Enzyme Wave

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Rice vinegar - A Japanese traditional fermented seasoning -

Enzymes for Yeast Extract Production

Food Additives GMP

AMANO

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From the Editor,

As introduced in previous Enzyme Wave articles (Vol. 2), the world exposition, Expo 2005 AICHI, is scheduled to open in Aichi Prefecture, where the Amano head office is located, from March 25 through September 25, 2005.
In order to accommodate the large number of visitors expected from all over the world, many projects have been planned and commenced to provide new exhibitions and attractions and also to improve the transportation system. The biggest project in the transportation systems is the construction of a new airport, the Central Japan International Airport, to serve as the new gateway to Aichi Prefecture.
CENTRAIR, as the new airport is called, will be located on a reclaimed island approximately 2 kilometers offshore Tokoname City, which is roughly 35 kilometers south of Nagoya. It is targeted to open in 2005, just before the opening of EXPO 2005. Convenient access from Nagoya and other cities in Aichi will be achieved by express trains and expressways. Thanks to its central location in Japan, visitors arriving at Centrair will find the rest of Japan easily accessible through the domestic air service network, the Tokaido Shinkansen ("bullet" train), and the Tomei and Meishin Expressways. Centrair is expected to be the new gateway not only to Aichi, but to all of Japan, promoting the movement of people, goods and information between Japan, and the rest of the world. We are looking forward to welcoming our guests soon though this new international airport.

Rice vinegar
- A Japanese traditional fermented seasoning -

Introduction

The oldest fermented food product created by man is an alcoholic beverage, and the oldest fermented seasoning is "vinegar." The raw materials of vinegar are mainly cereals and fruits. There are different kinds of traditional vinegar made from agricultural products compatible to the respective environments and climates of different places in the world, and they add variation to the respective food cultures. There are various kinds of cereal vinegar, including rice vinegar in East Asia including Japan, barley vinegar in Korea, and malt vinegar in England, and there are also varieties of cereal vinegar made from millet, barnyard millet, corn and the like. As for fruit vinegar, in addition to wine vinegar and apple vinegar found mostly in Europe and the United States, there is coconut vinegar in Southeast Asian countries. Though a great variety of vinegar for food is produced at present in Japan, the traditional vinegar in Japan is "rice vinegar."

The history of rice vinegar in Japan

Humankind has long known that harvested cereals and fruits change into alcohols due to fermentation when they are stored for a long time and that they become acidic with a longer period of storage. They have regularly used vinegar thus produced as a basic seasoning that makes their dietary life richer.

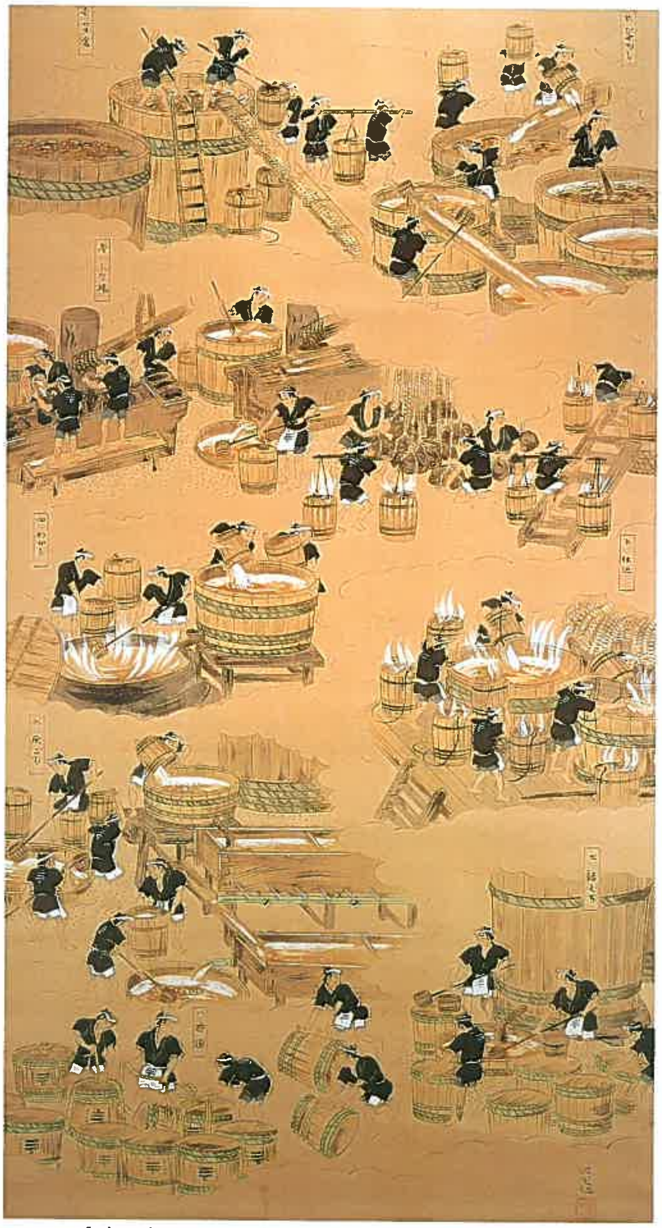
The first recorded appearance of vinegar for food occurred in Babylonia in ca. 5,000 B.C. The ancient record indicates that vinegar of fine quality was made from the pressed juice of date fruits. In the 5th century the brewing technology of vinegar was introduced into Japan at present day Osaka, where it was transmitted from China together with a brewing method for sake. Vinegar came to be produced in various parts of Japan in the 17th century. Through out Japanese history, vinegar was used not only as a basic seasoning but also combined with other seasonings to contribute to the development of a wide-ranging Japanese sense of taste. "Sushi" is a typical Japanese food that uses vinegar, and the word is derived from "sushi" which means acidic. It is thought that present day Sushi originated from acidic fish dishes formerly called sushi. The present form of sushi (nigiri-zushi) became popular in Edo (present-day Tokyo) in the 17th century. A reason for its popularity was that new technology was developed to produce lees vinegar from residual sake lees (solid matter) after brewing sake, and this lees vinegar greatly enhanced the taste of nigiri-zushi. In the early 19th century the production of rice vinegar significantly increased because of the greater availability of rice as a raw material and the introduction into Japan of the scientific principles of acetic acid fermentation, by which vinegar is made from sake, from Europe and the United States. In present-day Japan, vinegar for food use has expanded its repertory, covering cereal vinegar, fruit vinegar and brewed vinegar in response to the diversification of consumer needs. Recently, various healthy properties of "vinegar" have been revealed, and thus vinegar is now seen as not only a seasoning but also as a food for realizing a healthy dietary life.

"sushi (nigiri-zushi)" a typical japanese food

Production of rice vinegar

The first step in rice vinegar production is the preparation of saccharified rice starch by steaming rice with water and adding amylase found in rice koji at a constant temperature. In recent years, saccharification treatments using various enzyme agents have been conducted to simplify the complex manufacturing process of making rice koji. Alcohol fermentation is then performed by inoculating yeast (*Saccharomyces cerevisiae*) in a sugar solution obtained by mild filtration of the saccharified rice product. After fermentation, acetic acid fermentation is conducted by mixing seed vinegar prepared by culturing acetic acid bacteria (*Acetobacter pasteurianus*. etc.).

Major acetic acid fermentation methods include the surface fermentation method and the deep fermentation method. The surface fermentation method, also called the static culture method, is a traditional Japanese process in which acetic acid bacteria are propagated on the liquid surface of an alcohol fermented solution in a box-type fermentor of 1 m



a scene of rice vinegar manufacture at old days (shown a process flow from top to bottom)

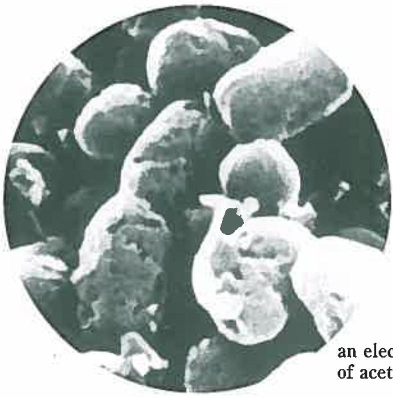
in depth. After keeping it warm for several days a clean pellicle of acetic acid bacteria (shown in picture) is formed on the liquid surface and oxidization begins. After acetic acid fermentation for about 2 weeks, the mixture is filtered, and the filtrate is left in place to allow maturing for about one month. During the storage, unstable macromolecules agglutinate, and the flavor becomes stable. After maturation is over, the product is filtered, sterilized and bottled. As shown in the table, rice vinegar thus produced has a high sugar and amino acid content, and is rich in extracted components and flavor. Therefore, it is the most suitable seasoning for "sushi" and other Japanese dishes.
The deep fermentation method is also called the aeration and agitation culture method. In this method, vinegar is efficiently produced through aeration and stirring of "shikomi" fluid because acetic acid bacteria are aerobic bacteria. Though it is not very popular for production of rice vinegar, it is frequently used for production of alcohol vinegar and cereal vinegar, in which extracted component content is low. In this case, the alcohol solution is put into a large tank made of stainless steel and equipped with aeration and cooling devices. A nutrition source for fermentation such as yeast extract is added if necessary, and acetic acid bacteria cultured in a small device are inoculated. Then air is blown directly into the tank as fine bubbles to promote fermentation. Fermentation begins several days after the transfer to the large tank, and the degree of acidity reaches 10 to 12% in 2 days or so. As for alcohol vinegar, this deep fermentation method has made it possible to produce high concentration vinegar containing 21% acetic acid.

It is also possible to use a semi-continuous fermentation method, in which half the product that has reached the targeted acidity and quality is taken off as a finished product and new material fluid is added to restart fermentation.

The flavor and health benefits of rice vinegar

The taste and aroma of rice vinegar are created by a complex intertwining of components related to taste, such as amino acids, organic acids, sugars and salts, and many flavor components; among these components, organic acids are important substances for expressing taste. The most abundant organic acid is volatile acetic acid, but nonvolatile organic acids, such as citric acid (the acidic component of citrus fruits), are generally present at 1 to 20% of the total amount of acids. The taste of the final product differs depending on the kinds of organic acids present and the balance in their amounts.
The most abundant amino acid is glutamic acid, which softens the acidity of rice vinegar and increases its flavor. In addition, glycine, lysine, alanine, aspartic acid, valine and other amino acids are also present. Sugars present include
In addition, glycine, lysine, alanine, aspartic acid, valine and

a clean pellicle of acetic acid bacteria in a box-type fermentor



an electron micrograph
of acetic acid bacteria

other amino acids are also present. Sugars present include glucose and fructose, which are energy sources of acetic acid bacteria, maltose, ribose, mannose and additional sugars. Sugar alcohols such as glycerine and sorbitol are also present in small amounts. Aside from acetic acid, the flavor component most detectable in rice vinegar is ethyl alcohol, a source of acetic acid. Esters are believed to contribute greatly to the generation of specific flavors in different kinds of vinegar; ethyl acetate, isobutyl acetate and isoamyl acetate are esters common to most vinegar for food use.

Rice vinegar as a seasoning, in combination with other seasonings such as miso and soy sauce, produces the unique taste of Japanese dishes described as "awasezu".

Vinegar is not only a seasoning but has been known for a long time to have functional effects on health. The effects have been proven to be due to the presence of its major component, acetic acid. These beneficial effects include the following:

• Improvement of appetite

The acidic taste of vinegar acts on the feeding center in the brain to improve the appetite by stimulating the senses of taste and smell.

• Acceleration of calcium absorption

Vinegar has the effect of drawing out the calcium contained in foodstuffs and causing it to be absorbed efficiently into the human body.

• Effect of recovery from fatigue

Vinegar can quickly restore the human body from a state of energy deficiency to its normal state.

• Control of blood pressure

A person with hypertension can bring his blood pressure close to the normal value by continuous intake of vinegar.

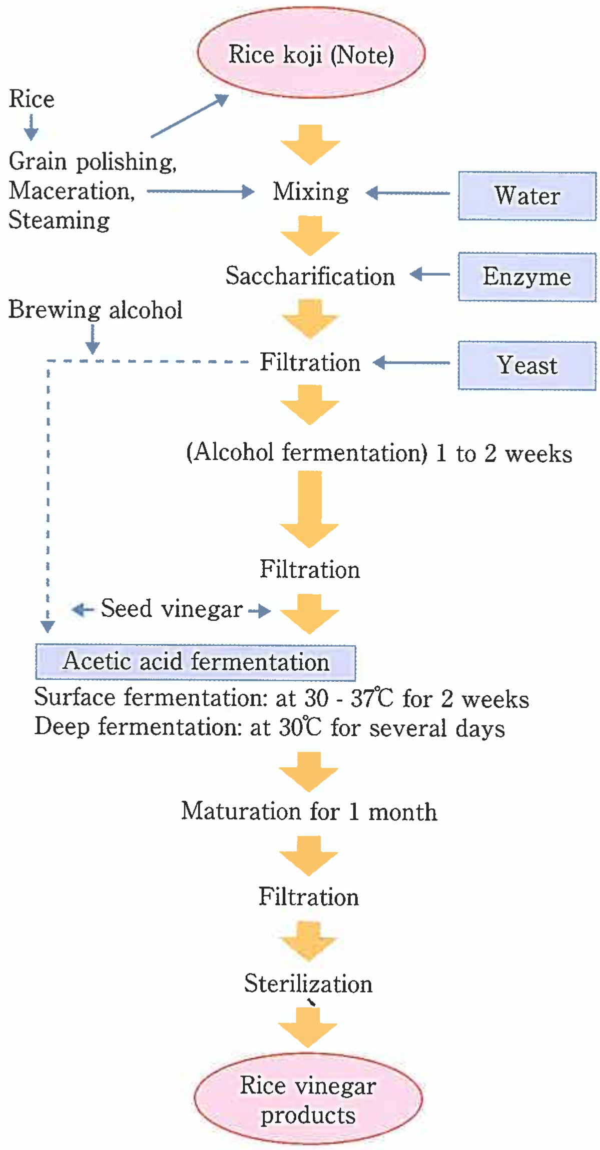
Post-genome era and vinegar for food

Acetic acid, which is the main component of vinegar for food, is produced by the oxidization of ethanol (generated by yeast alcohol fermentation) in which acetic acid bacteria consume oxygen in the air to oxidize ethanol. Recently, progress in the genomic analysis of microorganisms is remarkable. The genome of the acetic acid bacteria (*Acetobacter polyoxogenes*) used for production of vinegar for food has been analyzed and the existence of approx. 3,300 genes composing approx. 3.26 Mbp has been sequenced. Based on the information of gene sequences obtained, the functions of nearly half of the genes have been revealed. As the search for unknown genes advances, the findings of the studies will be utilized for quality control and advances in the production of vinegar.

Acknowledgment

We would like to thank Mitsukan Group Corporation (the biggest producer of vinegar in japan) for their helpful advice and valuable pictures.

General manufacturing process of rice vinegar



(Note) Refer to Enzyme Wave Vol. 1

Enzymes for Yeast Extract Production

Yeast extract is a soluble concentrate extracted from yeast and contains nutritional components including amino acids, nucleotides, vitamins, and minerals. Yeast extract is used in the food industry as a flavor enhancer, as a component of media for cell growth in the fermentation industry, as well as in pet foods, cosmetics materials, plant nutrition products, etc..

Yeast extract is generally manufactured by two methods, autolysis or the enzymatic treatment of *Saccharomyces* species (Brewer's yeast, Baker's yeast) and *Torula* yeast. Autolysis is the traditional method for production of yeast extract and is prepared by the degradation of yeast with their own endogenous enzymes. Yeast extract made by exogenous enzymatic treatment under controlled conditions is characterized as a flavor enhancer and is rich in delicious flavor components such as the ribonucleotides 5'-Guanylic acid (5'-GMP:guanosine-5'-monophosphate) and 5'-Inosinic acid (5'-IMP:inosine-5'-monophosphate), amino acids and peptides, as shown in Table 1. These components of yeast extract contribute to a delicious taste and enhance flavors that make it a good seasoning. Recently, enzymatic yeast extracts have been commercialized and extensively used as new flavor enhancers in the food industry.

At Amano, various enzymes for yeast extract production are available, as shown in Figure 1. Enzymatic yeast extract, rich in flavor enhancer nucleotides such as 5'-GMP and 5'-IMP, is produced in three steps:

1. Yeast cell lysis and RNA extraction
2. RNA hydrolysis
3. Conversion of 5'-AMP to 5'-IMP

Table 1:Comparison of properties of yeast extract prepared by Autolysis and Enzymatic Hydrolysis

	properties
Autolysis	The yeast smell is strong.
	Free amino acids rich.
	Classic manufacturing method
	Replacement of HVP
Enzymatic Hydrolysis	The yeast smell is few.
	Small peptides rich
	New manufacturing method
	The extraction of a useful element can be designed.
	5'-nucleotides rich
	Flavor enhancers rich such as 5'-GMP and 5'-IMP
	Amino acid pattern near beef extract
	Replacement of Seasoning

Enzyme YL-NL "Amano" for Yeast Lysis

YL-NL "Amano" contains a neutral protease, produced by *Bacillus subtilis* fermentation, capable of lysing yeast cells by hydrolysis of the proteins in the yeast cell wall. YL-NL "Amano" lyses Brewer's yeast, Baker's yeast and *Torula* yeast as shown in Table 2. This result demonstrates the wide rage utility of YL-NL "Amano" independent of the type of yeast. Yeast lysates prepared by YL-NL "Amano" are more clear compared to lysates prepared by β-glucanase and in addition the lysates are much less bitter.

Table 2:The Yeast Lysis Effect of YL-NL "Amano"

YL-NL "Amano" (w/yeast dry weight)	Lysis ratios of yeast cells(%)		
	Brewer's yeast	Baker's yeast	*Torula* yeast
0.0 (%)	14	15	27
0.1	52	40	70
0.3	63	44	78
0.5	72	56	80
1.0	80	67	81
2.0	82	74	87

Enzyme RP-1 for RNA hydrolysis

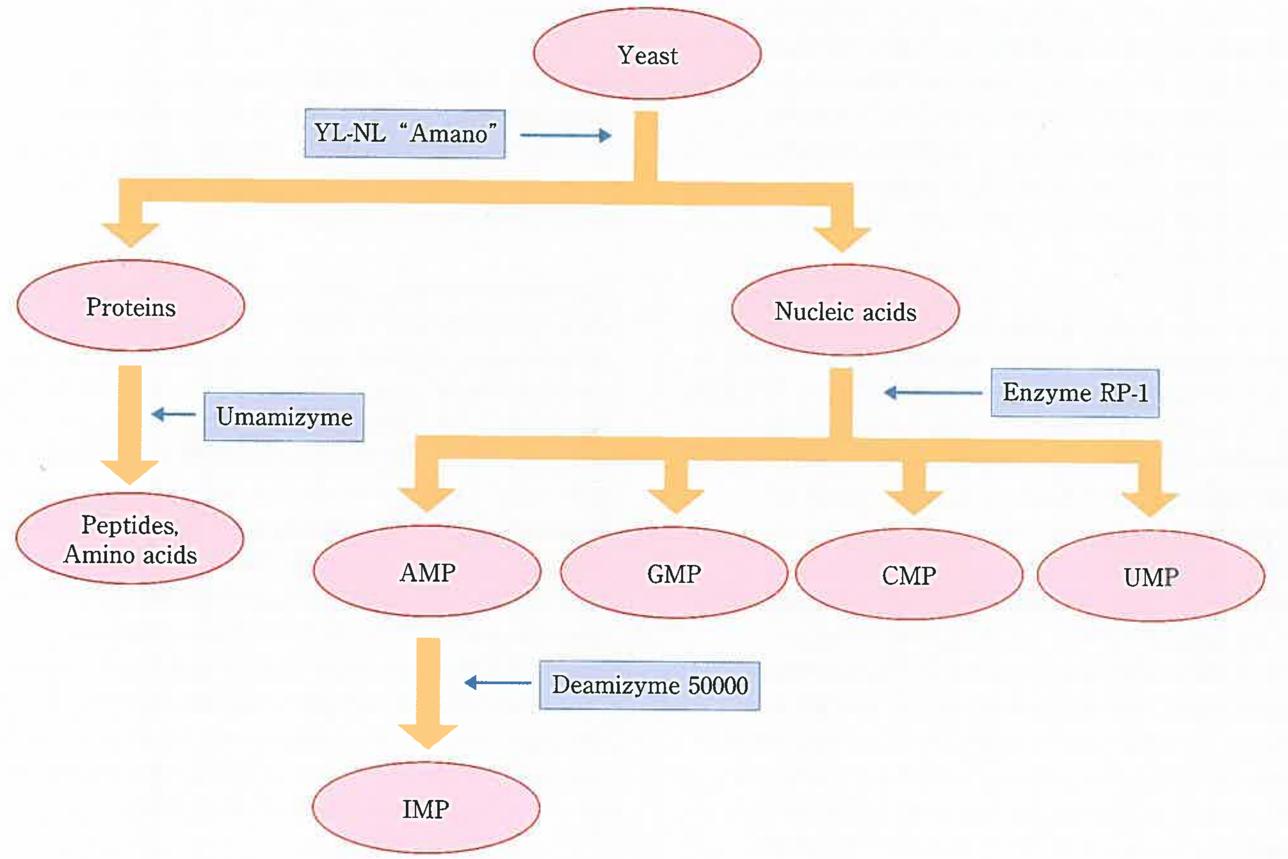
Enzyme RP-1 is a 5'-phosphodiesterase preparation produced by *Penicillium citrinum* fermentation. Enzyme RP-1 hydrolyzes RNA to 5'-nucleotides at a high level yielding adenosine-5'-monophosphate (5'-AMP), cytosine-5'-monophosphate (5'-CMP), uridine-5'-monophosphate (5'-UMP) and the flavor enhancer 5'-GMP. The advantages of Enzyme RP-1 include: absence of bacteria contamination because of the high reaction temperature (70℃), no side activity of other enzymes (phosphatase and 3'-nuclease) at 70℃ and addition of zinc sulfate is not necessary to activate nuclease activity. (In contrast, zinc sulfate is always added to malt root extract nuclease.)

Deamizyme 50000 for conversion of 5'-AMP to 5'-IMP

"Deamizyme 50000" is a 5'-adenylic deaminase produced by *Aspergillus melleus* fermentation. This enzyme has strong 5'-adenylic deaminase activity, which converts 5'-AMP into the flavor enhancer 5'-IMP as shown in Figure 2. Yeast extract containing 5'-IMP shows intense flavor enhancement, which cannot be produced by Autolysis.

Not only 5'-GMP and 5'-IMP but also amino acids (especially glutamic acid) and peptides play an important role as flavor enhancers in yeast extract. In combination with 5'-GMP and 5'-IMP, amino acids (glutamic acid) have

Figure 1 : Typical manufacture Scheme of Yeast Extract Using Amano enzymes

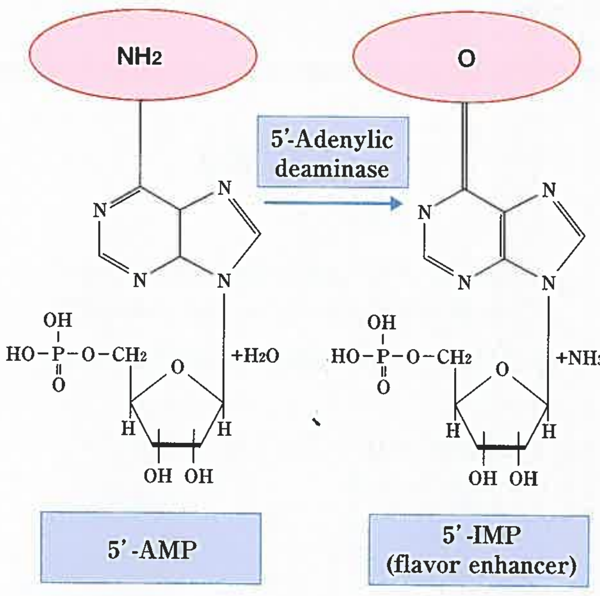


a synergistic effect on flavor enhancement; moreover, amino acids and peptides are useful for the creation of new reaction products by the interaction of amino acids and sugar (Maillard reaction)

Umamizyme and Peptidase R are suitable for the production of small peptides and amino acids including glutamine from yeast proteins. In addition, **"Glutaminase Daiwa"** can convert glutamine to the flavor enhancer glutamic acid.

Amano enzymes for yeast extract production have proven to be valuable in the creation of new flavor enhancers.

Figure 2 : Conversion of 5'-AMP to 5'-IMP by 5'-Adenylic deaminase



Food Additives GMP

Food additives are important functional substances for processed foods, and are playing an active role in terms of the sanitation of foods. In Japan, food additives are classified into 4 groups; Designated Food Additives, Existing Food Additives (including enzymes), Natural Flavoring Agents and Others. Enzymes were classified as Existing Food Additives in 1995 by the Ministry of Health, Labor and Welfare.

Recently, greater manufacturing and quality control of food additives is required. Current quality control is based on manufacturing control as defined in HACCP and ISO 9000; thus, the quality of a product is assured by the strict control of production processes and consumers looking for quality in products need to be concerned about the reliability of the supplier.

Because of the keen interest in the quality of food and food additives by consumers, the Japan Food Additives Association* (JAFA) has prepared a self-standardized manufacturing code and quality control code for food additives, the *Food Additives GMP*. The draft was issued in December, 2000. Subsequently, JAFA has conducted since October 2002 the voluntary Food Additives GMP Accrediting System in order to popularize the Food Additives GMP.

The Food Additives GMP is composed of the following concepts derived from pharmaceutical GMP and ISO 9000: Top executive leadership, Clarity of organization and responsibilities, Integrated documentation of quality information, Documentation of quality rules, Procedures for corrective action of quality failures, Self-inspection of management, Training and Recording.

The Food Additives GMP specifies that a Head (Food Sanitation Supervisor) supervises a Manufacturing Control Manager and Quality Control Manager, and specifies the responsibilities for the Self Inspection Manager and Educational Training Manager.

The documentation system includes: Product Standard Code, Manufacturing Control Standard Code, Manufacturing Hygiene Control Standard Code, Quality control Standard Code, Self-Inspection Procedure, Training Procedure, Action Procedure for Complaint, Action Procedure for Recall, Nonconforming Product Control Procedure, Corrective Action Procedure, Deviation Control Procedure, Calibration Procedure, Cleaning Procedure, Change Control Procedure.

Amano is a member of the Japan Food Additives Association, and is actively working with the Enzyme Committee. Amano and task members are currently working to create new guidelines for the registration of enzymes as food additives while taking into consideration harmonization with international standards.

*Japan Food Additives Association(JAFA)is composed of companies and associations that are involved in production, import, sales and utilization of food additives.

Please visit
http://www.jafa.gr.jp for more information about JAFA.

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