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March 18, 1994

Dear Dr. Raiten,

According to your request, we searched if any published paper exists on minute impurities in MSG. Finally we found a paper related to trace MSG impurity, written by governmental scientists in the Central Customs Laboratory, Ministry of Finance of Japan. They tried to differentiate the origin and raw materials of MSG to protect the patent rights and custom duty. (Acetic acid fermentation and the synthetic method mentioned in this 1977 paper are long gone.) As it was reported in Japanese in the Bulletin of the Central Customs Laboratory, English translation was done by the Japan Information Center of Science and Technology (JICST) and attached to the original:

Deki M, Echizen A, Temma T(1977), Minor Components in Monosodium Glutamate, *Kanzei Chuo Bunsekishoho*, 17, 59-62

Completely independent of the paper mentioned above, I enclosed HPLC charts of MSG and various foods analyzed recently in our Food Research Laboratories for your reference. The base line slightly rises at the time of buffer solution change as shown in Fig. 1. It is the baseline fluctuation and is not peaks of amino acids.

Docket No 92N-0391
FASEB # 811
3/18/94
fax

Please remind again that one should not lose the sight of the fact that foods consist of half million chemicals and a food additive is a much purer substance intended to be mixed with the foods.
Minute trace 'impurities', if any, should be understood in this context when you discuss its biological effect.

I hope the information provided would be of your use.

Sincerely yours,

Yoshi-hisa Sugita, Ph. D.
Chief Executive Officer

enclosure
YS/yo

Bulletin of the Central Custums Laboratory, 17 (1977)

Note

Minor Components in Monosodium Glutamate

Mituo DEKI, Akira ECHIZEN and Teruo TEMMA

1. Introduction

Monosodium glutamate (MSG) is well known as the first seasoning to be industrialized in Japan. Since MSG was manufactured by Suzuki in 1908, it has played an important role as a flavor enhancer in the food industry. At the beginning, MSG production was vulnerable to unstable supplies of raw materials and price fluctuations because MSG was manufactured by an extraction process using imported agricultural products including wheat, soybean, corn as raw materials. In order to overcome these shortcomings, research on a new method of MSG production not based on the extraction process was carried out since the earliest times. After World War II, expansion of demand of MSG elicited many studies on MSG production, resulted in the development of a synthetic method from acrylonitrile in 1952, followed by the establishment of a fermentation process in 1956, which was a turning point in the production of the seasoning.

Although it had long been known that some microorganisms extracellularly accumulated free amino acids, the first production of L-glutamic acid on an in industrial scale was achieved by Kita[1] using *Cephalosporium sp.* Since then, research on production of glutamic acid using microorganisms has developed vigorously in Japan. Industrialization of glutamic acid using *Micrococcus glutamicus* was

established by Kinoshita et al.[2] and many patents on MSG production using microorganisms followed in Japan.

With the establishment of industrial production of MSG by the use of microorganisms, most MSG in Japan is currently produced by fermentation process and a little is produced by extraction or synthetic methods. Since MSG production by fermentation is protected by patent rights in Japan as mentioned above, imported MSG produced in violation of Japanese patents for the fermentation method must be controlled as a material coming under Art. 21 of The Customs Tariff Law. Therefore, it is necessary to distinguish imported MSG according to manufacturing process. In this study, trace amino acids and trace metals contained in MSG produced by various process were determined and possible methods for differentiation were discussed.

2. Experimental Method

2.1 Determination of amino acid content

The amount of trace amino acids was determined by bioassay[9]. Alanine, cysteine and histidine were analyzed using *Leuconostoc citrovorum* ATCC8081, arginine, aspartic acid, glycine, leucine, isoleucine, lysine, methionine, phenylalanine, serine tyrosine and valine using *Leuconostoc mesenteroides* ATCC8042 and threonine using *Streptococcus faecalis* ATCC8043. A commercially available medium for quantitative analysis of powder type amino acids was used and optical absorbance at 660 nm was measured after 24 hour culture at 37°C. The amount of each trace amino acid was obtained using standard curves prepared for each amino acid separately.

2.2 Determination of calcium and magnesium content

To one gram of each sample dissolved in distilled water, a drop of HCl was added and filled up to 100 ml with distilled water. Calcium and magnesium were measured by an atomic absorption spectrometer (Perkin-Elmer type 303 equipped with premix burner) and the contents of the samples were obtained from the respective standard curves. The conditions for measurement were as follows.

(Table)

3. Results and Discussion

3.1 Trace metals in MSG

Table 1 shows the contents of calcium and magnesium in MSG produced by fermentation process using various sugars as a raw material. As shown in this table, MSG produced by fermentation of acetic acid contained calcium at 20-50 ppm (mean: 31 ppm). When starch was used as a raw material, the calcium content was in the range of 120-190 ppm (mean 160 ppm), 5-fold higher than that of MSG produced from acetic acid. The calcium content of MSG produced from glucose by fermentation was similar to that from acetic acid, whereas the calcium content of MSG produced from molasses ranged from 480 to 680 ppm, significantly higher than that produced from acetic acid or starch. The high calcium content of MSG produced from molasses was thought not due to differences in the manufacturing process, but probably due to molasses itself used as the raw material. It is well known that a large amount of calcium is found in cane-molasses, byproduct of crystal sugar production, in which lime treatment is involved in the manufacturing process.

When mixture of starch and molasses was used as a raw material for fermentation, calcium content of MSG decreased compared with the product from molasses alone. Calcium content

was around 250 ppm in MSG produced from beet-molasses as a raw material that contained around half the amount of calcium compared with cane-molasses. This value was nearly identical to that found in MSG produced from starch. Therefore, it is difficult to discriminate MSG produced from mixture of starch and cane-molasses from that produced from beet-molasses. However, since the calcium content of MSG extracted from gluten or produced by fermentation from acetic acid is markedly different from that of MSG produced from beet-molasses, we have shown that it was possible to recognize MSG derived from molasses from its calcium content.

Besides calcium, magnesium and lead are detected in MSG. Seto[4] has reported on the trace lead in MSG. The magnesium content was in the range of 15~30 ppm in MSG produced from molasses or starch by fermentation, and there was no difference between the two materials. Magnesium content was around 10 ppm in MSG produced from acetic acid, slightly higher than around 3.5 ppm measured in MSG produced by extraction.

3.2 Trace amino acids in MSG

Various kinds of amino acids are detected as impurities in MSG in the market manufactured by extraction or fermentation processes. The kind and amount of amino acids are different according to the manufacturing process. Narui et al.[5] analyzed the level of trace amino acids in commercially available MSG products and suggested that it is possible to infer the manufacturing process from the contents of sulfur-containing amino acids.

The contents of trace amino acids in MSG in the market, with known manufacturing processes and raw materials, were determined and the data are shown in Table 2.

The total amount of trace amino acids was around 1.9% in MSG produced from hydrolysates of soybean or gluten by extraction process, while it was no more than 0.05~0.08% in MSG produced by fermentation regardless of the raw material used. Thus, these marked differences in trace amino acids contents between the extraction and fermentation processes means that the quantitation of these trace amino acids would make it easy to distinguish between the extraction process and the fermentation process.

Meanwhile, even in the fermentation process, there were differences in the kinds of trace amino acid impurities according to the raw materials used. In the case of MSG from molasses, alanine was highest among the trace amino acids, followed by arginine, glycine, aspartic acid, leucine, lysine and others. As shown in Fig. 1, MSG produced from acetic acid by fermentation exhibited a similar tendency, but arginine was lower and leucine was higher compared to MSG from molasses. In MSG produced from a mixture of molasseses and acetic acid, arginine and leucine were low and aspartic acid was high. However, the type of trace amino acids as impurities was affected more by the strain of microorganism used and the trace additives in the culture medium, etc, than by the kind of raw material used for fermentation. Therefore, it seems difficult to infer the raw material for fermentation from the behavior of trace amino acids.

4. Abstract

The manufacturing process and the raw material of MSG were inferred from the contents of calcium and magnesium in the product. MSG produced from cane-molasses by fermentation contained calcium at 500 ppm or more, 10-fold higher than that in MSG produced from soybean and gluten by hydrolysis and extraction.

MSG produced from starch by fermentation had a mean calcium content of 160 ppm, and in MSG produced from acetic acid by fermentation, it was around 30 ppm, nearly equal to the value of MSG produced by extraction. These results show that MSG produced from molasses and starch by fermentation process could be distinguished from MSG produced by extraction process by calcium content. Magnesium content exhibited a similar tendency to that of calcium and it was approximately 25 ppm for MSG from molasses. On the other hand, the amount of trace amino acids showed a high value of 1.9% in MSG produced by extraction process, but the values were 0.05~0.08% in fermentation products regardless of the kind of raw materials used. The levels of trace amino acids in extraction products were higher than in the fermentation products, especially, leucine, alanine, arginine and threonine were high. There was no big differences in trace amino acid contents between fermentation products of acetic acid and molasses except some differences in the contents of arginine, glycine, leucine, lysine and threonine.

As the charts and tables were written in English, they were omitted from this translation.