DETERMINATION OF MONOSODIUM GLUTAMATE IN MEAT PRODUCTS

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Abstract

The monosodium glutamate is present in numerous foods, for example soups and meat products, because it is an excellent enhance of the flavor. Its determination is important, because it is related with food sanitary quality. In this work a new methodology has been developed to determine monosodium glutamate in hamburgers based on high performance liquid chromatography (HPLC). A method for separation of monosodium glutamate followed of derivatizing with dinitrophenyl reagent is presented. The DNP-amino acid was eluted with 25 % acetonitrile – 75 % glacial acetic acid 1 % (v/v). It is detected at 254 nm. This method is simple, fast and can be used in quality control food laboratory.

Resumen

El glutamato monosódico está presente en numerosos alimentos, por ejemplo sopas y productos cárnicos, debido a que es un excelente resaltador del sabor. Su determinación es importante, pues está relacionada con la calidad higiénica del alimento. En este trabajo se ha desarrollado una nueva metodología para determinar glutamato de sodio en hamburguesas, basada en la cromatografía líquida de alta presión (HPLC). En primer lugar se separa la sal sódica del aminoácido y se emplea el dinitrofenil como reactivo derivatizador. La elución se realiza con el siguiente sistema: 25 % de acetonitrilo- 75% de ácido acético glacial 1 % (v/v). La detección se realiza a una longitud de onda de 254 nm. El método desarrollado es simple, rápido y puede ser aplicado en los laboratorios de control de calidad de alimentos

Introduction

In Argentina hamburger consumption is very common especially for children and adolescent and it has increased since the radicacion of fast food restaurants, well known as fast food. Argentine Food Regulations defines the hamburger as "the product elaborated with minced meat, added salt, pepper, monosodium glutamate (MSG) and ascorbic acid, the lipid content it is not superior at 20 %" [1]. Glutamate is naturally present in foods containing protein. It can be found in a free form or bounded to the other amino acids in proteins. Nowadays, MSG is obtained by fermentative process using molasses, starch, sugar beets or sugar cane as raw material. It is an important additive for enhancing the food flavor. This additive harmonizes with the salt and acid flavors, and its contribution with the sweet food is poor. It is only in this free form that glutamate can enhance a food's flavor.

The amount of glutamate used to enhance the flavor in food such as meat, birds, soup, sauce and sausage oscillates between 0.20 and 1.00 % (w/w). It has self-limitation

because when the concentration is superior to 1.00 % (w/w) the effect on food flavor decreases.

Lately, investigations of sensorial physiologists from Europe, United States, and Japan indicate that this additive has the fifth basic taste defined as "unami" [2]. In Argentine it is considered as additive included in "Resolution 587" incorporated as resolution of Mercosur GMC 086/96 annex A of Official Bulletin (14/5/98) [3]. The fact that it is classified in this way indicates that there is not maximum limit to be added, but there are some bibliographic references that indicate that above certain amount MSG has possible toxic effects [2, 4] and it is associated with some myocardial and hepatic diseases [5]. Taking into account the above mentioned we consider that glutamate quantification in food is useful and can be used to evaluate food safety quality. There are a lot of methods to determine this additive in foods for example, amperometric [6], enzymatic [7], spectrometric [8] and liquid chromatographic techniques [9] so they can be used to determine this additive in meat and meat product. All those methods suffer from a number of drawbacks, such as sample preparation procedures too troublesome and mainly economic disadvantages. This emphasizes the necessity of a simple and convenient analytical method for accurate and reliable measurement of monosodium glutamate. The objective of this publication is to describe a new instrumental methodology, using high performance liquid chromatography to determine monosodium glutamate in hamburger.

Materials and method

Apparatus: Laboratory mixer (diameter 3.18 mm). Chromatograph high performance liquid (HPLC): Konick-500. Analytical Column: reversed phase ODS-Hypersil (5 um), 200 um x 4.6 mm. Hewlett- Packard. U.V. visible detector: KNK-029-757. Konick. Registrer C-R6A, Chromatopac. Shimadzu.

Reagents: All chemicals used were analytical reagent grade. Trichloroethylene. Sodium bicarbonate (5 % w/v). 2,4 dinitrofluorobenzene (DNFB). Diethylether. Hydrochloric acid (6 M). Methilic alcoholic. L-glutamic aminoacide (SIGMA). L- glutamic acid standard: 500 mg/ 100 mL.

Raw material: we used commercial hamburgers bought at different supermarkets of Bahía Blanca (Argentina).

Sample Preparation: 250 g of hamburger were processed with a laboratory mixer to obtain a homogenous sample.

Assay procedure: The proposed method has two steps: a) MSG isolation and b) chromatographic analysis.

a) Isolation of monosodium glutamate from food: A technique developed by Agulló et al [10] was employed to obtain a water extract in which the monosodium glutamate will be separated from the rest of the hamburger constituents. Aliquots of sample (20.0 g) were extracted by homogenizing with 20.0-30.0 mL of distilled water. The resulting slurry was filtered through a Buchner filter and a metallic vacuum trap. This procedure was repeated several times until 100 mL was collected. A 10 mL aliquot of this extract and 2.0 mL of trichloroethylene were added to a centrifuge tube, stirred and centrifuged at 3000 rpm for 15 minutes. The organic phase is discarded and the aqueous phase is used to chromatography analysis.

b) High Performance liquid chromatography analysis (HPLC).

c) Pre-column derivatization: to detect the analite at 254 nm it was necessary to derivatize the glutamic acid. There are several derivatizing reagents as dinitrophenyl (DNP), phenylthiohydantion (PTH), ortophtaldehyde (OPA), and dinitroflorobenzene (DNFB) and dansyl chloride (DNS). In this work an adaptation of Reedy methodology [11] was used. The pH of the supernatant was adjusted to 7.50 - 8.00 by adding appropriate amount of sodium bicarbonate 5%. A small aliquot sample (0.50 mL) was transferred to a test tube and 10 μL of 2,4-DNFB was added, then the mixture was shake in the dark at 40°C for 3 hours. The excess of DNFB was removed by extracting it with diethyl ether. The remaining aqueous fraction was acidified by 50 μL of 6 M hydrochloric acid and DNP-amino acid was extracted with diethyl ether until the ether no longer became colored. The ether was evaporated and the residue was taken up in 0.50 mL of methanol and injected into the HPLC apparatus.

d) HPLC Conditions: All chromatographic runs were made at ambient temperature (25°C) using a solvent flow rate of 1.2 mL/min. The ultraviolet detector was set at 254 nm. The mobile phase was preparing with 25% acetonitrile and 75% glacial acetic acid (1% w/v).

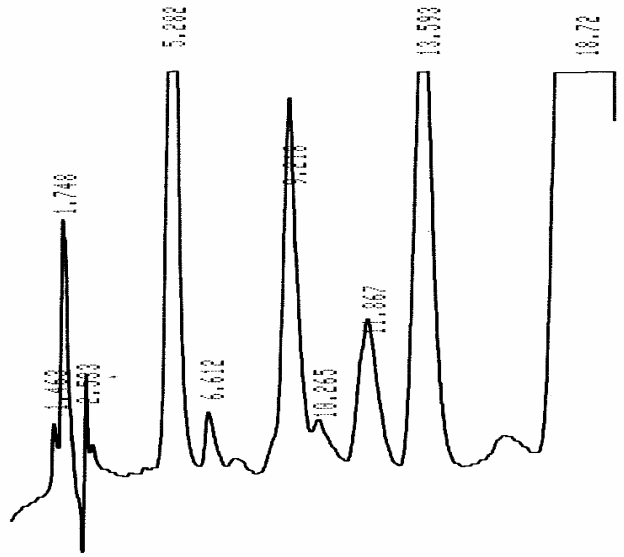

Figure 1: *Chromatogram of standard amino acid (retention time of glutamic acid: 9.218)*

Quantification study

Calibration curve: different aliquots of standard solution were subjected to pre-chromatography derivation and processed as the sample. The concentration range used was from 14.45 to 144.50 mg monosodium glutamate/100 g hamburgers.

Recovery assay: a sample spiked with a known amount of standard solution was processed using the same conditions above mentioned.

Quantification: it was necessary to use the values obtained in calibration curve, considering the peak, which retention time (TR) agrees with the standard solution retention time (9.2 min)

Results and discussion

Initially, we attempt to separate the free amino acid and to detect it at 240 nm without derivatization, but it was unsuccessful. The problem might have been the low sensitive at this wavelength due to low U.V. extinction coefficient of the amino acids. To solve this inconvenient, we decided to use the derivatization technique proposed by Reedy [11] to allow detection in U.V. region, after chromatographic separation.

Optimum HPLC solvent: three different solvent systems were used: 50% acetonitrile-50% acetic acid 1% (system 1); 50% methanol - 50% water (system 2) and 25% acetonitrile - 75% glacial acetic acid 1 % (v/v) (system 3). The system 3 was the most efficient mobile phase to quantify the monosodium glutamate. MSG peak identification was performed by comparing with the standard retention time (9.2 min). Two chromatograms, one of the standard amino acid and another of the sample, are shown in figure 1 and 2 respectively.

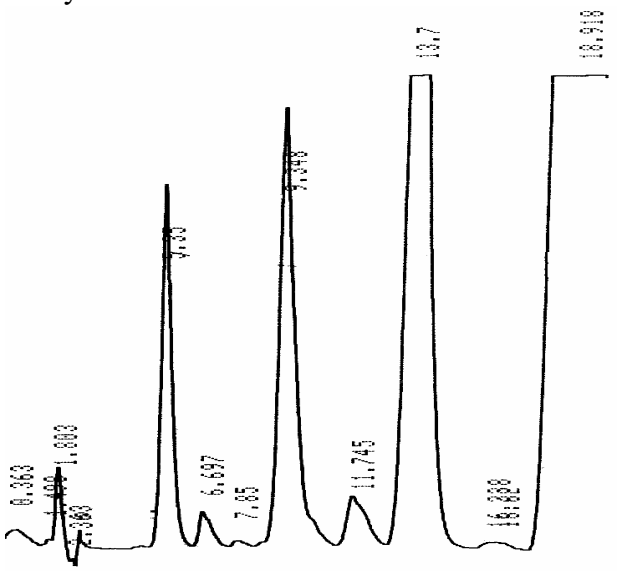

Figure 2: *Chromatogram of an hamburger sample acid (retention time of glutamic acid: 9.348)*

Monosodium glutamate concentration was determined by using a calibration curve (peak area versus concentration of different amount of monosodium glutamate/100 g hamburger). The regression equation was y = 4547.9 x - 39453 and the correlation coefficient was $r^2 = 0.96$. The mean recovery of 99.6% is obtained with a coefficient of variation of 0.10% (Table 1).

The concentrations of this additive in different samples of hamburger obtained with the proposed method are shown in Table 2.

The results of ANOVA analysis indicate a significant difference (P = 0.05) between the monosodium glutamate concentrations in the hamburgers analyzed. It is in concordance with the fact that the Argentine Food Codex does not establish an allowed value for this additive.

Table 1: *Recuperation assay. The values are means of three determinations.*

mg MSG/100 g hamburger	mg MSG/100 g hamburger.	
	Added	Recovered
105.8	28.5	134.2

Table 2*: Values of MSG in hamburger. The values are means of three determinations.*

Samples	mg MSG/100 g hamburger
1	100.5 ± 0.10
2	100.7 ± 0.15
3	130.3 ± 0.15
4	217.9 ± 0.10
5	178.9 ± 0.20

Conclusion

We used a simple and rapid technique to separate MSG from the rest of hamburger component. This procedure was in agreement with the chromatographic method used to determine this additive. Both techniques are reproducible and accessible to be used in quality control food laboratory.

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