background, the National Research Institute of Brewing (NRIB) was established within the Ministry of Finance in 1904.

Specially designated sake

Special designation is a sake classification system that fulfills the "standards for the labeling of the production method and quality of sake" established by the National Tax Agency JAPAN. There are eight classes in all.
As well as these designations, there are many different kinds of sake with different names. Please refer to our "Glossary of Terms on Sake Bottle Labels" for details.

special designation	ingredients※1※2	rice-polishing ratio (*seimai-buai*) ※3	percent of rice for making *koji*	other features※4
ginjo-shu	rice, rice *koji*, distilled alcohol	up to 60%	at least 15%	*ginjo* making, good characteristic flavor and appearance
daiginjo-shu	rice, rice *koji*, distilled alcohol	up to 50%	at least 15%	*ginjo* making, excellent characteristic flavor and appearance
jummai-shu	rice, rice *koji*	–	at least 15%	good flavor and appearance
jummai-ginjo-shu	rice, rice *koji*	up to 60%	at least 15%	*ginjo* making, good characteristic flavor and appearance
jummai-daiginjo-shu	rice, rice *koji*	up to 50%	at least 15%	*ginjo* making, excellent characteristic flavor and appearance
tokubetsu-jummai-shu	rice, rice *koji*	up to 60%, or special process	at least 15%	excellent flavor and appearance
honjozo-shu	rice, rice *koji*, distilled alcohol	up to 70%	at least 15%	good flavor and appearance
tokubetsu-honjozo-shu	rice, rice *koji*, distilled alcohol	up to 60%, or special process	at least 15%	excellent flavor and appearance

*1. The rice used has to be pass an inspection based on Agricultural Product Inspection Law indicating a certain level of quality
*2. The amount of distilled alcohol usage should not exceed 10% of rice weight
*3. The rice-polishing ratio has to be indicated on label.
*4. Definition of *ginjo* making: Usually refers to the process of using rice with a low rice-polishing ratio and low temperature fermentation to create the characteristic flavor of *ginjo-shu*

Special designations and their specifications

Sake Brewing

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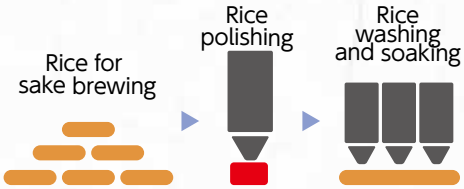
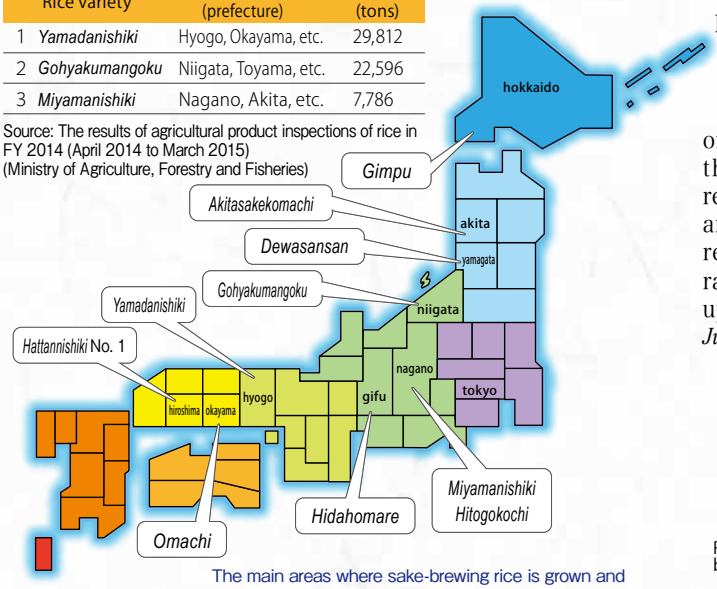


Rice for sake brewing

Sake is an alcoholic beverage made from rice. Currently, about 300 varieties of rice are cultivated in Japan. Some varieties of them are used to brew domestic sakes.Some kinds of rice classified by the Agricultural Product Standards as “brown rice for sake brewing” are also referred to as “sake-brewing rice.” They have superior properties that make them the most suitable for being used as the ingredient for brewing sake.Among the sake-brewing rice varieties, the most cultivated variety was *Yamadanishiki*. In addition to *Yamadanishiki*, many varieties of sake-brewing rice are grown, throughout Japan.

Rice variety	Main harvested site (prefecture)	Amounts (tons)
1 *Yamadanishiki*	Hyogo, Okayama, etc.	29,812
2 *Gohyakumangoku*	Niigata, Toyama, etc.	22,596
3 *Miyamanishiki*	Nagano, Akita, etc.	7,786

Source: The results of agricultural product inspections of rice in FY 2014 (April 2014 to March 2015) (Ministry of Agriculture, Forestry and Fisheries)

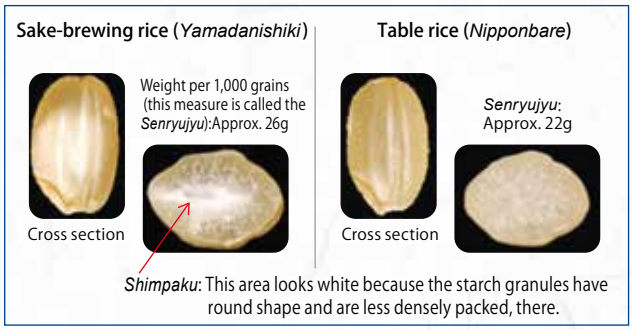


The main areas where sake-brewing rice is grown and the amounts inspected

Sake-brewing rice has large grains and often has an opaque white core called *shimpaku* in the center of each grain.

Sake-brewing rice is lower in protein content than table rice. The grains break down easily during the long, low-temperature fermentation process, resulting in good and consistent fermentation.Polished white rice, not brown rice, is used to make sake.

The outer part of brown rice contains lots of lipids, minerals and protein. Too much of these components will cause unpleasant flavor. That’s why these parts must be removed by rice polishing.

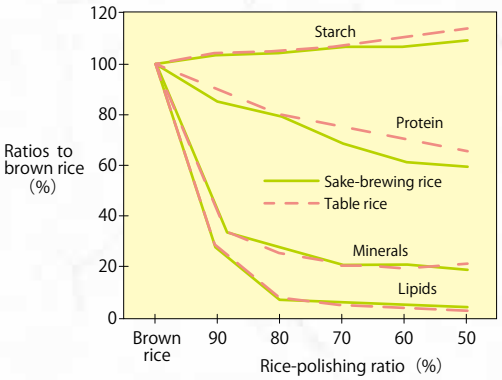


Comparison of sake-brewing rice and table rice

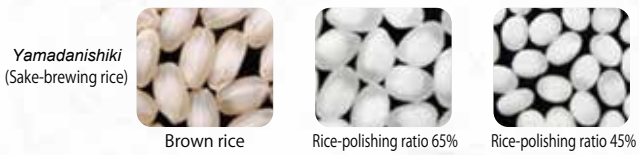
The following equation states the rice-polishing ratio, used to show the degree of polishing.

$$\text{Rice-polishing ratio} = \frac{\text{Weight of the white rice}}{\text{Weight of the brown rice}} \times 100(\%)$$

The table rice we normally eat has a rice-polishing ratio of approximately 90%. This means 10% of the exterior of the grain has been removed as bran. We consume the remaining 90% as white rice.The specially designated sakes are classified according to the rice-polishing ratio. They are required to be made of rice of the following rice-polishing ratios: *Honjozo-shu*, up to 70%; *ginjo-shu* and *jummai-ginjo-shu*, up to 60%; *daiginjo-shu* and *jummai-daiginjo-shu*, up to 50%. *Jummai-shu* is a special case with different requirements.



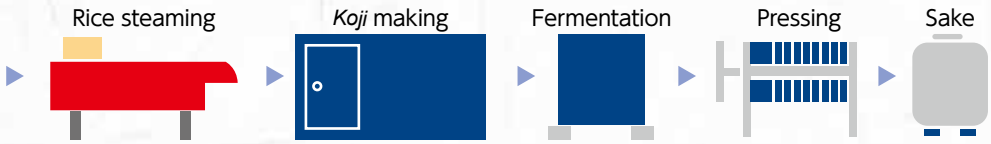
Changes in proportions of components caused by rice polishing



Yamadanishiki

Yamadanishiki is a sake-brewing rice that was cross-bred in Hyogo Prefecture.*Yamadanishiki* was developed in 1936 through 13 years of cultivation tests after cross-breeding at the Agricultural Experiment Station, Hyogo Prefecture.All of Japanese *Toji* (chief sake brewers) especially prefer to work with this variety of rice because it produces good tasting sake with good physical properties.It grows up to 130cm and the ears bow archwise at the time of harvest.*Yamadanishiki* accounts for 33% of the sake-brewing rice harvested in Japan. Approximately 71% of it is produced in Hyogo Prefecture (the result of agricultural product inspections of rice in FY 2014).

Ears emerge, blooming | Flowers | Just before harvest



Rice processing

Washing, soaking and steaming rice are referred to as rice processing.Well steamed rice is the basis of good sake brewing. It is a process sake brewers are very sensitive to.The moisture content in steamed rice has a significant impact, almost single-handedly determining the quality of the finished sake.Too much moisture content will cause the rice to break down too much during fermentation, and therefore produce too many components of taste.If the correct balance between the decomposition level of the steamed rice and the alcoholic fermentation by yeast is not achieved, it becomes difficult to fine-tune the flavor of the sake.

In order to produce steamed rice with the appropriate moisture content, first we must precisely control the amount of water to soak the rice before steaming.For the purpose, we have to ascertain the quality of the rice, measure the water temperature, try a test-washing, and determine the correct length of time to soak the rice. By precise control we mean achieving a ±1% level of accuracy of the amount of water absorbed.Controlling the water content of rice by means of curtailing soaking time is referred to a *Gentei-Kyusui* (limited water absorption).Especially for *ginjo* making, the entire brewing staff joins the process of washing rice, soaking it, and draining it, in order to hit the precise water absorption target.

Washing

Rice grains that are especially highly polished (with low rice-polishing ratio) tend to crack easily. This type of rice has to be washed with cold water quickly and carefully so as not to crack the grains.Once the grains are cracked, it will be difficult to adjust the amount of water absorption.

Left: Soaking (letting rice soaked so that it absorbs water)
A black tray is floated on water to check the water absorption.

Water

Approximately 80% of sake is water.In order to make delicious sake, we must obtain very high quality water that does not contain iron, because iron causes coloration of the sake.This section describes the water used by sake brewers in the two largest sake-brewing areas in Japan: *Miyamizu* (*Miya* water) in *Nada* and *Gokosui* (*Goko* water) in *Fushimi*.

Miyamizu in Nada

In the latter part of the Edo era, Mr. Tazaemon Yamamura, who had sake breweries in *Uozaki*, *Kobe*, and also in *Nishinomiya*, wanted to find out why his *Nishinomiya* brewery always produced higher quality sake than his *Uozaki* brewery. He changed *toji* (chief sake brewers) and tools of *Uozaki* with those of *Nishinomiya*, but this did not provide reliably good results. In 1840, when he brought water from *Nishinomiya* to *Uozaki* to make sake, the *Uozaki* brewery produced good quality sake.Rainwater on *Rokko* Mountain infiltrates into the soil and flows out of springs around the *Nishinomiya* Shrine. This spring water is the secret for the production of excellent sake.From that time, sake brewers in *Nada* began to use *Nishinomiya* water.People abbreviated the water from *Nishinomiya*: "*Miya* water" – *Miyamizu*.*Miyamizu* is hard water. Sake made in Nada using this hard water has a dry and crisp taste, so it is referred to as *Otoko-zake* (Men's sake).

Stone monument at the birthplace of *Miyamizu* (Nishinomiya City).

Gokosui in *Fushimi*

Fushimi in Kyoto City is a land blessed with a large amount of good quality underground water.One place where this water emerges from the ground is at *Gokonomiya*, a shrine near *Momoyama-goryou-mae* Station on the *Kintetsu Kyoto* line. The water from this spring is called *Gokosui*.This shrine was named after *Gokosui* (perfumed water) by the Emperor *Seiwa*, because water with a pleasant aroma sprung up in this shrine on September 9, 862.In *Fushimi*, in addition to *Gokosui* (also called *Iwai-no-Mizu*) there are six other sites where good water can be found. They are called the "seven wells of *Fushimi*."*Gokosui* is medium-hard water; that is, it is softer than *Miyamizu* in *Nada*. Sake made with this water is smooth and silky, so it is called *Onna-zake* (women's sake).

Gokosui
(*Fushimi-ku* in Kyoto City)

	Uozaki	*Nishinomiya*	*Gokosui* in *Fushimi*	tap water in *Tokyo*
pH	6.9	7.1	6.4	7.5
Calcium	29	51	8.4	–
Magnesium	3.5	7.3	5.5	–
Hardness	87	157	44	43
Sodium	11	25	11	3.1
Potassium	2.8	9.8	1.9	–
Chloride	10	35	7.3	1.8
Phosphoric acid	0.3	4.4	–	0.0
Iron	<0.005	<0.005	–	0.12

Unit: mg/L (except for the pH)

Comparison of the qualities of water from *Uozaki*, *Nishinomiya*, *Fushimi*, and *Tokyo*.

The World Health Organization (WHO) Drinking Water Quality Guidelines:
Soft water: Hardness of 60 mg/L or less
Medium-hard water: Hardness of 60 to 120 mg/L
Hard water: Hardness of 120 to 180 mg/L

Source: The Journal of Brewing Society of Japan: Vol. 87, p.909 (1992), the Bureau of Waterworks Tokyo Metropolitan Government (2015).

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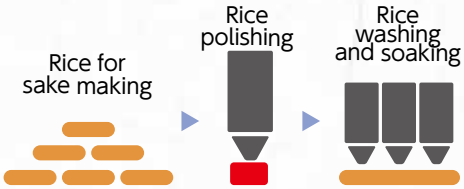
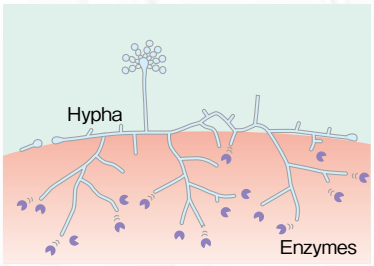
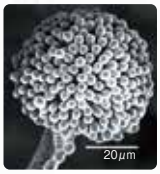


Koji mold (*Aspergillus oryzae*)

Koji mold has been named the National Fungi of JAPAN by the Brewing Society of Japan. It has been used to produce sake since ancient times.Besides being used for sake brewing, *koji* mold is also used for making such traditional Japanese products as *shochu* (a Japanese white spirit), *awamori* (an Okinawan spirit made with black *koji* only), miso, soy sauce, vinegar, and pickles.



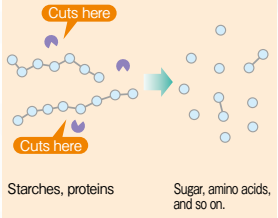


Koji mold spores (Also known as conidia Photo by Moriyuki Kawauchi)

After inoculated on the surface of steamed rice, the spores of *koji* mold germinate and extend their hyphae.The tips of the hyphae secrete enzymes.

Koji is cultured *koji* mold on rice, barley, beans, and so on. Rice *koji*, used for making sake, is of course grown on rice.*Koji* mold digests the rice using enzymes, converting starch to glucose. Then yeast eats the glucose to carry out the process of alcoholic fermentation.

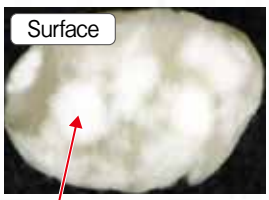
Enzymes are functional proteins that break down various substances.



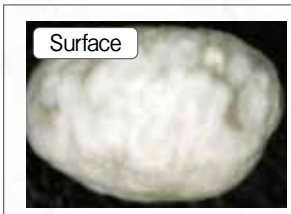
Making *koji* (*seikiku*)

After washing, soaking and steaming of rice, the next step in brewing sake is making *koji*.

There are two well-known features for growing *koji*.One is the *Sohaze-Koji* condition. This is when the *koji* is entirely covered with hyphae of *koji* mold called *haze* which looks like white. The other is called *Tsukihaze-Koji*. This *koji* has some *haze* area on the surface which has grown into the steamed rice. Both of them are considered to be good *koji*.Generally *tsukihaze-koji* is used when brewing *ginjo-shu*.*Tsukihaze-koji* is especially rich in glucoamylase, which breaks down starch into glucose, and it does not have so much protease, which breaks down protein into amino acids that impart taste.Therefore, the *tsukihaze-koji* can supply enough glucose to nourish the yeast, permitting a long lasting ferment at a low temperature. Sake made with this *koji* turns out a light and delicate taste.



Tsukihaze-koji
(Higher enzymatic activity, Light and delicate sake taste)



Sohaze-koji
(Higher enzymatic activity, Rich and full-taste sake)

Tsukihaze-koji and *sohaze-koji*

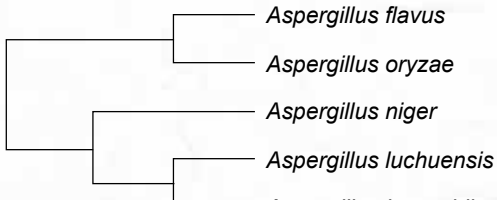
The safety of *koji* mold

Koji mold, which has been named after the National Fungi of JAPAN, can be classified into three types, according to color.Yellow *koji* mold (*Aspergillus oryzae*) is chiefly used for brewing sake. Black *koji* mold (*Aspergillus luchuensis*) is chiefly used for brewing *shochu* and *awamori*. White *koji* mold (*Asperigillus kawachii*) is a mutant strain of black *koji* mold.There are actually many mutant strains of each *koji* mold.

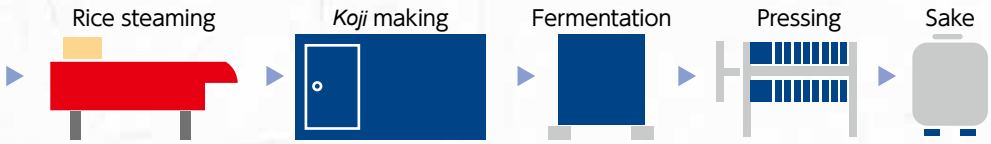
Yellow *koji* mold grown on rice has been used since ancient age for brewing sake.In 1960, it was reported in the UK that some strains of *Aspergillus flavus*, a species related to yellow *koji* mold, produces aflatoxin, which is a kind of mycotoxin. So, it came to a matter that even yellow *koji* mold might produce aflatoxin.NRIB immediately began to research this issue. In 1971, we clearly determined that the yellow *koji* mold group is different from the *Aspergillus flavus* group, and that yellow *koji* mold does not produce aflatoxin.This research proved that sake made with yellow *koji* mold is safe to consume.Then, in 2009, research at the genetic level proved that yellow *koji* mold does not generate aflatoxin.

Also black and white *koji* molds had already been confirmed to be safe.It was reported in 1994 that some strains of *Aspergillus niger*, a relative of these molds, produces ochratoxin, a kind of mycotoxin.We analyzed black *koji* mold, white *koji* mold, and *Aspergillus niger* in 2009 on the genetic level. We proved that black *koji* mold and white *koji* mold belong to a different group from that of *Aspergillus niger*, and that they do not produce ochratoxin or aflatoxin. We continuously conduct research on the safety of not only *koji* mold but also yeast and sake itself, as well as the effects of sake on human health.

Koji mold (From the left), yellow, white and black *koji* molds



Aspergillus phylogeny



Excessively steamed rice is unable to grow good *koji*. Sake brewers control moisture content and temperature to produce *sohaze-koji* or *tsukihaze-koji* according to the aimed quality of sake. It takes approximately two days to grow *koji*.

On the first day, the steamed rice is cooled down to the appropriate level and brought into the *koji*-making room. Then the steamed rice is spread on a table called *toko*, and *koji* mold spores are seeded across the entire surface.After the spores are seeded, the steamed rice is covered with a cloth to maintain the correct temperature and humidity. The rice rests for that first day on the *toko*. During this stay, the *koji* mold spores germinate.The first day is referred to as the *toko* period.

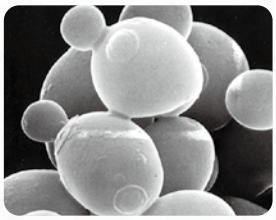
On the second day, the *koji* being produced is transferred into shallow trays, called *koji-bako* and *koji-buta*.This transfer task is referred to as *mori*.Amount of *koji* for a *koji-bako* is 15 to 30 kg, and for a *koji-buta* is 1.5 to 2.5 kg.As the hyphae of the *koji* mold grow, they generate heat, so the brewers have to control *koji* temperature to avoid it rising too much.The second day, when the *koji* mold is kept in the trays, is called the *tana* period.

Koji making (from the left, the *koji-bako* and *koji-buta*)

When the temperature of the *koji* reaches 40 to 43 degrees Celsius, and after 43 to 45 hours (or sometimes more than 48 hours when using the *tsukihaze-koji* process) has elapsed since the rice was brought into the *koji*-making room, *koji* making is complete.Good *koji* has a soft elasticity when grasped by the hand and it does not stick to the hand after being released.With good *koji*, you experience a light sweet taste and an aroma like roasted chestnuts.

Yeast

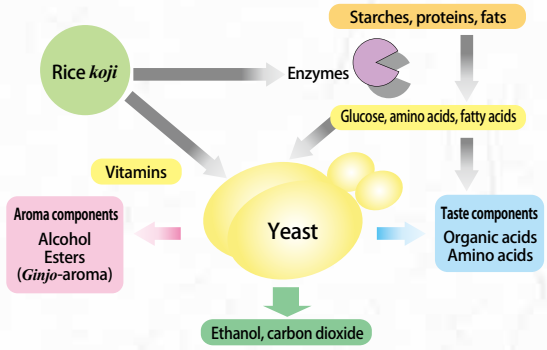
The alcohol in sake is made by yeast.There are many kinds of yeast living in the natural environment. Natural yeasts grow on fruit juice when a fruit is injured.Also there is wild yeast adhering to brown rice and white rice grains.There are many kinds of yeast. Some of them are used for making bread, others for making soy sauce and for making miso.The yeast called *Saccharomyces cerevisiae* is the chief one used for brewing sake. The term "saccharomyces" comes from the words for sugar and mushroom in Greek. The term "cerevisiae" comes from the Latin for beer.



Sake yeast

Yeast grows by the budding and separating of daughter cells from mother cells.
During sake brewing, yeast multiplies as much as 20 thousand times. One gram of fermentation mash finally contains approximately 200 million yeast cells.

Yeast generates ethanol and carbon dioxide gas from the glucose that the *koji* made by breaking down the starch in the rice. Yeast also produces many flavor components, in addition to ethanol.



The functions of *koji* mold and yeast in sake brewing

The aroma and taste components produces by yeast vary with each yeast type, so the aroma and taste of sake depend on the specific yeast used.Therefore, the selection of purified yeast from ferments of good sake batches has been carried out, accompanied by fermentation tests.Since 1906, the yeast thus selected has been distributed as "*Kyokai* sake yeast" by the Brewing Society of Japan.

One ampule (approximately 10 mL) in the photo contains approximately 20 billion yeast cells. If you ferment one ton of rice using this one ampule for starter culture and then fermentation mash, you can make approximately 1,200 bottles (using 1.8 L bottles) of *jummai-shu*.

Kyokai sake yeast (ampule)

Kyokai sake yeasts are numbered in ascending numerical order according to their launching. Nowadays, K-6, K-7, K-9, K-10, K-11, K-14, and K-1801 are often used. These yeasts are closely related,genetically. However, each sake has a different aroma and taste. They are used selectively to attain a desired goal regarding the qualities of the sake being produced.It is said that the aroma of *ginjo-shu* has become especially fine since "*Kyokai* sake yeast K-7" came into use (isolated in 1946).K-7 features a vigorous fermentation and pronounced aroma. Even nowadays this yeast is still widely used for brewing sake.From toward 1975, "*Kyokai* sake yeast K-9" has played a major role in the production of *ginjo-shu*. Its wonderful aroma as well as mild and smooth taste has established the style of *ginjo-shu*.Recently, "*Kyokai* sake yeast K-1801" is being used, featuring an apple-like ethyl caproate aroma and a mild taste with low acid production. Unique yeasts bred in the various prefectures are used to make many types of sake, including *ginjo-shu*.

Sake Brewing

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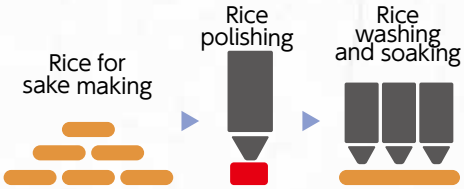


Shubo (Starter culture)

After the *koji* making has finished, the next step is to prepare the sake starter culture. Just after the starter culture is prepared, wild yeasts remain in the culture, in addition to desirable sake yeast, like *Kyokai* sake yeast. While the rice *koji* is being made, these wild yeasts increase as well. However, some wild yeasts (for example, film yeasts) produce unfavorable components that smell like paint thinner or vinegar, and others stop the process of alcoholic fermentation. For sake brewing, we must use excellent yeast that ferments at a low temperature, generates a great deal of alcohol, and results in sake with a good flavor. The propagation process of selected yeast, *shubo*, ensures a good fermentation of sake.

What features are necessary for starter culture? First, starter culture should contain a great deal of yeast. Second, starter culture has to have the appropriated level of acidity. An acidic condition helps prevent the increase of bacteria that can spoil the main fermentation. This phenomenon is similar to the process of making pickles, which is a way of storing vegetables in vinegar to prevent their spoilage by bacteria. There are two methods for making the starter culture acidic: using a lactic acid fermentation process, and using lactic acid specially prepared for brewing. The representative method of using a lactic acid fermentation process is called "*kimoto* (*kimoto* starter culture)." The representative method of using lactic acid is called "*sokujo* (*sokujo* starter culture)."

Kimoto

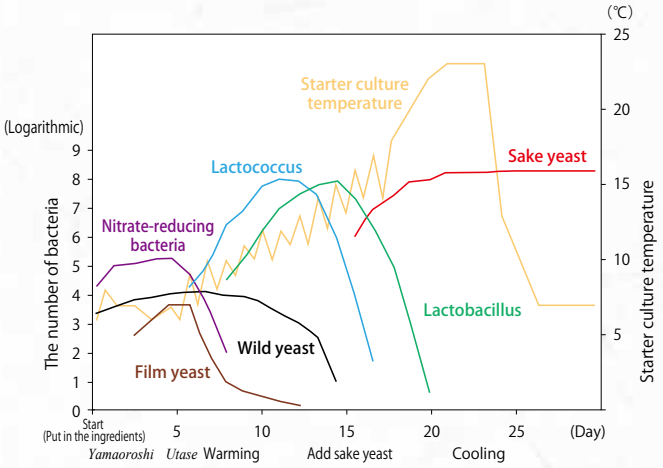
Kimoto is the technique developed in the Edo era (1603 to 1867) for growing yeast using natural microorganisms.The first step in the *kimoto* process is to put 14 to 15 kg of steamed rice, *koji*, and water into a tub, called *hangiri*, at a temperature of 8˚C.After a few hours, the rice grains swell with absorbed water. The workers stir the mixture inside of this tub well, every a few hours.

Yamaoroshi (tub (*hangiri*) and paddle (*kai*))

On the next day, they grind the rice using a tool shaped like a paddle,called *kai*.This grinding operation is called *Yamaoroshi*.It is very hard work for two or three workers to grind a tub of rice in a cold room, three times a day.After it is well ground, they move the mash into a tank, where it's kept for 3 days at 6 to 7˚C.This period is referred to as *utase*. Nitrate-reducing bacteria that were in the starter culture gradually change the nitrate in the water to nitrous acid.This nitrous acid suppresses the multiplication of the wild yeasts.

Five or six days after the ingredients were initially put in the tank, the warming of the mash begins.A cask full of hot water is put into the tank, kept for 2 to 3 hours and then removed. This process is repeated at an increasing rate of approximately 1˚C per day.

As the temperature rises, the lactic acid bacteria gradually become active and start to produce lactic acid.The combined actions of the lactic acid and nitrous acid cause the film yeast and wild yeast to die off.Then, as the lactic acid increases, the mash becomes so acidic that



The transitions of microorganisms in a *kimoto*-style starter culture

The *kimoto*-style starter culture was a great technique. This allowed brewers to grow pure sake yeast by the intelligent use of the natural transitions of microorganisms, where the types and numbers of microorganisms change as shown in the figure above.

the nitrate-reducing bacteria also die off, and the remaining nitrous acid is converted.

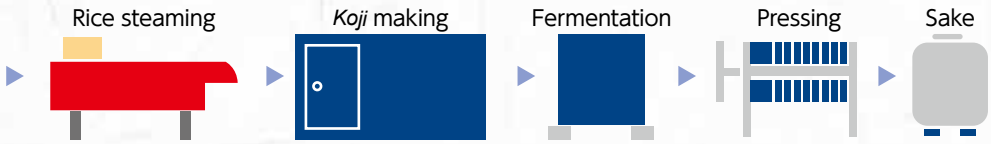
As the acidity increases further, even the lactic acid bacteria find themselves in an unfavorable environment, and they die off, as well.At this stage, the amounts of sugar and amino acids in the mash have increased, and so the mash is the ideal environment for growing of the sake yeast, which is well able to tolerate acidity.

Warming(In this process, the workers turn a warmer with their hands.)

Therefore, now is the time to add previously cultured sake yeast to the mash.In the past, the natural yeast that was specific to each brewery grows there, or the brewer retained some already fermented starter culture to add to his mash, continuing his established line of yeast.During the last half of the starter culture process, the remaining lactic acid bacteria are killed by the alcohol that is being generated by the yeast. As a result, finally only sake yeast remains in the culture.

Yamahai (*Yamahai* starter culture): a modified type of *kimoto*

Some bottlers of sake have *Yamahai* or *Yamahai-Shikomi* written on their labels.*Yamahai* is the abbreviation for *yamaoroshi-haishi*. This name was chosen to indicate that *yamaoroshi*, the most arduous work in the *kimoto* process, had been done away with. In the *yamahai* method, steamed rice is broken down by *koji* enzymes, not by the physical effort of the workers using their paddles.This process saves the time, labor, tools and space needed to grind the rice during the *kimoto* operation. This method was developed in 1909 by our institute.There is not much difference between the *kimoto* and *yamahai* processes, in terms of the final quality of the sake or the character of the yeast used.



Sokujo

Kimoto was a very successful technique established by our predecessors in the era when they did not know that sake was made by microorganisms. However, some lactic acid bacteria that would remain in their starter could spoil the sake. These harmful bacteria may multiply if the starter culture-making operation is not appropriately controlled.In the past, it had often happened that a warm winter resulted in contamination of the starter culture by harmful bacteria, and the fermentation mash would be spoiled.The mash can no more properly ferment due to infection than rice or vegetable can grow due to disease.Another consideration involved in the starter culture-making process was that much care and labor was required during the long period of four weeks to create the *kimoto*.

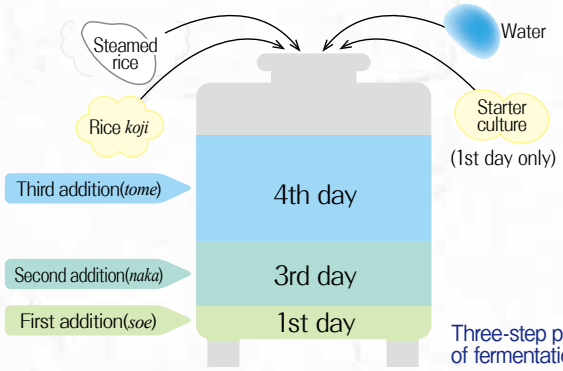
Then, in 1909 we developed the method to produce starter culture by adding very pure lactic acid (brewing lactic acid) to subdue the growth and activities of microorganisms. Simultaneously the *yamahai* process was developed.With *kimoto*, it takes two weeks or so to produce lactic acid by lactic acid bacteria. Using very pure lactic acid saves this period.This method produces starter culture much faster, so it is called *sokujo* (rapid production of starter culture).Currently, most sake is made through this *sokujo* process.

Moromi (Fermentation mash)

Fermentation mash is prepared by the addition of water, rice *koji*, and steamed rice to a starter culture, and then actual sake fermentation starts.

The size of a batch of sake manufacture is shown by the weight of the white rice used. A small batch is one of about a hundred kg, and a large batch can be 10 tons or more.Normally, a fermentation tank has a capacity three times the volume of the amount of rice used. If a sake batch is 10 tons or more, a fermentation tank with a capacity of 30 kL or larger is needed to hold the fermentation mash.The standard relationship between the steamed rice and the rice *koji* in a fermentation tank is 77 (rice) plus 23 (rice *koji*) (where the white rice is measured in kg), plus 130 liters of water.

The whole vat of fermentation mash is not prepared at once. It is prepared in successive stages, by dividing the total into three portions, prepared over four days.This process of preparing the fermentation mash with gradually increasing three portions is referred to as *sandan-jikomi* (three-step preparation of fermentation mash).This is done to prevent a delay in the production of alcohol by diluting the yeast too much, and to prevent the growth of unwanted bacteria.



Three-step preparation of fermentation mash

The 1st day is referred to as *soe* (first addition). The 2nd day is *odori* (the next day after *soe*), which is when the workers wait for the yeast to grow.The 3rd day is *naka* (second addition) and the 4th day is *tome* (third addition).

Low temperature fermentation is appropriate for brewng sake.The low temperature controls the activity of yeast and allows the alcoholic fermentation to progress steadily, resulting in finer quality and more delicious sake.

Fermentation takes place at 10 to 18°C generally.About one week after the third addition, fermentation becomes active. Then, fermentation settles down after three weeks, when the alcohol has reached 18 to 20 percent.

Foaming yeast and non-foaming yeast

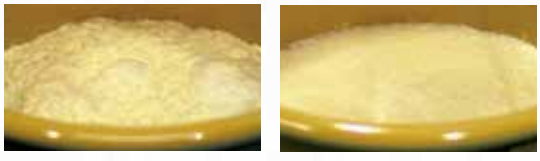
Normally, sake yeast produces a layer of foam that covers the surface of the fermentation mash from 4 to 10 days after the third addition.This was a characteristic of sake yeast. However, non-forming yeast was reported independently by NRIB and the Hiroshima Regional Taxation Bureau in 1916.

No foam layer means that brewery workers do not need to clean foam off their tank walls.They also do not need to worry about overflowing foam during active fermentation.In case of non-foam-generating-mash, fermenting tank could accommodate more mash than foam-generating-mash by the volume of form.There are many benefits to using non-foaming yeast, but manufacturers did not consider using it when it was first discovered.

Then, in 1963, the demand for sake exceeded the existing production capacity, and people saw that non-foaming yeast could contribute to increasing production capacity within the same facilities.

The staff at NRIB developed a breeding method of non-forming yeast, and eventually succeeded in obtaining a mutant non-foaming yeast from "*Kyokai* sake yeast K-7."In 1971, this mutant yeast was named "*Kyokai* sake yeast K-701," and it went into distribution.Now, a great deal of sake is brewed with non-foaming yeast. This research was a real game-changer for the sake industry to find a way "to create" a new yeast, in addition to "to select."

Then, we continued researching into foam made by yeast, and identified the *AWA1* gene that is responsible for forming of yeast.On the basis of this finding, we are now able to grow other non-foaming yeasts, because we can check various yeasts for the presence and expression of the *AWA1* gene.


Foaming yeast　　Non-foaming yeast

Pressing and finishing

Joso (Pressing)

Pressing is the process used to separate the matured fermentation mash into sake (the liquid) and sake cake (the solid).Sake cakes consist chiefly of the rice, *koji* and yeast remaining after the fermentation.During this pressing process, it is important to keep the temperature low, avoid too much pressure, and avoid the sake's contact with the air as much as possible.Sake, as well as other alcoholic beverages, deteriorates in quality through the effect of oxygen in the air.There are various methods to press sake. One method is to put the fermentation mash into a tube-shaped cloth bag called *saka-bukuro* and hang it so the sake drips out. Another method is to use a pressing device such as a traditional press (*fune*) or an automatic filter press for sake.


Fune (a traditional press)

When you use a *fune*, you lay some cloth bags filled with fermentation mash at the bottom of the *fune* and then stack more cloth bags on top.Stacking up the cloth bags causes the weight of themselves to press the fermentation mash. The naturally pressed sake drains out of a tap.The first-running sake is cloudy, but as the cloth weave is filled with residual solids it functions as a fine filter so that the later-running sake becomes transparent. The first-running sake is referred to as *arabashiri* (free run sake).Once the speed of sake being naturally pressed reduces, we use a press.At the top of the *fune* hydraulic unit is equipped, that presses the fermentation mash by gradually raising the pressure on the cloth bags.The flavor of drained sake changes little by little, depending on the time elapsed and differences in pressure level, even though it is coming from the same run of fermentation mash.


Cloth bags holding fermentation mash are stacked on the *fune*.

When pressing using an automatic filter press for sake, the fermentation mash pours in between vertical plates to which filtration cloths are attached. High-pressure air is forced into rubber bags attached to the plates. These rubber bags inflate and apply pressure to the fermentation mash from both sides.


Automatic filter press for sake

Oribiki (Removal of sediment), filtration

Soon after pressing, sake is a cloudy white, as shown in the upper right.The cloudiness is caused by fine particles of yeast, rice, and rice *koji*. This is referred to as the *ori* (sediment).

Oribiki is the process of removing the sediment from the sake after pressing.The sake yeast in the sediment is still alive after pressing. It can restart fermentation within a certain temperature range, or go into self-digestion (resulting in its death). In addition, the components in sake will be changed over time by the enzyme activities derived from *koji* and sake yeast. Leaving the sediment in the sake for a long time may increase the amino acids and sugar, causing deterioration of the sake's quality.Generally, after pressing, sake should be stored at a low temperature. When the sediment sinks, it is removed from the sake.The first removing is generally done five to ten days after pressing.The amount of sediment will depend on each pressing method used. Only a small amount of sediment remains after pressing using an automatic filter press for sake. Therefore, some brewers dismiss the process of sediment removal after using this pressing method. In this case, any remaining sediment is removed by a filtration machine.


Sake soon after pressing (free run sake (*arabashiri*)
When the sake is stored at a low temperature, and white hazes settle to the bottom of the bottle.

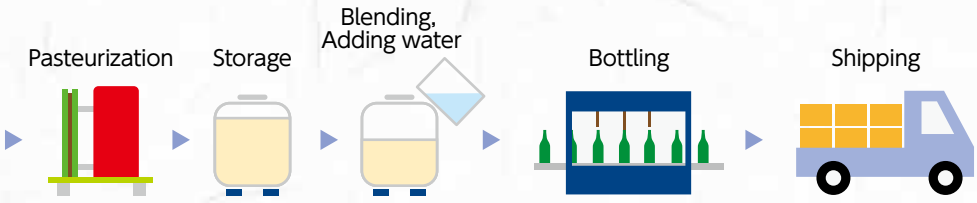
Filtration is used to remove fine particles (sediment and microorganisms) suspended in sake through a fine mesh filter.There are different filtration methods specific to respective purposes: filtration to remove sediment, filtration after activated carbon treatment to adjust flavor, and final filtration performed just before bottling.


Filtration machine

Activated carbon for brewing is added into sake to adjust flavor.There are a lot of extremely tiny pores on the surface of the activated carbon, which absorb coloring matters, bitter taste and smell constituents.Coloring matters and off-flavor constituents are removed owing to physical properties of activated carbon. Therefore this technique enables us to prevent coloration and over-maturation of sake caused by heat and light.Just after being pressed, the color of sake is light yellow. After activated carbon treatment and filtration, the sake is almost colorless and quite transparent.Whether or not to use the activated carbon and how much is that used are subject to the target quality of the sake.

Hi-ire (Pasteurization)

In pasteurization process, sake is heat-sterilized to assure microbial stability and product quality.Freshly pressed sake has a unique fresh deliciousness. However, the enzymes derived from the *koji* and sake yeast are still active.Fresh sake is quite unstable in quality. Over time, it is likely to get sweeter and develop an offensive smell, called *namahine-ka* or *mure-ka*.These enzymes cannot be removed by sediment removal and filtration.In addition, *hiochi* bacteria (some kinds of lactic acid bacteria that are tolerant to alcohol) can grow in fresh sake, deteriorating its quality.This is why the sake is



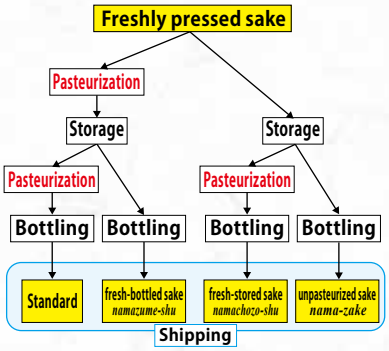
heated to about 60 to 70°C, which kills the *hiochi* bacteria and inactivates the enzymes. This stabilizes the product's quality.

In general, sake goes through two pasteurizations steps.The first pasteurization is performed before storage, and the second one is done just before or after bottling. There are two methods of pasteurization. One is to apply heat to sake by heat exchangers equipped between hoses linking tanks and bottle-fillers. The other is to heat bottles filled with sake.


Heat exchanger (for pasteurization)

Usually, heat exchangers are used to pasteurized sake.On the other hand, heating sake in bottles provides so precise temperature control that this method is especially used for *ginjo-shu*.Heating in the bottle prevents loss of the fruity flavor, and so the typical flavor of *ginjo-shu* is well retained.

All the same, there are also non-pasteurized sake brands.A sake stored, bottled, and shipped without any pasteurization is called unpasteurized sake (*nama-zake*). Typically, *nama-zake* is protected from quality degradation by cold storage or special filtration.This lets you enjoy the fresh taste of sake owing to being unpasteurized.In another variation, there is also sake that is pasteurized before storage, but not before bottling.This is referred to as fresh-bottled sake (*namazume-shu*).A sake that is not pasteurized before storage but is pasteurized before bottling is referred to as fresh-stored sake(*namachozo-shu*).



Number of pasteurizations and classifications of sake

Storage

After it is first pasteurized, usually sake is stored in a storage tank.In the storage tank, the sake sits quietly and matures. This will increase its good matured different flavor and smoothness from freshly pressed sake.It is important to keep the tank temperature quite constant, to promote stable maturing.

Blending, adding water

Blending is the process of mixing sakes from in different tanks. Even if the same rice, *koji* mold, and yeast are used, the components of sake may vary because of the variation in rice treatment and temperature profiles during the *koji* making and fermentation.In order to ship stable quality sake throughout the year, manufacturers blend different runs of sake.Blending is carried out when the sake is moved from storage tanks to as the next filtering and bottling processes.

Water may be added to sake in order to adjust its alcohol content.Just after pressing, sake has relatively high alcohol content, around 18 to 20%. Water is added to reduce alcohol content level to 15 to 16%, which is suitable for drink.The same high quality water as brewing water is used to dilute sake.

On the other hand, there is also sake without being diluted.This is referred to as undiluted sake (*genshu*).In general, *genshu* has a rich taste, but you should be careful not to drink too much! It has a high alcohol content.

Bottling, shipping

Bottling is the final process of brewing sake as a commercial product.Particular attention must be paid to the maintenance of a hygienic environment, to prevent contamination by foreign material and microorganisms.Therefore, some breweries clearly separate the bottling area from the manufacturing area where *koji* mold and sake yeast are handled, or place the bottling area in a separate location (or even another building).

Special machines are used for bottling to fill the bottles up to the specified amount.Pasteurization is performed at bottling, except when brewing unpasteurized sake (*nama-shu*) and fresh-bottled sake (*namazume-shu*). When heated sake was bottled,it pasteurizes both the bottles and the sake in the bottle, and the air is pushed out of the bottles by the steam emitted from the sake, thus decreasing the amount of oxygen inside.Therefore, this prevents deterioration by oxidation as well as by bacteria.


Pasteurization of bottles


Filling machine

After bottling, labels are put on the bottles. The sake is now a completed as a commercial product.The labeled sake bottles are packed in cardboard boxes or special plastic crates for shipping.

Enjoying sake

The maturation period and flavor change

Hiyaoroshi, freshly pressed sake

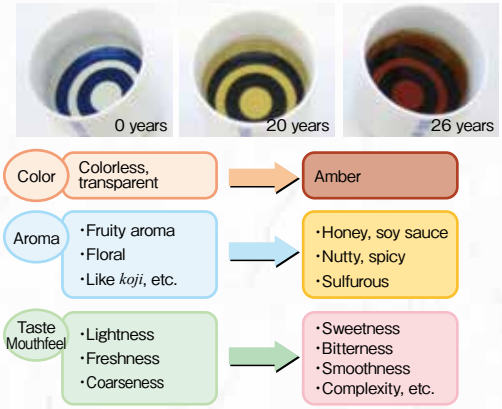
Typically, sake is held for maturation in a tank or bottle for from six months to a year, and then shipped throughout the year.In general, higher temperature promotes faster maturation. In the autumn, after being stored throughout the summer, sake is moderately mature and has a mild flavor.A specific sake called *hiyaoroshi* is shipped during the autumn.Although there is no legal definition of *hiyaoroshi*, it is a kind of *namazume-shu* that has been pasteurized before storage, and that is not re-pasteurized when bottled and shipped in the autumn.

Some sake is shipped without being aged.Such freshly pressed sake can be found at sake breweries and in liquor shops from the winter to the spring.Generally these fresh, un-matured forms of sake are unpasteurized (*nama-shu*).

Long aged sake

Before storage technology was well developed, it was very difficult to retain the flavor of sake at an acceptable level for over a year.Recently, thanks to the development of modern technology and devices of storage, sake with unique flavors obtained after long intentional storage is made.They are what is called long aged sake.

Even if it's pasteurized, sake stored for a long time changes its color to bright yellow and eventually to amber.This is because the reaction of sugar and amino acids in the sake creates a colored matter called melanoidin.At the same time, the aroma changes to a complex one with nuance of caramel, honey, nuts, and spices.This is believed to be the result of the reaction of sugar and amino acids which combine through the Maillard reaction, as well as from the break-down of amino acids.The flavor gradually becomes smoother. Due to some increase in bitter-tasting components, the sake develops a complex flavor and rich body, and it has a feeling of expanding volume that is different from the sake before storage.



The changes of characteristics during the storage of sake

Well-structured sake like *jummai-shu*, after being stored for a long time, can achieve a deep color, complex flavor, and rich body.Sakes with a delicate taste and aroma, like *ginjo-shu*, are often stored at a low temperature. After long storage, they have a light color, mild flavor, and a light and delicate taste.

Aged sake (*koshu*) is contrasted with new sake, and refers to sake brewed before the last brewing year (brewing year is from July 1st to June 30th of the next year).Among long-term stored sakes, those stored for an extremely long period are referred to as greatly aged sake (*daikoshu, ohgoshu*). Sakes stored longer than 5 years are sometimes called treasured sake (*hizo-shu*).

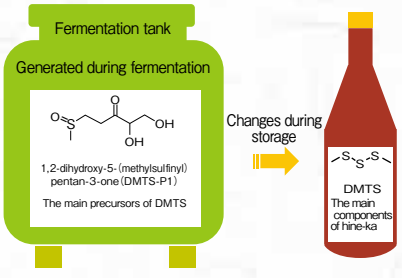
Off flavors in aged sake (*hine-ka*) versus a matured aroma

In the past, some aromas that developed in sake during storage and distribution were referred to as off flavors of aged sake (*hine-ka*). *Hine-ka* may contain various aromas. If, after a rather short storage and distribution period, the sake smells like *takuan*, a Japanese pickle, then the sake is considered to have deteriorated in quality.On the other hand, if a smell like caramel comes from long aged sake (held for a number of years and even decades), this is called the matured aroma (*jukusei-ko*). This aroma was recognized as a positive feature of long aged sake.However, off flavors and matured aroma were not well distinguished even by sake experts.

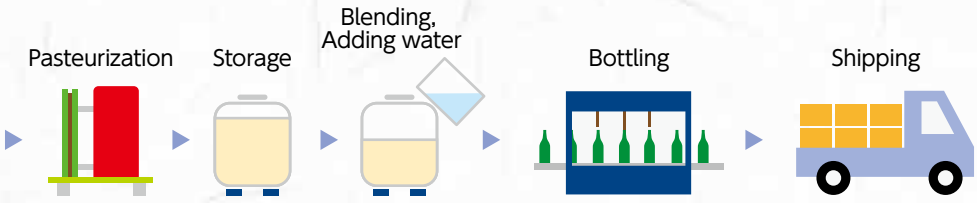
Therefore, our institute set about re-defining *hine-ka* as off flavors which are different from matured aroma, and began research into these two aromas.What we discovered is that *hine-ka* chiefly consists of dimethyl trisulfide (DMTS), while the caramel-like smell of matured aroma is related to sotolon, which is as known as the main flavor compound of aged sake.

We went on to research the cause of the *hine-ka* compound, DMTS, and found that yeast creates a precursor of DMTS (DMTS-P1). Also, various brewing conditions influence DMTS formation.

Even now, we are engaged in research into how to reduce DMTS in order to retain sake's quality for longer periods.



The DMTS-development process



Storing sake

In order to preserve sake's flavor, it is important to store it appropriately.In this section, we would like to explain how to store sake at home.

Light, high temperatures and oxygen are the enemies of sake.It is particularly the case that if sake is exposed to direct sunlight, high temperatures or sudden temperature changes, the sake's quality will suffer.Therefore, the principle is to store your sake in a cool, dark place.In addition, be careful and remember that even when a sake bottle is unopened, such strong odors as insecticide and soap can be transferred into the sake.

Make sure you store sake that are labeled "keep refrigerated," like *nama-zake*, in a refrigerator all the time.Enzymes are still active in *nama-zake*, so it will lose quality even faster than draft beer.Sakes other than those labeled "keep refrigerated" should be stored in a cool, dark place. Keep them in their original packages: the paper rolled around the bottle or the cardboard box.

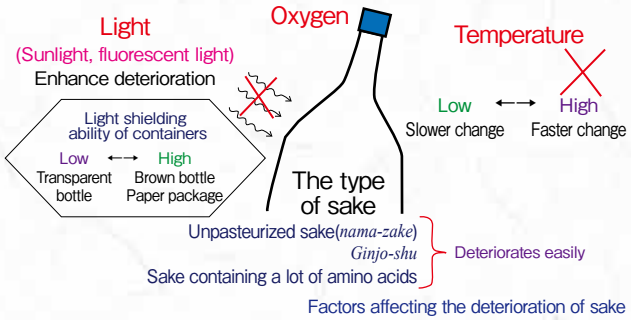
How should we store the once-opened sake?Of course, opened sake should be stored somewhere cool and not exposed to light. But besides that, it will also be negatively affected by the oxygen in the bottle.It is recommended that the opened sake should be drunk as soon as possible.

The mechanism of sake deterioration – the effects of light and temperature

It should be noticed that fermented liquor like sake, beer, and wine deteriorate more readily than distilled liquor like whiskey, *shochu* and vodka.

Why do these fermented liquors deteriorate easier than distilled liquors?It is because they contain much more such components as sugar and amino acids.These components do not evaporate by distillation. Therefore, they are not included in distilled liquor.Sugar and amino acids are the ingredients that make the characteristic flavor of sake. On the other hand, they can also cause unwanted change of color, aroma and taste.

You may find it hard to believe that light alone could cause deterioration of sake. Yet you know that ultraviolet rays in the light can have remarkable effects, like causing our skin to tan or even burn. It can trigger chemical changes of the components in sake.Therefore, change of color, deterioration of flavor, and increased bitterness are caused.



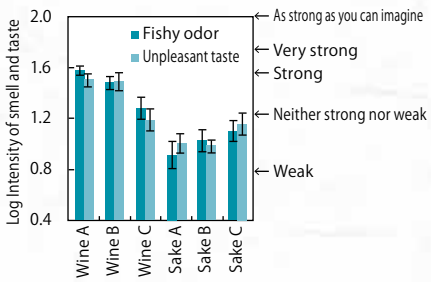
Factors affecting the deterioration of sake

The pairing of sake with different foods

It goes without saying that sake alone tastes good, but food enhance sake up to the outstandingly delicious level.By experience, we know that simple tasting Japanese foods like boiled *tofu* (*yudofu*) go well with sake.Each region has its own liking for tastes of sake ranging from sweet to dry, or from rich to light. Therefore it is well-known that each local sake goes well with its own dishes.In France, the relationship between a particular food and wine is referred to as "le mariage" (the marriage). In general, it is felt that white wine is good to drink with chicken and fish dishes, and red wine is good to drink with lean meat dishes.

There is a lot of information about the pairing of alcoholic beverages and foods, and there is also some understanding of the scientific reasons for these pairings. Below is some information from a research report from a leading Japanese liquor manufacturer: 1) White radish in *oden* (Japanese hodgepodge) is good with sake, but not good with either red or white wine. Camembert cheese goes well with wine. 2) Good pairing occurs when both the dish and the alcoholic beverages increase in positive flavor, and a pleasant aftertaste is created. Incompatibility results in an increased fishy smell or a bad taste experienced after tasting the dish. 3) Iron in wine accelerates the oxidation of polyunsaturated fatty acids such as the docosahexaenoic acid (DHA) contained in fish and shellfish, and this generates fishy odor compounds.

We are also carrying out the research about the pairing between alcoholic beverages and foods.We found that sulfurous acid in wine causes the fishy odor and bitterness from DHA contained in dried cuttlefish.If you try dried cuttlefish together with white wine, you will probably perceive a strong fishy smell. However, if you try it with sake, you may not perceive that smell much, or at all.This phenomenon occurs because sake does not contain sulfurous acid or iron. Thus, we have showed the scientific reason why sake goes well with the fish and shellfish that are the epitome of Japanese cuisine.



Degree of fishy smell and unpleasant taste when drinking alcoholic beverage while chewing dried cuttlefish.
(Labeled magnitude scale, mean value ± the standard error (n = 18))
Fishy smells and unpleasant tastes such as bitterness and harshness caused by combining a beverage with dried cuttlefish will be lower with sake than with wine.

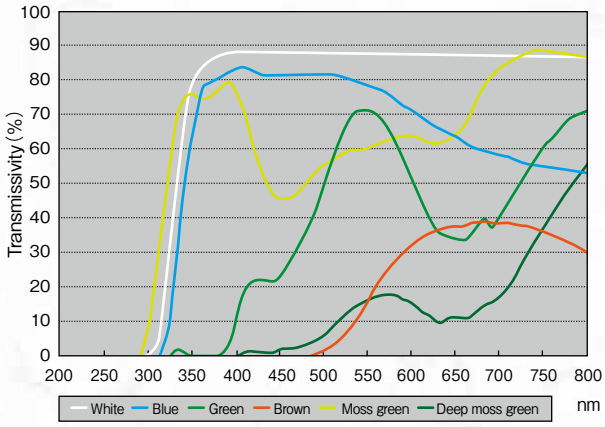
Enjoying sake

The odor that comes from exposure to light is referred to as light struck smell (*nikko-shu*). It should be noticed that not only sunlight, but also fluorescent lights, can cause slow deterioration of sake.

We also researched the relationship between the color of sake bottles and the effects of light.There are many kinds of light with different wavelengths. Humans can see light in the wavelengths from violet (approximately 380nm) to red (up to 780nm). Ultraviolet (UV) at wavelengths of 400nm or lower causes chemical reactions of some ingredients in sake.

The graph below shows the light-transmission characteristics of different color glass bottles.Brown bottles do not transmit light waves below the 450nm range that includes UV. Transparent (clear) bottles, blue bottles, and moss green bottles do transmit UV light.However, cans and paper-packaging do not transmit almost any UV light so that their contents are not affected by light.Lots of sake and beer are sold in brown bottles because they do not transmit UV rays.

On the other hand, high temperature causes the initiation of a browning reaction called the Maillard reaction in sake that contains a lot of sugar and amino acids; this reaction causes color changes and a sweet and toasted smell.Higher temperature enhances other reactions, and cause earlier deterioration.

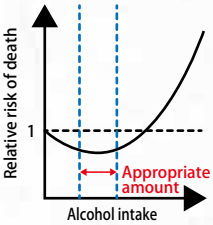


Transmitted light spectrum of colored bottles

Sake is made by means of the established technique that has been developed by sake brewers. It is delivered to customers at the high level of quality that sake-drinkers expect. The background of sake technology has been elucidated by research institutions, including ours.If you associated sake with its fruition of many years of scientific and technological development, you could find a much more distinguished taste of sake than you ever experienced.

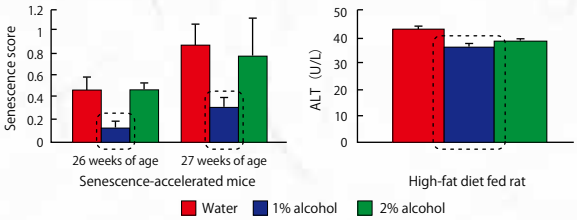
Alcohol and the J-curve effect

From ancient times, it has been said that alcoholic beverage is the best of all medicines, and it has been believed that drinking appropriate amounts will contribute to maintaining good health.On the other hand, imbibing large amount of alcohol can be hazardous as it factors into the development of various diseases, such as liver damage, cancer, and brain dysfunction.A variety of investigations are being carried out concerning drinking and health. There has been a report that found that people who drink alcoholic beverage in appropriate amounts have a lower death rate than those who do not drink at all and also lower than those who drink too much. The graph showing the amount of alcohol consumed relative to the death rate is known as the J-curve effect from the shape of the curve.However, this J-curve effect had not been tested by physiological experimentation, and there was no clear scientific basis for it.



J-curve effect

Therefore, we performed an experiment in which it gave small amounts of alcohol to animals, and investigated the effects on their health to verify the J-curve effect.Mice and rats who received low concentrations (1%) of alcohol were resistant to aging, as shown in the graphs below, and were not likely to suffer liver damage.These experiments suggested that a small amount of alcohol intake can be good for health. On the other hand, as the shape of the J-curve shows, excessive intake of alcohol is a factor disposing people to various diseases.Therefore, please remember that a “small amount of alcohol” is good for health is not the same as alcohol is good for health.Also, note that this result does not mean that non-drinkers should be forced to drink alcohol.



Relationship between alcohol intake and senescence scores (left), alcohol intake and ALT (Alanine Aminotransferase) (right)

The graph on the left shows that the senescence scores for mice given 1% alcohol were lower than those of the other groups (the plain water-fed group and the 2% alcohol-fed group). In the rat study results, shown on the right, the rat group fed a high-fat diet had less increase of the enzyme ALT (indicating reduced liver function) when the rats were given 1% alcohol than the other groups of rats did. Both studies seem to indicate that a small amount of alcohol is beneficial.

This research was conducted jointly by our institute, Hiroshima University, and the Brewers Association of Japan.

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