Chapter 2

Japanese Vinegar: Pair *'Komesu'* with *Sushi*. Drink *'Kurosu'* for Your Health

Rice vinegar is a traditional seasoning used in China, Japan and Korea. Japanese rice vinegars are slightly milder and sweeter than Western vinegar, therefore it is more appropriate for making *sushi*. In this chapter, I write about Japanese vinegar, *kurosu* in particular, which became popular in the past because it was said to promote health.

2.1 Vinegar, made from alcoholic beverages

The original meaning of Chinese characters, '酢' or '醋' which have come to mean vinegar now, was that vinegar is made (作) from *sake*, '酉', or alcoholic beverages, or to have been *sake* before. As these Chinese characters show, vinegar is made from alcoholic beverages. Therefore, people in Mesopotamia, Egypt, and China, who made alcoholic beverages, were able to produce vinegar more than 4,000 years ago. The character '酢' was used during the Han-dynasty (漢) (202BCE – 220 CE) in China, so it is supposed that the technique of vinegar fermentation was brought to Japan together with this character before Japanese envoys went over to China during the Sui dynasty (隋) (581~618).

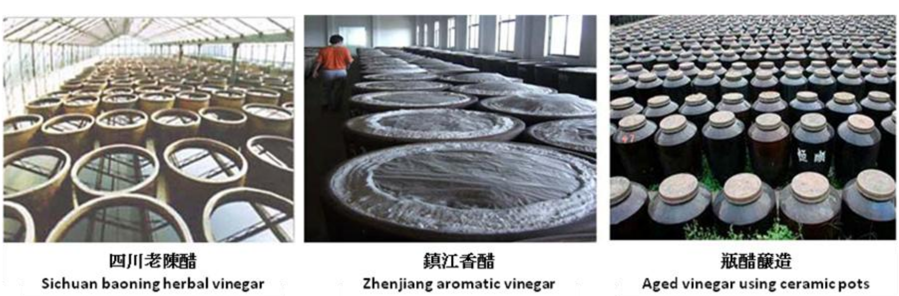


Fig. 2.1 *Traditional vinegar production with ceramic pots in China.*
(Pictures provided by Dr. F. Chen of Huazhong Agricultural University, China.)

In China, vinegar is still made mainly in ceramic pots (Fig. 2.1), and some Japanese vinegar breweries are also making vinegar with old-fashioned brewing style (Fig. 2.2).

Fig. 2.2 *Traditional vinegar production with ceramic pots in Japan.*
(Pictures provided by Mr. Nakano of Tobaya-suten Co., Toyama, Japan.)

2.2 The Qimin Yaoshu and the origins of vinegar

The Qimin Yaoshu, represented as 斉民要術 in Chinese characters, is said to be the oldest book written about food production and cooking. It was written during the Northern Wei Dynasty by the official Jia Sixie about 1,500 years ago (Fig. 2.3). It contains 23 vinegar production methods. Even now, Chinese vinegar is made from millet, barley, foxtail millet, glutinous millet, or sake lees rather than rice, which is preferred by the Japanese (Fig. 2.3). Instructions in the Qimin Yaoshu have precise descriptions: “You should use fresh water drawn from a well very early in the morning”, “Vinegar is destroyed by dropping single hair”. These remarks touch on hygienic education during the manufacturing process. We find other descriptions: “Jars should be covered and placed in the shade”, or “You should gauge the fermentation process of vinegar by the degree of appearance of patina on a bronze sword put on the cover of a jar”. This comment refers to measuring acidity, as they lacked our modern-day pH sensors. “White mold should be removed, or pushed to the bottom”. This description explains that white mold, or membrane-forming yeast, requires oxygen and dies if pushed to the bottom. Another description, “living organisms grow velvet coats in10 days”, shows the reader that the living organisms are living acetic acid bacteria. These descriptions tell us that ancient Chinese scientists already knew that living organisms were engaged in fermentation 1,300 years before Dr. Louis Pasteur discovered that fermentation is caused by living organisms.

Fig. 2.3 *Description of vinegar brewing in ‘Qimin Yaoshu’, the oldest agricultural book in China.*

The book was written by Jia Sixie in the Northern-Southern Dynasties (420-581 AD) and 23 different methods of brewing vinegars are recorded in detail. (Pictures quoted from, ‘斉民要術’, (eds.) S. Tanaka, R. Kojima and Y. Ohta, Yuzankaku Syuppan Co., Tokyo, 1997).

2.3 Rice vinegar made from Japanese *sake*

Figure 2.4 shows the production of *komesu* (amber vinegar) and *kurosu* (black vinegar) by using the method of static fermentation of a vinegar producing factory in the historical city of Nara, located in central Japan. Static fermentation is a traditional method without blowing air into a tank or stirring.

It is not a fast fermentation method, takes one month or more and requires fermentation by resting materials in barrels, jars, or containers of porcelain enamel. *Komesu* is made of polished rice, but *kurosu* is made of unpolished rice, with the husks still on the rice grain.

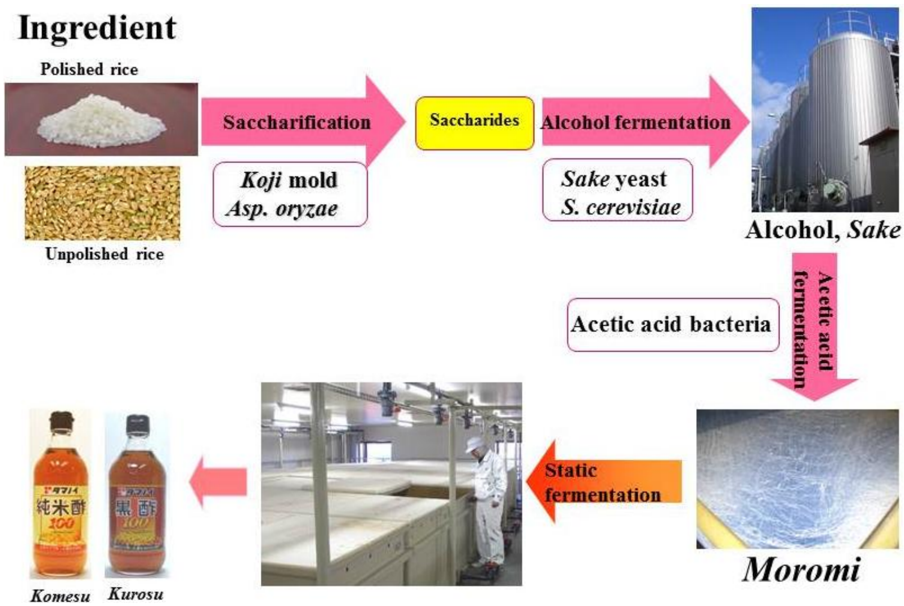


Fig. 2.4 *A flow sheet for Japanese rice vinegar fermentation.*

Komesu and *Kurosu* are produced from polished and unpolished rice, respectively, by the same process; saccharification of rice starch by *koji* mold and alcohol fermentation by *sake* yeast. An alcoholic liquid broth with vinegar wash and acetic acid bacteria called *moromi* is used to further the static fermentation process. (Pictures provided by Tamanoi Vinegar Co. Ltd. Nara, Japan.)

Unlike grapes used in winemaking, rice itself doesn't contain glucose, so the starch which is contained in rice should first be changed into saccharides, glucose, and maltose. This process is called saccharification. The starch in rice is degraded into glucose by amylase, an enzyme produced by *koji* mold. After that, this glucose is converted into alcohol by yeast enzymes. This process is called alcohol fermentation. Yeast by itself cannot convert starch into glucose, so starch must first be converted into glucose by the *koji* mold.

Fig. 2.5 *Transferring inoculation of a crepe pellicle of acetic acid bacteria.*

The crepe pellicles of acetic acid bacteria that cover the *moromi* surface are scooped up with a mesh bowl and gently floated into the pellicle layer of a new *moromi*. (Picture provided by Tamanoi Vinegar Co. Ltd., Nara, Japan.)

The above-mentioned processes are the same as those of Japanese *sake* production (Chapter 3). Japanese *sake* becomes sour if it is left to ferment for too long. This is because naturally occurring lactic acid bacteria metabolize sugar into lactic acid, or acetic acid bacteria metabolize alcohol into acetic acid. In Okinawa, an island located in the south of mainland Japan, there is a kind of vinegar called Ryukyu Koji Vinegar, which contains citric acid made with *Awamori koji* mold. It has a sour flavor and is healthy, but strictly speaking, it is not classified as vinegar. In producing vinegar, acetic acid bacteria play an important role in converting alcohol to acetic acid. This process is called 'acetification' or 'acetic acid fermentation'. Acetic acid bacteria cannot live without oxygen. Therefore, they float on the surface to access the air growing and forming a crepe-like skin. The crepe-like skin, or the living velvet coats described in the *Qimin Yaoshu*, is scooped up and transferred to new *moromi* and fermented. *Moromi*, which is a term concerned with Japanese *sake* fermentation, is a mixture that is created before filtering after saccharification and alcohol fermentation during the production of Japanese *sake*. The crepe-like skin of acetic acid bacteria are transferred to the new *moromi* (Fig. 2.5). As a result, alcohol in the *moromi* is converted to acetic acid by acetic acid bacteria.

Our ancestors passed on the traditional technique of producing *komesu* and *kurosu* for centuries. During that period, fundamental pasteurization as we know it was not performed.

2.4 What was the true character of bacterial pellicles kept up for one century

I was once asked to investigate which kinds of bacteria 100-year-old crepe-like bacteria skin contained. It seemed interesting, so I genetically analyzed them according to the methods of the time. We isolated around 200 samples of different bacterial strains contained in fermented *moromi*, and examined them using Polymerase Chain Reaction (PCR) with several special fragments of DNA sequences. This method allowed us to examine the minor differences in DNA sequences between species and strains.

PCR was an epoch-making tool that amplifies a piece of a small amount of DNA and generates tens of thousands of copies of that DNA. Life science has developed remarkably after the discovery of PCR. This method is used for DNA profiling, used in criminal investigations for identifying an individual by a bloodstain or hair left at the crime scene.

The bacteria identified from the genetic analysis were classified into groups A and B (Fig. 2.6). We classified the bacteria of 16SrRNA base sequences from both groups as the bacterial species *Acetobacter pasteurianus* (Fig. 2.7).

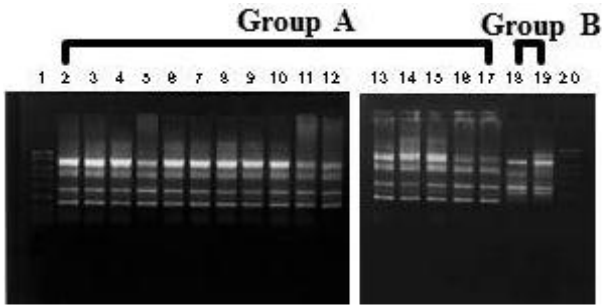
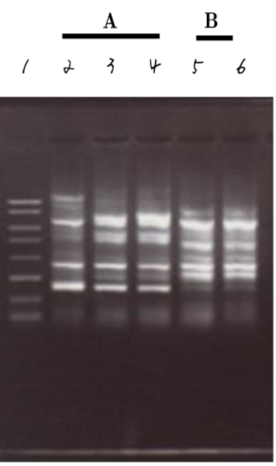


Fig. 2.6 *Analysis of acetic acid bacterial species isolated from Kurosu fermentation by the ERIC-PCR method.*

(K. Nanda et al., Applied Environmental Microbiology 67: 986-990, 2001).



1. Molecular size marker

2. *Acetobacter pasteurianus* ATCC33445^T

3~6. Isolated strains from *moromi* mash

Fig. 2.7 *Identification of acetic acid bacterial strains during fermentation of kurosu.*

Isolated strains 3 and 4 from the surface of moromi mash were the same as the type cultural strain, *Acetobacter pasteurianus* ATCC33445^T. (K. Nanda et al., Applied Environmental Microbiology 67: 986-990, 2001).

Scientific names of biological species are written in Latin, and may appear difficult at first. *Acetobacter* is a genus name and *pasteurianus* is a species name. For example, the scientific term of modern human beings is *Homo sapiens sapiens*.

2.5 Recent taxonomy uses molecular biology

I hope you will go on reading this chapter, taking a look at Figure 2.8, even if it appears difficult. I feel that it helps us understand the life was made beautifully. I have already told you that we found a bacteria species with the 16SrRNA base sequence. How did this become possible?

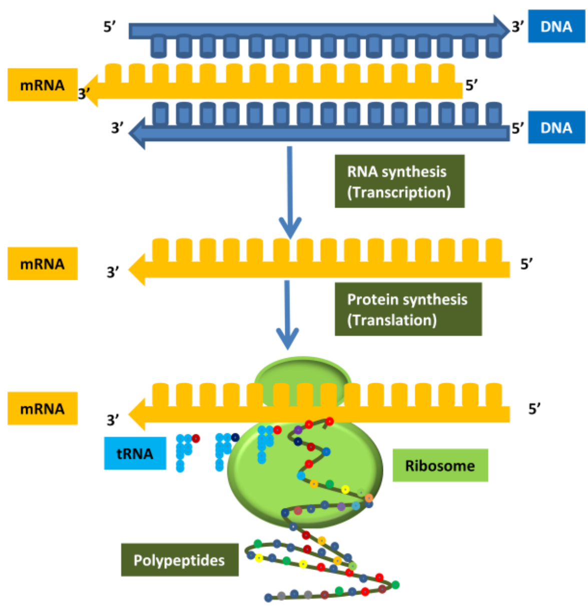


Fig. 2.8 *Basic genetic information from DNA to RNA to proteins.*

In all living cells, genetic information is streamed down from DNA to RNA (transcription) and then from RNA (mRNA) to proteins (translation). Amino acids carried by tRNA are transferred into polypeptides (protein) in ribosomes, devices for protein synthesis. DNA and RNA are elongated from 5’ terminal to 3’ terminal of the chains.

All living organisms without exception have ribosomes which perform the important task of forming proteins by translating information of messenger RNA (mRNA) into amino acid sequences. Ribosomal RNA (rRNA) contained in this ribosome is essential for living organisms, so they have been preserved throughout evolution. Minor variations in base sequences can be seen according to species. We can find out genus and species of a living organism by determining this rRNA sequence. 16SrRNA is a sequence of rRNA that can be found in all bacteria. Living organisms from bacteria to human beings have the same molecular mechanisms of transferring the DNA information to protein. As seen in Figure 2.8, they transcribe the information of DNA to mRNA (transcription) and translate it into amino acid sequences of proteins in ribosomes (translation). When information of mRNA is translated into amino acid sequences, tRNA, stuck with an amino acid, acts. 'tRNA' is an abbreviation for transfer RNA. Ribosomes are consisted with rRNA and ribosomal proteins. rRNA in the ribosome leads mRNA and tRNA to the correct position to elongate amino acid chain of protein and catalyzes the translocation to peptides, which are short amino acid chains.

Most genetic information of living organisms is recorded in DNA. However, there are exceptions, such as RNA, the influenza virus, or AIDS virus. It was found out that RNA itself is a catalyst and now we think that RNA took form at the beginnings of life. However, RNA is unstable, so it is thought that DNA, which is similar to RNA but stable, took its place.

During 1950-1970, when molecular biology progressed rapidly, we came to understand the structures and their mechanisms of living organisms as beautiful and rational.

2.6 My research at NIH

I began my molecular biological research of rRNA at the National Institutes of Health, Bethesda, Maryland, shortly after I first came to the USA in 1970. I succeeded in producing rRNA in a test tube similar to that in living cells and studied the regulation of RNA synthesis. In those days, life science was being advanced through the study of the *Escherichia coli* bacterium. The viruses (phages), which infect the bacteria were studied to elucidate the mechanisms of life at the molecular and genetic level. However, in those days, the genetic structures of higher organisms including human beings, plants, yeast and *koji* mold were not explained clearly.

Recombinant genetic technology or genetic engineering was born as a result of molecular biology in 1973. This technology resulted in rapid expansion of life science and led to new progress. I would like to introduce the remarkable results of life science elucidated by genetic engineering, but it is not directly related to Japanese foods, I will skip it for now.

2.7 Traditional fermentation techniques passed on for generations

During 1 month of vinegar fermentation by acetic acid bacteria, Group A strains were predominant and group B strains appeared a little towards the end of fermentation. I found that the bacterial strains of *komesu* and *kurosu*, whose living bacteria on the surface of *moromi* have been passed on for a century, were composed of an entirely uniform acetic acid bacterium named *Acetobacter pasteurianu*s. This result demonstrates that vinegar producers carefully kept the technique of preventing various bacterial contaminations.

Soon after that, I submitted the result of my research to the American Society for Microbiology, and American scientists valued my paper as the first example which elucidated traditional fermentation scientifically, and accepted my paper in an influential journal[*1].

2.8 Traditional Italian ingredients from the Emilia Romagna Region

A professor at Reggio Emilia University, Italy, invited me to give a lecture at the first international conference on acetic acid bacteria and vinegar. I was the first that examined the bacterial flora present during acetic acid fermentation in detail in spite of the large number of vinegar producing companies in the world.

Fig. 2.9 *Reggio-Emilia, an old town in Italy.*

I would like to introduce a few famous Italian cities where traditional foods are produced. First, Reggio Emilia (Fig. 2.9), where this conference was held, is a city in northern Italy, in the Emilia Romagna region. Bologna, Modena and Parma are near Reggio Emilia. Bologna is well known for its rich gastronomical tradition and universities. The University of Bologna, the oldest university in Europe, is in this city. On the 901st anniversary of the foundation of the University, I gave a lecture on genetic manipulation of lactic acid bacteria to the pharmaceutical students of the University in the old lecture theater, where dissection of human cadavers were held many years ago. Modena is very famous for its production of balsamic vinegar. Parma is famous for Parma ham and Parmigianino Reggiano Cheese, known as Parmesan Cheese in the USA (Fig. 2.10). After the international meeting in Reggio Emilia, I visited this city, for I had been asked to give a lecture by an acquaintance of a professor of the University of Parma. My topic was on biological remediation technologies, not on vinegar. At the time, I was mid- research on this subject. Our research theme was to clean up the contamination of heavy metals like cadmium from rice fields by using a leguminous plant, Chinese milk vetches.

Fig. 2.10 *Parmesan cheese produced in Parma, Italy.*
A master produces parmesan cheese in his cheese factory in Parma.

2.9 Traditional Italian balsamic vinegar

Italy takes pride in their balsamic vinegar, traditional wine vinegar. Until the 19^{th} century, it was produced monopolistically by the aristocrats of the region, the House of Este Dutch. I had a lucky chance to see the process of balsamic vinegar production. First, they put pressed grapes into big barrels (Fig. 2.11). Grapes contain grape sugar, glucose, and native grape yeasts convert grape sugar into alcohol. This alcohol is converted by acetic acid bacteria living in the barrels into acetic acid, or vinegar. The barrel-heads are then covered with cotton cloth. Moisture evaporates, so the vinegar is concentrated and thus transferred to small casks little by little every year. Finally, the vinegar ages and becomes sweet, delicious, and thick. Unless it is aged more than 12 years, it is not authorized as true balsamic vinegar. This certainly justifies the expensive price of traditional balsamic vinegar. If you buy it cheap, it is probably not traditional balsamic vinegar. 'Aceto' and 'balsamic' mean vinegar and good perfume, respectively in Italy.

Fig. 2.11 *Traditional Balsamic vinegar production in Modena, Italy.*

New cooked grape must is sequentially transferred from a large barrel to a small barrel set every year. In the barrel inhabitant yeasts and acetic acid bacteria ferment grape sugar to alcohol and acetic acid (vinegar), respectively. None of the product of the traditional balsamic vinegar may be withdrawn until the end of the minimum aging period of 12 years.

I was served ice cream with balsamic vinegar on it by an Italian girl at the welcome party. I was surprised to know that ice cream goes well with vinegar. It goes without saying that vinegar goes well with meat. I am sure that balsamic vinegar is also good for health.

It was through these experiences that I came to be regarded as a specialist in vinegar and I subsequently published two books about vinegar[*2, 3].

2.10 Why is vinegar good for our health?

Is it true that *kurosu* (Fig. 2.12) is healthy? Vinegar has been used as a remedy all over the world, in Europe, the Islamic world, China, and Japan. Vinegar was thought to be useful for treating inflammatory diseases. In Europe, cider vinegar as well as wine vinegar is used as a flavor agent and also to preserve foods. It is popular as a folk medicine and is suggested as a remedy to various diseases, not only obesity and arthritis but also for asthma, coughs, diarrhea, colitis, eczema, and hair loss.

Fig. 2.12 *Monitoring the kurosu fermentation process in a ceramic pot.*
(Picture provided by Sakamoto Brewing Co. Ltd., Kagoshima, Japan.)

Recently, scientific research has been made public as to the effects of *kurosu*. Results show that drinking *kurosu* every day for 12 weeks caused lower blood pressure in mild hypertensive subjects (24 men and 47 women) (Fig. 2.13). Other research has been pursued as to the effects of preventing the growth of cancer cells on mice (Fig. 2.14) and the anti-cancer effects against cultured cancer cells were found out.

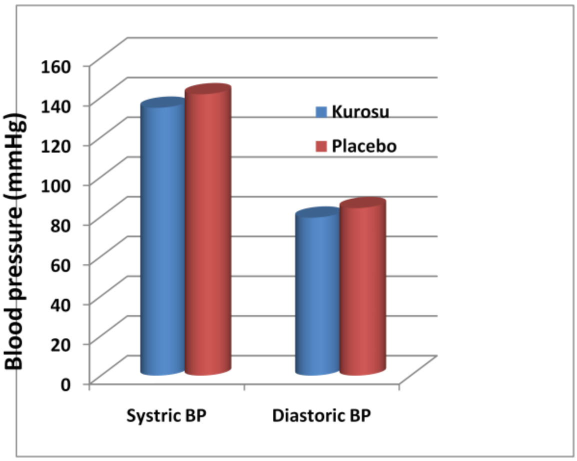


Fig. 2.13 *Hypotensive effect of kurosu on mild hypertensive subject.*

A randomized double blind, placebo-controlled study was conducted on 71 subjects (24 men and 47 women; average age 47.4) with mild hypertension without treatment. Subjects were given 7.5 mL (250 mg acetic acid) of each drink per day for 12 weeks. (S. Sugiyama et al., Japanese Pharmacology & Therapeutics 36: 429-444, 2008, in Japanese).

This research has been pursued through the collaboration of large Japanese vinegar companies and universities. European and American vinegar production companies have not made forays into this kind of fundamental research. These Japanese company researchers may have been influenced by their experimental training and senior and master theses.

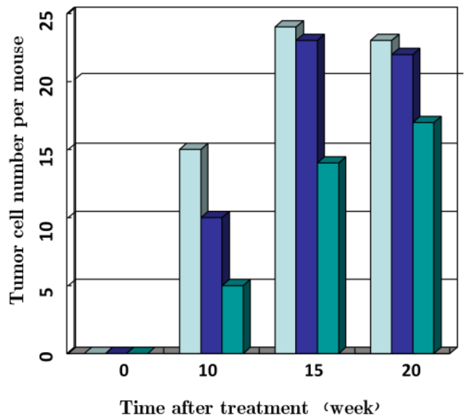


Fig. 2.14 *Inhibition of tumor cell growth by oral feeding of kurosu extracts in mice.*

Bar graphs from left to right: TPA (carcinogenic agent) treatment, TPA + kurosu (0.1 mg/0.1 ml), and TPA + kurosu (1 mg/0.1 ml) were orally fed and then determined tumor cell numbers at 0, 10, 15 and 20 weeks after the administration. (S. Nishidai et al., Bioscience Biotechnology and Biochemistry, 64: 1909-1914, 2000; K. Nanda et al., Journal of Experimental Clinical Cancer Research, 23: 69-75, 2004).

The difference between *komesu* and *kurosu* is as follows; *komesu* is made of polished rice, whereas *kurosu* is made of unpolished rice. These rice vinegars are produced by the static fermentation method, by which ingredients are fermented and aged slowly and carefully. It is not produced by the fast fermentation method known as the Orleans method, by which air is blown in tanks and materials are fermented rapidly. Figure 2.15 shows traditional *kurosu* fermentation using ceramic pots, and it is known as '*Fukuyama Kurozu'* of Kagoshima Prefecture, which is located in southern Japan. Fermentation which uses pots involve various kinds of *koji* mold, yeasts, lactic acid bacteria, and acetic acid bacteria because saccharification of unpolished rice starch, alcohol fermentation, acetic acid fermentation and maturing are done simultaneously (Fig. 2.16). In these traditional fermentations, and probably in traditional balsamic vinegar fermentation too, various kinds of microbes communicate and work together to form a microbial community called a 'biofilm'.

Fig. 2.15 *Ageing of Fukuyama-Kurozu production in Kagoshima, Japan.*

In ceramic pots, steamed rice, *koji* mold and water are mixed and fermented for several months using the combinational parallel fermentation process. Yeasts and acetic acid bacteria are in the ceramic pots. (Picture provided by Sakamoto Brewing Co. Ltd., Kagoshima, Japan).

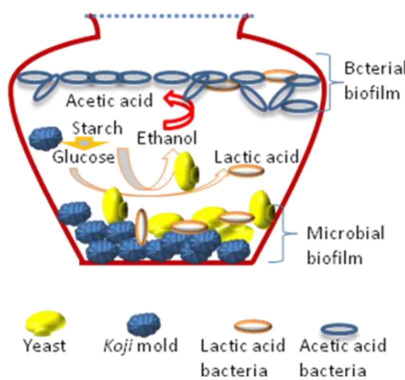


Fig. 2.16 *Schematic representation of microbial community formation in Fukuyama kurozu fermentation.*

Koji mold, yeasts and lactic acid bacteria are floating or sink and form biofilm at the bottom of the pot and acetic acid bacteria form biofilm on the surface of fermented *moromi.*

Kurosu contains amino acids, lipids and organic compounds like vitamins taken from rice bran and rice grain husks. It also contains 5-aminolevulinic acid (ALA), which can activate mitochondria, used as source of chemical energy. The amount of ALA in *kurosu* is 10 times the amount found in spinach or bell pepper and 7 times that of soy sauce or *natto*. According to previously published reports, *kurosu* has the following health benefits:

1. anti-inflammatory effects,
2. prevents lipid peroxidation,
3. lowers blood sugar levels,
4. promotes metabolism and prevents obesity,
5. lowers blood pressure,
6. promotes absorption of calcium in cells and prevents osteoporosis,
7. anti-cancer effects.

As these effects were known well, *kurosu* has become very popular among Japanese people several years ago. Chocolate which uses Japanese *kurosu* instead of balsamic vinegar is popular among London food connoisseurs. However, there is no custom of drinking vinegar in Europe or America. It is mainly used as salad dressing. Since many kinds of dressing are available at restaurants in Western countries Asian people sometimes feel it difficult to choose dressings. When I am asked what dressing I want, I make it a rule to answer, "Thousand Islands, please".

2.11 Rice vinegar demand will rise with *Sushi*

Although vinegar is produced all over the world, Japanese rice vinegar is being exported abroad. There is no rice vinegar in foreign countries, which is indispensable to *sushi* rice because *sushi* requires mild rice vinegar. However, total exports are valued at about 14 million USD. I am sure this amount will increase in the near future.

Like balsamic vinegar was once protected from imitation balsamic vinegar produced in countries other than Italy by certification from the Italian government, I think Japanese *kurosu* should be protected internationally, labeling, "This *kurosu* was produced from rice by static fermentation and aging". I hope Japanese *kurosu* will become popular internationally as a health beverage.

2.12 Summary

Japanese rice vinegars, *komesu* and *kurosu* are made of polished and unpolished rice, respectively. These rice vinegars are produced by the static fermentation method, by which ingredients are fermented with *koji* mold, yeast and acetic acid bacteria and are then set aside to age. *Komesu* is amber and it has mild sweet-sour taste, and is used for making *sushi*. *Kurosu* contains many amino acids, lipids and physiologically active compounds. These compounds include vitamins and 5-aminolevulinic acid taken from rice bran and rice grain husks. Thus, *kurosu* has the following health merits: anti-inflammatory effects; prevention of lipid peroxidation, obesity and osteoporosis; lowering of blood sugar levels and blood pressure; and potential anti-cancer effects. As these effects came to be well-known, *kurosu* has become a very popular health drink among Japanese people.

[*1] Nanda, K., Taniguchi, M., Ujiki, S., Ishihara, N., Mori, H., Ono, H., and Murooka, Y., Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. *Applied Environmental Microbiology*, 67: 986-990 (2001).

[*2] Stefano Mazza and Yoshikatsu Murooka, Vinegar through the time. In Vinegars of the World (eds. Paolo Giudici, Lisa Solieri), p. 17-39, Springer Milan (2008).

[*3] Murooka, Y., Vinegars in China, In Function and Science of Vinegars (eds. Japanese Society for Acetic Acid Research) (in Japanese), p. 21-30, Asakura Shoten, Tokyo (2012).