Original paper

Rapid Determination of Low-level Sulfite in Dry Vegetables and Fruits by LC-ICP-MS

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This paper describes a liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) method for the determination of low-level sulfite in dry vegetables and fruits. Aqueous 0.1% solution of formaldehyde was chosen as extraction solution for releasing the bound sulfite and forming the more stable hydroxymethanesulfonate (HMS). Sulfite was separated using an anion exchange column with isocratic elution by a 50 mmol•L⁻¹ ammonium nitrate in 0.1% formaldehyde solution (pH 7). Sulfite was detected as $^{32}$S$^{16}$O$^+$ by ICP-MS in DRC O₂ gas mode. In the optimum conditions, the linearity of the method is 0.05 – 5 mg•L⁻¹ for sulfite, and the detection limit is 0.02 mg•L⁻¹. The results demonstrated that the method achieved acceptable quantitative recoveries of 78.5% to 91.6% with relative standard deviations (RSDs) < 5%. Compared to the AOAC official method, the proposed method presented advantages of rapid and sensitive.

Keywords: Sulfite, dry vegetables and fruits, LC-ICP-MS

Introduction

Sulfite salts or sulfur dioxide are widely used as an additive in food and beverages to prevent oxidation, microbial spoiling, and to control enzymatic browning reaction during the food producing and storage process (Danilewicz, 2003; Lee and Shibamoto, 2002; Thorpe and Baynes, 2003). However, the excessive intake of sulfite is harmful to humans. Previous work demonstrated that daily consumption of sulfite exceeded 50 mg•kg⁻¹ body weight might cause many diseases, such as headaches, nausea and diarrhea (Suh et al., 2007). Due to potential health concerns, the use of sulfur dioxide and sulfite as additives in food is legally restricted in USA, EU and China. The Food and Drug Administration (FDA) of United States even requires sulfite warning on the label of foods if in the concentrations of sulfite exceed 10 mg•kg⁻¹.

Dry vegetables and fruits which are removed most of water from fresh product have less loss of nutrient substances. Compared to fresh product, dry product has much longer storage life. However, some producers may add a certain amount of sulfite to extend the dry product shelf period even in the poor storage environment. Those who claim to be the feature of “no preservatives” or “totally natural” in dry vegetables and fruits may consider to be exaggerated. So a reliable, sensitive method for sulfite analysis in dry vegetables and fruits is required. The method should be sensitive enough for covering the concentration range below 10 mg•kg⁻¹.

Various methods have been applied to determine sulfite in foods. Classical and official method of China National Standard (CNS, 2003) and Association of Official Analytical Chemists (AOAC, 1995) are based on the optimized Monier-Williams (OMW), which utilize the distillation of the samples under acidic condition and then analyzed by iodine or acid/base titration. However, these methods usually required the complicated sample preparation processes and cannot be used for fast or high-throughput analysis. Moreover, for...
dry vegetable and fruit samples, the OMW method tends to overestimate sulfite levels because of the presence of abundant volatile acidic compounds (Pizzoferrato et al., 1998; Zhong et al., 2012). In recent years, many new methods have been reported, including electrochemistry (Chen et al., 2012; Isaac et al., 2006; Karimi-Maleh et al., 2012), ion chromatography (Koch et al., 2010; Zhong et al., 2012), liquid chromatography (Pizzoferrato, 1998; Theisen et al., 2010; S. Wang et al., 2007; Zuo and Chen, 2003), optical chemical sensors or biosensors (Alamo et al., 2010; Dinckaya et al., 2007; Filik and Çetintaş, 2012), flow injection (Navarro et al., 2010; Ruiz-Capillas and Jiménez-Colmenero, 2009; Tzanaras et al., 2009). Above-mentioned methods indeed greatly shorten the analysis time. The limit of detection (LOD) was even lower than 0.1 mg·L⁻¹ in sample solution with some methods. However, by these methods, complicated operations and reagents, self-made equipments were required which prevent them from being applied widely in rapid determination of sulfite in food samples.

In recent years, liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) technology has been well developed for the determination of some metal and metalloid species due to its easy connecting to LC. Dynamic reaction cell (DRC) technique with O₂ makes the determination of total sulphur and sulphur species easier (Bandura et al., 2002; Lin and Jiang, 2009; Schaumlöffel et al., 2007; Stürp et al., 2006; M. Wang et al., 2007; Yang and Jiang, 2004), which eliminate the serious isobaric interferences from O²⁻, N³⁻ and S²⁻ (abundance: 95.018%). However, there still have some issues need to take into account when applying LC-ICP-MS for the determination of sulfite in food matrix sample.

Our work aimed to develop a simple, sensitive and reliable method for the determination of sulfite in dry vegetables and fruits. The separation was achieved on an anion-exchange column followed by ICP-MS detection. In the process, extraction conditions were optimized, and potential matrix interference was discussed.

Materials and Methods

Reagents and standards All of the chemicals used were of analytical-reagent grade. All solutions were prepared with deionized water. Sodium sulfite, sodium sulphate, sodium thiosulfate, ammonium nitrate and formaldehyde were purchased from SCRC (Shanghai, China). Sulfur-containing amino acids was purchased from local farmer’s market and supermarket in Hangzhou, China. Samples were comminuted by stainless steel grinding mill. An amount of 1.00 g of the dry sample was weighed into a 50-mL polypropylene centrifuge tube. First, 20 mL 0.1% formaldehyde (v/v) (pH 7, adjusted by 20% ammonia) was added to the tube, then the mixture shaken well and extracted by ultrasonic for 15 min before it was filtered with a 0.22 μm tetrafluoroethylene membrane filter for further LC-ICP-MS analysis. All samples were prepared and determined in the same day.

Sulfite analysis by LC-ICP-MS LC-ICP-MS was used for identification and determination of sulfite. The chromatographic separation was performed on a Hamilton PRP-X100 anion exchange column (250 × 4.1 mm i.d., 10 μm). A 100 μL aliquot of the sample was applied to the column, and an isocratic elution at a flow rate of 1.5 mL·min⁻¹ was used. The mobile phase was composed of 50 mmol·L⁻¹ ammonium nitrate (pH 7, adjusted by 20% ammonia) in 0.1% formaldehyde (v/v) in 1.0% formaldehyde (v/v). The instrumental parameters of ICP-MS measurements were as follows: RF power was set to 1400 W, analog stage voltage, pulse stage voltage and lens voltage were 1670 V, 1055 V and 6.80 V, respectively. The data acquisition parameters were: dwell time: 500 ms, sweeps per reading: 1, readings per replicate: 750. High purity oxygen (99.9999%) was used for dynamic reaction cell (DRC) and the DRC was optimized to the following parameters: RPa: 0, Rpq: 0.8, cell gas: 0.8 mL·min⁻¹, QRO: 6.5 V, CRO: -1.3 V, CPV: -16 V.

Safety considerations In this study, 0.1% formaldehyde solution was used as extraction solution and mobile phase. Formaldehyde has been classified as a known human carcinogen (cancer-causing substance) by the International Agency for Research on Cancer and as a probable human carcinogen by the U.S. Environmental Protection Agency. Researchers wishing to conduct similar experiments are urged to exercise great care in sample handling and to ensure that sample lines, dilution apparatus, and pump exhausts have no leaks and are properly exhausted into appropriate waste air and water handling systems.

Results and Discussion

Stability of the extraction solution To solve the substantial sulfites loss during extraction process, formaldehyde was used to convert sulfite into a more stable hydroxymethanesulfonate (HMS).
Low-level Sulphite Determination by LC-ICP-MS

Fig. 1. SO$_3^{2-}$ (0.5 mg•L$^{-1}$) in different pH formaldehyde solution with ultrasonic extraction.

Long time stability experiment has been done in our research. The results were shown in Fig. 1. It showed that during the extraction process, lower pH was advantageous to improve the sensitive of the SO$_3^{2-}$ determination, but it was unstable due to oxidation happening after longtime extraction. While higher pH would sacrifice the determination sensitivity of SO$_3^{2-}$, it was advantageous to stability of the HMS. The concentration of formaldehyde less affected the stability of the HMS when the concentration >0.1% (v/v). So, considering formaldehyde to the environmental impact, 0.1% (v/v) formaldehyde in alkaline solution (pH 11) was selected in this experiment. Applying this solution to extract SO$_3^{2-}$ from dry vegetable and fruit samples, it was found that after 15 minutes extraction, all experimental samples tend to be stable.

Optimization of the DRC instrument It is well known that $^{16}$O$^{16}$O, $^{14}$N$^{16}$O and $^{18}$O$^{18}$O interfere greatly with sulfur isotope ($^{32}$S). In this work, DRC system with oxygen gas (O$_2$) was employed to reduce the background interferences in ICP-MS analysis. In DRC system, sulfur reacted with O$_2$ to form $^{32}$S$^{16}$O$^+$, which eliminating the interferences of isobars. The RPq and cell gas (O$_2$) flow parameters were of major importance to affect the operation of the dynamic reaction cell. When the RPq value was 0.45 and O$_2$ flow was 0.8 mL•min$^{-1}$, best S/N (the ratio of single to noise) was received. Since $^{32}$S$^{16}$O$^+$ was chosen as the quantitative signal, the interference of $^{46}$Ca$^+$ should be taken into account. Dry vegetables and fruits contain a considerable number of water-solubility Ca ions. Researchers (Yang and Jiang, 2004) have shown that the significant interference of $^{46}$Ca$^+$ to $^{32}$S$^{16}$O$, when they had similar quantities in water solution. Fortunately, in our study, it had no signal peak after separating by LC system, even the concentration of Ca increased to 100 mg•L$^{-1}$ in water solution.

Interference by water-soluble sulfocompound Except SO$_3^{2-}$, some water-soluble sulfur compounds, such as sulfate (SO$_4^{2-}$), thiosulfate ($S_2O_3^{2-}$), sulfur-containing amino acids (taurine, methionine, cysteine and cystine) which could be found in dry vegetables and fruits may coelute with SO$_3^{2-}$. Fifty mmol•L$^{-1}$ ammonium nitrate (NH$_4$NO$_3$) was chosen as the mobile phase. The pH value of mobile phase was irrelevant to the separation of the SO$_3^{2-}$ from SO$_4^{2-}$ and $S_2O_3^{2-}$. However, for sulfur-containing amino acids, when the pH value decreased to 5, the peaks of sulfur-containing amino acids overlapped considerably with that of SO$_3^{2-}$. By increasing the pH to 7, the retention time became longer resulting in adequate separation of sulfur-containing amino acids and SO$_3^{2-}$. In this pH condition, SO$_3^{2-}$ was well separated from sulfate (SO$_4^{2-}$), thiosulfate ($S_2O_3^{2-}$) and sulfur-containing amino acids (Fig.2). The total runtime was approximately 5 minutes.

In this experiment, different types of dry vegetable and fruit samples with different matrix were tested in our experiment. All these samples didn’t contain any sulfite (blank samples). The blank samples and blank samples added 0.5 mg•L$^{-1}$ sulfite were extracted by 0.1% formaldehyde, and tested by LC-ICP-MS. Typical chromatograms were shown in Fig.3 (a, b, c d). It showed that for different matrix samples, no interference peak appear at the retention time of SO$_3^{2-}$, and other sulfur compounds were well separated from SO$_3^{2-}$. In the meantime, the retention time of the sulfite in different matrix samples corresponded to that in the chromatogram of the sulfite standard solution (Figure 2). Extending the elution program time to 30 min, no other peak was found. Five minutes was long enough to separate SO$_3^{2-}$ to other interference

Table 1. Recoveries for the determination of sulfite in selected dry vegetable and fruit samples ($n = 6$)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (mg•kg$^{-1}$)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dry onion</td>
<td>ND$^a$</td>
<td>78.5%</td>
<td>2.8</td>
</tr>
<tr>
<td>dry jujube</td>
<td>ND$^a$</td>
<td>86.5%</td>
<td>2.5</td>
</tr>
<tr>
<td>dry mushroom</td>
<td>10.1</td>
<td>87.6%</td>
<td>4.3</td>
</tr>
<tr>
<td>dry garlic</td>
<td>8.55</td>
<td>84.2%</td>
<td>3.4</td>
</tr>
<tr>
<td>dry mango</td>
<td>65.8</td>
<td>89.0%</td>
<td>4.8</td>
</tr>
<tr>
<td>dry kiwi fruit</td>
<td>50.1</td>
<td>91.6%</td>
<td>3.9</td>
</tr>
</tbody>
</table>

$^a$ not detected

Fig. 2. Chromatogram of (1) SO$_3^{2-}$ (0.5 mg L$^{-1}$) standard solution coexisting (2) SO$_4^{2-}$, (3) S$_2$O$_3^{2-}$ (dashed line) and (4 + 5) sulfur-containing amino acid (solid line) exuding by 50 mmol•L$^{-1}$ NH$_4$NO$_3$ (pH 7).
This program was faster than reported ion chromatography methods (Koch et al., 2010; Zhong et al., 2012) and HPLC methods (Pizzoferrato et al., 1998; Theisen et al., 2010; S. Wang et al., 2007; Zuo and Chen, 2003).

Method validation  At the optimized conditions, the procedure was validated in terms of linearity, limits of detection and quantitation, precision, and accuracy.

The linear range of the established LC-ICP-MS method was $0.05 \rightarrow 5 \text{ mg}\cdot\text{L}^{-1}$ for sulfite, following the regression equation: $Y = 2.2 \times 10^6 X + 4.3 \times 10^3$. The limit of detection (LOD) as three times the SD from the blank samples ($n = 10$) with the addition of the SO$_3^{2-}$ concentration, which was close to the expected LOD. The limit of quantification (LOQ) was calculated as three times the LOD value.

The LOD and LOQ values of SO$_3^{2-}$ were 0.02 mg•L$^{-1}$ and 0.06 mg•L$^{-1}$, respectively. It should be possible to determine the concentration of the sulfite under 10 mg•kg$^{-1}$ from this LOQ.

Accuracy and precision of the analytical method were evaluated by means of recovery and relative standard deviation (RSD). The results are shown in Table 1 (the background contents of sulfite in dry mushroom, dry garlic, dry mango, and dry kiwi fruit were derived from sulfite added in the laboratory). The mean recoveries of the sulfite at three spiked levels were ranged from 78.5% to 91.6%, with RSD ranging from 2.5% to 4.8%.

Application to real samples and method comparison  The selected method was applied to analysis of sulfite in 4 laboratory added samples and 10 samples collected local from farmer’s market and supermarket in Hangzhou. Sulfite was found in 2 collected samples which were dry raisin (8.19 mg•kg$^{-1}$) and dry bamboo shoot (68.8 mg•kg$^{-1}$) respectively. These 2 samples with another 4 laboratory added samples were also determined by AOAC Official Method 990.28 for method comparison. Student’s $t$ test was employed to estimate the significance of values. For high-level sulfite samples, at the 95% confidence level, $|t_{calc}|$ is 2.120, and $|t_{calc}|$ values were lower than $|t_{crit}|$ (Table 2), there was no significant difference between the analytical values by the proposed and official methods. For low-level sulfite samples, $|t_{calc}|$ values were higher than

![Chromatograms of different blank matrix dry vegetable and fruit samples](image)
Table 2. Comparison of LC-ICP-MS method and AOAC official method on quantifying sulfite in selected dry vegetables and fruits samples (n = 9)

| Sample               | LC-ICP-MS method | AOAC official method | \( |t_{\text{calc}}| \) value |
|----------------------|------------------|----------------------|----------------|
| dry raisin           | 8.19 ± 0.30      | 10.9 ± 1.4           | 4.166          |
| dry bamboo shoot     | 68.8 ± 1.8       | 71.7 ± 1.8           | 2.014          |
| dry mushroom (laboratory added) | 10.1 ± 0.5 | 13.7 ± 2.4           | 6.301          |
| dry garlic (laboratory added) | 8.55 ± 0.23 | 11.0 ± 1.7           | 3.167          |
| dry mango (laboratory added) | 65.8 ± 1.6 | 67.6 ± 1.9           | 1.857          |
| dry kiwi fruit (laboratory added) | 50.1 ± 1.5 | 51.8 ± 1.2           | 1.978          |

\( |t_{\text{calc}}| \) which meant significant difference between the analytical values by the two methods. Because by official method, abundant volatile organic acids in vegetable and fruit were also titrated with standardised NaOH, which probably caused the results higher (Pizzoferrato et al., 1998). On the other hand, high LOD (10 mg•kg\(^{-1}\)) of official method may also lead to significant difference.

Conclusion
In this study, a novel LC-ICP-MS has been developed for the determination of sulfite in dry vegetables and fruits. The proposal method was able to eliminate the interferences effectively, and showed highly selective. The proposed method show favourable recovery and RSD when determination of sulfite under 10 mg•kg\(^{-1}\). Rapid separation with very low detection limit was the most advantage of the proposed method, and it was successfully applied for the determination of sulfite in dry vegetables and fruits.

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References


