FOOD SCIENCE

UNDERGRADUATE EXPERIMENT

Quantification of Monosodium Glutamate in Beef, Chicken, and Vegetable Bouillon Cubes Using Benchtop NMR Spectroscopy

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INTRODUCTION

Monosodium glutamate, often colloquially referred to simply as MSG, is a commonly used cooking additive capable of imparting umami flavors into a variety of dishes (**Figure 1**).[1] MSG is synthetically mass-produced, but also occurs naturally in its glutamic acid form in many different foods, such as seaweed, tomatoes, cheese, and anchovies, among many others.[2] While it is a popular misconception that its ingestion can cause a variety of negative side-effects, numerous studies have shown that MSG poses no health risks and is generally recognized as safe by The United States Food and Drug Administration (FDA).[3]

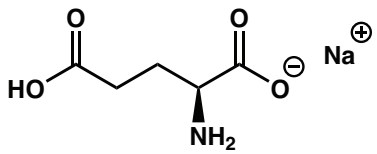

Figure 1. *Chemical structure of monosodium glutamate (MSG).*

A convenient way of adding rich umami flavors to recipes involves using concentrated bouillon cubes, typically offered in different varieties such as beef, chicken, and vegetable. One of the main ingredients contained in these products is MSG. In this sample experiment, we take advantage of quantitative nuclear magnetic resonance (qNMR) spectroscopy to quantify the MSG content in beef, chicken, and vegetable bouillon cubes, using a simple extraction with chloroform, followed by water.

Experimental

The samples prepared herein were analyzed on instruments operating at ^{1}H frequencies of 60 MHz and 100 MHz. For this study, the following chemicals were used as received and without further purification: MilliporeSigma: chloroform (≥99%), sodium formate (99.998% trace metals basis); Deutero GmbH: deuterium oxide (99.9%). Beef, chicken, and vegetable bouillon cubes were purchased from a local grocery store.

The following acquisition parameters were used for all analyses: 60 MHz: spectral width, 30 ppm; number of points, 8192; scan delay, 48 s; number of scans, 16; spectral center, 5 ppm; dummy scans, 0;

pulse angle, 90°; gain: auto; acquisition time, 4.3 s; 100 MHz: spectral width, 30 ppm; number of points, 16384; scan delay, 48 s; number of scans, 16; spectral center, 5 ppm; dummy scans, 0; pulse angle, 90°; gain: auto; acquisition time, 5.3 s. The longest T_1 time for these samples was determined to belong to the internal calibrant, sodium formate, and was measured to be approximately 10.4 seconds. Typically, an interscan delay (acquisition time + scan delay) of at least five times this value is used for accurate quantification in NMR.[4] As such, an interscan delay of at least 52 seconds was desired.

A stock solution of sodium formate was prepared by accurately weighing approximately 60 mg of sodium formate in a 1 mL volumetric flask and dissolving in D_2O. The sodium formate acts as an internal calibrant to quantify the MSG, as its purity is known, and its signal in ^{1}H NMR does not overlap with other signals. Furthermore, it is soluble in the medium of analysis and is compatible with the other species in solution.[4]

Sample Preparation

A single bouillon cube was carefully unwrapped and weighed. Approximately 100 mg of the cube was accurately weighed in a 1 mL centrifuge tube. To this tube, 0.6 mL of chloroform were added, a vortex mixer was used to thoroughly dissolve the organic components, then the suspension was centrifuged. The supernatant was carefully decanted, an additional 0.6 mL of chloroform were added, then the vortexing, centrifuging, and decanting steps were repeated, for a total of three extractions. The remaining off-white solid was dissolved in 740 µL of D_2O, filtered through a small plug of Celite® directly into a 5 mm NMR tube, and 60 µL of the sodium formate stock solution were carefully added (approximately 3.6 mg of sodium formate added). The contents of the NMR tube were well mixed before placing the sample inside the spectrometer for analysis. This process was repeated for the preparation of two additional samples, and each sample was analyzed in triplicate at both magnetic fields, allowing for confirmation that the method performs well under both repeatability and reproducibility conditions.

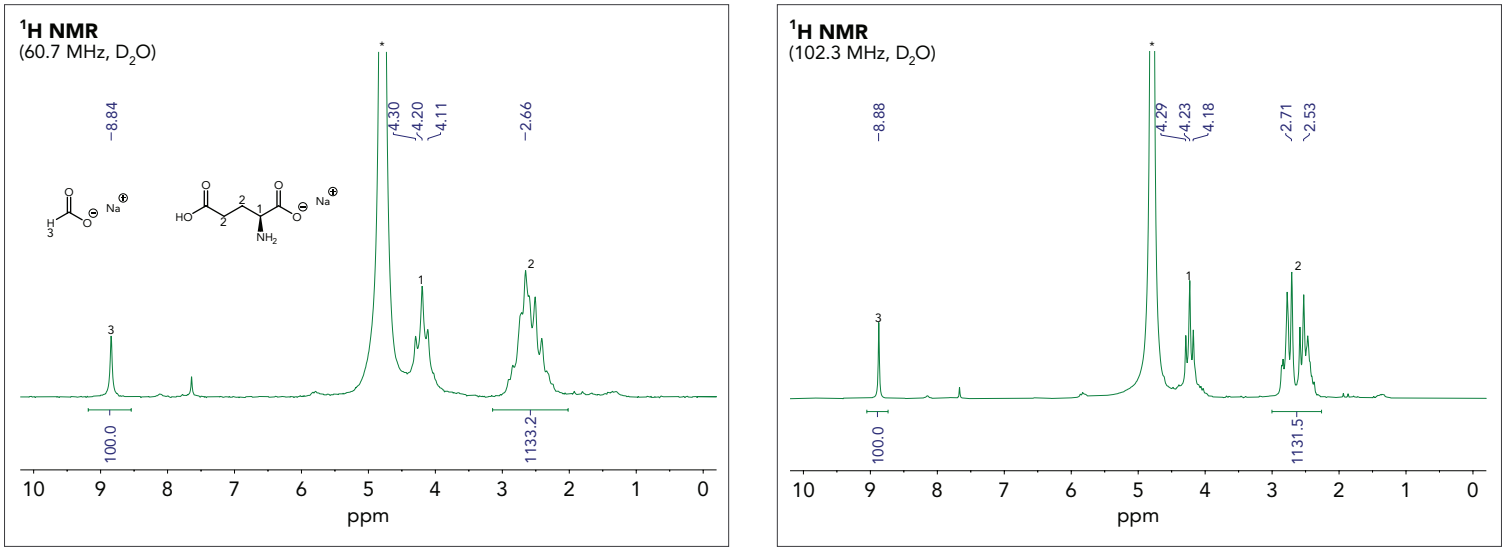

Figure 2. ^{1}H NMR spectra of extracted beef bouillon cubes with added sodium formate (internal calibrant) in D_2O at 60 MHz (left) and 100 MHz (right). The structures are shown, and their corresponding signals used for qNMR are labeled. The asterisk represents the residual solvent peak for HOD.

Results

Examples of ^{1}H NMR spectra obtained at both 60 MHz and 100 MHz are presented in **Figure 2**. While the peak dispersion observed at 100 MHz is larger than at 60 MHz, the same quantitative information is contained in both spectra, and the same integration values can be obtained. While the methine signal (1) in MSG could be used for qNMR, it overlaps slightly with the residual water signal, and the integration values would be overestimated as a result. Thus, the methylene signals (2) were used for quantification purposes.

The results of all the analyses performed on the bouillon cubes are summarized in **Table 1**. Overall, the quantified amounts of MSG were found to be the same for the different cube types across all runs and on both instruments. The relative standard deviation (RSD) values are low for all samples. The MSG content in the beef bouillon cube analyzed in this study was found to be approximately 7.6% by weight, compared to 15.9% for chicken and 20.3% for vegetable. Based on the initially recorded masses of the cubes, these correspond to approximately 820 mg of MSG per cube for beef, 1913 mg for chicken, and 2224 mg for vegetable.

***Table 1**. Summary of the results obtained for the qNMR analysis of MSG in beef, chicken, and vegetable bouillon cubes. Each assay is presented as an average of triplicate analyses, and the RSD values are included in parentheses.*

Monosodium Glutamate		
Beef Bouillon[a]		
Magnetic Field	**Assay**	**MSG (wt%)**
60 MHz	1	7.6 (0.1)
	2	7.6 (0.1)
	3	7.6 (0.1)
100 MHz	1	7.6 (0.1)
	2	7.6 (0.1)
	3	7.6 (0.1)
Chicken Bouillon[b]		
60 MHz	1	15.9 (0.1)
	2	15.9 (0.2
	3	15.9 (0.1)
100 MHz	1	15.9 (0.1
	2	15.9 (0.2)
	3	15.9 (0.1)
Vegetable Bouillon[c]		
60 MHz	1	20.3 (0.1)
	2	20.3 (0.0)
	3	20.3 (0.1)
100 MHz	1	20.3 (0.1)
	2	20.3 (0.1)
	3	20.3 (0.2)

[a]Average of averages, $\bar{\bar{x}}$ = 7.6% for both 60 MHz and 100 MHz. Average of RSD values, $\overline{RSD}$ = 0.4% (60 MHz) and 0.1% (100 MHz).
[b]Average of averages, $\bar{\bar{x}}$ = 15.9% for both 60 MHz and 100 MHz. Average of RSD values, $\overline{RSD}$ = 0.2% (60 MHz) and 0.1% (100 MHz).
[c]Average of averages, $\bar{\bar{x}}$ = 20.3% for both 60 MHz and 100 MHz. Average of RSD values, $\overline{RSD}$ = 0.1% for both 60 MHz and 100 MHz.

Conclusions

The work presented herein provides a method for students to learn about the quantification of ingredients in certain food products. Specifically, MSG in beef, chicken, and vegetable bouillon cubes was quantified, and the results agree across all samples and between both the 60 MHz and 100 MHz NMR instruments used in this study. Overall, this approach makes use of the powerful capabilities of benchtop NMR technology and can address many of the accessibility issues commonly encountered with traditional high-field NMR instruments. Furthermore, this experiment provides students with an opportunity to learn about qNMR and to obtain their own results in a matter of minutes, without having to rely on an instructor to operate the instrument on their behalf.

References

(1) (a) Stańska, K.; Krzeski, A. *Otolaryngol. Pol.* **2016**, *70*, 10–15.
(b) Wifall, T. C.; Faes, T. M.; Taylor-Burds, C. C.; Mitzelfelt, J. D.; Delay, E. R. *Chem. Senses* **2007**, *32*, 161–172.
(2) (a) Yamaguchi, S.; Ninomiya, K. *J. Nutr.* **2000**, *130*, 921S-926S.
(b) Shigemura, N.; Shirosaki, S.; Sanematsu, K.; Yoshida, R.; Ninomiya, Y. *PLOS ONE* **2009**, *4*, 1–9.
(c) Kochem, M.; Breslin, P. A. S. *PLOS ONE* **2017**, *12*, 1–9.
(3) Zanfirescu, A.; Ungurianu, A.; Tsatsakis, A. M.; Nițulescu, G. M.; Kouretas, D.; Veskoukis, A.; Tsoukalas, D.; Engin, A. B.; Aschner, M.; Margină, D. Compr. *Rev. Food Sci. Food Saf.* **2019**, *18*, 1111–1134.
(4) Pauli, G. F. *Phytochem. Anal.* **2001**, *12*, 28–42.

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