

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.

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.

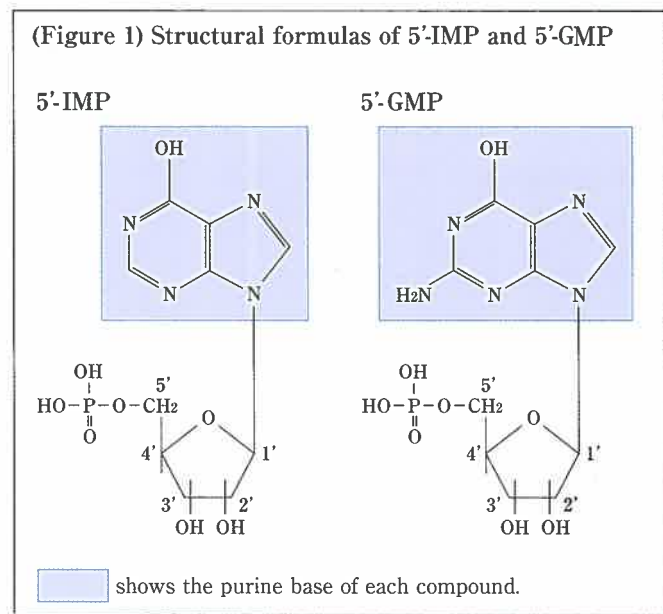
Production Methods of 5'-IMP and 5'-GMP

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 <i>Bacillus subtilis</i> and chemically phosphorylated into 5'-IMP. Sodium 5'-guanylate is produced using <i>B. subtilis</i> . Or 5'-Amino-4-imidazolecarboxyamidoriboside (AICA-R) is produced with <i>B. megaterium</i> , 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 <i>Brevibacterium ammoniagenes</i> . 5'-XMP (xanthylic acid) is produced using <i>Brev. ammoniagenes</i> and converted into 5'-GMP with <i>Brev. ammoniagenes</i> .



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

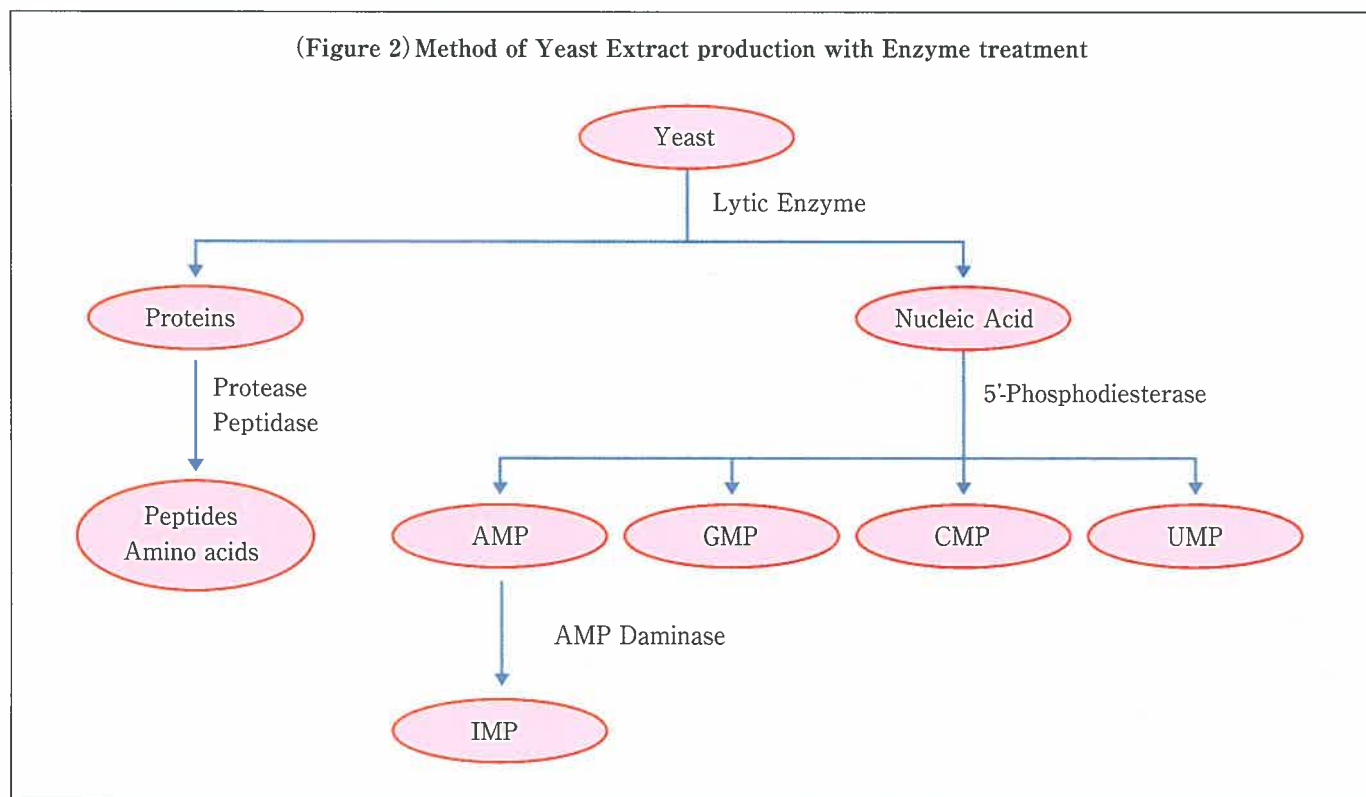
Yeast extracts are classified into two groups based on their extraction methods: one is extracted with enzymes or hot water and contains a high volume of 5'-IMP and 5'-GMP, and the other by autolysis of yeast cells (See Table 4).

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 <i>umami</i> and <i>kokumi</i> / 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 <i>kokumi</i> 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	1,850	1,900	1,910	1,910	36.4
Total		5,420	5,485	5,900	5,300	100.0