Enzyme Wave

Volume 7
2004

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AMANO

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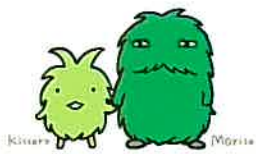
From the Editor,

The international exhibition, EXPO 2005 AICHI, is scheduled to open in Aichi Prefecture, Japan on March 25, 2005. One hundred twenty-five countries and eight international organizations have decided to participate in the exposition (as of March 2004).

With "Nature's Wisdom" as the main theme, and "Nature's Matrix," "The Art of Life," and "Development for Eco-Communities" as subthemes, the expo sites are steadily taking shape. A Global House, where there are surprises and touching moments in store, and a main stage (The EXPO Plaza) for grand global interchanges are under construction in the Nagakute Area. The construction of a pavilion and an area designed to make new history is making steady progress in the Seto Area. A frozen mammoth is scheduled to be displayed for the first time in the world. Morizo and Kiccoro, who are dryads symbolizing nature, will play active roles as mascots for EXPO 2005 AICHI.

Fossilized microbes were found in a rock in Africa, so it is assumed that microbes developed 3.5 billion years ago, a billion years after the earth was formed. In comparison, mammals originated 220 million years ago, and humankind came into being more than 2 million years ago. Viewed in the light of a food chain, decomposed microbes are utilized by plants and transferred from herbivorous animals to carnivorous animals (including human beings). In addition, microbes also decompose waste generated by people, as well as dead animals and plants, and return them to the natural environment. So they play a part in a recycling-orientated society and contribute maintaining the global environment.

Amano Enzyme Inc. produces and sells enzymes with the help of microbes and human technology and makes contributions to human health and food culture. Indeed, Amano performs corporate activities based on our concept in line with Nature's Wisdom, the main theme of EXPO 2005 AICHI. As a sponsor, Amano supports EXPO 2005 AICHI.



愛・地球博
EXPO 2005 AICHI JAPAN

Photo on the cover:

A carp streamer, called *koinobori*, is an ornament made of fabric in the shape of a carp five meters or more in length.

In Japan, the Boys' Festival, called *tangonosekku*, is held on the fifth of May in celebration of boys' health. Because a carp symbolizes a child's success in life, people put up carp streamers in the garden and pray for their children's growth.

Katsuobushi
- A typical Japanese fermented seasoning -

It is no exaggeration to say that *katsuobushi* (dried bonito) typifies the taste of traditional Japanese foods.
Among international dishes, people enjoy a variety of broths made from foods that contain ingredients for flavor; boiling these foods enhances the flavor. We call this kind of broth *dashi* in Japan. Typical broths include *shirumono* (soup) in Japanese cuisine, *tang* in Chinese cuisine, and bouillon and soup stock in Western cuisine **(Table)**.
Many delicious dishes with compound flavors can be made by using broth for seasoning with other ingredients that add flavor. Typical ingredients for *dashi* include *katsuobushi* (dried bonito), *niboshi* (made of boiled and dried sardines), kelp, and *shiitake* mushrooms; they are used for different types of dishes in various parts of Japan. Among them, *katsuobushi dashi* (extract of dried bonito) can be said to be the origin of Japanese cuisine.

What is *katsuobushi* ?

Katsuobushi is produced by boiling bonito meat **(Photo 1)**, removing the bones, smoking, shaving the surface of the meat, applying mold for fermentation, and finally sun-drying. A bonito of five kilograms yields about 800 grams of dried bonito. A total catch of bonito reaches 300,000 to 400,000 tons annually.
Historically, the first mention of dried bonito can be seen in 1,300-year-old Japanese documents. A fermentation procedure like the current method of applying mold was added to the process of drying bonito meat about 300 years ago. With the process of drying bonito fully developed, many cookbooks that use dried bonito as a seasoning have been published. Bonitos have been consumed by the Japanese people as a "good-luck" fish since ancient times. Today, bonitos are used in wedding ceremonies, presents given to guests at housewarming parties, and for gifts. It is not too much to say that Japanese cuisine has been greatly influenced by dried bonito.

Process of *katsuobushi*

Dried bonito is known by different names according to the season the fish is caught, the fishery, and the different characteristics of processing used. Two traditional forms of bonito are *kamebushi* and *honbushi*. *Kamebushi* is prepared from two dried bonito made from the right and left halves of a small bonito while *honbushi*, is prepared from four dried bonito made from the right and left halves of a big bonito that is further cut into the dorsal side and the abdominal side. More than six months can pass from the time the fresh fish are unloaded to the time dried bonitos are shipped.

Photo 1: Bonito (Skipjack tuna)
The scientific name of the bonito is *Katsuwonus pelamis*. The fish is called skip jack in Canada and striped tuna in Australia. Bonito is found in the temperate zone and the tropical zone of the world's oceans. Bonito can grow up to one meter, but typically bonito up to 50 centimeters are caught in large quantities.

The process of making *honbushi* is briefly outlined below:

1. Cutting bonitos (*Namagiri*)
First, the fish heads are cut off. Then, dorsal skins, organs, and fatty meat are removed, and the bonitos are cut into portions.

2. Dividing bonito meat (*Aidachi*)
The bonitos are cut along the central fishbone into the dorsal side and the abdominal side. Small bonitos are processed as *kamebushi*.

3. Laying bonitos in a basket (*Kagotate*)
The bonitos have been cut in a way so that they will not break into pieces while cooking. They are put in a container or basket with good thermal conductivity before proceeding with the following process of *Shajuku*.

4. Putting bonitos in a cooker (*Shajuku*)
Eight to ten baskets are piled up, and the bonito meat is boiled for 60 to 90 minutes. This will kill enzymes and delay decomposition as well as solidify protein so the bonito meat can be easily processed. In addition, the water content of the bonito meat will be reduced for easier drying.

Table : Major broths of the world

	Type	Ingredients for flavor
Japanese cuisine Dashi (Broth)	○Kelp: Kelp is used for soups with seafood seasoned with salt and tofu when the flavor of ingredients should not be destroyed.	· Glutamic acid
	●**Dried bonito: Dried bonito is widely used for Japanese cuisine. (Photo 3)**	· Inosinic acid, glutamic acid, and peptides
	○Dried small sardines: Dried small sardines are used as a thick broth for meso soups and boiled and seasoned vegetables.	· Same as above and glutamic acid peptides
Western cuisine Soup stock Fond de veau	○Soup stock with chicken bones	· Inosinic acid and glutamic acid
	○Soup stock with vegetables (onions, carrots, celery, cabbage, etc.)	· Glutamic acid
	○Fond de veau: Beef shanks with bones, calves' bones and flavored vegetables are fried and boiled to bring out the flavor.	· Inosinic acid and glutamic acid
Chinese cuisine Tang (Broth)	○*Ji tang*: Ginger, onions and water are added to a whole chicken and boiled for a long time.	· Glutamic acid and inosinic acid
	○*Rou tang*: Ham and pork loin are boiled with ginger, onions, and water for a long time.	· Inosinic acid
	○Xiang lu tang: Dried shiitake mushrooms are soaked in water to reconstitute them. Onions and ginger are added to the reconstituted mushrooms and boiled over low heat to make broth.	· Guanylic acid and glutamic acid

5. Removing bones (*Honenuki*)
After boiling the bonito the bones, excess skin, scales and subcutaneous fat are removed in a water tank.

6. Smoking (*Baikan*)
Smoking (*baikan*) further hastens the drying of the fish.

7. Shaving (*kezuri*)
The dried bonitos collect tar during the smoking process which is removed with any fat content that forms at the surface by shaving the fish.

8. Applying mold (*kabitsuke*)
After removing the tar, the dried bonitos are sun-dried for several days, packed in casks or wooden boxes, and put in a fermentation room where mold is applied for fermentation. The water content will be further reduced because mold grows all over the surface of the dried bonitos. In addition, fat will resolve, and flavor ingredients will condense.

9. Sun-drying (*Nikkan*)
The dried bonitos that were treated with mold are sun-dried, and the mold is temporarily removed. After cooling, the dried bonitos are put into the fermentation room again and covered with more mold. This process is usually

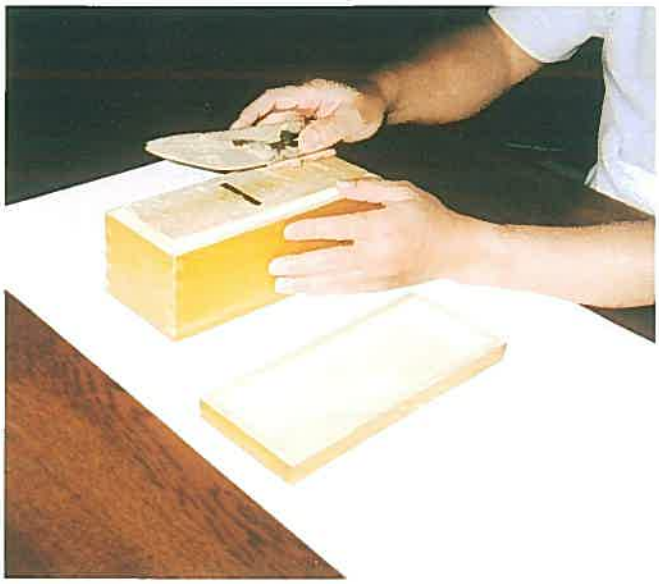


Photo 2: Shaving a ***katsuobushi.***

repeated four times in order to finish *honbushi*.

The mold that is applied to dried bonitos belongs to the *Aspergillus glaucus* group. The mold is very useful for resolving fat, but it is not useful for resolving protein. Lipase, which is secreted from mold, is a fat-decomposing enzyme. It resolves fat, forms fragrant alcohols, and mellows the smoking smell so that the flavor characteristic of dried bonito will be created. Lipase also decomposes bad-smelling ingredients such as trimethylamine so that dried bonitos will be transformed into flavor ingredients without the bad smell typical of fish.
Mold hyphae are seen about 50 to 500 μm from the surface layer of a dried bonito. Mold spores are localized in a layer of 20 to 120 μm outside the surface layer. Neither mold hyphae nor mold spores exist in the inner portion of dried bonitos.

Taste of *Katsuobushi* (flavor ingredient)
Dried bonito is a highly nutritious food that is mostly protein (70%) and contains a very high content of the amino acid histidine. Dried bonito is also rich in vitamins, especially the Vitamin B group and Vitamin D, and also omega 3 fatty acids, such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), which are known to promote good health.
The primary flavor ingredient of dried bonitos is inosinic acid. Inosinic acid does not create a strong flavor by itself but when an aromatic amino acid, such as sodium glutamate, is present its full flavor is brought out.

Preparation of broth from *katsuobushi*
With a water content of slightly over 10 percent, dried bonito can be said to be one of the driest foods in the world. Therefore, to use dried bonito, it should be shaved with a plane called a kanna to a thickness of 0.1 mm to 0.5 mm **(Photo 2)**. The shavings of dried bonito, called kezuribushi, are also available at stores.
To make broth, dried bonito is usually used with kelp. There are various ways of making broth according to the region of Japan, the type of dish being prepared and of course on the chief preparing the dish. A basic way to make broth is the following: first, kelp is put into water in a pot and then the pot is heated. The kelp should be taken out just before the water comes to a boil. Dried bonito shavings (a weight equal to about three percent of the boiled water) should be added and boiled for five to ten minutes. After removing from the heat, the broth should be put through a piece of cloth in order to remove insoluble matter.

Photo 3: Noodles sup with ***katsuobushi dashi.***

Digestive Enzymes Improve Nutritional Status in the Elderly

Digestive Enzymes Improve Nutritional Status in the Elderly

A link between nutritional status and health among the general population is well documented; for the elderly this link is even more pronounced with nutritional health directly affecting life satisfaction and life expectancy. Poor nutrition among the elderly varies in different cultures and is highly influenced by social and economic conditions as well as the physical activity and participation of the elderly in society. In addition, the capacity to chew decreases in the elderly, which leads to a reduction in digestive and absorptive efficiency, resulting in compromised nutrition. Two indicators of nutritional status are the concentrations of serum albumin and HDL-cholesterol. The effect of digestive enzymes on the level of these two indicators in the elderly will be presented below.

Serum Albumin and HDL-Cholesterol Levels Are Markers for Vital Human Functions

Serum albumin accounts for about 60% of the serum proteins found in human blood and functions as a transport protein that convey various nutrients throughout the body. The normal range for serum albumin in human blood is 4.0 - 5.0 g/dL (BCG method). A low level of serum albumin decreases the effect of antibiotics and under conditions of malnutrition or starvation serum albumin serves as a source of nutrition.

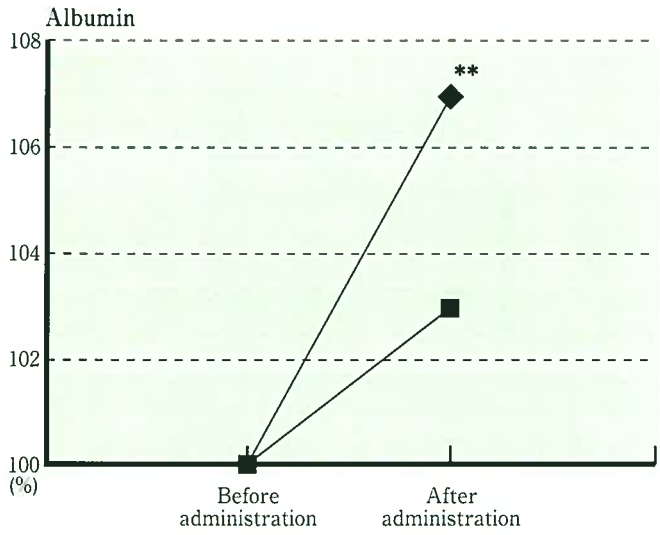
HDL-cholesterol removes excessive cholesterol from cells and transports it to the liver, where it is metabolized and eliminated. Blood levels of 70 mg/dL or more in Western countries and 90 mg/dL or more in Japan confer what has recently been described as the "long life syndrome".

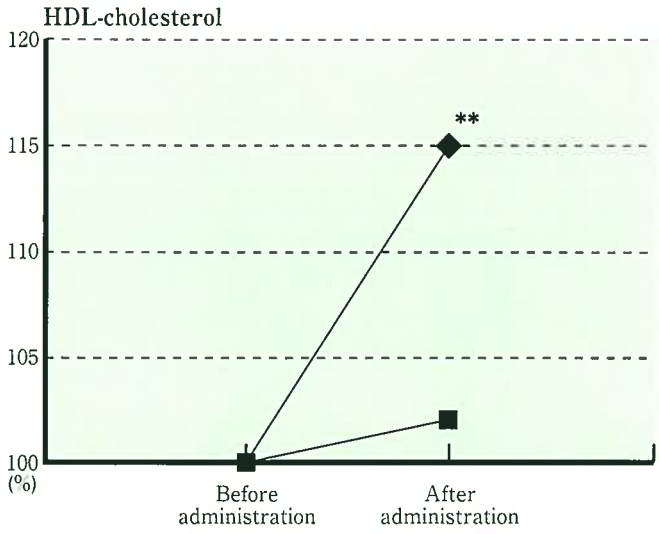
Average age of administration and control groups

		Administration group	Control group
Average age (years)		76.2±13.7	77.5±5.1
Sex	Male	4	3
	Female	9	8

Administration of Commercially Available Digestive Enzyme Preparations

Elderly residents (excluding hospitalized residents and long-term absentees) at a nursing home in a suburb of Tokyo were divided into two groups so that the sex, age, ADL (activities of daily living), and degree of obesity (weight/height) were comparable between the two groups. The residents and their families gave their informed consent to the study. One group of residents was given a total of 9 tablets of a commercially available digestive enzyme preparation daily in three doses for 6 months, the other group received a placebo. The digestive enzyme tablets contained 135 mg of Biodiastase 2000, 30 mg of Lipase AP12, and 90 mg of Newlase. No significant differences were observed between the two groups in terms of food intake, body weight, total cholesterol, serum iron, urea nitrogen, zinc, tocopherol, uric acid, GPT, hemoglobin, blood pigment, hematocrit, red blood cell count or white blood cell count. However, the serum albumin and HDL cholesterol levels were significantly increased in the administered group.





Conclusion

We conclude that administration of digestive enzyme preparations may significantly improve the serum albumin and HDL-cholesterol levels in malnourished elderly people and thereby result in an improvement of their nutritional health and general well-being.

Shibata et al. Geriat. Med., **37**, 1355-1359 (1999).

Changes in body weight for administration and control groups

Variable		Before administration	After administration
Body weight (kg)	Administration group	45.2±8.4	45.7±7.9
	Control group	41.1±6.0	40.6±5.1

Changes in blood albumin and HDL-cholesterol levels for administration and control groups

Variable		Before administration	After administration	Significance
Albumin (g/dL)	Administration group	3.98±0.21	4.24±0.26	**
	Control group	3.88±0.45	4.00±0.71	
HDL-cholesterol (mg/dL)	Administration group	46.5±14.2	53.5±16.6	**
	Control group	53.7±15.8	54.6±17.9	

** P<0.001

Digestive Enzyme Preparations Commercially Available

"Biodiastase 2000" is multiple digestive enzymes preparation containing amylase, protease and cellulase, which is manufactured by unique fermentation of a strain belonging to *Aspergillus oryzae*, extraction, purification and standardization with a suitable diluent.

"Lipase AP12" is a digestive enzyme preparation, which contains lipolytic enzyme. "Lipase AP" is manufacutured by unique fermentation of a strain belonging to *Aspergillus niger*, extraction and purification and standardization with a suitable diluent. Lipids are indispensable nutrient as energy source. The ratio of lipids in energy intake has been increasing according to recent National Nutrient survey, as the dietary life has been changing to European and American style.

"Newlase" is digestive enzyme preparation, which contains acid protease and lipase which is manufactured by unique fermentation of a strain belonging to *Rhizopus niveus*, extraction and purification and standardization with a suitable diluent. "Newlase" has both proteolytic activity and lipolytic activity, and shows digestive activity not only in stomach but also in intestine.

Progress in Japan on the genomic analysis of molds

Progress in Japan on the genomic analysis of molds
The effort in Japan to analyze the genome of molds is currently focused on *Aspergillus oryzae*, which has been utilized to brew traditional Japanese fermented foods, such as sake, miso and soy sauce, for more than a thousand years*.

The genome of *A. oryzae* is currently being mapped by Expressed Sequence Tag (EST) analysis; the total number of ESTs has reached about 17,000, with 6,700 clusters determined by the end of year 2000. It is estimated that at least 5,000 independent EST clones have been isolated. The number of genes represented by the EST clones is believed to be about 40% of the total genes in the *A. oryzae* genome.

Whole-genome sequence analysis of *A. oryzae* was initiated in August 2001. Whole-genome shotgun sequencing procedures have resulted in the accumulation of sequence data representing about 6 times the length of the entire genome of *A. oryzae*. Analysis of the data revealed a unique sequence of 37 million base pairs, which indicates that at least 95% of the whole genome sequence has been determined. Based on this data, together with the results of EST analysis (5,000 clones) as described above, and prediction of gene identification, we estimate that the total number of genes in the genome of *A. oryzae* to be about 13,000.

Since April 1998, an international consortium has performed whole-genome analysis of *A. nidulans*, followed by analysis of other molds, including *A. fumigatus*, *A. niger*, *Neurospora crassa*, etc. in the U.S. and Europe. The Japanese group and the American and European groups have exchanged their genetic data to allow more accurate prediction of the individual gene functions studied by each group, as well as more efficient and unified annotation of individual genes.

The total number of known genes in the *A. oryzae* genome exceeds the earlier estimate by about 40%, which indicates that *A. oryzae* is a very valuable genetic resource. We anticipate that *A. oryzae* will make more contributions to industry when the functions of the genes of *A. oryzae* are revealed by post-genome sequencing techniques such as gene expression analyses by DNA microarrays and proteomic analyses.

* Enzyme Wave No. 1, 2 and 4