Enzyme Wave

Volume 8
2004

Lactic Acid Fermentation and Traditional Japanese Foods

Arteriosclerotic Diseases and Blood Cholesterol Measurements

Incorporated Status for Japanese National Universities

AMANO

Contents

Lactic Acid Fermentation and Traditional Japanese Foods·····2

Arteriosclerotic Diseases and Blood Cholesterol Measurements····5

Incorporated Status for Japanese National Universities·····7

The Art of Communication

In mid-April, I left the UK after staying there for three-and-a-half years to take a new assignment at our head office in Japan. I enjoyed my time in the UK and found it very impressive. A particularly memorable incident occurred one day in the souvenir shop in the British Museum. I met an elderly lady who wanted to buy some souvenirs. She asked the sales assistant what they cost, and the sales assistant replied. The lady informed the sales assistant that she wanted to buy the items. The sales assistant asked if the lady needed to have them gift-wrapped. The lady said no, but she needed a plastic bag. The goods were put in a plastic bag, as she expected. The elderly lady paid with a GBP10.00 bank note, and the sales assistant gave her the plastic bag and the change with thanks. In return, the lady said thank you very much.

This type of scene happens all over the world and would be ordinary without my additional comments. If I say this conversation was done in two languages, maybe many people will be impressed. The old lady was Japanese and could speak only Japanese, and the sales assistant was a British lady who spoke English.

This is a typical example of good communication without a common language. If we are determined to make our counterparts understand, they will do their best to understand. Good communication will result; as the old proverb says, "Where there is a will, there is a way."

Next spring, we will have the 2005 World Exposition, in Aichi, Japan, along with the construction of a new airport, Centrair (New Central Japan Airport). We are expecting to have more visitors than ever from all over the world. When you come to Japan, better communication will make your stay more enjoyable, with or without a common language.

Lactic Acid Fermentation and Traditional Japanese Foods

Introduction

Fermented foods add complexity to our eating habits. Fermented foods are made from milk, vegetables, grains, and other foods, and their scents and tastes are modified by microbial fermentation. Lactic acid bacteria are involved in many kinds of fermented foods: yogurt, cheese, sour bread, pickles, alcoholic drinks, soy sauce, and miso. Yogurt and cheese are the first foods people think of when discussing foods made by lactic acid fermentation. According to records, people in Japan ate a dairy food similar to cheese during the Asuka and Nara eras more than 1300 years ago. It was called *daigo* and is considered to be a food like condensed milk or butter oil. When Buddhism, which forbids meat-eating, was introduced into Japan, people gradually gave up manufacturing these dairy products. Therefore, details of these foods in the literature are sketchy. In this issue, we introduce steeped foods as traditional Japanese fermented foods in which lactic acid fermentation plays an especially important role.

What are steeped foods?

Steeped foods are divided into two types, depending on whether or not fermentation by microorganisms is an essential part of the process. When fermentation is involved, foods such as vegetables, fruits, fishes, meats, etc. are pickled in salt, rice bran, sake lees, soy sauce, miso, vinegar, etc. When microorganisms are not involved, these foods are simply pickled in seasoning soup. Steeped foods have become an important element of many cultures such as in Korea (kimchi), China (zatsuai), Europe (pickles) and other countries **(Photo 1)**.

Nuka-miso pickles

Japanese steeped vegetables in which lactic acid fermentation plays an important role are summarized in **(Table 1)**.

Pickling vegetables in *kome-nuka* (rice bran powder produced from polishing brown rice) is unique to Japan. *Kome-nuka* kneaded with salt, malted rice, soybeans, sake lees, etc. is called *nuka-miso* or *nuka-doko*. Cucumbers, eggplants, turnips, and other vegetables pickled in *nuka-miso* are a favorite Japanese side dish.

Inside *nuka-miso*, lactococci such as the *Pediococcus* genus proliferate at the beginning of the fermentation. Thereafter, as acid concentration increases, lactobacilli such as *Lactobacillus plantarum* and *Lactobacillus brevis* predominate. Lactic acid produced by these bacteria prevents contamination and growth of other unwanted bacteria and adds a sour taste to the pickles. In addition, B vitamins and other nutrients diffuse from *kome-nuka* into *nuka-miso* and provide additional nutrients not contained in fresh vegetables. Also, *kome-nuka* proteins are degraded into amino acids and add various flavors and tastes to *nuka-miso* pickles.

Different methods of preparing *nuka-miso*, each with a different taste referred to as "mother's taste", have been handed down within individual households.

Photo 1 : A scene of steeped food shop

Table 1. Japanese steeped vegetables in which lactic acid fermentation is involved.

Steeped in	Name of food	Preparation
salt	takana-zuke, pickled plum, tsubo-zuke, kan-zuke, nozawana-zuke, pickled shallots, etc.	Vegetables are steeped in salt with other minor ingredients with or without pretreatment overnight or for several months.
kome-nuka	takuan-zuke, nuka-miso pickles, etc.	Vegetables are dried in the sun or salt and steeped in kome-nuka with other minor ingredients.
non-salt	goishicha, awabancha, sunki, etc.	Leaves are steeped after pretreatments with boiling and steaming for rigorous lactic acid fermentation.
others	suguki, etc.	Special pretreatment is necessary, followed by heating and maturing.

Source: Lactic acid bacteria: Secrets of fermented foods for good health by Michio Kozaki

Takuan

Takuan is made of white radish pickled in *kome-nuka* and became popular in Japan during the Edo era. Freshly cropped white radish contains more than 90% water, and it is necessary to reduce the water content before pickling in *kome-nuka*. Takuan is divided into two types depending on the method used to reduce the water content: dried *takuan* and salt-pressed *takuan*. The manufacturing process is illustrated in **(Figure 1)**. *Takuan* is prepared by washing radishes; drying them in the sun to remove water **(Photo 2)**; steeping them in a large amount of salt (rough pickling), then in a small amount of salt (intermediate pickling); and finally steeping them finally in *kome-nuka*, salt, and seasonings (final pickling). Lactic acid bacteria appear during the process of rough pickling and then grow during the intermediate and final pickling processes. They add a specific sour taste and flavor to *takuan*. Since drying in the sun depends on the weather, it is not appropriate for mass production at a factory. In contrast, salt-pressing, a method to remove water from radishes by steeping them in salt, does not depend on the weather.

Steeped tea leaves

Teas are classified as green tea, oolong tea or black tea depending on how soon after picking the enzymes in the leaves are inactivated by heating. The three kinds of tea are called non-fermented tea, partially fermented tea and fermented tea, respectively. Fermentation in this case is performed by the enzymes in tea leaves and differs from that performed by microorganisms.

Special types of non-fermented tea like (*awabancha* in Tokushima) involve lactic acid fermentation. This type of tea is characterized by its sour taste. To prepare *awabancha*, the tea leaves are boiled and the broth is saved for later use, and the then leaves are placed in a tea roller to damage them mechanically. The damaged leaves and the broth are stuffed into a barrel, and a heavy stone is placed on the top of the cover to press the leaves. Thereafter, the leaves are dried in the sun.

A steeping process called "*akutai*" is commonly included in preparing the above kinds of tea; when this is included, these special types of tea are also called pickled tea leaves. *Akutai* provides an important place for lactic acid fermentation, which adds a sour taste and flavor to steeped tea leaves. *Lactobacillus plantarum* is among the primary lactic acid bacteria that grow during steeping process.

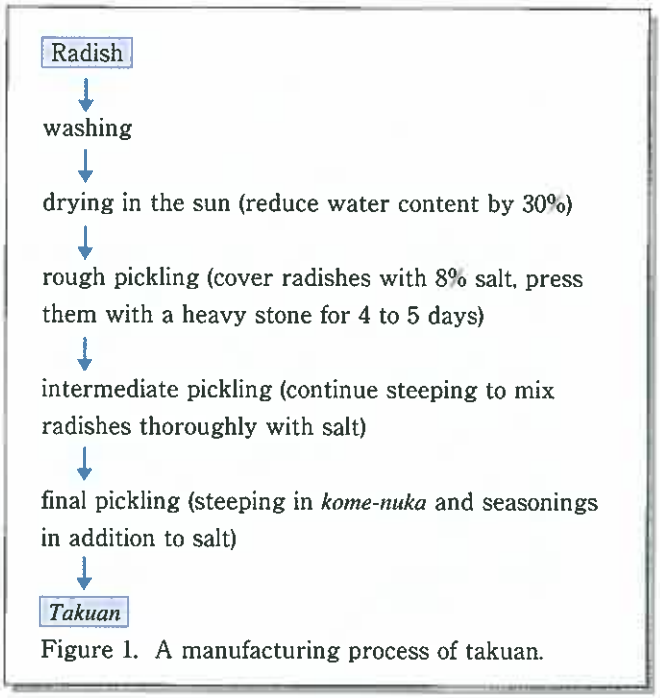

Figure 1. A manufacturing process of takuan.

Photo 2 : Drying of white radish in the sun.

Steeped fishes

Japanese steeped fishes in which lactic acid fermentation plays an important role are summarized in **(Table 2)**. These foods are prepared by steeping salt-embedded fishes with steamed rice or *kome-nuka*. The specific flavor that characterizes steeped fishes comes from lactic acid fermentation during the process of steeping fishes with steamed rice. Preparation of steeped fishes is characterized by its long period of rice steeping ranging from 4 months to 2 years. During this process, organic acids such as lactic acid, acetic acid, isovalerian acid, propionic acid and butylic acid, and extracts obtained from microbial fermentation and by autodigestion of fish meat are produced which create the flavor and sour taste specific to steeped fishes.

Concluding remarks

Lactic acid can guarantee better preservation of fermented foods in addition to better flavor. Lactic acid bacteria have been used as a medicinal preparation for controlling intestinal function. Lactic acid obtained by lactic acid fermentation is now being used as a raw material to develop polylactate, which has been familiar to us from antiquity, provides us with an enriched and healthy diet and may offer in the future the possibility for novel applications in new fields.

Table 2 . Japanese steeped fishes in which lactic acid fermentation is involved.

Name of food	Species of fish	Preparation
funa-zushi	crucian	Fishes are steeped in salt, then with steamed rice for more than 4 months.
nare-zushi	mackerel, saury, spotted shad, sweetfish, etc.	Fishes are steeped in salt, then with steamed rice for 7 to 10 days. Different fishes are used in different districts.
i-zushi	salmon, herring, trout, sandfish, etc.	Fishes are steeped in salt, then with steamed rice, malted rice, sake, and sweet sake seasoning. Vegetables such as carrot, cucumber, and ginger and seaweeds such as agar-agar and sea tangle are added. They are sometimes steeped with herring roe and salmon roe.
heshiko	sardine mackerel	Fishes are steeped in salt, then with kome-nuka and malted rice.
nuka-zuke	herring puffer fish	Fishes are steeped in salt, then with kome-nuka and malted rice.
	puffer fish ovary	Ovaries are steeped in salt for more than 2 years, then with kome-nuka for more than 1 year. The ovary contains tetrodotoxin, but finished products can be eaten.

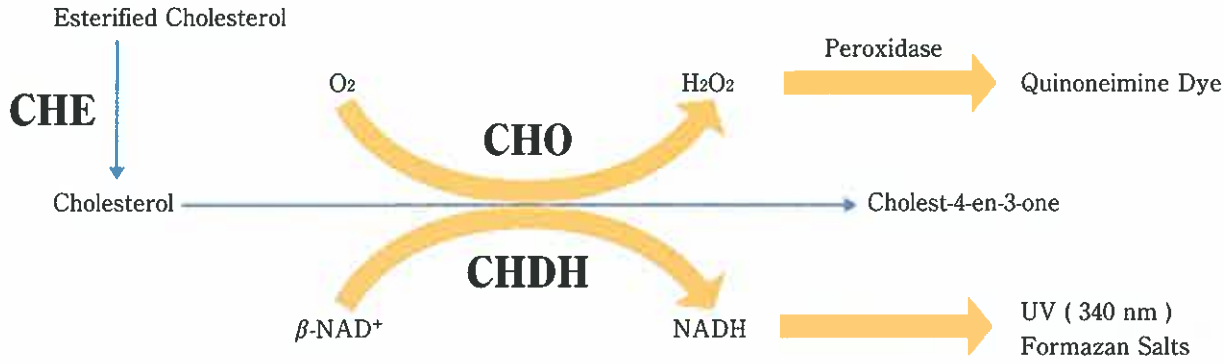

Figure 1. Enzymatic Determination of Cholesterol.
CHE , Cholesterol esterase ; CHO , Cholesterol oxidase ; CHDH , Cholesterol dehydrogenase.

Arteriosclerotic diseases such as cerebral infarction and ischemic heart diseases (myocardial infarction and angina pectoris) are one of the primary causes of death not only in European and American countries but also in Japan.
A large-scale epidemiological study indicated that obesity, aging, smoking, hyperlipidemia, and diabetes are the risk factors for arteriosclerotic heart diseases. A number of studies enabled us to draw a rough picture representing the process of arteriosclerosis from the accumulation of cholesterol in the wall of blood vessels to the manifestation of arteriosclerotic diseases.

Since the discovery of the relationship between blood cholesterol concentration and arteriosclerotic diseases, measuring cholesterol has been one of the most widely performed medical tests. This article will focus on how to measure blood cholesterol.

Transportation of blood cholesterol

Cholesterol is a component of the biomembrane and serves as a precursor for bile acid, steroid hormones, and vitamins. Cholesterol is absorbed from the diet through the small intestine or synthesized in the liver and transported to peripheral tissues via the blood stream. Since cholesterol is almost completely insoluble in water, it is transported via lipoproteins.

Lipoproteins are spherical granules encapsulated with a monolayer membrane consisting of phospholipids, cholesterol, and proteins. Cholesterol esterified with fatty acid and triglyceride constitutes the hydrophobic core of the sphere. The surface of the granule is composed of proteins, phospholipids, and the hydrophilic part of cholesterol, which makes the entire granule a blood-soluble, transportable lipid.

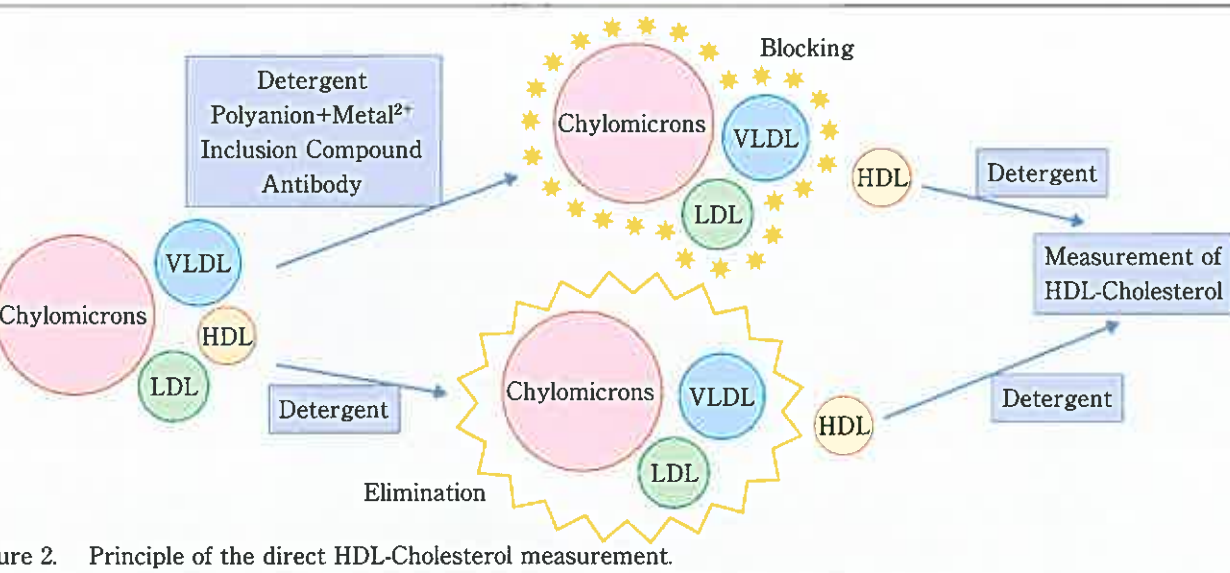

Figure 2. Principle of the direct HDL-Cholesterol measurement.
HDL, High-density lipoprotein ; LDL, Low-density lipoprotein ; VLDL, Very low-density lipoprotein.

Among lipoproteins, high-density lipoprotein (HDL) protects against atherosclerosis, but low-density lipoprotein (LDL) induces atherosclerosis. Therefore, to evaluate risk for atherosclerosis, it is necessary to separate HDL or LDL from other lipoproteins so that the concentrations of cholesterol contained in HDL and LDL can be measured separately.

Quantification of blood cholesterol

There are two methods for enzymatic measurements of cholesterol: the system using cholesterol oxidase (CHO) and the system using cholesterol dehydrogenase (CHDH). In the CHO system, hydrogen peroxide generated by the reaction catalyzed by CHO is mixed with a hydrogen donor and coupler to create color (quinoneimine pigment) by the action of peroxidase. In the CHDH system, NADH generated by the reaction catalyzed by CHDH is measured by absorption in the ultraviolet part of the spectrum or is coupled with electron mediators such as diaphorase and phenazinium compounds to form formazan salts, which can be measured by absorbance in the visible part of the spectrum **(Figure 1)**.

Distinguishing between cholesterol in HDL or LDL relies on 1) surfactants, 2) a variety of additives, and 3) specificity of enzymes used [cholesterol esterase (CHE), CHO, CHDH]. We optimize these factors individually when we measure cholesterols within HDL or LDL that possess various molecular sizes, surface characteristics, and molecular ingredients.

Blood total cholesterol concentration is measured by performing an enzymatic reaction after solubilizing all lipoproteins with surfactants. Esterified cholesterol is converted to cholesterol by the action of CHE to remove the fatty acids bound to cholesterol, and then cholesterol concentration is determined by the enzymatic reaction as described above.

The concentration of cholesterol within HDL is determined by the direct measuring method. In the direct measuring method, either the surface of the lipoproteins other than HDL are blocked by surfactants, clathrate compounds (cyclodextrin sulfate, calixarene), chemical modification enzymes, antibodies, etc. to inhibit enzymatic cholesterol oxidation, or cholesterol contained in the lipoproteins other than HDL is degraded. As the next step, a surfactant that can exclusively solubilize HDL is added so that cholesterol within HDL is measured. However, the above-described blocking method cannot completely block the cholesterol existing at the surface of the lipoproteins other than HDL; we therefore add polyanions and Mg^{2+} for complete blocking **(Figure 2)**.

Concluding remarks

Management of the risk factors described previously is essential to prevent arteriosclerotic diseases. To manage hyperlipidemia, especially hypercholesterolemia, it is necessary to monitor continuously. We believe our method to measure cholesterol within lipoproteins, especially HDL and LDL, as described in this article can contribute in some way to the possible future self-monitoring of blood cholesterol.

Table Amano's Diagnostic Enzymes for Cholesterol Determination

Product name	Origin	Activity
Cholesterol esterase "AMANO" 3	*Pseudomonas* sp.	≧ 15 u/mg
Cholesterol oxidase "AMANO" 1	*Burkholderia* sp.	≧ 1.5 u/mg
Cholesterol oxidase "AMANO" 6	*Microorganism*	≧ 10 u/mg
Cholesterol dehydrogenase "AMANO" 5	*Nocardia* sp.	≧ 5 u/mg

Incorporated Status for Japanese National Universities

The 89 national universities in Japan have long been a vital underpinning for the growth of the country, serving as centers of intellectual leadership in society through the development of human resources and academic pursuits. However, as the Japanese society has undergone major transformations, there have accordingly been changes in what is expected of national universities. Therefore, the issue of necessity of reforms in national universities has been attracting increasing attention, while administrative and financial reforms have been of growing importance as a political issue facing the Japanese government. Against this background, a comprehensive review was undertaken to examine how Japanese national universities had been functioning over the past few years, resulting in the decision that from April 2004 all national universities in Japan would become independent administrative institutions.

All Japanese national universities, which used to form part of the central administration of the state, have been incorporated as independent institutions, in compliance with the National University Corporation Law, which was enacted in July 2003. This means that national universities, which had been nothing more than internal organizations of the Ministry of Education, Culture, Sports, Science and Technology of Japan, have begun to be run independently and autonomously by their own management, led by their respective President and Chairman of the Board.

In response to this change in the management of national universities, an "Administrative Council" and an "Education and Research Council" have been set up in each national university to discuss managerial issues and to discuss issues concerning education and research, respectively. Each national university has thus assumed a duty of accountability to society, as well as the responsibility to appropriately reflect the voices of society. This is why it has been stipulated that people from outside the university must account for at least one half of the members of the Administrative Council in each university. The aim of this is to infuse those traditionally closed organizations with private-sector vitality and thereby let the market mechanism work.

The National University Corporation Law clearly provides that the Japanese government shall continue to assume responsibility for the implementation and financial aspects of education and research at national universities. Each national university is obliged to pass a financial audit by a third party, while being given considerable discretion over budget allocations, promotion of industry-university collaboration, etc. Their medium-term operating goals and plans are more specific than those in the past, and their performance is now subject to assessment by a third party, such as the "National University Evaluation Committee."

The incorporation of Japanese national universities this time is expected to encourage competition between university teachers, for example, through the fact that as a result of this reform, inventions made by academic and research staff will belong to their institutions. Particularly, less renowned national universities are actively setting out clear policies to "survive in competition with other universities by developing their own distinctive strengths." This reform is generally highly regarded as the right move to make for national universities in the face of intensifying international competition in the 21st century.