

## Estimation of Monosodium Glutamate by Modified HPLC Method in Various Pakistani Spices Formula

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**Summary:** The concentration of monosodium glutamate (MSG) was evaluated in ten samples of formulated spices in Pakistan. The samples were analyzed by modified HPLC method after water extraction and derivatization of MSG by dinitrofluorobenzene (DNFB), using reversed phase C<sub>18</sub> column, mobile phase was consisted of methanol: water (1:1) followed by UV detection at 254 nm. The standard curve of derivatized MSG of 500, 250 and 125 µg/mL was plotted to determine the quantity of MSG in spices samples. The peak of MSG was identified by comparing it with retention time of MSG standards, that is, 8.6 min. MSG concentration in different samples was in the range of 2.6-7.7%, showing a wide range of added MSG. Glutamate contents in the samples were in the permissible limit established by the European Directive, 95/2/CE. Furthermore, MSG levels in food prepared by the formulated Pakistani spices samples are also less than the largest palatable dose of MSG for humans.

Keywords: MSG, Glutamate, HPLC, Spices.

### Introduction

Monosodium glutamate (MSG) is the sodium salt of the non-essential amino acid glutamate, one of the most abundant amino acids found in nature. Animal proteins may contain about 11 to 22% by weight of glutamate while plant proteins contain as much as 40% glutamate [1]. Glutamate is thus found in a wide variety of foods and in its free form has been shown to have a flavor enhancing effect [2]. MSG has a characteristic taste called umami which is considered distinct from the four other basic tastes (sweet, sour, salty and bitter). Umami has gained widespread acceptance as a fifth basic taste [3] and therefore, glutamate is often deliberately added to foods – either as the purified MSG salt or as hydrolyzed protein. Previously, MSG was made from wheat gluten and defatted soybean cakes but now normally obtained by the fermentation of carbohydrates (molasses from sugar cane or sugar beet, as well as starch hydrolysates) using bacterial or yeast cultures. The least solubility of glutamate is the characteristic which makes its purification easy [4].

The optimal palatability concentration for MSG is between 0.2 – 0.8% (w/w) and its use tends to be self-limiting as over-use decreases its palatability [5]. The largest palatable dose for humans is about 60 mg/kg body weight [6]. However, there are some studies showing certain amount of MSG has possible toxic effects [7] and may be associated with myocardial and hepatic

diseases [8]. Therefore, MSG as a food ingredient has been the subject of health studies.

In the late 1960s, numerous case reports appeared in the scientific literature describing 'MSG symptom complex'. Regarding this, a Federation of American Societies for Experimental Biology (FASEB) compiled a report on behalf of FDA that concludes that MSG was safe for most people when eaten at customary levels. However, it also said that, based on anecdotal reports, some people may have an MSG intolerance thus causing 'MSG symptom complex' which are considered representative of the acute, temporary and self-limited reactions to oral ingestion of MSG [9], symptoms may include burning sensations in the back of the neck, forearms, chest, facial pressure/tightness, chest pain, headache, nausea, palpitation, numbness in back of neck, radiating to arms and back, tingling, warmth, weakness in face, temples, upper back, neck and arms, bronchospasm (observed in asthmatics only), drowsiness and weakness. In some recently conducted studies, the most frequently reported symptoms were headache, numbness/tingling, flushing, muscle tightness and generalized weakness [10, 11]. Prevalence of these symptoms are suggested to be 1–2 % of the general population.

Considering the MSG as a risk factor, this study was aimed and designed to screen the Pakistani

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spices formulations for MSG levels and we quantified MSG concentration using HPLC by modification of the method of [12]. Therefore, we have developed a convenient and reliable method for the determination of MSG from species.

## Results and Discussion

### Optimization of Solvent System

Rodriguez [12] used acetonitrile: glacial acetic acid 1% (v/v, 1: 3) as the mobile phase which did not work in our work environment and did not give any peak of standard MSG. After trying many reported mobile phases (data not given), 50% methanol: 50% water was selected and was shown compatible with our system. Therefore, this solvent system was used for chromatographic separation of MSG in all the samples.

### Standard Curve

Serially diluted standard MSG solutions (1000, 750, 500, 250, 125 and 100  $\mu\text{g/mL}$ ) were derivatized with DNFB and subjected to HPLC for separation. All the running conditions were same as samples. The retention time of standard was 8.6 min unlike that of Rodriguez [12] where it was 9.2 min, the difference may be due to different solvent systems used which increased the solubility of the test compound in the samples. The standard curve was plotted between peak areas versus different concentrations of MSG standard in  $\mu\text{g/mL}$  as shown in Fig. 1. The regression equation calculated was ( $y = 40656x - 39778$ ) with the correlation coefficient  $r^2 = 0.992$ . The representative chromatograms of monosodium glutamate standards (125, 250 and 500  $\mu\text{g/mL}$ ) are also shown in Fig. 2.

### Quantification of MSG in Spices Samples

Derivatized samples of 10 brands of spices were subjected for chromatographic separation followed by the detection at 254 nm. The samples were run in triplicate and the calculated percentage (w/w) was taken as a mean of three values, the Average Deviation was also taken. The peak of MSG was identified by comparing it with retention time of MSG standards viz. 8.5-8.7 min. The respective concentrations of MSG in the samples were calculated with the help of standard curve. The percent concentrations of MSG observed in different brands (A-J) are presented in Fig. 3. The MSG concentration in different spice samples were found

in the range of 2.7-8.8 % showing a wide range of added MSG but among them the most prevalent normal levels were 3.0-5.0%. This is the first data of MSG in formulated spices used to prepare Pakistani food.

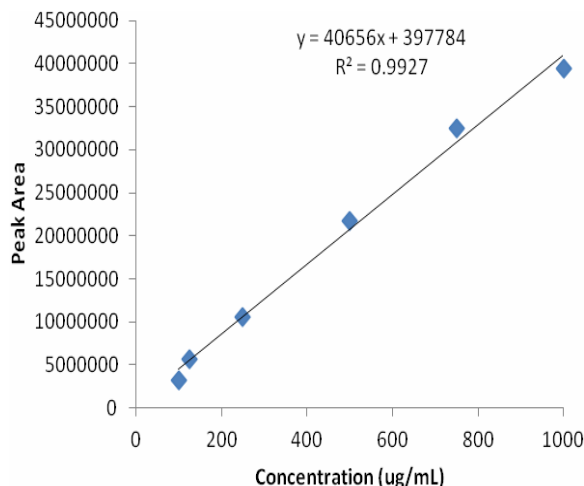


Fig. 1: Standard curve of Monosodium Glutamate.

Although the MSG is not considered as deterioration component of spices powder due to its stable chemical nature, it is single amino acid, the care was taken that the shelf life of the products used is 01 year and at the time of experiment, expiry date was more than 06 months ahead. Other constituents are also spices and have no interacting effect on MSG. The product was kept at room temperature during the experiment as recommended by manufacturer. The protein was also estimated by Biuret method in all the samples but no significant protein was found in any of them (data not shown). This proved that the glutamate concentration in spices only represented the MSG added in them and not because of any hidden source (hydrolyzed proteins). Our data is also comparable with the data of added MSG in salad dressing samples in which glutamate contents were between 0.266- 0.753 % [13]. It was considered that salad dressings were not containing hydrolyzed or meat protein which was the same in case of spices we used. In one of the report, quantification of MSG in hamburgers commercially available in Argentine markets was 0.1-0.2% [12]. Main reason we observed is that the hamburgers were ready to eat and therefore the MSG quantity found in them was per serving. However, in case of spices, they are used to prepare 0.5-1.0 kg meat or vegetable item and so the MSG was diluted 10 times which was 0.3-0.50% in prepared food. This level of MSG is comparable with the MSG levels in ready to eat hamburgers and salad dressings.

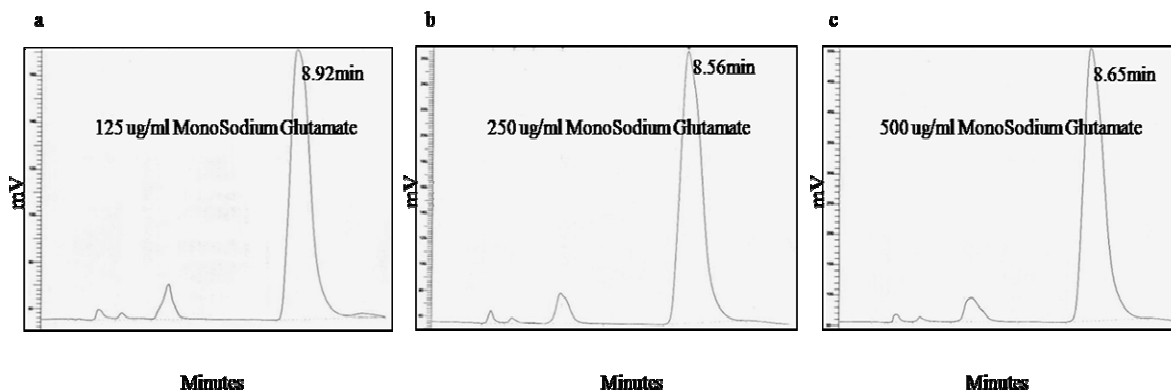


Fig. 2: Representative chromatograms of Monosodium Glutamate Standards. a) 125 µg/mL, b) 250 µg/mL and c) 500 µg/mL.

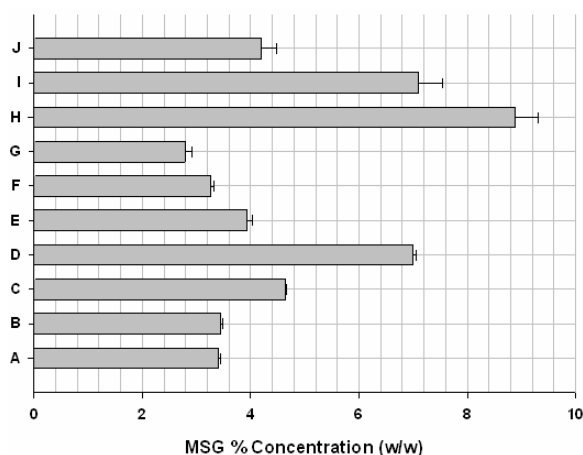


Fig. 3: Percent Concentration (w/w) of Monosodium Glutamate in Various Pakistani Spice Brands.

Another reason may depend on the developed taste of population of two nations, Pakistani citizens preferred spicy and savory foods with added meat taste. According to European Directive, 95/2/CE, it was established that the glutamate contents in products ready for consumption should not exceed the limit of 10 g/kg of food product, in the present study, the spices sample showed the presence of 27-88 g/kg glutamate which as stated above was reduced to 10 times in prepared food. The obtained value falls well within the established limit at the time of consumption, although for seasoning and spices, there are no established specified maximum levels of MSG. Further, the palatable dose of MSG ranged between 0.2-0.8 percent and the largest palatable dose of MSG for humans is 60 mg/kg body weight [6], the range we observed in spices is within this range. If the average body weight is considered 60 kg, the suitable amount of MSG would be <3.6 g MSG per meal while the normally consumed MSG is far less than this value.

Scientific Committee of Food (SCF) in 1991 decided not to use L-glutamate for pregnant women and children under 12 weeks of age [14].

## Experimental

### Samples and Reagents

MSG standard (99%) was purchased from Vedan Enterprises; 2,4-dinitro-1-fluorobenzene was bought from Merck; sodium bicarbonate was from BDH, hydrochloric acid used was from BioM Laboratories, Cerritos, USA; diethylether was purchased from Fluka and methanol from Sigma-Aldrich. All the reagents used were of analytical grade except HPLC grade methanol. Commercial samples of famous ready to use spices brands purchased from the local market and were specific for preparing different food recipes.

### Standard and Sample Preparation

Stock standard of MSG (5 mg/mL) was prepared in deionized water. The stock was diluted to make the working standard solution which was further diluted serially in deionized water to obtain 1.0, 0.75, 0.5, 0.25, 0.125 and 0.1 mg / mL of MSG to make standard curve. pH was adjusted to 7.8 using sodium bicarbonate (5 % w/v).

Ten spices samples were weighed to make 5 mg / mL solution in deionized water and the 10 mL solutions were filtered by Whatman filter paper No. 1. The filtrates containing isolated MSG were collected and the pH was adjusted to 7.8. Both the standard MSG and the MSG isolated from samples needed pre-column derivatization. Among several derivatizing agents, we chose DNFB [12]. Aliquot of 0.5 ml of standard solutions and samples were transferred to a test tube and 10 µL of DNFB was added. The mixtures were shaken in water bath for 3 hours at 40°C in the dark. Excess of DNFB was

removed by extracting with 0.5-1 mL diethyl ether. Remaining aqueous solution was acidified with 50  $\mu$ L hydrochloric acid (6M) before extracting DNP-amino acid with diethyl ether. The extraction continued until the ether no longer gave color. The traces of ether were evaporated and the leftover residue was collected with 500  $\mu$ L methanol out of which 20  $\mu$ L of each sample and standard was injected for chromatographic analysis.

#### Instrumentation and HPLC Analysis

Samples were run on HPLC (Perkin Elmer, Series-200) equipped with a manual injector and a 20  $\mu$ L loop and a UV/visible detector. Reversed phase C<sub>18</sub> analytical column Kromasil (5  $\mu$ M, 25 x 0.46, Teknokroma) was used. Sonication of samples and standards was performed by Sonicator (Branson-3510) while pH was adjusted by digital pH meter (PCSIR, Pakistan). Samples were separated with mobile phase, consisting of methanol: water (1:1) with a flow rate of 1.2 mL / min at ambient temperature 25°C and peak was detected at 254 nm.

#### Conclusion

In conclusion, a modified HPLC method is used to quantify MSG in spices using single organic mobile phase. Data shows that MSG levels per meal consumed by Pakistani people are within the range established by European Directive (10 g/kg of product or prepared food) [15] and also within the palatable range for human (60 mg/kg body weight). It is concluded that using these branded spices; Pakistani population may not face the health problems like headache, nausea, palpitation, drowsiness etc. which are the representative symptoms of MSG intolerance.

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