Enzyme Wave

Volume 9
2005

Nucleic Acid Seasoning

Enzyme Modified Cheese (EMC)

JABEE (Japanese Accreditation Board for Engineering Education)

AMANO

Contents

Nucleic Acid Seasoning······2
Enzyme Modified Cheese (EMC)······5
JABEE (Japanese Accreditation Board for Engineering Education)····7

From the Editor,

The key words for Aichi in 2005 seem to be "future" and "world," as shown by Expo 2005 and the new airport CENTRAIR (introduced in vol.7 and vol.6 respectively). In contrast, the beautiful garden "Tokugawa-en," with associated key words "past" and "Japan," was opened in Nagoya in 2004.

Tokugawa-en was built in 1695 as a retirement house of Tokugawa Mitsutomo, who was Owari's second-generation domain lord. In 1931, the residence was donated to Nagoya and opened to the public; since then, it has been used as a general park. In 2004, it was renovated as a Japanese garden in the style of a major daimyo (a feudal load) garden during the Edo period. These gardens are characterized by a symbolically condensed landscape of Japanese nature in which a pond represents an ocean.

Tokugawa-en is attached to the Hosa Library and the Tokugawa Art Museum. Both the museum and the library feature objects inherited from the first Tokugawa shogun, Tokugawa Ieyasu, at the core of their collections.

If you visit Tokugawa-en, you will enjoy it beauty and learn about the traditional Japanese "samurai culture."

KUROMON
This building reflects the architecture of the old samurai residence.

TORA NO O
The river expresses the canyon's beauty.

Nucleic Acid Seasoning

Introduction

There are four types of seasonings that improve the taste of processed foods and dishes: amino acids, nucleic acids, organic acids, and inorganic acids

"*Umami*" is a term that expresses the savory or meaty taste of food. *Umami* was not included in the four former basic elements of taste, sweetness, saltiness, sourness, and bitterness because it was unique to Japan. However, it is now recognized worldwide and is included in the five current basic elements of taste. The word "*umami*" comes from "*dashi*," which is a food base that provides the underlying taste in Japanese cooking. It corresponds to Western soup stock and Chinese *tsuotan*. Japanese people have traditionally made *dashi* from kelp, bonito flakes, and dried shiitake mushrooms.

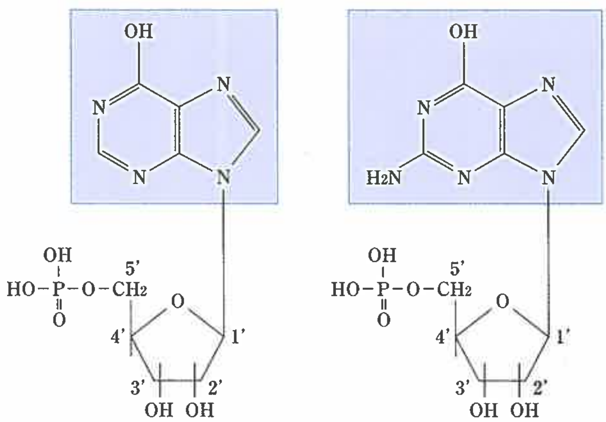
IMP and GMP Structure and Taste

What specific part of food provides the *umami* taste? Kikunae Ikeda found in 1908 that the *umami* component of sea tangle is monosodium glutamate (MSG), an amino acid. Shintaro Kodama discovered in 1913 that the *umami* substance of bonito flakes is 5'-inosinic acid (5'-IMP), and Akira Kuninaka found in 1960 that the *umami* component of shiitake is 5'-guanylic acid (5'-GMP).

Both 5'-IMP and 5'-GMP are ribonucleotides, components of ribonucleic acid (RNA). Nucleotides consist of pentose, phosphoric acid, and a base (purine or pyrimidine). Both IMP and GMP have three isomers, depending on whether a phosphate is esterified at the 2', 3', or 5' position of the pentose.

(Figure 1) Structural formulas of 5'-IMP and 5'-GMP

5'-IMP　　5'-GMP



shows the purine base of each compound.

Only 5'-IMP and 5'-GMP (as shown in Figure 1) meet the following three requirements for an *umami* taste:

① Purine (adenine or guanine) base
② The purine base has an OH group at the 6' position.
③ The pentose has a phosphate at the 5' position.

Production Methods of 5'-IMP and 5'-GMP

There are three production methods for 5'-IMP and 5'-GMP: nucleic acid degradation, fermentation/synthesis, and direct fermentation. See Table 1 for descriptions of these methods. The fermentation method currently predominates because of its low cost.

In recent years, an enzymatic phosphorylation method has been established as an industrial manufacturing process in which 5'-IMP and 5'-GMP nucleotides are synthesized from the nucleosides inosine and guanosine, using pyrophosphoric acid as the phosphate donor.

(Table 1) Production methods for 5'-IMP and 5'-GMP

Nucleic acid degradation (Enzymatic method)	The RNA of yeast obtained from sulfite waste liquor or molasses is degraded with 5'-phosphodiesterase to produce a mixture of 5'-AMP, 5'-GMP, 5'-CMP, and 5'-UMP. Then, tasteless 5'-AMP is changed into 5'-IMP using an AMP deaminase. The product is purified using an ion-exchange resin to obtain 5'-IMP and 5'-GMP.
Fermentation/ synthesis ① IMP ② GMP	Inosine is produced by fermentation using *Bacillus* subtilis and chemically phosphorylated into 5'-IMP. Sodium 5'-guanylate is produced using *B. subtilis*. Or 5'-Amino-4-imidazolecarboxyamidoriboside (AICA-R) is produced with *B. megaterium*, from which guanosine is obtained by chemical synthesis and converted into 5'-GMP.
Direct fermentation ① IMP ② GMP	5'-IMP is produced by direct fermentation using *Brevibacterium ammoniagenes*. 5'-XMP (xanthylic acid) is produced using *Brev. ammoniagenes* and converted into 5'-GMP with *Brev. ammoniagenes*.

Various nucleic acid seasonings

Nucleic acid seasonings are divided into two types: high purity IMP and GMP, and yeast extracts containing IMP and GMP at high concentration.

① Nucleic acid seasoning

Nucleic acid seasonings include single component seasonings and compound seasonings. The single component seasoning is 5'-IMP, 5'-GMP, or a mixture of the two. The compound seasoning is a mixture of nucleic acids and amino acids and is further classified by the blend ratio of nucleic acids **(See Table 2).** As *umami* is synergistically strengthened by combining 5'-IMP and 5'-GMP with MSG, most nucleic acid seasoning products are compound seasonings. In Japan, low- and high-nucleic acid compound seasonings are mainly sold for home use.

(Table 2) Classification of nucleic acid seasonings currently available

Classification	Ingredient
Nucleic acid seasoning	5'-IMP, 5'-GMP, sodium ribonucleotide
Low-nucleic-acid compound seasoning	Sodium ribonucleotide (1%-2.5%)
High-nucleic-acid compound seasoning	Sodium ribonucleotide (6%-12%)

Compound seasonings have been commercially available in Japan since 1961 and are used in home cooking, processed foods, and dishes at restaurants. Nucleic acid seasonings are manufactured not only in Japan, but also in foreign countries. The world demand is 130,000 t, and the average market growth is about 7% **(See Table 3).**

② Yeast extract

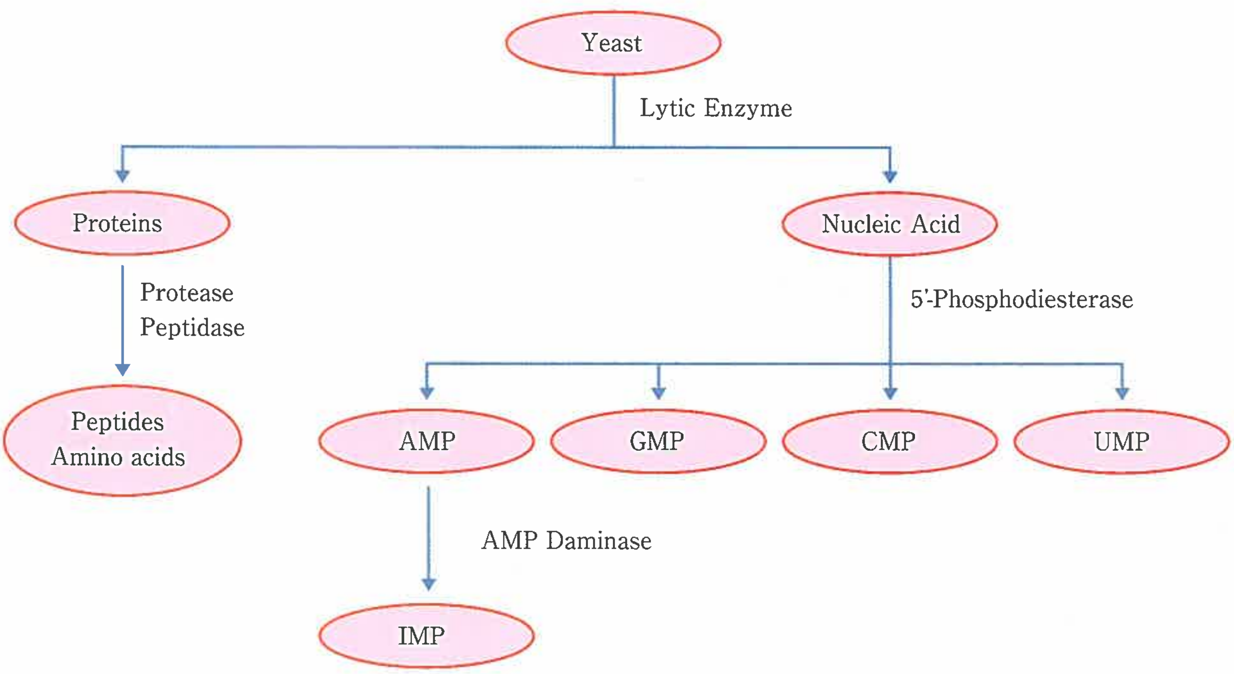
Yeast extracts are classified into two groups based on their extraction methods: one is extracted with enzymes or hot water and contains a high volume of 5'-IMP and 5'-GMP, and the other by autolysis of yeast cells **(See Table 4).**

Production of yeast extract

In enzymatic treatment, yeast cells are degraded with a cell wall lysing enzyme and subjected to extraction, and RNA in the extract is treated with the nucleic acid degrading enzyme 5'-phosphodiesterase to obtain a mixture of 5'-AMP, 5'-GMP, 5'-CMP, and 5'-UMP. Using an AMP deaminase, the tasteless 5'-AMP is converted to 5'-IMP with *umami* flavor to produce a yeast extract that contains 5'-IMP and 5'-GMP at a high rate **(Table 2. Figure 2).**

The starting material for yeast extract is baker's yeast, brewer's yeast, and *Torula* yeast. The RNA content is 4%-8% for baker's yeast and brewer's yeast and exceeds 17% for some *Torula* yeasts.

(Figure 2) Method of Yeast Extract production with Enzyme treatment



A yeast containing a high amount of nucleic acids is used to produce a yeast extract with high nucleic acid content. Enzyme treatment yields 5'-IMP and 5'-GMP rich yeast extract which provides good seasoning that cannot be produced by other methods. Also, the addition of protease and peptidase catalyzes the degradation of protein, resulting in the production of an amino acid and peptide rich yeast extract. For detailed practical uses of enzyme treatment, see Topics in Enzyme Wave Vol. 6.

Autolysis, in which the yeast cell wall is lysed by the enzymes of the yeast cells, is the oldest method that is commonly used. As nucleic acids are randomly degraded with the yeast's own phosphodiesterase and phosphatase, they are broken down into tasteless 2'- and 3'-ribonucleotides and nucleosides. Yeast extracts are low in nucleic acids with *umami* flavor when produced by this method. However, various proteases within the cells give a yeast extract with high amino acids.

Uses of yeast extract

Yeast extract contains protein, various vitamins, minerals, nucleic acids, glutathione, and dietary fibers. Yeast extracts are superior to single component seasonings because they produce the complex taste *umami* and "*kokumi*," or fullness. They are used as flavor enhancers in various foods throughout the world.

Yeast extract is categorized as a degraded natural seasoning, as are protein hydrolysates (HAP and HVP). However, HAP and HVP have safety concerns because the intermediate products MCP (monochloro propanol) and DCP (dichloro propanol) in the production process may be mutagenic. Because of this, yeast extracts have been promoted as an alternative to MSG and HAP/HVP.
Yeast extract has a beef taste. Because of BSE infection breakouts in recent years, it is used as an alternative to beef extract, and production has increased worldwide. The market size for yeast extracts is 40,000 t in Europe, 20,000 t in North America, 20,000 t in Asia, 5,000 t in Oceania, and 10,000 t in Japan.

Summary

Microorganisms and their enzymes are largely involved in the production of natural seasonings, such as nucleic acids and amino acids. The contribution of microorganisms and their enzymes to our life, including the production of seasonings, has been increasing. As their functions are further clarified, a safer and wider variety of seasoning agents will be produced to enrich our eating experience.

(Table 4) Comparison of the characteristics of yeast extract between enzymatic degradation and autolysis

Production method	Characteristics
Enzymatic degradation	Weak yeast odor Rich in nucleic acids that provide taste Strong *umami* and *kokumi*/ deep taste due to peptides Low amino acid liberation rate, high peptide content Protein degradation rate of 20% - 40%
Autolysis	Strong yeast odor Small amount of nucleic acids that provide taste Moderate *kokumi* High amino acid liberation rate Protein degradation rate of 50% - 60%

(Table 3) Changes in the estimated demand of nucleic acid seasonings and demand composition

Investigation by Nikkan Keizai Tsushinsya (Units: ton and %)

Category/Year		2000	2001	2002	2003	Composition
Export		2,600	2,650	3,000	2,400	45.3
High-nucleic-acid compound seasoning (8%-12%)	Home use	150	145	140	130	2.5
	Business use	350	350	345	340	6.4
	Total	500	495	485	470	8.9
Low-nucleic-acid compound seasoning (1%-2%)	Home use	90	90	85	80	1.5
	Business use	100	100	170	170	3.2
	Total	190	190	255	255	4.7
Mixed ingredients	Business use	260	250	250	250	4.7
Single ingredient	Business use	1,850	1,900	1,910	1,910	36.4
Total		5,420	5,485	5,900	5,300	100.0

Enzyme Modified Cheese (EMC)

About Cheese

One of the major dairy products is cheese. Cheese is a fresh food made by lactic acid bacteria and enzymes acting on milk. It is consumed throughout the world, particularly in America and Europe. As an efficient source of calcium, cheese is gaining in popularity, and global consumption of it is increasing. There are two types of cheese: natural cheese, which is ripened during manufacturing; and processed cheese, in which natural cheese is processed and pasteurized. There are many varieties of natural cheese, depending on the region where it is produced and the method of production, and each has a characteristic flavor (aroma and taste). Although processed cheeses do not have as much flavor as natural cheeses, they have some advantages over natural cheeses, such as longer shelf-life and a consistent flavor that can be adjusted for local tastes.

Cheese as Flavoring

Although natural cheese is commonly eaten as it is, the use of cheese for cheese-flavored snacks and sauces has increased in recent years. Natural cheese requires a ripening process, which increases the cost and therefore makes it unsuitable for use in mass-produced, inexpensive foods. Instead of using natural cheese, chemically synthesized flavorings can be used to add cheese flavor to food. These days, natural foods are more favored by consumers, so flavorful but low-cost natural cheese is in demand. Natural cheeses that have a shorter ripening process are relatively inexpensive; however, they have the disadvantage of bland flavor. In order to solve this problem, enzymes are used to enhance the flavor of natural cheese.

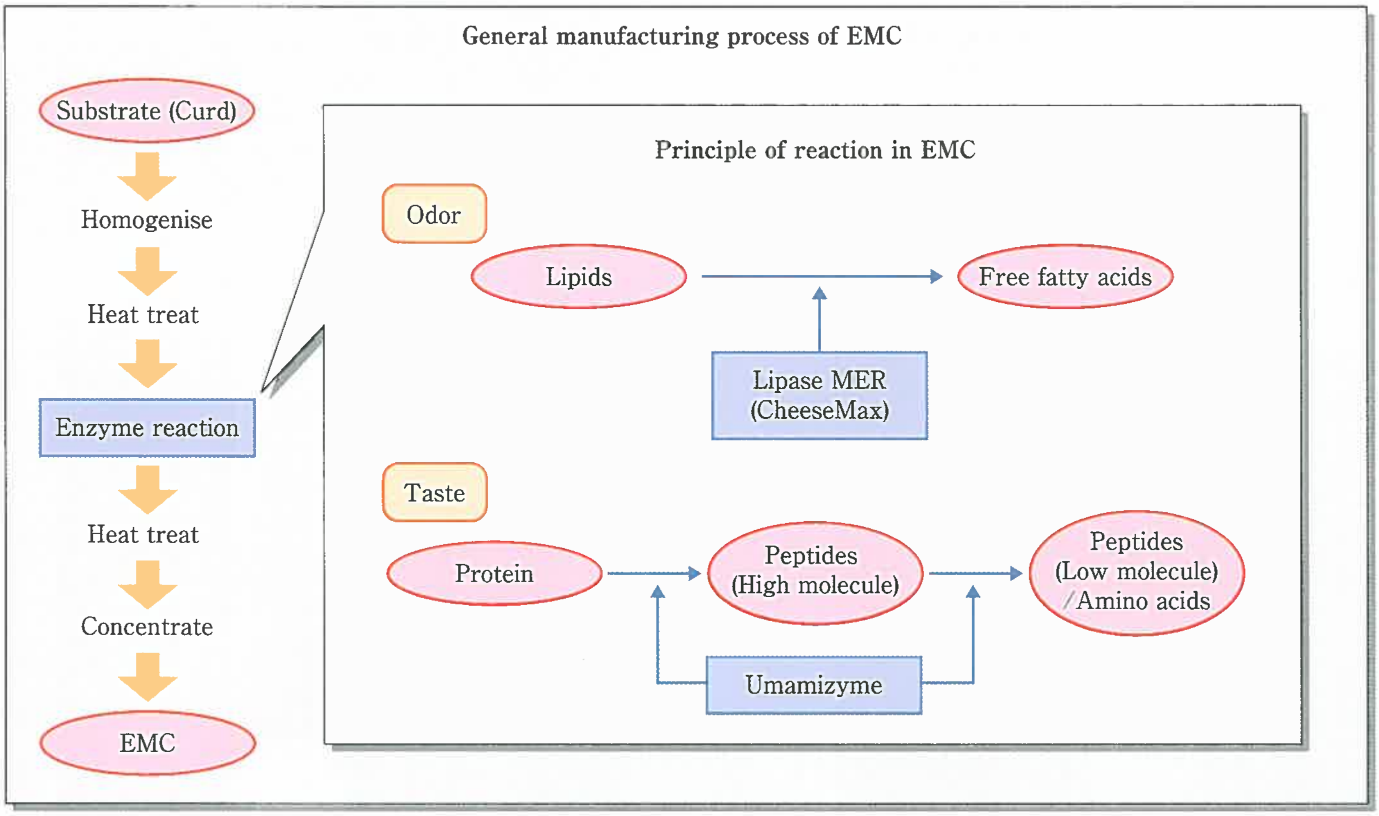
EMC

Cheese with flavor enhanced by enzyme treatment is called Enzyme Modified Cheese (EMC), and its use is increasing. It is not commonly eaten as it is but used as a flavoring to add cheese flavor to food. Pre-ripened curd or under-ripened natural cheese is used as an ingredient. After the flavor is enhanced by the enzymes' reactions, the cheese is processed into powder or paste and then added to processed foods.

What types of enzymes are suitable for the production of EMC? Basically, enzymes with reactions that are related to the ripening process of natural cheese are used.

Use of Lipase in EMC

Lipases are extensively used to enhance the flavor of EMC because the fatty acids formed in the decomposition of milk fat are important for flavor. Generally, short-chain fatty acids have a cheese-like aroma, middle-chain fatty acids have a butter-like aroma, and long-chain fatty acids have a



soap-like aroma; therefore, short-chain fatty acids are one of the key components in cheese flavor. Lipase (pregastric esterase), which is obtained from the forestomachs of calves, kids, lambs, or the like, has been used for a long time in natural cheese production. Pregastric esterase specifically liberates short-chain fatty acids and is widely used in EMC production. However, due to recent problems such as BSE and foot and mouth disease, pregastric esterase is not the best choice for EMC. Instead, lipase from microorganisms is growing in popularity. The advantages of using the microorganism-derived lipase include low cost, compliance with kosher regulations, and production of a wide variety of flavors, depending on the types of lipase used. A new product of our company, Lipase MER "Amano," (CheeseMax for the U.S. market) is a microorganism-derived lipase that increases the amount of short-chain fatty acids in EMC and produces an aroma which is very similar to that of pregastric esterase.

Use of Protease in EMC

Proteases are often used to intensify the taste. The properties required for EMC proteases include the ability to liberate many low-molecular peptides and amino acids, which create *umami* or savory flavor, and impart a less bitter taste. Bitterness comes from hydrophobic amino acids contained at the ends or within peptides. Using peptidase in combination with protease can effectively reduce bitterness by decomposing amino acids. One of our products, Umamizyme, is a combined enzyme preparation of protease and peptidase; it increases the amount of amino acids without creating bitterness, making it an appropriate enzyme for EMC production. Furthermore, Umamizyme not only intensifies the taste but also has other effects, such as softening the ingredients of EMC and enabling manufacturing with highly concentrated material. This improves the efficiency and workability in the production process.

Future of EMC

These days, cheddar, blue, and other types of EMC with flavors similar to natural cheese are produced. The flavor of EMC, however, lacks a quality found in natural cheese. This is probably because the enzyme reactions involved in the ripening of natural cheese are so complex that some flavor components cannot be formed solely through the action of protease and lipase. Accordingly, a variety of approaches are followed in EMC production, such as combining many enzymes or using lactic-acid bacteria fermentation in combination with enzymes. Our company has data to produce EMC with flavor profiles similar to those of natural cheeses. We are also continuing to research and develop new enzymes for EMC in addition to protease and lipase.

JABEE (Japanese Accreditation Board for Engineering Education)

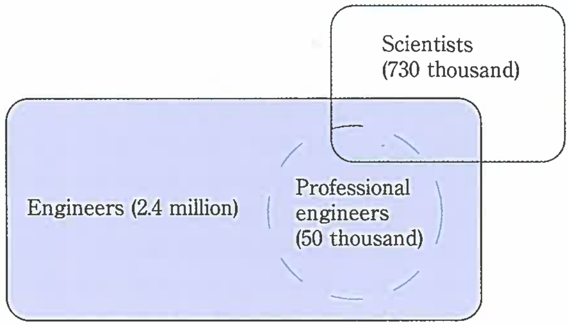
Bioengineering is a field of study that applies physics and chemistry to biology. JABEE (Japanese Accreditation Board for Engineering Education) has intensified its activities to ensure that engineers in Japan's bioengineering field are equal to those in the international community.

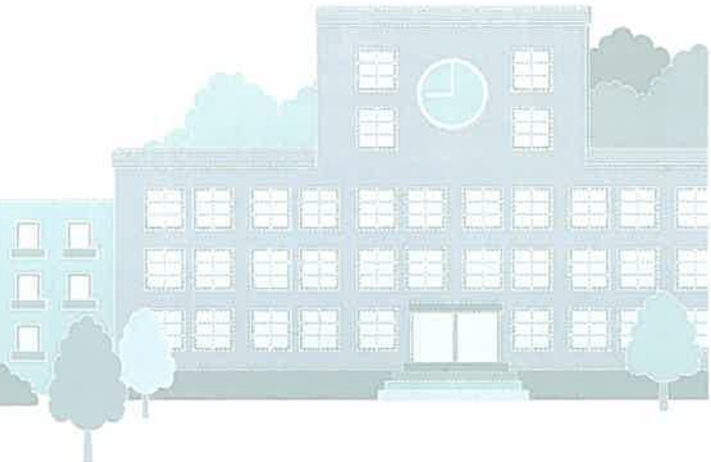
JABEE is a professional accreditation system that utilizes external organizations to evaluate the engineering education programs provided by higher education institutions such as universities. The system accredits programs that meet the demanding level of the international community. Completing a JABEE-accredited program certifies the qualifications of the institution, just as ISO accreditation provided to factories certifies their qualifications.

The Washington Accord (WA) guarantees the international comparability of engineers. JABEE is now a provisional associate member of WA and is preparing for formal entry. After JABEE joins WA, Japan's engineers who have completed a program accredited by JABEE can be evaluated on equal terms with their counterparts who have completed programs in the major Western countries. This will pave the way for Japanese engineers to become professional engineers in the U.S. and chartered engineers in the U.K.

The figure below shows the number of Japan's technical experts sorted by title. The total number of technical experts is 2.400 million. In the West, the job title "engineer" is used only for those who have become qualified, which is equivalent to "professional engineer" in Japan (currently 50 thousand). Those who complete a program accredited by JABEE are exempted from the first-stage test to become professional engineers under the designation by the Minister of Education, Culture, Sports, Science and Technology.

Number of Japan's technical experts sorted by title





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June.2005