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A survey on the presence of free glutamic acid in foodstuffs, with and without added monosodium glutamate

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Abstract

A survey on free glutamic acid (Glu) content in a variety of foods (broths, soups, sauces and salad dressings), with and without added monosodium glutamate (MSG), was carried out. A simple procedure, involving a dilution step for liquid samples or homogenization with 0.1 N HCl for solid and slurry samples, followed by derivatization with *o*-phthaldialdehyde, HPLC separation on C18 column and spectrofluorometric detection, was employed to quantify Glu, as well as a number of other free amino acids and biogenic amines. Broths and soups with added MSG had Glu contents of 92.7–341 mg/100 g. The highest amounts of Glu in foods with no added MSG were found in products containing hydrolyzed proteins (up to 129 mg/100 g). None of the products ready for consumption exceeded the limit of 10 g/kg of food, established by the European Directive, 95/2/CE [European Parliament and Council Directive (1995). No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. *Official Journal, L061*, 1–40].

Relatively high amounts of biogenic amines were found in marmite (77.3 mg/100 g of putrescine and 32.2 mg/100 g of tyramine) and soy sauce while broths and soups showed generally low amounts of biogenic amines, putrescine being the most represented.

Keywords: MSG; Glutamic acid; Biogenic amines; Free amino acids; HPLC

1. Introduction

L-Glutamic acid (Glu) is a widespread amino acid present in foodstuffs as the free and protein-bound form. Foods containing large amounts of free Glu (tomatoes, mushrooms and cheese) are traditionally used to obtain savoury dishes (Giacometti, 1979; Yamaguchi & Ninomiya, 2000). Only the free form of Glu, in its L-configuration, presents flavour enhancing properties, and, for this reason, it is widely used as a flavour enhancer in the food industry, particularly in the form of the monosodium salt. Monosodium glutamate (MSG) gives the typical aroma "umami", recognized as the fifth basic taste, very similar to "meat aroma" or "broth aroma" (Bellisle, 1999). MSG can be added "pure" or as a "hidden ingredient" of yeast extracts or hydrolyzed proteins, both containing high percentages of Glu (Hegenbart, 1998; Nagodawithana, 1992).

There is no complete agreement about the safety of MSG, even though the Food and Drug Administration (FDA) includes it among the substances generally recognized as safe (GRAS). A report from the Federation of American Societies for Experimental Biology (FASEB, 1995), identified two groups of people susceptible to high MSG doses: a group intolerant to high quantities of MSG and asthmatics. Even though MSG has been identified as a determining factor of "Chinese restaurant syndrome" and migraine (Bader, 1992; Rajda, Tajti, Komoróczy, Seres, & Klivényi, 1998; Schwartz, 1988; WHO, 1987), properly conducted double-blind studies among individuals who claimed to suffer from the syndrome, have failed to demonstrate an unequivocal relationship between "Chinese restaurant syndrome" and MSG consumption (Geha et al., 2000; WHO, 1987).

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Free L-Glu is the most abundant free amino acid in the brain and one of the major excitatory neurotransmitters in the mammalian central nervous system (CNS). When brain glutamate concentration rises above physiological levels, it becomes toxic to the neurons containing glutamate receptors (Blandini & Greenamyre, 1998; Monno, Vezzani, Bastone, Salmona, & Garattini, 1995; Roy, Peyton, & Spencer, 1995).

Glutamate ingested with the meal is readily metabolised. High doses of MSG given by gavage or parenteral administration are able to produce lesions of the CNS in rodents and primates. Administration with food (particularly in the presence of carbohydrate), lowers the peak plasma levels attained; peak plasma levels are also concentration-dependent and limited by unpalatability at high concentrations (Bogdanov, Tjurmina, & Wurtman, 1996; Monno et al., 1995; WHO, 1987).

It has been suggested that excitatory amino acids (glutamic and aspartic acid) might play a central role in the pathophysiology of Parkinson's disease (Blaylock, 1997). Furthermore, different authors have reported MSG as a cofactor or an aggravating factor for other neurodegenerative diseases, e.g. Alzheimer's, amyotrophic lateral sclerosis and Huntington's Chorea (Bader, 1992; Meldrum, 2000; Nelson, Matkin, Longstreth, & McGuire, 2000).

With regard to legislation (Walker & Lupien, 2000), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1987 and the Scientific Committee of Food (SCF) of the Commission of the European Community (EC) in 1991, stated a not-specified acceptable daily intake (ADI) for L-Glu and relative salts. For precautionary reasons it was decided not to use this additive for pregnant women and children under 12 weeks old. According to Blaylock (1997), due to deficiency of the blood–brain barrier, foetuses and infants can be exposed to an excess of intracellular Glu from the circulation. In 1995, a directive of the European Commission (95/2/CE) on food additives fixed a limit of 10 g/kg for the sum of L-Glu and salts present in food products, except for unprocessed foods, baby foods (for which Glu and salts use are not allowed) and "seasoning and spices" (for which no maximum level is specified).

Fernandez-Flores, Johnson, and Blomquist (1969) proposed a formaldehyde titration procedure for Glu quantification after its separation on an ion-exchange column from an aqueous extract. Later, minor modifications were made to the separation and purification steps and different detection methods were proposed to improve sensitivity (Sporns, 1982). Reversed-phase HPLC was also used for Glu separation after derivatisation with phenylisothiocyanate, followed by UV detection at 254 nm (Daniels, Joe, & Diachenko, 1995) or after derivatization with *o*-phthaldialdehyde (Beljaars, van Dijk, Bisschop, & Spiegelenberg, 1996) to convert Glu to a fluorescent complex. Enzymatic analysis was also applied, giving good sensitivity with rapid sample preparation (Chapman & Zhou, 1999; Janarthanan & Mottola, 1998; Skurray & Pucar, 1988).

The aim of this work was to carry out a survey on the content of free Glu in several kinds of foods (broths, soups, sauces and salad dressings), with or without added MSG, present in the European and US market. A simple HPLC method, previously used for simultaneous determination of free amino acids and biogenic amines (Moret, Populin, Conte, & Cosens, 2005), was employed for this purpose. Data concerning biogenic amines are also considered.

2. Materials and methods

2.1. Samples

Different kinds of food products were purchased from the European and US markets: broths (homemade or canned products from the market), soups (ready-to-eat soups, condensed soups and creams), soup bases (bouillon cubes, pastes and granulated powders), sauces and salad dressings.

2.2. Chemicals

Aspartic acid, glutamic acid, asparagine, serine, histidine hydrochloride monohydrate, glutamine, arginine hydrochloride, glycine, threonine, alanine, γ-aminobutyric acid, tyrosine, valine, methionine, tryptophan, phenylalanine, leucine, isoleucine, lysine hydrochloride and homocysteic acid (internal standard), were purchased from Sigma–Aldrich (Steinheim, Germany). Biogenic amine standards: tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, 1,7-diaminoheptane (internal standard) were purchased from Fluka (Buchs, Switzerland).

A stock standard solution of amino acids and biogenic amines was prepared by adding an accurately weighed amount of each standard (ca. 50 mg) to a 50 ml flask and diluting to volume with water. A fresh diluted (1:400) working standard solution was prepared weekly for calibration.

All solvents used were HPLC-grade.

2.3. Sample preparation

According to the nature of the sample, different preparation procedures were followed.

Liquid samples (ready-to-eat broths) were simply diluted prior to derivatization and HPLC analysis. Soup bases (bouillon cubes, pastes and granulated powders), were dissolved in boiling water (following the instructions on the label) and then treated as a liquid sample. In the case of more complex samples, such as soups containing pieces of vegetables, salad dressings and sauces, a representative amount of the sample was homogenized with a mixer and, a 5 g amount of the resulting slurry was extracted in a mixer (2 min) with 100 ml of 0.1 N HCl containing a known amount of internal standards. The resulting homogenate was centrifuged at 20,000×*g* for 20 min (4 °C) with a

Cryofuge 20-3 centrifuge (Hereaus, Karlruhe, Germany) and the aqueous layer was collected. If necessary, the extract was filtered through paper and diluted before derivatization.

o-Phthaldialdehyde (OPA) derivatives were prepared by adding 250 µl of a ready to use OPA reagent solution (Sigma–Aldrich) to a 50 µl aliquot of the diluted extract. The mixture was vortexed for 1 min and injected 2 min after the addition of the OPA reagent.

2.4. HPLC analysis

HPLC determination was performed with an instrument consisting of two Pro Star solvent delivery pumps, model 210 (Varian, Palo Alto, CA, USA) equipped with a manual injector and a 20 µl loop. The column was a C18, Kromasil (LabService Analytica, Bologna, Italy), 250 × 4.6 mm i.d., 5 µm particle size, thermostatted at 30 °C.

The separation of OPA-derivatives was performed with a mobile phase, consisting of 370 ml of water plus 90 ml of phosphate buffer at pH 7.0 as solvent A, while solvent B was acetonitrile. The gradient elution programme was held at 13% of B for 15 min, ramped at 50% of B (40 min) and then at 85% of B (60 min) and held until the end of the run (62 min) with a flow rate of 0.8 ml/min. Detection was performed with a spectrofluorometer, model FP-2020 (Jasco, Tokio, Japan) set at 330 nm (λ excitation) and 440 nm (λ emission).

3. Results and discussion

3.1. General

Samples were analysed for their Glu contents employing a method used in a previous work (Moret, Populin et al., 2005). Except for valine, that co-eluted with methionine and leucine, that co-eluted with *iso*-leucine, the method here applied allowed quantification of free Glu and all the other free amino acids present in the standard mixture, as well as biogenic amines. Fig. 1 shows the HPLC trace obtained from the analysis of a soup base sample (SB 4) without added monosodium glutamate.

3.2. Glutamic acid

Tables 1 and 2 show the concentrations of Glu in a variety of food products, with and without added MSG, from both the Italian and US markets. In the case of bouillon cubes, pastes, and salad dressings, the Glu concentration was expressed as the weight of the product ready for consumption, prepared following the instructions on the label. Glu percentage (of the sum of the other quantified amino acids) is also shown together with the estimated MSG intake per standard serving (calculated according to indications on the label). In the case of homemade broths it was considered that one serving corresponds to 250 ml.

In products with added MSG, Glu was always the most represented free amino acid with percentages between

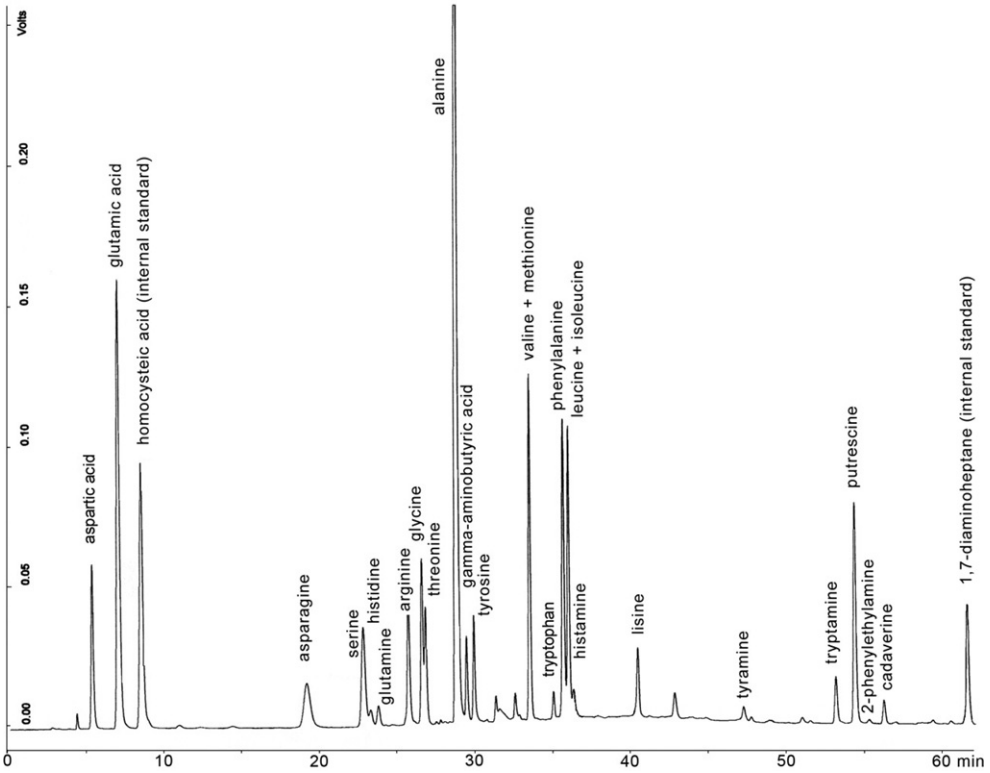

Fig. 1. HPLC chromatogram (spectrofluorometric detection) of a soup base sample (SB 4) without added monosodium glutamate.

Table 1
Glutamic acid (Glu) contents of broths (BR), soups (SO) and soup bases (SB)

Sample code	Ingredients	Added MSG	Origin	Glu (mg/100 g product ready to use)	Glu (mg per serving)	Glu (% on total free AA)
BR 1[a]	M	No	EU	5.1	12.7	12.1
BR 2[a]	M	No	EU	1.5	3.7	6.5
BR 3[a]	M, V	No	EU	40.3	101	6.5
BR 4[a]	M, V	No	EU	12.7	31.6	9.1
BR 5[b]	M, V, Y	No	USA	27.2	68.0	19.6
BR 6[b]	M, V, Y	No	USA	33.8	84.5	21.1
BR 7[b]	M, Y	Yes	USA	162	405	71.6
BR 8[b]	M, Y	Yes	USA	118	295	65.3
BR 9[b]	M, V, Y, H	Yes	USA	187	468	80.9
BR 10[b]	M, V, Y, H	Yes	USA	242	606	78.5
SO 1[c]	V, Y	No	EU	39.7	99.3	14.9
SO 2[d]	V, Y	No	EU	29.2	73.0	12.9
SO 3[c]	V, Y	No	EU	45.5	114	15.8
SO 4[c]	V	Yes	EU	176	439	47.0
SO 5[c]	V	Yes	EU	284	710	61.1
SO 6[c]	V, Y	Yes	EU	221	554	59.7
SO 7[d]	V, Y	Yes	EU	208	521	49.4
SO 8[c]	M, V	Yes	EU	223	558	59.2
SO 9[e]	M, V, Y, H	Yes	USA	267	667	50.6
SO 10[e]	M, V, Y, H	Yes	USA	280	701	56.3
SO 11[d]	M, V, Y, H	Yes	USA	341	853	86.6
SO 12[d]	M, V, Y, H	Yes	USA	316	790	78.8
SB 1[f]	V	No	EU	0.3	0.8	8.8
SB 2[f]	V, H	No	EU	129	322	47.7
SB 3[g]	V, Y	No	USA	8.2	14.5	30.3
SB 4[f]	V, Y	No	EU	7.8	19.5	24.6
SB 5[f]	V, Y	No	EU	46.5	116	24.7
SB 6[f]	V, Y, H	No	EU	10.1	25.3	19.5
SB 7[h]	V, Y, H	No	EU	67.7	169	22.5
SB 8[g]	M	No	EU	1.3	3.3	5.9
SB 9[g]	M	No	EU	2.8	2.2	4.3
SB 10[g]	M, Y	No	USA	9.2	18.3	16.0
SB 11[h]	M, V, H	No	EU	87.8	220	47.5
SB 12[h]	M, V, Y, H	No	USA	19.0	38.0	34.9
SB 13[f]	V	Yes	EU	178	445	91.6
SB 14[f]	V	Yes	EU	146	291	77.4
SB 15[h]	V, Y	Yes	EU	236	589	86.6
SB 16[h]	V, Y	Yes	EU	295	737	86.8
SB 17[h]	V, Y	Yes	EU	339	848	86.6
SB 18[h]	V, Y	Yes	EU	276	691	86.0
SB 19[h]	V, Y	Yes	EU	294	735	87.9
SB 20[f]	V, Y	Yes	EU	133	332	81.1
SB 21[f]	V, Y	Yes	EU	92.7	232	83.9
SB 22[f]	V, Y, H	Yes	EU	115	288	92.7
SB 23[h]	M, V, Y	Yes	EU	315	788	78.8
SB 24[h]	M, V, Y	Yes	EU	323	808	86.8

AA, amino acids; M, meat; V, vegetables; Y, yeast extract; H, hydrolyzed vegetable proteins.
[a] Home prepared broth.
[b] Canned liquid broth.
[c] Ready to use soup.
[d] Cream.
[e] Condensed soup.
[f] Granulated powder.
[g] Paste.
[h] Bouillon cube.

47.0% and 92.7% (on average 76.5%). Among the different types of products analyzed, soup bases and salad dressings with added MSG showed the highest percentages of Glu (76.8–92.5%).

Food products with no added MSG presented a larger variability, with percentages ranging from 4.3% to 47.7% (on average 18.9%). Samples with no added MSG, containing protein of only vegetable and/or animal origin, had

Table 2
Glutamic acid (Glu) contents of sauces and salad dressings

Sample code	Ingredients	Added MSG	Origin	Glu (mg/100 g product ready to use)	Glu (mg per serving)	Glu (% on total free AA)
SS	V, H	No	USA	677	102	20.2
TE	V, Y, H	No	USA	60.0	9.0	17.4
MA	V, Y	No	USA	836	33.4	10.7
SD 1	M, V	Yes	USA	291	246	89.1
SD 2	V	Yes	USA	753	784	90.0
SD 3	V	Yes	USA	462	481	87.0
SD 4	V	Yes	USA	266	252	81.9

AA, amino acids; SS, soy sauce; TE, teriacky sauce; MA, marmite; SD, salad dressing; M, meat; V, vegetables; Y, yeast extract; H, hydrolyzed vegetable proteins.

very low Glu percentages (between 4.3% and 12.1%), while the presence in the formulation of hydrolyzed proteins (and to a lesser extent of yeast extract) contributed to an increase of the Glu percentage. Both these ingredients are considered as "hidden sources" of MSG and for this reason the FDA has proposed that the phrase "contains glutamate" be added to the label, in addition to the listing of the common or usual name of hydrolyzed protein ingredients that have significant levels of glutamate. As demonstrated by samples SB 2 (47.7% of Glu) and SB 11 (47.5%), particularly high percentages of Glu can be reached with the addition of hydrolyzed proteins. Glu content of hydrolyzed proteins depends of course on free amino acid composition of the proteins used: it is known, for example, that gluten has a high percentage of Glu.

Glu contents comprised between 92.7 and 341 mg/100 g for products with added MSG and between 0.3 and 129 mg/100 g for products with no added MSG, were found for broths, soups and soup bases. The four salad dressing samples with added MSG had Glu contents between 266 and 753 mg/100 g. Except for teriacky, sauce samples had very high Glu contents: 677 mg/100 g for soy sauce and 835 for marmite (both of these products had no added MSG). The products containing vegetable and/or meat proteins showed the lowest Glu contents, while higher contents were found in products treated with hydrolyzed protein. With respect to the corresponding food products with no added MSG, those with added MSG presented, on average, a Glu content 6–7 times higher.

None of the products ready for consumption exceeded the limit of 10 g/kg of food product established by the European Directive, 95/2/CE. However, some bouillon cubes, concentrated bases and salad dressings exceeded the 10 g/kg when the Glu content was expressed on the product as purchased from the market (data not shown), but not when it was expressed on the product ready for consumption. Anyway these products are included in the category "seasoning and spices" for which no maximum level is specified.

The oral ED_{50} for production of hypothalamic lesions in neonatal mouse is about 500 mg/kg body weight by gavage (Walker & Lupien, 2000; WHO, 1987). The amount ingested with a single meal is far from being of concern for human health, even when different products with high MSG loads are consumed during the same meal. The largest palatable dose for humans is about 60 mg/kg body weight with higher doses causing nausea (WHO, 1987). The amounts of Glu per serving ranged from 232 to 853 mg for products with added MSG and from 0.8 to 322 mg for products with no added MSG. It can be concluded from these data that, while in the case of an adult, it is very difficult to exceed the 60 mg/kg body weight, in the case of a child of 12 kg body weight (2 years old), it is possible in some cases (samples with more than 720 mg of Glu per serving) to exceed the dose able to cause nausea with a single serving.

Compared to soup samples of European origin, US soup samples with added MSG generally presented a slightly higher MSG load.

3.3. Biogenic amine

Biogenic amines are low molecular weight organic compounds that can be found in relatively large amounts in fermented foods as a consequence of enzymatic decarboxylation of the corresponding amino acids. Their presence in vegetables and meat products has also been associated with food spoilage (Moret, Smela, Populin, & Conte, 2005) and, for this reason, they can be used as a marker of freshness.

Putrescine was generally the most represented biogenic amine with amounts ranging from trace to 10.1 mg/100 g of product ready-to-eat in broths, soups and soup bases. Compared to soup bases with no added MSG (pastes and bouillon cubes), which contained, on average, 0.4 mg/100 g of putrescine, larger amounts (on average 5.1 mg/100 g) were found in soup bases (bouillon cubes and granulated powders) with added MSG. Low amounts of putrescine were also found in homemade broths (on average 0.4 mg/100 g). Tyramine concentrations not exceeding 1.0 mg/100 g and lower amounts of cadaverine and histamine (generally not exceeding 0.2 mg/100 g) were found in broths, soups and soup bases.

As expected, marmite, a concentrated yeast extract (77.3, 32.2 and 19.6 mg/100 g of putrescine, tyramine and cadaverine, respectively) and soy sauce, a fermented product (44.9, 3.9 and 33.9 mg/100 g of putrescine, tyramine

and cadaverine, respectively) had the highest biogenic amine load.

4. Conclusion

Results of this survey showed that none of the products with added MSG exceeded the European limit of 10 g/kg of product. Due to the presence, among ingredients, of hydrolyzed proteins, probably rich in Glu, some of the products with no added MSG presented relatively high Glu contents (approaching those of some products with added MSG). This confirms the necessity to declare on the label the presence of glutamate, when "hidden sources" are used. The higher biogenic amine content (possible marker of food spoilage) found in broth and soup samples with added MSG, with respect to the corresponding products without added MSG, seems to supports the idea of the Italian Association of Consumers "Altroconsumo" according to which this flavour enhancer is often used to mask ingredients of poor freshness.

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