

Lactic acid bacteria

Genus name: Lactobacillus, Tetragenococcus
Typical fungus: Lactobacillus casei, Tetragenococcus halophilus



Bacteria with which the history of fermentation began

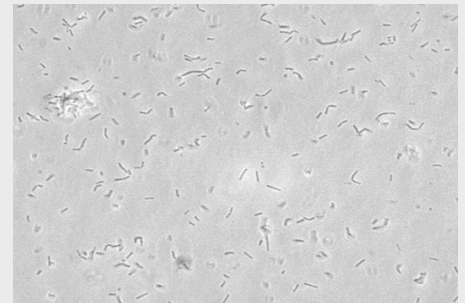
Lactic acid bacteria is the generic name for bacteria that consumes sugar to produce lactic acid, and exist everywhere in the world of nature, as with yeasts. It is believed that the history of fermentation begins with livestock milk fermented by lactic acid bacteria thousands of years ago. Dairy products such as yogurt, cheese, and butter are produced by adding lactic acid

bacteria to milk to cause fermentation. Lactic acid bacteria are also indispensable for producing miso, soy sauce, and pickles. For example, in the pickle-making process, lactic acid bacteria adhering to the surface of vegetables take sugar contained in the vegetables, and yeasts adhering to the vegetables also take part in fermentation. This combination produces flavors

unique to pickles. Tetragenococcus halophilus is a salt-tolerant lactic acid bacterium that is indispensable for producing salty fermented foods such as miso and soy sauce. Lactic acid bacteria are facultative anaerobic (they dislike oxygen), and lactic acid fermentation takes place inside foods.

Bacillus subtilis natto

Genus name: Bacillus
Typical fungus: Bacillus subtilis



Tough bacteria that survive special environments

Bacillus subtilis natto is a bacterium that inhabits rice straw, dry grass, and fallen leaves, and has high propagating power. When exposed to high or low temperatures, dry conditions, undernourished, or placed in other severe environments, it forms a special cell structure called a spore and goes into a dormant state. When placed in an environment suitable for propagation, the spores can germinate back into vegetative

cells.

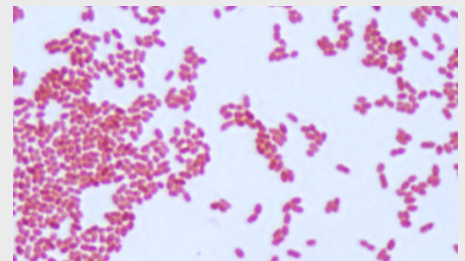
A typical fermented food produced by Bacillus subtilis natto is itohiki (stringy) natto. The substance that makes viscous itohiki natto is a high polymer comprised of γ -glutamic acids, a type of amino acid, connected in a specific way, which makes Japanese natto what it is. The umami of natto is produced by a strong enzyme in Bacillus subtilis natto during fermentation.

When boiled soybeans are packed in

rice straw and kept at a certain temperature, Bacillus subtilis natto inhabiting in the rice straw propagates and produces highly nutritious itohiki natto. The wisdom of taking advantage of the characteristics of Bacillus subtilis natto was discovered through experience by the Japanese.

Acetic acid bacteria

Genus name: Acetobacter
Typical fungus: Acetobacter aceti



Unusual bacteria that like oxygen and alcohol

Acetic acid bacteria is the generic name for bacteria that change alcohol into acetic acid, and are indispensable for producing vinegar. The strong acidic taste and acrid smell of vinegar come from acetic acid. Acetic acid bacteria adhere to flowers or fruits, and float in the air. Generally, fermentation does not require oxygen, but acetic acid bacteria cannot grow without oxygen. Acetic acid bacteria carry out acetic acid fermentation, in which they

use oxygen to oxidize alcohol to acetic acid. Acetic acid bacteria are acid resistant and produce acetic acid to create an acidic environment. This prevents other microorganisms from entering the environment and enables dominant fermentation. Japanese rice vinegar is made by fermenting sake with acetic acid bacteria. Wine vinegar is made by fermenting wine with acetic acid bacteria.

Komagataeibacter xylinus is a type of

acetic acid bacteria and forms a thick film in the process of fermentation. This characteristic can be used to make the milky, jelly-like nata de coco, which is made by fermenting coconut water with K. xylinus. The solids floating in Kombucha, a lightly carbonated drink made by fermenting tea, are also made by the same characteristic of K. xylinus.

2-1 History of fermented foods

It is believed that the world's first fermented food is a yogurt-like food, made unexpectedly in Central Asia approx. 6,000 years ago from milk left out in a dry grass field. Having obtained knowledge of fermentation, human beings produced various fermented foods in different countries around the world by taking advantage of the local climate and foodstuffs grown there through trial-and-error processes. Following the discovery of microorganisms by Leeuwenhoek, a Dutch lens polisher, in the 17th century, fermented foods underwent a revolution through the power of science. Let's trace the history of fermented foods until this revolution and the discovery of microorganisms.

Birth of fermented foods

Fermented foods can be seen in various regions around the world, and have been produced and developed through experience, influenced by the local climate and the technologies for preserving foodstuffs grown there. In Europe, where dairy farming was popular, many of their fermented foods were made of milk. It is said that the phenomenon of curdling milk was found unexpectedly by a desert traveler, who noticed that the milk had curdled in a water flask made of a goat stomach. Europe's cool climates and abundance of milk lead to the development of technologies that make use of this phenomenon to produce cheese and yogurt. In grape-growing regions, wine was born, and in island countries surrounded by the sea, fermented foods using fishery products commonly appeared. In Japan, where rice growing has been popular, various fermented foods using rice were born.

Ancient Egyptian wall paintings show that bread making had already taken place at that time. It is unknown whether the fermentation there was carried out intentionally. However, considering that kneaded flour swells via fermentation by the action of yeast in the air, which changes sugar into alcohol and carbon dioxide, it is supposed that the ancient Egyptians had naturally experienced the phenomenon of fermentation. It is also believed that grape juice was naturally fermented into wine. It can be assumed that sweet juice left to stand came to produce alcohol and was found to have a pleasant flavor and make people feel pleasant when drunk, and that is how alcohol culture was born. It is believed that wine had already been brewed in the country of Georgia around 7000 to 5000 B.C. Every country in the world has its own alcohol culture based on the foodstuffs grown in their local climate. When wine is fermented with acetic acid bacteria, vinegar can be made. Similarly,

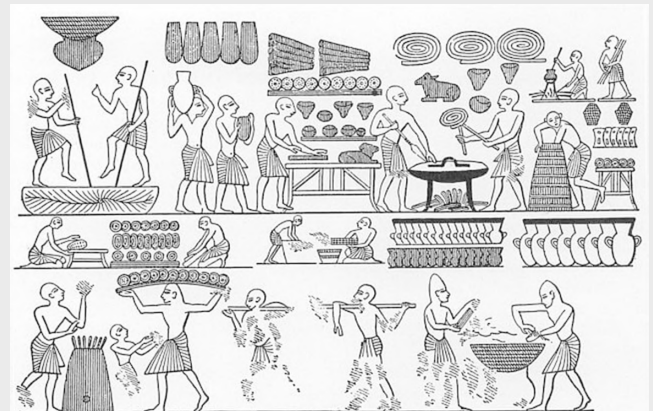
looking at various regions around the world, regions with an alcohol culture produced vinegar made from the same foodstuffs as the alcoholic drinks they produced.

Originally, fermented foods were born from techniques for preserving valuable foods. If foodstuffs are not properly preserved, putrefactive bacteria grow. If they are preserved by adding salt added, the osmotic pressure effect prevents putrefactive bacteria from inhabiting the food. However, salt-tolerant yeast or lactic acid bacteria, which are not commonly seen, can grow in the presence of high salt concentrations, and thus, continue to carry out fermentation. As a result, a unique flavor that is different from the original foodstuff is produced, changing the taste. Fermented foods were born from these experiences of our ancestors.

Discovery of microorganisms and beginning of zymology

In the 17th century, Leeuwenhoek, a Dutchman, discovered the existence of microorganisms. This discovery provided a great opportunity for human beings to come to know the involvement of microorganisms in fermentation. Leeuwenhoek observed various microscopic organisms with a microscope he had made himself and shared his findings. Owing to his observation, human beings came to know for the first time that microscopic organisms exist in the world. This is the beginning of microbiology.

It is Pasteur, a French microbiologist, who greatly contributed to the development of zymology. Pasteur scientifically explained the phenomenon of fermentation. He demonstrated in an experiment using a well-known swan neck flask (*1) that rot is not caused without an external factor and



Bread making depicted on the tomb of Ramses III (circa 1221-1156 B.C.)
https://commons.wikimedia.org/wiki/File:Ramses_III_bakery.jpg?uselang=ja

that rot is caused by an invisible factor (microorganisms in the air). He also established the pasteurization technology and contributed to the production of safe-to-drink wine (*2). In addition, another of Pasteur's big achievements is that he succeeded in separating acetic acid bacteria from wine that turned sour, or wine vinegar, as the responsible bacteria for the first time.

Koch, a German known to have discovered cholera bacteria and tubercle bacteria, is another great contributor. Koch succeeded in separating pathogens from animals infected with anthrax. Koch's method for separating microorganisms has been used as an important method in subsequent microbial studies. Hansen, a Dane, and his associates invented a pure culture method for yeast by applying Pasteur's theory, and achieved the innovation of separating and culturing beneficial yeasts for beer production based on this method.

Thanks to the discoveries and inventions by these great figures, human beings can today safely eat fermented foods.

*1 A flask with a thin and long neck extending in a lateral direction. By bending the neck like a swan neck in the middle, the flask can prevent microorganisms in the air from entering it.

*2 In the presence of alcohol, just keeping grape juice at a low temperature for a short period of time has a bactericidal effect. This technology is the same as hiire, a process in brewing sake in Japan where the sake is gently heated to sterilize the liquid before preservation, and it is believed that this hiire process had taken place 300 years before Pasteur's discovery.

2-2 The Japanese and Fermentation

Japan is sometimes called one of the world's largest producers of fermented foods. Japan has a unique fermentation culture born from the fusion of a humid climate specific to East Asia, the varied topographies and abundant nature surrounded on all sides by the sea, and the sharp eyes of our ancestors. This unique culture is based on *Aspergillus oryzae*, a mystical mold, and koji made by this mold. Here, let's look at the background and characteristics of Japanese fermentation culture from a historic perspective.

Asian regions with a hishio culture

Fermentation technologies introduced from China have played an important role in the background of Japan's fermentation culture. Soybeans originated in China, where there are many soybean-based fermented foods. Around 700 B.C. (in the Zhuo Dynasty period), [hishio], which are salted and fermented foods and is considered to be the original form of soy sauce, had already been made (Hishio is generally written as “醬” in kanji, and is also written as “比之保”). There are different types of hishio: kusabishio (salted and fermented vegetables), kokubishio (salted and fermented cereals), uobishio (salted and fermented fishes), and shishibishio (salted and fermented meat). Basically, when foodstuffs are salted, water is carried out of the cells by the osmotic effect of salt. It is thought that these salted foodstuffs would begin to ferment with time and the resultant infusion had been used as a fermented seasoning. Then, kusabishio evolved into pickle-like foods and kokubishio into soy sauce and miso.

It is said that hishio was introduced to Japan around the 4th to 6th century. Miso was called “hishio” and was like salted soybeans at that time. Rice miso was born by using koji, and then spread across the country. The origin of the word [miso] was written as [未醬] in kanji. “未” means yet-to-be, and [未醬] means yet-to-be-completed [hishio]. It was probably changed to [味噌] and then to the present kanji characters [味噌]. Soy sauce was the liquid collected from the surface of miso (called “tamari”) at that time, and was a by-product of making miso. This liquid was developed into soy sauce using koji. Uobishio was made by salting small fishes and fermenting them over a long period of time into a liquid, and production declined since after the Heian era. However, some uobishio products, such as Shotsuru in Akita Prefecture, Ishiru in Ishikawa Prefecture, and Ikanago soy sauce in Kagawa Prefecture, have been handed down until today.

In not only Japan but also other Asian regions based on rice farming and fish

eating, kokubishio and uobishio had been used since a long time ago. Fermented seasonings similar to miso that have been handed down until today include Chinese hishio seasonings such as doubanjiang (chili bean sauce) and tianmianjiang (sweet bean paste), and South Korean gochujang (Korean red chili paste). Thai nam-pla and Vietnamese nuoc mam are kinds of uobishio. In addition to Japanese soy sauce and miso, these are seasonings that exactly represent Asian regions with a fermentation culture.

Koji and the Japanese

In Japan, where rice farming was popular, fermented foods using rice were developed using fermentation technologies introduced from China. The underlying technology for producing these fermented foods is [koji], and rice koji, made of rice, was used to make sake and soy sauce. The process of making soy sauce, miso, sake, vinegar, or other fermented foods by applying fermentation is called [brewing], and Japanese brewing technologies are characterized by its use of koji. The importance of koji in the process of brewing can be seen in the procedures for brewing soy sauce, miso, and sake. In brewing soy sauce, [koji making comes first, stirring comes second, and heating comes third]. In brewing miso, [koji making comes first, boiling comes second, and brewing comes third]. In brewing sake, [koji making comes first, sake mash making comes second, and brewing comes third]. Koji is an epoch-making invention by Japan, and forms the backbone of Japan's fermentation culture.

Originally, [麴], the kanji character for koji, was created in China, and refers to rice, barley, wheat, soybeans, or other cereals inoculated with a fermentation mold. In Japan, the Japanese original kanji [糴] is also used, where “米” means rice, and refers to rice inoculated with *Aspergillus oryzae*. This provides evidence that rice koji was born in Japan.

Koji in China is made by grinding wheat, barley, or other cereals into powder, kneading it together with water, molding the mixture into a mochi (rice cake) shape, and cultivating a fermentation



Inside the koji room, where koji is made
Courtesy of: Fukuoka Soy Sauce Shop
(Iga City, Mie Prefecture)

mold, which may be *Rhizopus* or *mucor* inhabiting in China. For this reason, this koji is called [mochi koji]. In Japan, on the other hand, *Aspergillus oryzae* is used for koji. *Aspergillus oryzae* is cultivated on rice grains to make bara (granular) koji. This difference is that bara koji takes advantage of the characteristics of the fungus used. The mochi koji style is suitable for *Rhizopus* and other molds of the same genus, which grows its elongated roots deep into the mochi, drawing nutrition from it and easily propagating inside the mochi. However, *Aspergillus oryzae* cannot easily propagate on mochi. *Aspergillus oryzae* likes rice, and attaches to the rice surface and grows elongated spores there rather than elongated roots deep into the rice. In Japan, the bara koji style takes advantage of these characteristics of the mold to develop a method to make koji from rice grains.

In Japan, efforts have been made to pursue safer and more stable fermentation. As a result, our ancestors found that to make quality koji, it was important to use a quality fungus, and then, [seed koji dealers] appeared as a type of business which handled quality *Aspergillus oryzae*. If wood ash is added to *Aspergillus oryzae* spores, the potassium in charcoal helps the fungus to propagate while undesirable bacteria that dislike alkaline environments can be controlled. Our ancestors found that pure *Aspergillus oryzae* spores could be obtained by this method based on the knowledge of these characteristics of the fungus and charcoal.

Aspergillus oryzae sp. molds in the natural world are toxic. Experientially, our ancestors stored quality *Aspergillus oryzae* used for producing sake, and succeeded in growing it as a pure culture and successfully separating the safe *Aspergillus oryzae*. This is how *Aspergillus oryzae* was [domesticated], and only safe, beneficial fungi came to be used for producing fermented foods in Japan. Technologies that form the basis for today's biotechnologies have been handed down to us continuously since ancient times in Japan.

2-3 Diversity of fermentation cultures – Difference between the East and the West

Fermentation is closely related to the climate of the region, the microorganisms inhabiting it, and foodstuffs harvested there. Bread, cheese, wine, and Japanese soy sauce, miso, and sake are all fermented foods, but are made by using different microorganisms and different methods. Japanese miso and soy sauce were developed based on fermentation technologies introduced from China, and are deeply related to Chinese fermentation culture. Different countries make different fermented foods, but the fermentation cultures behind them can be divided roughly into the fermentation culture of the East and that of the West, which have their own respective characteristics.

Multiplication-based and addition-based fermentation cultures

The eastern areas of the world, including East Asia’s China and Southeast Asia, has hot and humid climates, where putrefactive bacteria easily grow and foodstuffs easily rot. For this reason, the technology of salting foodstuffs, such as vegetables and fishery products, had developed to preserve them for longer periods of time. During long-time preservation, fermentation is started by fungi or bacteria that act even in the presence of salt, such as lactic acid bacteria. Various pickles, such as Japanese pickles, Chinese Szechuan pickles, and Korean kimchi pickles, and various kinds of fish sauce (present form of uobishio) used in Southeast Asia are fermented foods made by combining salting and fermentation.

What is also important to note about fermentation in the East is fermentation using [molds]. The East has hot and humid climates that are suitable for molds to propagate, and is abundant with rice, barley, wheat, soybeans, and other cereals molds are fond of. Fermented foods made

by using molds can be found in each of these regions, such as cereal-based alcoholic beverages including sake, Shaoxing rice wine, and makgeolli, and seasonings including doubanjiang, gochujang, soy sauce, and miso. By involving molds as well as other fungi and bacteria such as lactic acid bacteria, acetic acid bacteria, and yeast in fermentation, unique, complex, and sometimes peculiar tastes that combine saltiness, sourness, and umami are produced.

On the other hand, in the western areas of the world, including Europe and the areas around the Mediterranean Sea, which have relatively dry climates, there was no need to salt foodstuffs to prevent putrefactive bacteria from growing. Typical fermented foods made in these areas include bread and beer made from wheat and barley, respectively, alcohol beverages made from fruits, and dairy products such as cheese and yogurt, and the production of these foods involves relatively fewer kinds of fungi and bacteria and is based on simple fermentation. For example, when the sugar in milk is decomposed by lactic acid bacteria, sour lactic acid is produced. When the lactic acid is left to stand, it curdles into yogurt.

Similarly, when the sugar in grapes is changed by yeast to produce alcohol, wine can be made. By using a certain fungus or bacterium with a certain foodstuff, a certain fermented food can be made. Based on this idea, clear and theoretical fermentation cultures have developed.

Generally, food cultures in the West are based on the idea of extracting the positive qualities of foodstuffs and blending them, which can be said as the idea of addition. On the other hand, food cultures in the East are based on the idea of making the most of or bringing out the natural tastes and flavors of foodstuffs, and involving different kinds of fungi and bacteria in the process, which can be said as the idea of multiplication. People in the West may think that fermentation cultures in the East are kind of mysterious, especially the way in which the molds used are largely invisible. This shows that fermented foods are inseparable from the climates where they are produced. Savor wine and feel the climate of the region where it was made. Savor pickles and think about the living of people in the region where they were made. Every fermented food has a story.

Colum

Tradition of fermentation in Japan

Microorganisms act on materials to change them into other materials beneficial to human beings. This is fermentation. Fermentation in Japan has a deep history, and its strong footprints have been left in Japan’s oldest history books.

[Harima no Kuni Fudoki (topography of Harima Province)] has a story saying, [Dried boiled rice offered to Iwa no Okami (great god) got wet and moldy. Sake made by brewing it was presented as niwaki (sacred sake), and a drinking party was held]. This story is considered to be the most widely believed theory about the origin of koji used in Japan. The setting of this story is believed to be present Niwata-jinja Shrine located in Shiso City, Hyogo Prefecture, and legend has it that sake was made using warm water present behind the main shrine. The same book also has a story saying, [Michinushihime no Mikoto, a goddess, gave birth to a child whose father was unknown. To find

which god was the father, paddy fields were made and sake was brewed. When offered the sake, the child poured it for one of gods gathered. Thus, they could find the father].

[Kojiki (Records of Ancient Matters)] has a story saying, [Susanoo no Mikoto, a god, let Yamata no Orochi (big snake with eight heads), which was extremely fond of sake, drink sake to eliminate it. The god cut off the eight heads and tail of the mortally drunk and sleeping big snake]. The sake that the snake drank was [Yashiori no Sake], which is written as “八塩折之酒” in kanji. “八” means many, “塩” means matured moromi soup, and “折” means repeat. A description that the process of brewing was repeated 8 times can be found in the history book, and it can be assumed that the sake was very tasty. Koji in Japan has such an ancient origin, and has been handed down the ages, which lets us realize how great fermentation is.

3-1 Fermented seasonings and sake

Miso and soy sauce are two seasonings indispensable to dining tables in Japan. They are both fermented seasonings that are made mainly of soybeans and originated from technologies introduced from the Chinese continent. Over their long histories, various kinds of miso and soy sauce have been produced to suit the climates and taste preferences of various regions in Japan, and they are today world-renowned seasonings. Sake, which is called “seishu” under the Liquor Tax Act, is based on the Japanese people’s rice-eating culture and *Aspergillus oryzae*, which are combined with delicate technologies for manipulating microorganisms that our ancestors developed. It is worthy to be called the [national drink] of Japan. Here, details about these fermented products, including their histories and methods for producing them, are introduced.

Miso (Soy paste)

Infinite variations produced by the different climates of different regions

Miso is a solid salty fermented seasoning made by fermenting soybeans in the presence of a high concentration of salt. In the Japanese Agricultural Standards (Act on Japanese Agricultural Standards) (*), miso is defined as a [semisolid material made by adding koji based on rice, barley, or another cereal (or koji made of soybeans) to steamed soybeans, mixing salt, and allowing the mixture to ferment and mature].

The history of miso

It is believed that miso originated from hishio and kuki (boiled and mashed soybeans with added salt), which have been known from ancient times in China. Hishio refers to salted and fermented foods. There are different types of hishio, such as kusabishio (salted and fermented vegetables), uobishio (salted and fermented fishery products), and shishibishio (salted and fermented beef, pork, etc.). Asia, with its rice-farming culture, saw the development of kokubishio (salted and fermented beans or soybeans). Typical seasonings that originated from hishio include doubanjiang (chili bean sauce) and touchi

(fermented black beans) in China and gochujang (Korean red chili paste) in South Korea.

In Japan, a food modeled after hishio and kuki was introduced through the Korean Peninsula approx. 1300 years ago. It was like today’s soybean miso, represented by the kanji characters “味噌.” This is believed to be the origin of miso. It is said that the kanji characters “味噌,” which is pronounced “miso,” started to be used in the Heian era. The kanji character [噌] was created in Japan, and is only used in the kanji characters “味噌,” indicating that miso is Japan’s own food. After that, Japan’s own rice miso using rice koji was developed in the Japanese rice-eating culture, and spread across the country.

In the Heian era (794 – 1185), miso was eaten as-is, as a side dish, or used as a seasoning put on tofu (bean curd) or vegetables, and was considered as an expensive food habitually eaten by the upper-class. This kind of miso is called [name miso (miso eaten as it is)] or [okazu miso (miso eaten as a side dish)], and still today, there are various kinds of miso eaten in this way. Popular ones include kinzani miso, tai miso, iriko miso, and sankai miso. These are regional specialties or they reflect regional eating habits.

Miso soup was invented in the Kamakura era (1185 – 1333). Miso soup is said to be a great product invented by Japanese food culture. Unlike soup popular in other countries, miso soup is not eaten alone but always eaten with rice. [Ichiju-issai],

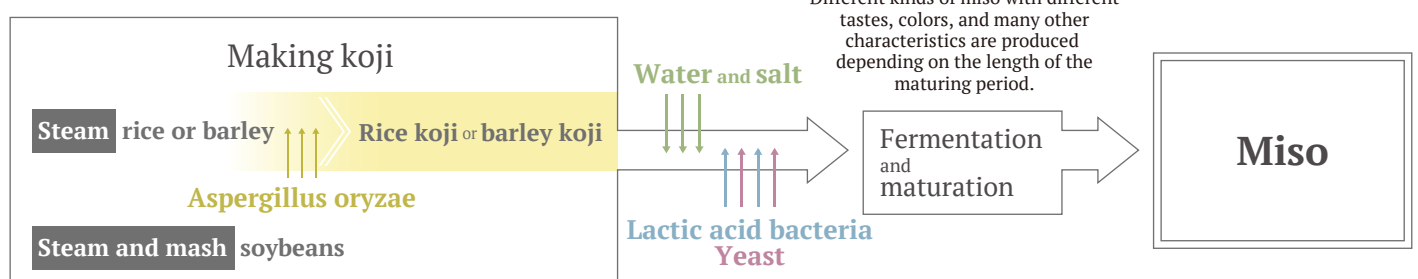


the one soup plus one dish (or three-dish) meal style comprised of cooked miscellaneous cereals, miso soup, and a pickle, was established in this era.

In the Sengoku period (1467 – 1573), military commanders around the country used miso as army provisions because of its good keeping quality and nutritiousness, and thus, miso came to be made widely around the country under the leadership of feudal lords. [Oensogura], built in the castle town of Sendai Aoba by Masamune Date, is believed to be Japan’s first miso factory. The taste of miso produced in the factory has been handed down as [Sendai miso]. Shingen Takeda, who built the basis for [Shinshu miso], and Ieyasu Tokugawa, the founder of the Edo Shogunate, are known to have been extremely fond of miso soup, which helped to develop the miso culture in Edo.

*1 This is a system based on the Act on Japanese Agricultural Standards (“JAS Act”), an act enacted in order for the Japanese government to specify standards on foods and agricultural, forestry, and marine products and on methods for handling these products (“JAS standards”). Under this system, food products and agricultural, forestry, and marine products can be labelled with a JAS mark, which certifies that they satisfy JAS standards, or advertisements of businesses handling these products can contain a JAS mark.

Process of producing rice miso and barley miso



At least 1300 years have passed since hishio and kuki were introduced, during which various kinds of miso that use or reflect different local ingredients, climates, and eating habits in different regions in Japan have been produced, and have played a great role in creating local food cultures.

Variety of miso

Miso is roughly divided into four kinds: [rice miso], [barley miso], [soybean miso], and [blended miso]. These are categorized into [ordinary miso]. [Rice miso] is classified into the three kinds of ama (strongly sweet) miso, amakuchi (moderately sweet) miso, and karakuchi (salty) miso by the amount of koji and salt used, and into three more kinds by color: white miso, red miso, and light-colored miso (medium-colored miso).

Ama miso is made in a short period of 5 to 20 days, and is very sweet due to the sugar content derived from rice koji. Ama miso is divided into two types by color: white ama miso and red ama miso, which is due to the difference in the method used for processing the soybeans.

[Red ama miso] is brownish-red, glossy miso represented by [Edo-style ama miso], and has a unique flavor produced by the combination of the savory aromas of koji and soybeans. On the other hand, [white ama miso] is a vivid light yellowish-white, and is characterized by a unique flavor that comes from rice koji. [Saikyo miso] made in Kyoto is typical miso of this kind, and [Sanuki miso] made in Kagawa Prefecture and [Fuchu miso] made in Hiroshima Prefecture are also well-known miso of this kind. Ama miso is commonly used for miso-based dishes or miso sauce rather than for miso soup, and has a short shelf life.

Amakuchi miso is also called “chuama (medium-sweet) miso.” [Aijiro miso] made in Shizuoka Prefecture and [Gozen miso] made in Tokushima Prefecture are well-known miso of this kind, and miso of this kind is also produced in the Setouchi seashore region.

Karakuchi miso is made over a long brewing period, and the rice miso that is produced in the largest amounts is karakuchi miso. Karakuchi miso comes in light and red colors. The color shade depends on the method used for processing the soybeans used as the ingredient and the flavor depends on the length of the aging period. Represented by [Shinshu miso], light-colored karakuchi miso is bright light yellow, and has a refreshing flavor unique to fermentation, and a light and plain taste. Represented by [Sendai miso], red karakuchi miso has a vivid brownish-red color and a pleasant flavor resulting from a long period of aging, and presents a robust and strong taste produced by a harmony of umami and saltiness.

[Barley miso], which is also called “inaka (country-style) miso,” was originally made by farmers for their own consumption, and its industrial production takes place in limited areas such as the Kyushu, Shikoku, and Chugoku regions and some parts of the Kanto region.

It is [soybean miso] that is made the same way as the earliest kinds of miso, and it is considered the original form of miso. Soybean miso does not use rice or barley, and is made by turning all ingredient soybeans into koji. Other kinds of miso use bara (granular) koji while soybean miso uses molded koji made by pounding steamed soybeans into a ball. It contains salt at a concentration of 10% to 11%, which is somewhat lower for salty miso. It is dark brownish-red, and presents a

unique flavor, with a robust and rich umami and a slightly astringent taste. Soybean miso is produced only in Aichi Prefecture, Gifu Prefecture, and Mie Prefecture, and miso produced there is named “Haccho miso,” “Nagoya miso,” “Sanshu miso,” “Tamari miso,” etc.

In addition to these three kinds of miso, other kinds of miso collectively called blended miso are also subject to the quality labeling standards under the Act on Japanese Agricultural Standards. Blended miso includes miso made by mixing rice miso, barley miso, and/or soybean miso as well as miso made by mixing rice koji, barley koji, and/or soybean koji and brewing the mixture.

Miso other than ordinary miso is called [processed miso], and is divided into [brewed name miso], which includes Kinzanji miso and hishio miso, and [processed name miso], which includes tekka miso, tai miso, and yuzu miso, according to the production method. [Brewed name miso] is made from dehulled soybeans, barley, salt, vegetables, and other ingredients by fermentation and maturation. [Processed name miso] is made by adding other ingredients, such as vegetables, fishery products, sugar, and spices, to ordinary miso and heating and kneading the mixture, and is available in many varieties.



Soybean koji, vital for making soybean miso
Courtesy of: Marukajozo (Toyota City, Aichi Prefecture)

Classification of miso and major areas of production

Type of miso	Classification by taste or color		Percentage of koji	Salt concentration	Major areas of production
Rice miso	Ama miso	White	15~30	5~7	Kinki region, Chugoku region, Setouchi
		Red	12~20	5~7	Tokyo
	Amakuchi miso	Light-colored	8~15	7~12	Shizuoka, Kyushu
		Red	10~15	11~13	Tokushima and other regions
	Karakuchi miso	Light-colored	5~10	11~13	Kanto-Koshinetsu, Hokuriku and other regions of the country
		Red	5~10	11~13	Kanto-Koshinetsu, Tohoku region and other regions of the country
Barley miso	Amakuchi miso	Light-colored	15~25	9~11	Kyushu, coastal areas of Japan Sea in the Chugoku region
	Karakuchi miso	Red	8~15	11~13	Kyushu, Shikoku, northern part of Kanto
Soybean miso	Karakuchi miso	Red	Whole quantity	10~12	Chukyo (Aichi, Mie, and Gifu Prefectures)

Typical miso colors

Rice miso (salty/red)	
Rice miso (light-colored)	
Rice miso (sweet/white)	
Soybean miso	
Barley miso	

[Nihon no Dentoshoku Jiten] (Encyclopedia of Japanese Traditional Foods)(Edited by Nihon Dento Shokuhin Kenkyukai (study group), Asakura Publishing Co., Ltd.)The figure in page 249 was partially changed.

Shoyu (Soy sauce)

Magical liquid that contains 300 kinds of flavors in a single drop

Shoyu is a fermented seasoning in the form of a clear liquid. It is made by soaking koji made of soybeans in salt water to allow fermentation and maturation to take place. Since shoyu is rich in glutamic acid, an umami component, and other amino acids, it can be used in cooking to add a salty taste as well as umami to dishes and make them tasty. Of these different kinds of shoyu, koikuchi shoyu has the richest flavor. There are more than 300 known flavor components of shoyu, which include caramel, vanilla, rose flower, banana, apple, matsutake mushroom, smoky flavors, and many other flavors. Pouring only a single drop of shoyu on a dish wraps it in a soft shoyu flavor. The pleasant flavor increases the tastiness of the dish. Shoyu contains flavor elements from various foodstuffs, and thus goes well with various dish flavors.

The history of shoyu

Shoyu originated from hishio (jiang in Chinese) and kuki (chi in Chinese) in the Qin period in China or as early as over 2000 years ago. In [Qi Min Yao Shu] written in



Shoyu moromi Courtesy: Fukuoka Soy Sauce Shop (Iga City, Mie Prefecture)

[Moromi] is created by combining salt and water to koji. Salt-tolerant lactic acid and yeast grow and fermentation occurs to develop the unique flavor of shoyu.

the early 6th century, the technique for producing the original form of shoyu, or the supernatant of mamebishio (salted and fermented soybeans) called “Dou Jiang Qing”, is described.

Although there are various stories regarding the origin of shoyu in Japan, it is said that it was introduced together with Buddhism. According to the Taiho Code (701), it is recorded that various kinds of hishio were made of soybeans in Shoin, a facility for producing and storing hishio, established by the Imperial Household Department. [Engishiki], a book for codes and procedures on national rites and prayers written in the Heian era, which is approx. 200 years after that, refers to soup separated from hishio called “saisho,” which is today’s tamari (thick) shoyu.

On the other hand, there is another story that shoyu originated from tamari soup from miso spread by a Zen priest named Kakushin in the Kamakura era. Kakushin went to China, practiced ascetic exercises in the Kinzan-ji Temple, and learned how to produce miso. After coming back to Japan, he founded a temple, which later became Kokoku-ji Temple, in Yurasho, Kii Province, and spread the method for producing miso while performing missionary work. It is also said that the soup accumulated at the bottoms of vats in the process of miso production was found to be tasty and suitable for flavoring simmered dishes, and this soup is the origin of shoyu.

In the Muromachi era (1336 – 1573), industrial production of shoyu began in the Kansai region, which was the center of culture at that time. Until the beginning of the Edo era, shoyu made in the Kansai region was called kudari shoyu (shoyu brought from the capital), and became available in Edo. With the development of the city of Edo, Edo-style culture emerged, and koikuchi (dark-colored) shoyu that goes with tempura, eel kabayaki (broiled/grilled eels basted with a sweet sauce), foods boiled down in shoyu, and other Edo-style dishes was invented. Then, koikuchi shoyu replaced kudari shoyu, which had accounted for the majority of



Shoyu blends well with foods, whether through cooking or by dipping. It also works to eliminate the fishy scent of raw seafood.

shoyu in Edo until then, and became one of Edo’s major seasonings.

In the Kanto region, Noda and Choshi in Chiba Prefecture developed as Japan’s largest producers of shoyu partly due to their convenience for transportation and other favorable geographical conditions. Shoyu brewers were concentrated in certain production areas such as Noda, Choshi, Tatsuno (Hyogo Prefecture), and Shodo-shima Island (Kagawa Prefecture), and competed with each other on quality and developed shoyu into the staple of Japan’s food industry that it is known for today.

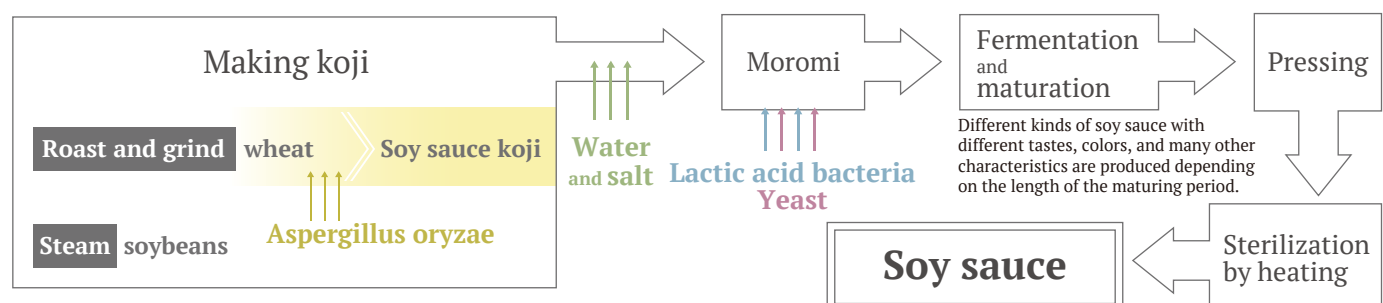
Shoyu is sold not only in Japan but also in more than 100 countries around the world, and now is one of the world’s best-known seasonings. For example, the word soybean originated from shoyu bean. It is also said that Louis XIV, the former king of France, who was well-known as a gastronome, habitually used shoyu as a secret ingredient.

Shoyu has been internationalized owing to efforts of leading shoyu manufacturers including Kikkoman Corporation, which penetrated the U.S. market earlier than others. Shoyu became familiar to Japanese Americans, American soldiers who was stationed in Japan and then returned to the U.S., and other people living there, and came into general use in the country. Shoyu was also integrated into American food culture as the base for teriyaki sauce.

Variety of shoyu

Under the Act on Japanese Agricultural Standards, shoyu is divided into five kinds:

Process of producing koikuchi soy sauce



koikuchi (dark-colored) shoyu, usukuchi (light-colored) shoyu, tamari (thick) shoyu, saishikomi shoyu (shoyu brewed in another finished shoyu product), and white shoyu.

Koikuchi shoyu accounts for more than 80% of shoyu produced across the country, and thus, shoyu generally refers to koikuchi shoyu. Koikuchi shoyu is made by cultivating *Aspergillus oryzae* in a mixture of soybeans and wheat in equal amounts to produce shoyu koji, and soaking it in salt water to allow fermentation to take place. The enzymes in *Aspergillus oryzae* cause the ingredients to decompose, and the shoyu koji melts into a sticky substance. Then, umami is produced. During this process, lactic acid bacteria grow and yeast carry out alcohol fermentation, producing a rich flavor.

Usukuchi shoyu is made by using a mixture of soybeans and wheat in equal amounts as the ingredients of shoyu koji. In the production process, steamed rice or amazake made of steamed rice is added to prevent the shoyu color from becoming dark. Thus, usukuchi shoyu is characterized by the production method making the shoyu color as light as possible. To slow down the fermentation and maturation speed, 10% more salt than

for koikuchi shoyu is used. Usukuchi shoyu was developed with the intent to keep the natural flavors and colors of foodstuffs used in dishes. It originated in Tatsuno, Hyogo Prefecture, and developed in close connection with kaiseki ryori (tea ceremony dishes), vegetarian dishes, and other traditional Japanese dishes.

Tamari shoyu is made by using shoyu koji that does not use wheat but is made almost only of soybeans. Tamari shoyu originated in the Tokai region of Aichi, Gifu, and Mie Prefectures, and tamari shoyu made in the Tokai and Hokuriku regions accounts nearly 80% of the nationwide shipment volume. Tamari shoyu requires a longer maturation period than koikuchi shoyu, and has a rich and unique flavor. It is often used as shoyu for sashimi or kabayaki, and also used widely to flavor a variety of foods such as rice snacks, foods boiled down in shoyu, and noodle soup.

Saishikomi shoyu is shoyu made by using a mixture of soybeans and wheat in equal amounts as the ingredient of shoyu koji, adding kiage shoyu (shoyu squeezed from moromi) instead of salt water in moromi, and brewing the moromi. Saishikomi shoyu originated from Kanro (nectar) shoyu from the Yanai region, Yamaguchi Prefecture. Since kiage shoyu is added in

the production process, saishikomi shoyu uses slightly less salt than koikuchi shoyu, has a thick taste and color, and is viscous. It is used as shoyu for sushi or sashimi or in sauces for eels.

White shoyu is made by using a mixture of a small amount of soybeans and wheat as the ingredient of shoyu koji, and strongly controlling the appearance so that it looks less dark-colored and less glossy through the production process. It originated in Hekinan City, Aichi Prefecture approx. 200 years ago, and is still now produced in this region. It has an extremely light color, tastes very sweet, and presents a unique koji flavor and amazake flavor. It is used for foods that should be plain or light-colored. For example, it is used for hot pot dishes, soup dishes, and needle soup or to process confectioneries, rice crackers, fish sausages, and pickles.



Koji for white shoyu
Courtesy of: Hichifukujozo (Hekinan City, Aichi Prefecture)

Characteristics and regions of production of shoyu

Type	Production method and characteristics	Region	Color
Koikuchi shoyu	Equal amounts of soybeans and wheat are mixed to create shoyu koji, which is soaked in salt water 1 to 1.2 times the volume of the koji to allow fermentation to take place. The fermentation and maturing period are approximately eight months. The moromi is stirred occasionally during fermentation to encourage yeast growth. After fermentation and maturing is complete, moromi is pressed to squeeze kiage shoyu, which is heat sterilized at 85°C or higher to create shoyu products. This shoyu is the most flavorful as the yeast has been fermented very well.	Various regions of the country	
Usukuchi shoyu	Usukuchi shoyu is made by using soybeans and wheat as the ingredients of shoyu koji. Amazake is added to make the shoyu taste mild. The salt concentration is relatively high, to suppress fermentation, and some ideas are introduced in the production process to prevent the shoyu color from becoming dark. To make good use of the original colors and flavors of foodstuffs, usukuchi shoyu has a mild color, umami, and flavor, but it contains more salt than koikuchi shoyu.	Kansai region	
Tamari shoyu	Tamari shoyu is made by using soybeans and a very slight amount of wheat as the ingredients. Soybeans are mashed into a sphere, from which koji is made. The koji is then fermented in a small amount of salt water. Tamari shoyu requires a longer maturing period than koikuchi shoyu. Tamari shoyu is rich in umami components, and has a rich, unique flavor. It boasts a rich flavor that works to eliminate the raw smell of fish and meat.	Tokai region (Aichi, Gifu, and Mie Prefectures)	
Saishikomi shoyu	Saishikomi shoyu is made by mixing equal amounts of soybeans and wheat to make shoyu koji and brewing (maturing) it in kiage shoyu rather than in salt water for a long period of time (1.5 to 2 years or longer). Saishikomi shoyu has a rich flavor, color, and umami.	Yamaguchi Prefecture, Sanin region to Kyushu region	
White shoyu	White shoyu is made by mixing wheat and soybeans at a ratio of approx. 9:1 to make shoyu koji and brewing it in water with a relatively high concentration of salt. The fermentation and maturing period is approx. 3 months. The process for coloring the shoyu does not take place or is completed in a very short period of time. White shoyu is light-colored, has mild umami and is not so robust, but has a strong sweet taste and flavor that come from koji. It is suitable for osuimono and chawanmushi (savory steamed egg custard).	Aichi Prefecture	

Sake

Product of biotechnology for skillfully manipulating microorganisms

Alcoholic beverages are made from a sugar-containing foodstuffs by alcoholic fermentation using a yeast. Wine is made by fermenting grape juice with a yeast, and what is important for wine is the quality of grapes used as the ingredient. Beer is made by saccharifying barley with malt and then fermenting it with a yeast. On the other hand, sake, which is the national drink of Japan and is called “seishu” (clear sake) under the Liquor Tax Act, can be said to be an alcoholic beverage made by manipulating microorganisms more skillfully.

The basic method for producing sake is as follows: First, steam appropriately polished rice, and cultivate *Aspergillus oryzae* on it to make rice koji. Add a yeast to small amounts of the rice koji and water, add a small amount of steamed rice, and cultivate the microorganism to make sake mash. Further add steamed rice, rice koji, and water to the sake mash in three parts, and allow the mixture to brew and ferment at a low temperature. Then, the rice starch is decomposed by the *Aspergillus oryzae*-derived amylase into glucose (saccharification), and the

yeast carries out alcoholic fermentation to finally produce sake. The yeast is allowed to carry out fermentation at a low temperature to keep a good balance between starch saccharification and yeast fermentation. There are two types of sake mash: one is yamahaimoto, which is made by adding yeast after wild lactic acid bacteria have grown well after brewing, and another is sokujomoto, which is made by adding commercially available lactic acid and yeast to moromi at the same time during brewing. Both types of sake mash were originated from the wisdom of allowing yeast to carry out fermentation in a pure environment by weeding out bacteria moving from koji, or brewing water, to moromi.

The alcohol concentrations of beer and wine are approx. 5% and 12%, respectively, while that of sake is as high as 20% (however, the alcohol concentrations of sake products are adjusted and decreased to approx. 15%). The high alcohol concentrations of sake are due to its specific production method, where high-concentration brewing using a very small amount of water or only a 1.2–1.3 water to rice ratio takes place, and the yeast is allowed to continue alcoholic fermentation by adding steamed rice, rice koji, and other ingredients in three parts (three-stage brewing) and allowing the saccharification of rice starch and fermentation to take place at the same time (parallel multiple fermentation).



Yeast used for sake are characterized by having a high alcoholic fermentation capacity and producing a fruity flavor like apple or banana called “ginjoka.”

Rice koji used for sake must have sufficient amounts of diastatic enzymes to dissolve the rice, saccharify the starch in moromi (a fermented pasty mixture of ingredients), and produce a large amount of glucose. This allows the yeast to actively carry out fermentation. Koji is made by cultivating *Aspergillus oryzae* on rice grains or solid culture. Liquid culture does not allow *Aspergillus oryzae* to produce sufficient amounts of enzymes. Especially, the genes of diastatic enzymes have been found by recent studies to be switched on only when they are cultivated on a solid medium. The sake production technology developed by our ancestors through experience can be said to be biotechnology for skillfully manipulating microorganisms.

Column

Tips for exporting fermented foods shared by a long-established shoyu brewery

Since washoku, traditional Japanese food culture, was registered as a UNESCO Intangible Cultural Heritage in 2013, it has been attracting increasing attention, and people around the world have come to know that it is nutritionally well-balanced and good for health. In addition, partly owing to the dissemination of information by those with a deep knowledge of food cultures through social media and other channels, Japanese fermented foods have also been gaining increasing attention. Especially, the export volume of shoyu increased more than two-fold to 48,090 tons in 2021 from 19,822 tons in 2012 (*). There has been steadily growing demand for shoyu from not only the U.S. and European countries, but also other countries. Although many products on these markets are made by leading manufacturers, shoyu breweries who still use traditional methods to produce shoyu are also expanding their business overseas through their own strategies.

“Unlike mass-produced shoyu products, shoyu brewed in wooden vats is a delicate product that directly reflects the tastes and sincerity of the craftsmen brewer making it. If a brewer goes overseas and directly conveys the appeal of their products, that will differentiate their products from other products,” says Mr. Kichigoro Fueki, the head of Fueki Shoyu, a long-established brewery founded more than 230 years ago (in Saitama Prefecture). Recently, they have been operating Shoyu Park, a park where visitors can learn about the process of producing shoyu, developing sweets using shoyu, and taking on other various challenges while keeping up the tradition of shoyu. They have so far sold their products in more than 10 overseas countries (as of 2022). They intend to further increase their outlets. “One of the advantages of shoyu is that it serves as a secret ingredient for dishes while keeping the natural tastes and flavors of other



Children learning about making shoyu at [Shoyu Park]. An actual shoyu brewery is open to the public where visitors can observe the shoyu production process and enjoy dishes using shoyu. In recent years, more and more foreign tourists have been visiting this facility.

ingredients. It is important for us to pursue the advantages of individual products and our strengths as a brewer and convey them,” Mr. Fueki says.

Such sincere attitudes of brewers should let people around the world know the appeals of shoyu and other Japanese fermented foods and create new Japanese food booms.

* The figures are based on the actual exports of agricultural, forestry and fishery products/foods (by item) for 2021 announced by the Ministry of Agriculture Forestry and Fisheries of Japan.
https://www.maff.go.jp/j/shokusan/export/e_info/attach/pdf/zisseki-23.pdf

3-2 Other typical fermented foods

Japanese foods are characterized by its cooking techniques, which preserve the flavors of the ingredients, and the Japanese diet based on one soup plus three dishes, healthy menus that make good use of umami, and fermented foods are deeply related to these characteristics. All of the fermented foods introduced here are deeply related to the background of Japanese food culture, and are indispensable to our daily diets.

Dried bonito

World's hardest fermented food responsible for creating the umami of Japanese foods

Dried bonito is a food made by smoking a bonito fillet, drying it well, and then spraying a mold on it to allow fermentation to take place. Thin shavings or powder of this dried bonito is used to prepare soup stock for various dishes such as miso soup and simmered dishes, or used as toppings for cold tofu, boiled greens with shoyu, etc. The umami and fragrant flavor produced by dried bonito are indispensable for Japanese foods.

A smoked and dried bonito fillet is called arabushi, and a fillet shaped by shaving the surface of arabushi is called hadakabushi. Hadakabushi is then sprayed with a mold to make karebushi, which is further sprayed with a mold several times to make honkarebushi.

The history of dried bonito

In Japan, bonito has been eaten since ancient times, and descriptions of dried bonito-like foods such as katsuo (hard fish) can be found in Kojiki (Records of Ancient Matters) written in the 8th century, the oldest existing history book of Japan. In the Engishiki written in the middle of the Heian era (around the 10th century), boiled and dried bonito appear as nikatauo (a fish boiled and dried hard). Nikatauo was dried by the sun in this era. In the Muromachi era, bonito came to be smoked and dried by using fire and smoke, and called katsuobushi, the present Japanese name for dried bonito. Shijoryu Hochogaki, a cookbook in the Muromachi era, uses the word Hanakatsuo (dried bonito shavings), and shows that hanakatsuo was used together with shoyu to flavor dishes.

It is said that spraying a mold after the process of roast-drying *1 was started in Tosa Province (Kochi Prefecture) in the mid-1600s. Dried bonito made in Tosa was called Tosabushi and was sprayed with mold only once. Subsequently, the process of spraying a mold came to be repeated to reduce the fishy smell, increase umami,

and produce the unique flavor, resulting in the current honkarebushi.

Production method

Dried bonito is produced as follows. First, cut off the head of a bonito, remove the internal organs, and then fillet it into three parts. If they are large in size, cut them into the dorsal part and abdominal part. Put the bonito fillets in a basket, place it in a cooking pot, stew*2 them at around 80°C for 60 to 90 minutes, allow them to cool, and then manually remove the bones. The fillets roast-dried once after these steps are called namaribushi. Repeat roast-drying about 10 times to produce completely black masses covered with a tarry substance. Shave off the tarry substance on the surface of this arabushi to turn it into hadakabushi, and spray it with a beneficial mold*3. Then, the surface will be covered with the mold in 1 to 2 weeks. Beneficial molds are characterized by having strong lipolytic ability, and weak proteolytic ability. Sun dry the masses with the mold on the surface, brush the mold off the surface, and spray the mold again. By repeating this last process of drying, brushing, and spraying the mold 4 times, honkarebushi can be obtained. It takes 60 to 80 days to make dried bonito from a whole bonito fish.

The greatest characteristic of dried bonito is its hardness. This hardness is because its water content has been reduced to approx. 15% by repeatedly roast-drying and spraying it with mold. Drying to the utmost limit creates an environment where microorganisms cannot grow, increasing its shelf life. In addition, the sprayed mold decomposes and removes fat, which prevents soup stock extracted from dried bonito from clouding, as seen in soup stock extracted from chicken or pork bone.

Dried bonito contains a rich amount of 5'-inosinic acid, an umami component. It is known that the combination of 5'-inosinic acid and glutamic acid, which is another umami component, creates a synergetic effect and enhances umami. Mixed soup stock extracted from both dried bonito and kombu seaweed is exactly the product of that synergetic effect. The value of dried bonito also lies in its flavor produced by



the processes of roast-drying and mold spraying. More than 400 kinds of flavor components, including alcohols, phenols, and carbonyl compounds, created through this process have been found.

Previously, special cutter boxes for dried bonito were used in general households, but today, dried bonito is sold in the form of shavings. However, the flavor unique to dried bonito is irreplaceable, and it is still true that dried bonito is indispensable for Japanese foods and Japanese people.

Other bushi types

Dried bonito has different names depending on the production area. For example, it is called "Tosa bushi" or "Satsuma bushi" in Kagoshima Prefecture, or "Izu bushi" or "Yaizu bushi" in Shizuoka Prefecture. They have different shapes, flavors, and other characteristics unique to the regions. Today, Kagoshima Prefecture and Shizuoka Prefecture are the two major production areas of dried bonito. Dried fishes other than bonito are also produced in various regions. These include dried soda or bullet tuna (bullet mackerel, frigate mackerel or sumasoda), dried tuna, dried mackerel, dried mackerel scad (mackerel scad or jack mackerel), dried round herring, and dried sardines (anchovies). With different color shades and flavors, these dried fishes are used to make soup stock for soup called "osuimono," miso soup, soba noodles, udon noodles, Chinese noodles, and other various dishes. Thus, they are supporting the tastes of Japanese food in various ways.

*1 Process of drying while smoking used for dried bonito. This process has the effects of concentrating umami, preventing rot, and killing fishy smells.

*2 Process of stewing raw bonitos after they are cut and trimmed. While they are stewed, water is poured so as not to stew in boiling water. This process has the effects of preventing the bonito from rotting, making them easier to dry, and locking in the umami.

*3 Eurotium species (Eurotium herbariorum and Eurotium repens) and Aspergillus glaucus, a mold related to Aspergillus oryzae, are used.

Natto

Well-balanced nutritional food with a unique, addictive smell and flavor

Japanese natto is first associated with itohiki (stringy) natto put on top of rice. Natto is a salt-free soybean product made by fermenting boiled soybeans with *Bacillus subtilis natto*, and is characterized by its unique smell and viscous substance produced by fermentation. It is extremely nutritious, and is today eaten together with rice across the country on a daily basis. Previously, however, it was eaten as a valuable source of protein in the frigid Tohoku region.

In Japan, there is another kind of natto called “shiokara (salted and fermented) natto” (or hama natto or tera natto). It is made by using soybean koji from inoculating *Aspergillus oryzae* rather than *Bacillus subtilis natto* in boiled soybeans.

Soybean koji is immersed in salt water to allow it to carry out lactic acid fermentation, and then dried into shiokara natto. It is believed that shiokara natto was modeled after kuki, which is Chinese salt-cured fermented soybeans and is considered as the origin of Japanese miso. It has a longer history than itohiki natto, and is believed to have already been made back in the Nara era.

It is unknown when people started making itohiki natto. Although there are various stories regarding its origin, all stories commonly indicate that it originated in the Asuka era (6th to 7th century) or the Heian era (9th to 11th century), and boiled soybeans wrapped in straw were unexpectedly found to emit an odor and have changed into a viscous, stringy state. In *Shojin Gyorui Monogatari*, a tale of vegetables and fishes written in the Muromachi era (14th century), Natto Taro Itoshige, a character apparently considered to personify itohiki natto, appears, which suggests that itohiki natto was already present at least at that time.



Until before the Edo era, natto was made at homes and eaten as an ingredient in soup dishes. In the Edo era, industrial production of natto began, and itohiki natto started to be put on top of rice at around this time. Itohiki natto contains rich amounts of vitamins such as vitamins B1 and B2, minerals, and calcium, is easy to digest and absorb, and thus, was a food suitable for Japanese people at that time, who lived on plain food.

There are salt-free fermented soybean foods similar to Japanese natto in other Asian countries, such as tempeh eaten in Indonesia and kinema introduced in Nepal and Bhutan, but it is only Japanese natto that is put on top of rice.

Mirin

Sake that evolved from a beverage into a seasoning

Mirin is a liquid, sweet fermented seasoning containing approx. 45% sugar and approx. 14% alcohol. It is also considered as one of Japan's national drinks like sake, and is often used in cooking together with shoyu as an alcohol-based seasoning that is indispensable for giving a sweet taste to Japanese foods.

When used in cooking, mirin has various beneficial effects. For example, it can be used to give a sweet taste or a glossy or shiny look to dishes, gives a soft but sharp sweetness to dishes, help liquid seasoning components to soak into foodstuffs, prevents foodstuffs from softening and falling apart during boiling, and remove fishy smells. These effects are mainly due to alcohol and sugars contained in mirin.

It is believed that mirin originated in a sweet alcoholic beverage called nerizake in the Muromachi era (14th to 16th century), or another sweet alcohol beverage called miirin and introduced from China in the Sengoku period (15th to 17th century). Komai Nikki, a diary of a man named Komai written in 1593, is the first book where the name mirin appears. Mirin was drunk as a sweet alcoholic beverage at

that time, but since very sweet mirin made by brewing glutinous rice and rice koji in shochu like today's mirin appeared in the late Edo era, it came to be used as a sweet seasoning. Many cookbooks published in the late Edo era carry recipes for various dishes using mirin, suggesting that mirin had become established as a basic seasoning at that time.

Mirin is made by adding shochu or an alcohol for brewing to steamed glutinous rice and rice koji and allowing the ingredients to ferment for 40 to 60 days. For this reason, production of mirin does not involve the process of alcoholic fermentation. The enzymes in *Aspergillus oryzae* are allowed to act in the presence of alcohol, which serves as a preservative, and as a result, sugar is produced by the saccharification of starch, and amino acids are produced by protein degradation. The reason that glutinous rice is used is that glutinous rice starch is less prone to retrogradation compared to non-glutinous rice starch, and is more susceptible to the action of the enzymes in *Aspergillus oryzae*. Moromi after fermentation is pressed into products, and the residue after the pressing is used as mirin lees for making pickles or in pickling media for making pickled fish or meat.

Glucose constitutes 80% to 90% of the sweetness components of mirin, but it contains other various sugars such as isomaltose, kojibiose, maltose, and



α -ethylglucoside. This gives mirin its complex, elegant sweetness.

Mirin is also called hon (true) mirin. There are seasonings similar to mirin such as seasonings that mimic mirin and fermented seasonings (cooking sake). Seasonings that mimic mirin do not contain alcohol and are made by blending additional sugars. Fermented seasonings are made by adding salt to a salt level of approx. 2% after alcoholic fermentation for making the products undrinkable.

Hon (literally, “true”) mirin falls under the category of alcoholic beverages, but is not suitable for drinking. It is used as a seasoning. There is another sweet alcoholic beverage that falls under alcoholic beverages but is used in cooking like hon mirin. It is called “akumochizake” and is brewed with a small amount of water and matured with wood ash added. As a result, a dark brown, sweet alcoholic beverage is produced. Akazake of Kumamoto Prefecture, Jizake of Kagoshima Prefecture, and Jidenshu of Shimane Prefecture are well-known akumochizake.

Vinegar

Driving force behind today's sushi

Vinegar is a liquid seasoning made from rice, barley, fruits, or other ingredients by fermentation with acetic acid bacteria. The origin of the word vinegar is vinaigre (vin+aigre) in French, which means sour wine. As suggested by the word origin, vinegar was made as a result of ethanol contained in an alcohol beverage being changed into acetic acid by acetic acid bacteria and turning sour, and thus has a close relationship to alcohol.

In the around 4th century, vinegar production technology was introduced from China to Japan together with the sake production technology. Rice vinegar first became popular in Japan, and the method of mixing rice koji, steamed rice, and water in a vat and allowing the mixture to ferment in a static state (static fermentation) was used until the Edo era (the late 19th century).

In the Heian era, a small dish containing vinegar was placed as a seasoning on nobles' dining tables together with salt and sake, and they ate dishes with these seasonings. It is believed that because there were no effective means of preserving foodstuffs at that time, foodstuffs had to be disinfected with seasonings. In the Kamakura and Muromachi eras, vinegared dishes such as namasu (thin strips of raw meat or fish dressed with vinegar) became popular. In history books describing the Edo era, there appear many descriptions of vinegar mixes made of vinegar and spices or miso.

It is the Heian era when a vinegar production method appeared for the first time in a Japanese book, and vinegar described in books published since then throughout the Edo era is mostly rice vinegar. When it comes to other kinds of vinegar, only several descriptions of sake vinegar (vinegar that was produced as sake but turned sour) can be found. This suggests that rice vinegar was developed in Japan since ancient times.

Production methods for vinegar

In Japan, production methods for vinegar are roughly divided into two types: one is the traditional surface fermentation (static fermentation) method, and another is the deep fermentation method (fermentation by stirring for aeration) used for the industrial production of vinegar products. The pot vinegar production method handed down in some regions, including Fukuyama, a town in Kagoshima Prefecture, is typical of the former method. This is the method of putting rice koji, steamed rice, and water in pots and leaving them outside for 6 months. In the pots, lactic acid fermentation and saccharification occur, and then when alcoholic fermentation starts, acetic acid bacteria soon form a thin film on the liquid surface and carries out acetic acid fermentation. This means that the enzymes in *Aspergillus oryzae* work as well as lactic acid bacteria, yeast, and acetic acid bacteria carry out fermentation sequentially in the pots, and then vinegar is produced through these processes. Acetic acid fermentation ends in six months, but the content in the pots is allowed to further mature for six months to three years. During this period, the liquid turns a dark golden brown. For this reason, vinegar produced by this method is called "kurozu (meaning "black vinegar")." Kurozu is made by using unpolished rice or rice that is not completely polished, and using 4.5 times more rice than general rice vinegar. As a result, kurozu is especially rich in amino acids.

On the other hand, the deep fermentation-based rice vinegar production method involves two fermentation steps. Sake is made from rice first, and then an acetic acid bacterial film is inoculated into the



Pot vinegar production being conducted through traditional surface fermentation (Kirishima City, Kagoshima Prefecture)
© K.P.V.B



sake to allow static acetic acid fermentation to take place. Grain vinegar made using sake lees obtained as a by-product of sake production is called kasuzu (lees vinegar). By maturing sake lees for a certain period of time, adding water, then inoculating an acetic acid bacterial film into the mixture to allow static fermentation to take place, kasuzu can be produced. As the sugar and amino acid content in sake lees increase during maturation, sake lees look red, which reflects the progression of the Maillard reaction (*) during fermentation and maturation. Dark red kasu vinegar is called "red vinegar".

Uses for vinegar

Among the various kinds of vinegar, it was rice vinegar that had been mainly developed until the Edo era in Japan. Since the Meiji era, various kinds of vinegar, including fruit vinegar, has become popular with the advancement of vinegar manufacturing technologies. Rice vinegar mixed with miso, soy sauce, soup stock, spices, etc. has been used in Japanese dishes. Rice vinegar is used to make vinegared rice, and kasu vinegar is especially said to go well with Edo-style nigirizushi (small rice balls with a slice of raw fish on them). Kasuzu, which is less expensive than rice vinegar, contributed to the spread of today's sushi and has grown in demand with the development of sushi.

Vinegar gives dishes a sour taste as well as removes unpleasant odors from foodstuffs, makes the colors of foodstuffs look bright, has a strong disinfectant capability, making and foodstuffs last longer. Thus, it has various beneficial effects when used in cooking and helps to preserve foodstuffs longer. In addition, recent studies have identified various functionalities of vinegar, and it has an increasingly wide variety of uses.

* A reaction that takes place between sugar and amino acids, etc. when heated and produces melanoidin, a brown substance

Pickles

Fruit of the wisdom from taking advantage of regional climates

Japanese pickles are divided into unfermented pickles and fermented pickles. Asazuke (lightly soaked pickles) and chomizuke (seasoned pickles) are made by soaking foodstuffs in salt, shoyu,

or other seasoning liquids for a short time to saturate them with the seasoning's taste, and are unfermented pickles. On the other hand, it is said that the root of fermented pickles is kusabishio, a kind of Chinese hishio made by salting and fermenting foodstuffs. Vegetables were salted to improve their shelf life and fermentation gave them a better flavor. Then, these vegetables came to be eaten as fermented pickles. This technique soon spread across Japan, and local fermented foods made by using local vegetables have



since developed.

In the process of making fermented pickles by salting, etc., lactic acid bacteria,

etc. adhering to vegetables can grow during salting, which increases the flavor unique to fermentation. First, as lactic acid bacteria grow, a decrease in the pH due to lactic acid makes it difficult for undesirable bacteria to grow. At the same time, the lactic acid bacteria come to lose their growth power, and acid-resistant lactic acid bacteria (of a kind different from the previous lactic acid bacteria) instead start to grow. As fermentation proceeds, the cells of the ingredient vegetables are broken down to allow them to have a softer texture, and the fermentation components

soak into them to increase sourness or umami, producing a refreshing flavor unique to fermentation.

Nukazuke (rice bran pickles) is another kind of pickles made by using rice bran (powder generated in the process of polishing brown rice into white rice). In a rice bran bed, which is generally used repeatedly for long periods of time to create generations of lactic acid bacteria, yeast, and other various bacteria and produce the complex microbial flora in nukazuke. For this reason, nukazuke has a complex and unique flavor.

Pickles have developed as the fruit of the wisdom for preserving valuable foodstuffs longer, and it is said that there are more than 600 kinds of pickles produced in various regions in Japan. In Japan, there are a variety of marinades and pickling beds and a variety of ingredients that can be pickled as well as a variety of appropriate pickling methods for such marinades and pickling beds. By combining these elements, Japan became one of the world's largest producers of pickles.

Amazake

Fermented drink called a drinkable intravenous drip

Amazake is a very sweet drink made by adding water to rice koji or to rice koji and rice and heating the mixture to cause it to undergo saccharification. "Zake" in amazake means sake, but it is alcohol-free and does not fall under the category of alcoholic beverages.

Nihonshoki (Chronicles of Japan), a history book written in the Nara era, contains a description of amanotamazake, which is closer to amazake rather than today's sake, and is considered to have been very sweet sake made of rice koji but contained a very small concentration of alcohol. It is said that amazake came to be drunk by ordinary people in the Edo era, and especially in summer, many amazake peddlers were seen on the streets because people at that time tried to survive the hot summers by drinking amazake due to its high nutritious value, so much so that it was called a drinkable intravenous drip.

Amazake is made by mixing rice koji for making miso and water, or equal amounts of rice koji, cooked rice, and water, and heating the mixture at 55 to 60°C overnight. Cooked glutinous rice is often used. If rice koji is available, amazake can be made easily at home. However, with the booming of koji in Japan, many brewers have recently come to sell amazake.

In the process of producing amazake, an *Aspergillus oryzae*-derived amylase acts on starch, which is the main component of rice, to produce a large amount of glucose, which is the main component of amazake. In addition, rice protein is degraded by an *Aspergillus oryzae*-derived protease to produce peptides and amino acids.

Amazake also contains B vitamins as metabolites of *Aspergillus oryzae* and dietary fiber that composes *Aspergillus oryzae* cells and rice. Since amazake contains a large amount of glucose and vitamins and minerals necessary for sugar metabolism, it can be said to be a drink that quickly helps us recover from fatigue.

In addition, rice-derived glucosylceramide and N-acetylglucosamine in *Aspergillus oryzae*, which promote the synthesis of



collagen and hyaluronic acid involved in helping our skin retain water, can be expected to provide beauty effects.

Amazake may also be made by using sake lees rather than rice koji, and amazake made of rice koji is called koji amazake, and amazake made of sake lees sakekasu amazake. Sakekasu amazake is made by dissolving sake lees in hot water and adding sugar, and may contain traces of some alcohol left in sake lees. The production of sakekasu amazake does not involve a fermentation process, but sakekasu amazake can also be said to be a fermented drink as sake lees is a by-product of brewing sake.

Column

Power of fermented seasonings to bring umami out of foodstuffs

Many basic seasonings used in Japan are fermented seasonings, and koji is indispensable for producing these seasonings. The main role of koji is to decompose foodstuff components. For example, in the process of producing amazake, the amylolytic enzymes produced by *Aspergillus oryzae* decompose rice starch into glucose, giving amazake its rich, sweet taste. In the process of producing shoyu, the proteolytic enzymes produced by *Aspergillus oryzae* decompose soybean protein and wheat protein into amino acids, which give rich umami to shoyu. Generally, protein and starch have no taste. They are decomposed by enzymes into amino acids or glucose, which produces umami or sweetness.

However, taste is complex. The 20 amino acids can be arranged and bonded in a certain sequence to make straight chains, which is folded into a giant protein molecule. The bonds holding the chain together

are called peptide bonds, and a series of several amino acids connected by peptide bonds is a peptide. Peptides are always contained in fermented foods. Proteolytic enzymes from *Aspergillus oryzae* can break this peptide bond and fragment proteins. These 20 amino acids include not only glutamic acid, which produces umami, but also glycine and alanine, which produce sweetness, and valine and leucine, which produce bitterness. Thus, amino acids have a variety of tastes, and when they connect to make a chain through peptide bonds, a completely different taste is produced. Different peptides of amino acids arranged in different sequences produce different complex and thick tastes or robust tastes. The rich and complex tastes of fermented foods come from peptides, and *Aspergillus oryzae*, which produces these peptides, is the source of fermented foods.