Colorimetric Determination of Monosodium Glutamate in Food Samples Using L-glutamate Oxidase

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Abstract A rapid, sensitive and simple chromogenic method was developed for quantitative determination of monosodium glutamate (MSG) in food samples. The method incorporated a glutamate oxidase and peroxidase. The liberated H₂O₂ from a glutamate sample as a result of enzymatic action was measured using 4-aminoantipyrine and phenol as a chromogenic reagent at 502 nm. Glutamate calibration curve was linear up to 125 mmol L⁻¹ with a detection limit of 2 mmol L⁻¹. The results of analyzing L-glutamate in various food samples using the approach were compared to those of a standard procedure employing HPLC method. MSG concentration in different samples ranged from 0.93 to 4.9 g/kg. This method is relatively sensitive and specific, without the need of pre-treatment for sample. Fig 5, Tab 1, Ref 25

Keywords MSG; colorimetry; L-glutamate oxidase; soup; noodle; hamburger

CLC TS207.3

Monosodium glutamate (MSG) is the sodium salt of non-essential amino acid. It is sold as a fine white crystal substance similar in appearance to salt or sugar. MSG is widely used as a flavour enhancer in a variety of food preparations (e.g. soup base, noodles, soy sauce, etc.) The most palatable concentration for MSG is between 0.2% and 0.8%, and the largest palatable dose for humans is about 60 mg/kg body weight.

MSG’s use has become controversial since 1980 and questions have been raised since then about its safety. It has been identified as a determining factor of (Chinese Restaurant Syndrome) [1] When certain people consume food that contains MSG, symptoms may occur such as headache, nausea, facial pressure, tightness, chest pain and warmth [2]. It has also been suggested that excitatory amino acids (glutamic and aspartic acid) might play a central role in the pathophysiology of Parkinson’s disease [3]. Different authors have reported MSG as an aggravating factor for other neurodegenerative diseases, e.g. Alzheimer’s, amyotrophic lateral sclerosis [4].

A number of non-enzymatic and enzymatic methods for the quantitation of L-glutamate in the metric have been reported to this date. The non-enzymatic methods are mainly based on chromatographic techniques [6-9]. However, these methods involve complicated and time-consuming procedures, and require extensive sample pre-treatment [10]. Several types of L-glutamate biosensors have been reported, including L-glutamate oxidase (L-GLOD), L-glutamate dehydrogenase (GDH) and L-glutamate decarboxylase (GDC) [10-12]. However, the GDC and GDH have some drawbacks due to poor substrate specificity and the requirement for expensive coenzyme such as NAD⁺. L-glutamate oxidase (GLOD) is used instead because it has relatively high substrate specificity compared to GDH and GDC and does not require additional coenzyme. GLOD is an enzyme that specifically catalyzes oxidative deamination of L-glutamate in presence of water and oxygen with the formation of α-ketoglutarate, ammonia and hydrogen peroxide [13-14] as follows:

\[ L-\text{Glutamate} + O_2 \rightarrow L-\text{GLOD} \rightarrow \alpha-\text{keto-glutarate} + NH_3 + H_2O \]

L-glutamate can be quantified either by substrate disappearance (O₂) [15], or by the product formation (NH₃ or H₂O). H₂O₂ has been detected by amperometric methods [16-17] or chromogenic reaction by using flow injection system [18] or test strip [19].

The aim of this research was to find a simple and rapid chromogenic method for determining MSG in food, without complicated sample pre-treatment or using advanced equipment. The principle of the chromogenic reaction in this study was as follows: (POD: peroxidase):

\[ 2H_2O_2 + \text{Phenol} + 4-\text{Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinone} + 4H_2C \]

1 Experiment

1.1 Reagents

L-GOD (L-glutamate oxidase from stryptomyces), HRP (peroxidase from horseradish), MSG, and aminoantipyrine were purchased from Sigma Aldrich (USA). Phosphate buffer (Na₂HPO₄ and NaH₂PO₄) and phenol were purchased from Panreac (Spain). Chromogenic reagent consisted of aminoantipyrine 0.11 g and phenol 0.043 g was made up in 100 mL of phosphate buffer (pH = 7.5).

1.2 Apparatus

The absorbance was measured by HITACHI UV-VIS spectrophotometer U-1800 (serial No. 5103534). For the HPLC analysis, precolumn derivatisation using ophthaldialdehyde (OPA) was carried out as described by Ryth-Rinder M et al. [20]

1.3 Standard preparation

Exactly 100.0 mg of MSG was dissolved in 100.0 mL volumetric flask. Dilution of the stock solutions were made by deionised water to yield 200, 150, 100, 75, 50, 25, 20, 10, 5 mg/L
of MSG standard solutions.

1.4 Preparation of food sample for analysis

Soup, noodle and hamburger were obtained from local restaurants and supermarkets in Damascus, Syria. All the samples were analyzed fresh. Soup and noodle samples were prepared by dissolving 50 mg in warm water (10 mL), incubated at 70 °C for 10 min, then allowed to cool to room temperature; solutions were shaken and centrifuged at 2,000 r/min for 15 minutes. Hamburger samples were prepared as follows: 250 g of hamburger were processed with a mixer to obtain a homogenous sample. Aliquots of samples (20 g) were extracted by homogenizing with 20-30 mL distilled water, the resulting slurry was filtered. This procedure was repeated several times until 100 mL was collected. A 10 mL aliquot of this extract and 2 mL of trichloroethylene were added to a centrifuge tube, stirred and centrifuged at 2,000 r/min for 15 minutes. The organic phase was discarded and the aqueous phase used. The samples were diluted appropriately for quantitation of glutamate, so as to ensure that the glutamate concentration fell within the linear calibration range.

1.5 Application of the bioreaction

The reaction mixture contained 0.5 mL of GLOD (0.2 U/mL), 0.2 mL of HRP peroxidase from horseradish (0.1 U/mL), 0.1 mL of chromogenic reagent and 0.3 mL of phosphate buffer (pH = 7.5). After pre-incubation for 2 min at 37 °C, 0.1 mL of standard glutamate was added, making the total volume 1.2 mL [21]. The absorbance at 502 nm was measured by spectrophotometer after incubation for 20 min at 37 °C with gentle shaking.

2 Results & Discussion

Many well-known chromogenic reagents are available with peroxidase for quantitation of H2O2 generated in biochemical reactions [22-23], including ABTS, TMB, o-phenylene diamine. However, many of these systems are light/air sensitive, carcinogenic or lacking appreciable water solubility. Phenol was selected in this work with system (4-AAp and phenol) maximum colour development observed at 502 nm in a phosphate buffer (pH = 7.5).

2.1 Effect of L-GLOD concentration on the reaction

In order to evaluate the effect of L-glutamate oxidase concentration on reaction, four amounts (0.02 U, 0.06 U, 0.1 U, 0.14 U) of L-GLOD in phosphate buffer solution were tested. The optimum amount for L-GLOD was 0.1 U, which gave a maximum absorbance (Fig. 1). Decrease in absorbance with increasing concentration of enzyme from 0.1 to 0.14 U may be due to blockage of the active site of the enzyme for higher concentration. This might prevent MSG to reach the active site of L-GLOD [24].

2.2 Effect of temperature on the reaction

Variation in absorbance at different temperature from 20 to 60 °C is illustrated in Fig. 2. With all other conditions constant, the maximum absorbance was at 37 °C. Above this temperature, a decrease of absorbance was observed, which may be due to the reduced concentration molecular oxygen in the medium and thermal deactivation of the enzyme at higher temperature [24].

2.3 Effect of pH on the reaction

The pH profile study was carried out through bioreaction at various pHs (5.0 - 9.0). All other conditions were kept constant. The buffers (0.1 mol/L) used were: acetate buffer (pH 5.0-6.0), sodium phosphate buffer (pH 6.0-7.5), and Tris-HCl buffer (pH 7.5-9.0). The optimum pH of the reaction was reported at pH = 7.5 (Fig. 3).

2.4 Calibration curve and linear range

The reaction with different glutamate concentration between 2 μmol L⁻¹ and 990 μmol L⁻¹ was investigated. The calibration curve was linear over 25-125 μmol L⁻¹ (R² = 0.997). The limit of detection (LOD) was 2 μmol L⁻¹. The concentration of L-glutamate can be estimated from the calibration curve (Fig. 4). The relative standard deviations (RSD) was 1.2% (N = 6).

Specificity of the reaction of L-glutamate was tested by applying the reaction on most possible interferents such as L-aspartate and D-glutamate. No colour or absorbance change was observed, showing that these substances did not interfere with the detection of L-glutamate.

In the recovery studies, food samples (soup, noodle,
and hamburger) extracts of known glutamate concentrations were spiked with glutamate standards. The spiked samples were analyzed in duplicate. Analysis of these samples resulted in recoveries in the range 95%-101%, indicating that the complicated matrices of these food samples did not interfere with the performance of the glutamate bioreaction.

2.5 Analysis of food Samples

In order to evaluate the suitability of this reaction for quantitation of glutamate, a variety of soup, noodle and hamburger from different manufactories were selected for analysis. The directive of the European Commission (95/2/CE) fixed a limit of 10 g kg⁻¹ for the sum of L-glutamate and salts present in food products, except for unprocessed and seasoning for which no maximum limit is specified [23]. As shown in Table 1 the average L-glutamate in the common food samples of our experiment ranged from 0.93-4.9 g/kg.

![Fig. 4 Calibration curve of and linear range of the reaction](image)

Table 1 Comparison of L-glutamate level in food samples obtained from local restaurants and supermarkets by using the bioreaction and HPLC method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average L-glutamate per whole packet (g/kg ± s)</th>
<th>Bioreaction</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream of mushroom soup (1)</td>
<td>4.6 ± 0.09</td>
<td>4.53 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Cream of mushroom soup (2)</td>
<td>4.9 ± 0.04</td>
<td>4.88 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Cream of mushroom soup (3)</td>
<td>4.5 ± 0.09</td>
<td>4.51 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Vegetable soup (1)</td>
<td>3.6 ± 0.11</td>
<td>3.59 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Vegetable soup (2)</td>
<td>3.3 ± 0.09</td>
<td>3.3 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Vegetable soup (3)</td>
<td>3.8 ± 0.11</td>
<td>3.79 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Chicken soup (1)</td>
<td>3.19 ± 0.11</td>
<td>3.06 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Chicken soup (2)</td>
<td>3.3 ± 0.04</td>
<td>3.1 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Chicken soup (3)</td>
<td>2.85 ± 0.12</td>
<td>2.87 ± 0.05</td>
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</tr>
<tr>
<td>Lentil soup (1)</td>
<td>0.93 ± 0.17</td>
<td>1.21 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Lentil soup (2)</td>
<td>1.1 ± 0.11</td>
<td>1.1 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Lentil soup (3)</td>
<td>0.93 ± 0.09</td>
<td>0.9 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Noodle soup (1)</td>
<td>2.76 ± 0.11</td>
<td>2.74 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Noodle soup (2)</td>
<td>4.65 ± 0.12</td>
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<tr>
<td>Noodle soup (3)</td>
<td>4.8 ± 0.14</td>
<td>4.79 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Noodle soup (4)</td>
<td>4.8 ± 0.04</td>
<td>4.77 ± 0.21</td>
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<td>Noodle soup (5)</td>
<td>4.75 ± 0.11</td>
<td>4.71 ± 0.19</td>
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<td>Noodle soup (6)</td>
<td>4.7 ± 0.04</td>
<td>4.73 ± 0.07</td>
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<tr>
<td>Hamburger (1)</td>
<td>1.35 ± 0.08</td>
<td>1.34 ± 0.06</td>
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</tr>
<tr>
<td>Hamburger (2)</td>
<td>1.3 ± 0.12</td>
<td>1.32 ± 0.11</td>
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</tr>
<tr>
<td>Hamburger (3)</td>
<td>1.7 ± 0.14</td>
<td>1.68 ± 0.17</td>
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<tr>
<td>Hamburger (4)</td>
<td>2.1 ± 0.06</td>
<td>2.06 ± 0.2</td>
<td></td>
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</tbody>
</table>

L-glutamate was determined from the homogenized sample of the entire package; for each sample, three subsamples were analyzed for their glutamate content.

The results of L-glutamate in the food samples using our new approach were compared to those using a standard procedure employing HPLC method. There was excellent correlation between the values obtained by the proposed method and by the standard reference method (Fig. 5).

3 Conclusions

This study based on the bioreaction for quantitation of MSG in food, established a method with several advantages over current ones. It is rapid and simple, and does not need pretreatment of the samples. The new approach involves application of the two reactions simultaneously in the same tube for determination of MSG in food.

Calibration curves are linear up to 25 μg with a detection limit of 0.5 μg. This method is more sensitive than other methods, especially those using FI and test strip. This is the reason why it can be used in clinical analysis (diagnosis in myocardial, neurological and hepatic diseases). The time needed for the complete colour development is 20 min. We thus concluded that this analysis can be operated by non-specialized technical staff in small laboratories.

References


Muslim NZM, Ahmad M, Heng LY, Saad B. Optical biosensor test strip for the screening and direct determination of L-glutamate in food samples [J]. Sens Actuators B, 2012, 161: 493-497


