Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 811–815
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Simultaneous determination of flavor enhancers in stock cube samples by using spectrophotometric data and multivariate calibration

Carolina C. Acebal, Adriana G. Lista *, Beatriz S. Fernández Band *

FIA Laboratory, Department of Chemistry, Universidad Nacional del Sur, Av. Alem 1253, 8000 Bahía Blanca, Argentina

Received 21 November 2006; received in revised form 17 May 2007; accepted 10 June 2007

Abstract

Spectrophotometric data followed by a suitable treatment of chemometric analysis were used for the simultaneous determination of monosodium glutamate (MSG), guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP) in stock cube samples, without any previous extraction step. By this way, the overlapping of the absorption spectra was resolved using a PLS-1 model. The concentration for experimental calibration matrix were varied between 5.03–34.2 μg mL^{-1} for IMP and GMP, and 448–1399 μg mL^{-1} for MSG. The relative errors of prediction (REPCV %) were 1.8, 2.8 and 3.1 for IMP, GMP and MSG, respectively.

To verify the accuracy of the proposed method a recovery study on real samples was carried out with satisfactory results (92–110%).

Keywords: Flavor enhancers; Stock cubes; Spectrometry; PLS

1. Introduction

Monosodium glutamate (MSG), guanosine 5′-monophosphate (GMP) and inosine 5′-monophosphate (IMP) are widely used as flavor enhancers in food. They are commonly added together in some meat products like meat, chicken and stew stock cubes owing to their synergistic effect (Schlichtherle-Cerny & Amado, 2002). This effect allows to decrease the required amount of them in food.

Ingestion of high concentrations of MSG causes the appearance of neurological diseases, mainly Parkinson and Alzheimer (Arruda, Filho, Montenegro, Araújo, & Silva, 2003). So, its concentration in food must be controlled.

IMP and GMP are purine nucleotides synthesized in the human body and play diverse roles in the cellular metabolism (Devlin, 1997). López Navarro, Bueno, Gil, and Sanchez Pozo (1996) suggested that dietary nucleotides are energetically advantageous to fulfill the liver need for nucleotides. However, people with high levels of uric acid in blood and urine must avoid food with this kind of compounds because the degradation of purine nucleotides leads to the formation of uric acid (Devlin, 1997).

The Argentine Food Code (CAA) (Código Alimentario Argentino, Capítulo VI, Artículo 440) establishes the maximum allowed quantities of these analytes to be added in stock cubes: 8 g dm^{-3} for MSG and 0.50 g dm^{-3} for both IMP and GMP.

The MSG determination has mainly been carried out by chromatographic (Anderson, Zaharevitz, & Strong, 1987), spectrophotometric (Khampha, Meevootisom, & Wiyakrutta, 2004) and fluorimetric (Chapman & Zhou, 1999) techniques using derivatization or enzymatic reactions. In recent years, the nucleotides IMP and GMP have been determined by using high performance liquid chromatography (HPLC) (Charpentier et al., 2005; Ferreira, Mendes, Gomes, Faria, & Ferreira, 2001).

The multicomponent analysis using chemometric techniques as partial least squares (PLS) for the spectrophotometric data (Arancibia, Martínez Delfa, Boschetti, Escandar, & Olivieri, 2005; Gómez González, Renedo, & Arcos Martínez, 2005; Zarei, Atabati, & Malekshabani,

* Corresponding authors. Fax: +54 291 4595160 (A.G. Lista).
E-mail addresses: alista@criba.edu.ar, usband@criba.edu.ar (A.G. Lista).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.06.009

2006) has been successfully applied . One of the most important advantages of multivariate calibration is the simultaneous determination of the analytes of interest in a mixture. Durán Meras, Muñoz de la Peña, Espinosa-Mansilla, and Salinas (1993) have developed a method for the simultaneous determination of IMP and GMP, in presence of MSG, using derivative spectrometry and PLS-1/PCR. Moreover, a previous extraction with hexane was carried out.

In this work, a novel spectrophotometric method for the simultaneous determination of IMP, GMP and MSG in stock cubes using a diode array detector and chemometric analysis was proposed. No previous sample treatment is required and a continuous flow system was used to increase the sample throughput and to reduce the sample manipulation.

2. Experimental

2.1. Apparatus

All spectra were obtained by using a Hewlett Packard 8452A diode array spectrophotometer, with a spectral bandwidth of 2 nm.

A Hellma QS flow cell of 18 μL and 1 cm optical path, and a Gilson minipuls-3 peristaltic pump were used.

All the reaction coils were made of PTFE tubing (i.d. 0.5 mm).

PLS-1 analysis was performed by applying Mat Lab program and MVC1 subroutine (Olivieri, Goicochea, & Iñón, 2004). Experimental design was carried out by using the Unscrambler v. 6.0 program.

2.2. Reagents

Analytical grade reagents and ultra pure water (>18 M Ω cm^{-1}) were always used.

0.50 g L^{-1} IMP (Fluka), 0.49 g L^{-1} GMP (Fluka) and 14.03 g L^{-1} MSG (Anedra) stock solutions were prepared by dissolving the appropriate amount of their solid drugs in distilled water.

A pH 10.0 buffer solution was prepared mixing 50.0 mL of 0.03 M $Na_2BO_4 \cdot 10H_2O$ (Mallinckrod) and 18.3 mL of 0.1 M NaOH (Anedra) and diluting to 100 mL with distilled water.

Six commercial stock cubes and a granulated meat flavored stock were purchased in different local supermarkets.

2.3. Sample preparations

Taking into account the enhancers' concentration in real samples, a suitable amount of them was weighed and diluted to 25.0 mL with water.

2.4. Calibration and validation sets for multivariate analysis

For training the PLS-1 model, a calibration set of ten standard solutions was prepared following a full factorial design with two centres. The concentration levels were 5; 34 μg mL^{-1} for IMP and GMP, and 450; 1400 μg mL^{-1} for MSG.

The external validation of the calibration models was achieved by using another full factorial design. Ten synthetic mixtures were prepared with concentrations within the range used for the calibration set.

2.5. Procedure

The continuous flow manifold is depicted in Fig. 1. In order to introduce standard mixtures or samples solutions into the system, a selection valve (SV) was used. These solutions merged with the buffer solution stream in the reaction coil (R) and reached the flow cell. 17 s after sample introduction, the flow was stopped and the absorption spectrum was recorded between 190 and 320 nm. Then, the flow was immediately restored. The total time for each sample was 18 s.

The sample solution was on line filtered. For this, the stream flew through a packed column (length 4.0 cm; internal diameter 0.7 cm) filled with acetate (stuff for cigarettes), placed before the SV.

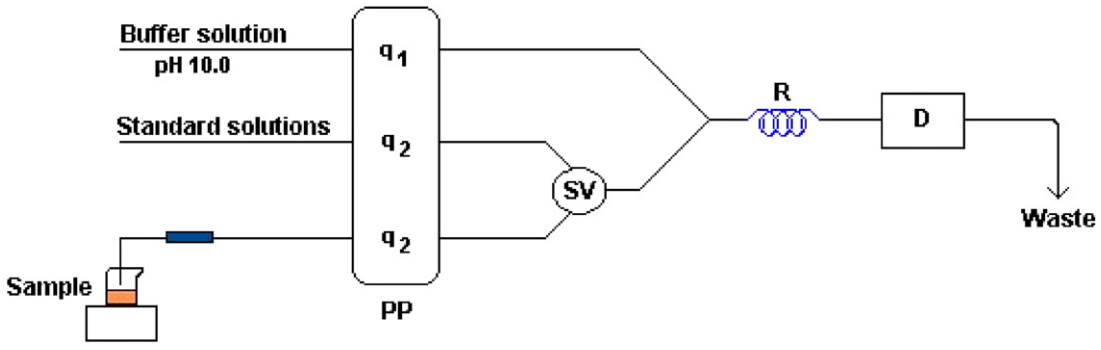

Fig. 1. Continuous flow system with a spectrophotometric detection. SV: selection valve; R: reaction coil; PP: peristaltic pump; C: acetate column; q1, q2: flow rates; D: diode array detector.

3. Results and discussion

IMP, GMP and MSG have native absorptions in the UV and their spectra present a serious overlapping. Therefore, the direct spectrophotometric quantification was not possible, thus a PLS calibration for their simultaneous determination was performed.

3.1. pH selection

The influence of pH on the absorption spectra of the analytes was thoroughly studied. A strong overlapped of the three analytes spectra was observed between 200 and 230 nm, when the pH was varied from 1.5 to 5.5. When the pH was increased from 6.0 to 11.5, the maximum of MSG at 210 nm augmented significantly, whereas the maxima of the other analytes remained constants. On the other hand, the overlapping of IMP and GMP spectra between 230 and 300 nm present similar characteristics along the pH range. So, an alkaline pH was selected for the simultaneous determination of the three analytes.

Another pH study was carried out be stepwise varying the pH values between 9.0 and 11.0 in 0.5 units. The pH 10.0 was selected because over this value no significant changes were observed in the spectra. Therefore, different buffer solutions (pH 10) were tested (sodium tetraborate decahydrate–NaOH, Britton Robinson, Glycine–NaOH). The sodium tetraborate decahydrate–NaOH buffer solution presented the optimum conditions. Fig. 2 shown the analytes spectra at pH 10.0 in this buffer solution.

3.2. Flow system variables optimization

The optimization of the continuous flow system variables was carried out by using the univariate method. For the study of each variable a calibration set was prepared and the PLS-1 model was applied to the spectral data. The reactor length was studied between 300 and 1180 mm and the optimum value was 600 mm. The studied range for the buffer flow rate was 1.10–3.43 mL min^{-1}, and for the sample flow rate was 0.58–1.8 mL min^{-1}. The selected values were 2.06 mL min^{-1} and 1.08 mL min^{-1}, respectively. In all cases, the optimum value was obtained taking into account the lowest relative error prediction (REP %) value for the analytes.

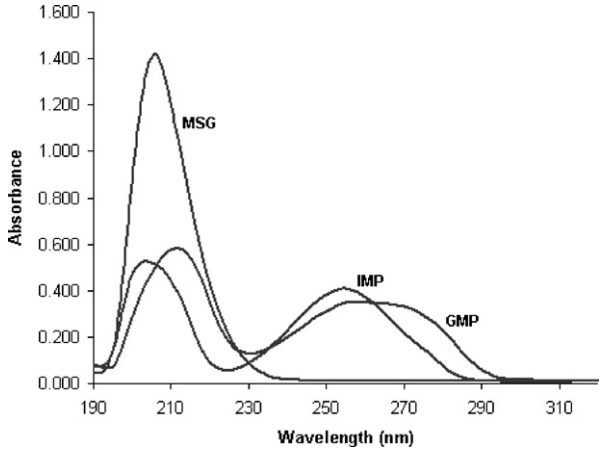

Fig. 2. Absorption spectra of the analytes at pH 10.0.

3.3. Statistical parameters

The absorption spectra for the analytes' mixtures were recorded from 190 to 320 nm. PLS model was developed in the PLS-1 model and the data were mean centered in order to remove constant background effects.

Table 1 shows the spectral ranges, the PLS-1 latent factors that have been chosen and the calibration statistical parameters obtained for each analyte.

The optimum number of factors was selected applying the leave-one-out cross-validation method described by Haaland and Thomas (1988) and the appropriated wavelength regions were selected from the spectra.

The relative mean root square error (RMSECV) and the relative error of prediction (REPCV) of the calibration were satisfactory and gave an indication of the data fit quality. The elliptical joint confidence region (EJCR) for each slope and intercept was examined. The ellipses for

Table 1
Calibration parameters

Parameter	IMP	GMP	MSG
Calibration results			
Concentration range ($\mu g\ mL^{-1}$)	5.028–34.190	5.028–34.190	447.6–1398.6
No. of factors	5	4	4
RMSE[b], CV [a] ($\mu g\ mL^{-1}$)	0.358	0.551	282
REP[c], CV [a](%)	1.8	2.8	3.1
Figures of merit			
LOD_k[d]($\mu g\ mL^{-1}$)	0.244	0.240	16.1
SEN_k ($mL\ \mu g^{-1}$)	1.2×10^{-3}	1.4×10^{-3}	4.6×10^{-4}
γ_k[e] ($mL\ \mu g^{-1}$)	1.2	1.4	0.46

Figures of merit for the simultaneous determination of IMP, GMP and MSG by spectrophotometry combined with PLS-1.

[a] CV: cross validation.

[b] RMSE (root mean square errors) = $\left[\frac{1}{I}\sum_1^I (c_{nom} - c_{pred})^2\right]^{\frac{1}{2}}$.

[c] REP (relative error of prediction) = $100 \times \frac{RMSE}{\bar{C}}$.

[d] LOD (limit of detection) = 3 × noise/sensitivity.

[e] $\gamma_k = (SEN_k/\|\delta r\|), \|\delta r\|$ is a measure of the instrumental noise and equal to 0.001.

Table 2
Analysis of real samples

Sample		IMP ($g\ dm^{-3}$)	GMP ($g\ dm^{-3}$)	MSG ($g\ dm^{-3}$)
Beef stock	A	0.000	0.000	11.93 ± 0.65
	B	0.087 ± 0.002	$0.004 \pm 0.000_2$	3.90 ± 0.09
	C	0.021 ± 0.002	0.008 ± 0.001	2.63 ± 0.09
	D	0.038 ± 0.001	0.025 ± 0.002	10.33 ± 0.27
	E	0.044 ± 0.002	0.019 ± 0.001	6.93 ± 0.32
Chicken stock	E	0.087 ± 0.001	0.021 ± 0.002	7.48 ± 0.35
Stew stock	E	0.044 ± 0.003	$0.009 \pm 0.000_3$	10.33 ± 0.27

A, B, C, D, E: different commercial brands.

Table 3
Recovery study in real samples

Sample		Added (g dm^{-3})			Found[a] (g dm^{-3})			R (%)		
		IMP	GMP	MSG	IMP	GMP	MSG	IMP	GMP	MSG
Beef stock		–	–	–	0.087 ± 0.002	0.0043 ± 0.0002	3.90 ± 0.09	–	–	–
	1	0.098	0.099	2.76	0.186 ± 0.040	0.108 ± 0.005	6.88 ± 0.56	101	105	108
	2	0.193	0.196	2.76	0.273 ± 0.010	0.195 ± 0.006	6.88 ± 0.47	97	97	108
	3	0.098	0.098	5.49	0.180 ± 0.020	0.106 ± 0.002	9.33 ± 0.31	95	104	99
	4	0.195	0.196	5.51	0.285 ± 0.010	0.198 ± 0.016	9.80 ± 0.30	102	99	107
	5	0.194	0.097	5.46	0.282 ± 0.040	0.101 ± 0.006	9.32 ± 0.71	100	100	99
Stew stock		–	–	–	0.044 ± 0.002	$0.009 \pm 0.000_3$	10.33 ± 0.27	–	–	–
	1	0.049	0.049	2.67	0.091 ± 0.003	0.057 ± 0.002	13.02 ± 0.99	96	98	101
	2	0.098	0.098	2.71	0.134 ± 0.004	0.099 ± 0.003	13.05 ± 0.28	92	92	100
	3	0.049	0.049	5.46	0.094 ± 0.007	0.060 ± 0.005	16.32 ± 0.30	102	104	110
	4	0.097	0.097	5.36	0.134 ± 0.011	0.100 ± 0.004	15.35 ± 0.44	93	94	94
	5	0.098	0.049	5.37	0.137 ± 0.009	0.059 ± 0.002	16.16 ± 0.58	95	102	109

1, 2, 3, 4, 5: different added ratio concentrations of the three enhancers.
[a] The results are averages of three replicates.

the three analytes include the theoretically expected values of (1,0), which indicated that the proposed methodology was accurate. The figures of merit are also summarized in Table 1, showing low detection limits for each analyte as well as high sensitivities.

3.4. Analysis of real samples

The proposed method was applied to six different commercial stock cube samples and a granulated meat flavored stock. Table 2 shows the analytes concentrations in the samples, expressed as required for CAA (8 g dm^{-3} for MSG and 0.50 g dm^{-3} for both IMP and GMP). Only three of the analysed samples have a MSG concentration higher than those recommended by the Argentine Food Code.

There is a reference method to determine only MSG concentration in this kind of samples. This method requires a laborious task because it involved a chromatographic separation with several elution steps for the analyte extraction and then, a potentiometric titration and its selectivity is so poor. Therefore, to validate the proposed method a recovery study was done.

For this purpose, an appropriate amount of two samples was weighed, spiked with the corresponding enhancers concentrations, diluted to 25.0 mL and introduced into the system. In Table 3 the different combination of analytes concentrations added to each sample and the obtained results for the recoveries are shown.

As can be seen, the recoveries are acceptable for this kind of products. The obtained results also showed that there are not interferences of other possible additives in the samples.

4. Conclusion

The importance of the proposed method is the simultaneous determination of IMP, GMP and MSG in stock cube samples without any pre-treatment. There are not evidence in the literature about the determination of the three analytes in real samples.

One of the advantages of the method is to have a manifold FIA extremely simple, totally accessible for the most routine laboratories. Moreover, owing to it is not necessary any sample pre-treatment a higher throughput was achieved.

Also, the only reagent used in the determination is the buffer solution, so the costs for analysis is lower.

The reproducibility and accuracy tests were successful and the recovery study showed that the proposed method yields satisfactory results.

Acknowledgements

Financial support from the Universidad Nacional del Sur is gratefully acknowledged. C. Acebal and B.S. Fernández Band acknowledge to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

Anderson, L. W., Zaharevitz, D. W., & Strong, J. M. (1987). Glutamine and glutamate: Automated quantification and isotopic enrichments by gas chromatography/mass spectrometry. *Analytical Biochemistry, 163*(2), 358–368.

Arancibia, J., Martínez Delfa, G., Boschetti, C., Escandar, G., & Olivieri, A. (2005). Application of partial least-squares spectrophotometric-multivariate calibration to the determination of 2-sec-butyl-4,6-dinitrophenol (dinoseb) and 2,6-dinitro-*p*-cresol in industrial and water samples containing hydrocarbons. *Analytica Chimica Acta, 553*(1–2), 141–147.

Arruda, N. J., Filho, J. L., Montenegro, M. C., Araújo, A. N., & Silva, V. L. (2003). Simple and inexpensive flow L-glutamate determination using pumpkin tissue. *Journal of Agricultural and Food Chemistry, 51*, 6945–6948.

Chapman, J., & Zhou, M. (1999). Microplate-based fluorometric methods for the enzymatic determination of L-glutamate: Application in measuring L-glutamate in food sample. *Analytica Chimica Acta, 402*(1–2), 47–52.

Charpentier, C., Aussenac, J., Charpentier, M., Prome, J., Duteurtre, B., & Feuillat, M. (2005). Release of nucleotides and nucleosides during yeast autolysis: Kinetics and potential impact on flavor. *Journal of Agricultural and Food Chemistry, 53*, 3000–3007.

Código Alimentario Argentino, Capítulo VI, Artículo 440 (Res. 125,25.1.82).

Chapter 12Devlin, T. (1997). *Purine and pyrimidine nucleotide metabolism* (4th ed.). *Textbook of Biochemistry with clinical correlations*. New York: Wiley-Liss (pp. 489).

Durán Meras, I., Muñoz de la Peña, A., Espinosa-Mansilla, A., & Salinas, F. (1993). Multicomponent determination of flavor enhancers in food preparations by partial least squares and principal componet regression modelling of spectrophotometric data. *Analyst, 118*, 807–813.

Ferreira, I., Mendes, E., Gomes, A., Faria, M., & Ferreira, M. (2001). The determination and distribution of nucleotides in dairy products using HLPC and diode array detection. *Food Chemistry, 74*(2), 239–244.

Gómez González, M. J., Renedo, O., & Arcos Martínez, M. (2005). Simultaneous determination of antimony(III) and antimony(V) by UV–vis spectroscopy and partial least squares method (PLS). *Talanta, 68*(1), 67–71.

Haaland, D. M., & Thomas, E. V. (1988). Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Analytical Chemistry, 60*(11), 1193–1202.

Khampha, W., Meevootisom, V., & Wiyakrutta, S. (2004). Spectrophotometric enzymatic cycling method using L-glutamate dehydrogenase and L-phenylglycine aminotransferase for determination of L-glutamate in foods. *Analytica Chimica Acta, 520*(1–2), 133–139.

López Navarro, A. T., Bueno, J. D., Gil, A., & Sanchez Pozo, A. (1996). Morphological changes in hepatocytes of rats deprived of dietary nucleotides. *British Journal of Nutrition, 76*(4), 579–589.

Olivieri, A. C., Goicochea, H. C., & Iñón, F. A. (2004). MVC1: An integrated MatLab toolbox for first-order multivariate calibration. *Chemometrics and Intelligent Laboratory Systems, 73*(2), 189–197.

Schlichtherle-Cerny, H., & Amado, R. (2002). Analysis of taste-active compounds in an enzymatic hydrolysate of deamidated wheat gluten. *Journal of Agricultural and Food Chemistry, 50*, 1515–1522.

Zarei, K., Atabati, M., & Malekshabani, Z. (2006). Simultaneous spectrophotometric determination of iron, nickel and cobalt in micellar media by using direct orthogonal signal correction-partial least squares method. *Analytica Chimica Acta, 556*(1), 247–254.