Determination of the Contents of Cr (III) and Cr (VI) in Man-Made Chromium-Enriched Foodstuffs by Graphite Furnace Atomic Absorption Spectrometry

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Abstract — A new application of graphite furnace atomic absorption spectrometry (GF-AAS) for the determination of the contents of Cr (III) and Cr (VI) in chromium-enriched foodstuffs was developed to evaluate the quality of commercial foodstuffs, including the nutrition and toxicity. Thenoyltrifluoracetonate (TTA) was used to form volatile complex with Cr (III), and separated from Cr (VI) as a vapor in the heated graphite furnace. The effects of sample digestion mode, pH, concentration of TTA, sonication time & temperature, and atomization conditions on the separation of Cr (III) and Cr (VI) were investigated. The limit of quantification (LOQ) of Cr (VI) was 0.003 mg/kg. The average recovery of Cr (VI) in chromium-enriched eggs was between 75% and 96%, and the relative standard deviation (RSD) was less than 11.5%. The developed method was also applied for a survey of the relative standard deviation (RSD) was less than 11.5%, and the average recovery of Cr (VI) in chromium-enriched eggs was between 75% and 96%, and the relative standard deviation (RSD) was less than 11.5%. The developed method was also applied for a survey of the contents of Cr (VI) in all samples were below its maximum contaminant level (MCL).

Keywords — Graphite Furnace Atomic Absorption Spectrometry, Volatilization of Cr(TTA)3, Chromium-enriched foodstuffs

I. INTRODUCTION

It is well known that the biochemical property and toxicity of chromium, mainly as Cr (III) and Cr (VI) in environment and biological systems, are highly dependent on its oxidation state [1]. Cr (VI) is known to be toxic and carcinogenic even at relatively low concentration[2-4]. On the contrary, Cr (III) is an essential micronutrient for human’s and animals’ health at low concentration[5,6]. The required intake of chromium is between 50 and 200 g/day for adults[7,8]. Deficiency of Cr (III) maybe cause diabetes, while excessive intake will bring many negative effects even for healthy persons. Though the traditional sources, such as rice, eggs, wheat and milk, are still the main providers of chromium, there is a trend of producing improved foods and nutriments with higher content of micronutriments through various methods [9, 10]. However, with the increasing of Cr (III) content in these foods, the content of toxic Cr (VI) may be elevated at the same time. Therefore, to make a more precise and accurate evaluation of the nutrition and potential toxicity of chromium-containing foodstuffs, the contents of Cr (III) and Cr (VI) is required to be determined respectively.

A variety of analytical techniques have been developed and utilized to determine the chromium content, including high performance liquid chromatography (HPLC) [11-13], atomic absorption spectrometry (AAS) [14-22], spectrophotometry [23-25], inductively coupled plasma atomic emission spectrometry (ICP-AES) [26-29] and microwave plasma atomic emission spectrometry [30]. With the low level of chromium and high content of matrix in most of natural samples (normally at g/L level), preconcentration techniques are required. In addition, because only the total chromium content can be determined by most analytical techniques, Cr (III) and Cr (VI) have to be separated in advance through some procedures, which are time-consuming, complex and sometimes costly. In 1992, Y. An et al.[31] developed a direct method, in which trifluoroacetylacetonates (TFA) was selected to form Cr(III)-(TFA)3, a volatile chelate, subsequently vaporized in heated graphite furnace, with Cr (III), for determination of chromium(III) content by AAS, based on the work of R. Moshier [32].

Considering potential different chelating ability of acetonates with Cr (III) and Cr (VI), thenoyltrifluoracetonate (TTA), instead of TFA, was applied as a chelating agent for Cr (III). Various effects, including sample digestion mode, pH, concentration of TTA, sonication time and temperature, and atomization conditions, on the separation of Cr (III) and Cr (VI) have been studied and discussed in this work. The developed AAS method was also applied for the determination of the contents of Cr (III) and Cr (VI) in several chromium-enriched

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foodstuffs. Our study shows the new method is accurate and robust, therefore is suitable to high throughput analysis in agriculture analytical service laboratories.

II. EXPERIMENT

Instruments

The content of trace chromium was determined by a Perkin-Elmer Model AA 600 graphite furnace atomic absorption spectrophotometer. Analyses were carried out by using pyrolytic graphite-coated electrographite tube. The Chromium hollow cathode lamp was operated at 25 mA. Chromium atomization was performed at a wavelength 357.9 nm and a spectral band-width 0.7 nm. The furnace was cleaned by raising the temperature to 2600 ºC and the graphite tube was allowed to cool down to 20 ºC after each analysis. The temperature program for the atomizer is shown in Table 1. The injected volume was 20 µL.

Table 1 Graphite furnace program for the determination of Cr

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature (ºC)</th>
<th>Ramp time (s)</th>
<th>Hold time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>110</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Preparation</td>
<td>600</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>1500</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Atomization</td>
<td>2400</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Clean</td>
<td>2600</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Reagents and Samples

All the solutions were made from analytical reagent grade chemicals and distilled and millipore filtered water with resistance of 18.2 MΩcm.

Cr (III) standard solution (1mg/mL, GBW(E)080121) was purchased from National Research Center (China) as certified reference material (CRM). Cr (VI) standard solution (1mg/mL) was prepared by dissolving 0.093 gram of K2Cr2O7 in 25 mL deionized water. Buffer solution was prepared by mixing 0.1 mol/L sodium acetate solution with 0.1 mol/L acetic acid solution and pH was adjusted to 5.7.

Thenoyltrifluoroacetone (TTA) alcohol solution (98%), K2Cr2O7 (99.8%), hydrochloric acid (36%-38%) were purchased from Tianjin Chemical Reagent industry (Tianjin, China); Natural nutritive chromium-enriched samples, including eggs, pine polley powder capsules, high calcium brain tonic capsules, zinc capsules, white coffee, nutrient super calcium power and tibet-garlic capsules, were provided by Tianjin Tianshi Biological Development Co., Ltd., Tianjin, China.

Sample Preparation

One gram (accurate to 0.0001 g) of selected sample was dissolved in 2 mL of hydrochloric acid solution and then ashed at 550 ºC in a crucible. After cooling down to ambient temperature, sample was mixed with 2 mL of 0.20 mol/L TTA alcohol solution and 2.5 mL of buffer solution and diluted to 10 mL with deionized water. The pH value was adjusted to 5.7; after 10 minutes of ultrasonication at 50ºC, the sample was left at room temperature for 1 hour.

Working Curve of Cr (VI)

The absorbency of Cr (VI) in standard solutions, in which the concentration of TTA was 0.20 mol/L, with concentration ranging from 0.001 mg/L to 0.010 mg/L was determined by GF-AAS. The working curve of Cr (VI) was achieved by plotting as absorbency against concentration.

Determination of Cr (III) and Cr (VI)

The absorbency of Cr (VI) in the prepared samples was determined by GF-AAS and converted into the concentration according to the working curve. Following the same method, the content of total Cr was determined without adding any TTA to the sample, and the difference between the contents of total Cr and Cr (VI) was that of Cr (III).

III. RESULT AND DISCUSSION

Selection of Digestion Mode

Three digestion methods, nitric acid, nitric acid – perchloric acid (v:v 1:4) and ash at 550 ºC, were used to treat the standard solutions prepared by mixing Cr (III) (5 µg/L) solution with Cr (VI) (5 µg/L) solution stoichiometrically as well as the above standard solutions with the sample added to. The concentrations of total Cr were subsequently determined by GF-AAS method mentioned in experimental part.

As shown in Table 2, treated with nitric acid and nitric acid-perchloric acid (1: 4) solution, Cr (III) was oxidized to Cr (VI) and the content of Cr (VI) measured by GF-AAS was equal to that of total Cr in solutions. While using the ash method, the measured contents of Cr (VI) were very close to the initial Cr (VI) contents in solutions , which indicate that there was no inter-conversion between Cr (III) and Cr (VI) during this pretreatment procedure. As a result, ash method at 550 ºC was selected to treat samples in our study.
Table 2 The effect of sample digestion on detection result

<table>
<thead>
<tr>
<th>Solution</th>
<th>Digestion method</th>
<th>Content of Cr (VI) (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (III) (5 µg/L) + Cr (VI) (5 µg/L)</td>
<td>HNO₃</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>HNO₃-HClO₄-550 ºC Ash</td>
<td>9.7 (µg/L)</td>
</tr>
<tr>
<td>Sample + Cr (III) (5 µg/L) + Cr (VI) (5 µg/L)</td>
<td>HNO₃</td>
<td>11.9 (µg/L)</td>
</tr>
<tr>
<td></td>
<td>HNO₃-HClO₄-550 ºC Ash</td>
<td>12.3 (µg/L)</td>
</tr>
</tbody>
</table>

**Effects on the formation of Cr (III)-(TTA)₃**

**Effect of pH**

The pH value of the solution plays a crucial role on complex formation between Cr (III) and TTA. A series of solutions containing 10 µg/L Cr (III), 2 mL of 0.20 mol/L TTA alcohol solution and 2.5 mL buffer solution were adjusted to pH values from 1 to 9. Then the absorbency of solution was determined by GF-AAS and converted into the volatilization of Cr (III) subsequently. Shown in Figure 1, the data indicate that the maximum volatilization of Cr (III)-(TTA)₃ was achieved at the pH ranging from 5 to 6. Thus the pH value of 5.7 was selected as the optimum.

**Effect of the concentration of TTA**

To investigate the effect of the amount of TTA on volatile complex formation, 2 mL TTA alcohol solution with different concentrations (0.05 mol/L ~ 0.3 mol/L) was added to Cr solutions containing 10 µg/L of Cr (III) and 2.5 mL buffer solution at pH 5.7. Then the mixed solutions were subject to the graphite furnace program in Table 1. It was found that the formation of Cr (III)-(TTA)₃ was not complete at low concentration of TTA. However, Cr (III) was chelated with TTA completely when the concentration of TTA increased to 0.20 mol/L and the maximum volatilization was achieved. At higher concentration, the absorbance signal tended to be stable which implied that excessive TTA had no adverse effect on the complex formation. Thus, the concentration of TTA 0.20 mol/L was selected for further experiment.

**Effect of sonication time and temperature**

A series of Cr (III) solution containing 10 µg/L of Cr (III) and 2.5 mL buffer were mixed with 2 mL 0.20 mol/L TTA solution and heated at different temperatures (30, 40, 50, and 60° C) and different periods of time (5, 10, 15, and 20 min) in an ultrasonic bath. The absorbency of Cr (III) was determined and plotted in Figure 2 (the effect of sonication time) and Figure 3 (the effect of temperature). The result indicated that when the temperature was higher than 40° C and the time was over 10 min, Cr(TTA)₃ was volatized completely and tended to be stable. Therefore the temperature and time for the ultrasonic bath in this experiment were selected to be 50° C and 10 min respectively.

![Fig.1 The effect of pH values on Cr(III) volatilization](image)

![Fig.2 The effect of sonication time on Cr(III) volatilization](image)

![Fig.3 The effect of temperature on Cr(III) volatilization](image)
The Effect of Graphite Furnace Atomization Conditions

**Selection of the pretreatment and pyrolysis temperature for the graphite furnace**

Considering the best separation of Cr (III) from Cr (VI), the temperature for the pretreatment and pyrolysis steps was changed during the ramp period of graphite furnace in order to obtain the optimal condition. The absorbencies of Cr (III) and Cr (VI) were determined and plotted against temperature in Figure 4. It is shown that the absorbance signal of Cr(III) falls gradually from 0.05 to 0.02 (close to the blank value) and tends to be stable at 600°C when Cr (III) was considered to be volatilized completely, while that of Cr (VI) remained basically unchanged before 1600°C. Hence 600°C and 1500°C were respectively selected as the temperature of pretreatment and pyrolysis in following study.

**Effect of pretreatment time**

To determine the optimal time for the pretreatment step at temperature of 600°C, the AAS signal was measured at different pretreatment time from 20s to 80s. The result showed that Cr (III)-TTA₃ was volatilized completely after 60 seconds and tended to be stable. Therefore, the pretreatment time for further experiment was decided to be 60 seconds.

The recoveries and relative standard deviations of Cr (VI) were discussed by determining chromium-enriched eggs. Considering that the health standard limit for chrome quantity in eggs is no more than 1.0mg/kg as specified in GB 14961-94(China), the quantity to be labeled was selected within the range from 10 to 1000 ng (Table 3). Corresponding to different labeled quantities the recoveries were shown in Table 3. The average recovery of Cr (VI) in chromium-enriched eggs was between 75% and 96%, and the relative standard deviation was less than 11.5%.

### Table 3 The recoveries and relative standard deviations of Cr(VI) in chromium-enriched eggs sample (n=10)

<table>
<thead>
<tr>
<th>Quantity Fortified (ng)</th>
<th>Average recovery (%)</th>
<th>RSD(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>75</td>
<td>11.5</td>
</tr>
<tr>
<td>20</td>
<td>81</td>
<td>9.7</td>
</tr>
<tr>
<td>200</td>
<td>92</td>
<td>6.3</td>
</tr>
<tr>
<td>1000</td>
<td>96</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Determination of the contents of Cr (III) and Cr (VI) in the chromium-enriched foodstuffs**

Among the various chromium-enriched foodstuffs provided by Tianjin Tianshi Biological Development Co., Ltd., Tianjin, China, ten kinds of samples were selected randomly to evaluate their nutrition and potential toxicity. The contents of Cr (III) and Cr (VI) in these ten samples, including chromium-enriched eggs, pine pollen powder capsules, high calcium brain tonic capsules, zinc capsules, white coffee, nutrient super calcium powder and four kinds of tibet-garlic capsules were determined for ten times under the optimal conditions, and the average values were listed in Table 4. All RSDs of the tests are less than 10% which indicated the developed method has a satisfied precision and accuracy.
Table 4 The contents of Cr(III) and Cr(VI) in the nutrimental foodstuffs (n=10)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Contents of Cr(III) (mg/kg)</th>
<th>RSD(%)</th>
<th>Contents of Cr(VI) (mg/kg)</th>
<th>RSD(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chromium-enriched eggs</td>
<td>0.12</td>
<td>4.56</td>
<td>0.005</td>
<td>6.87</td>
</tr>
<tr>
<td>Pine pollen powder capsules</td>
<td>1.14</td>
<td>2.33</td>
<td>0.010</td>
<td>6.94</td>
</tr>
<tr>
<td>High calcium brain tonic capsules</td>
<td>1.56</td>
<td>1.89</td>
<td>0.005</td>
<td>7.82</td>
</tr>
<tr>
<td>Zinc capsules</td>
<td>0.57</td>
<td>3.87</td>
<td>0.010</td>
<td>8.22</td>
</tr>
<tr>
<td>White coffee</td>
<td>0.78</td>
<td>2.01</td>
<td>0.005</td>
<td>8.67</td>
</tr>
<tr>
<td>Nutrient super calcium powder</td>
<td>0.74</td>
<td>2.87</td>
<td>0.010</td>
<td>9.64</td>
</tr>
<tr>
<td>Tibet-garlic capsules (CN6050801A)</td>
<td>1.24</td>
<td>1.98</td>
<td>0.008</td>
<td>9.56</td>
</tr>
<tr>
<td>Tibet-garlic capsules (CN6050801B)</td>
<td>1.04</td>
<td>1.99</td>
<td>0.005</td>
<td>8.22</td>
</tr>
<tr>
<td>Tibet-garlic capsules (CN6050801C)</td>
<td>1.08</td>
<td>1.77</td>
<td>0.005</td>
<td>6.87</td>
</tr>
<tr>
<td>Tibet-garlic capsules (CN6050801A)</td>
<td>1.83</td>
<td>2.97</td>
<td>0.010</td>
<td>6.94</td>
</tr>
</tbody>
</table>

a: n is the number of times of determination of Cr in each kind of sample.
b: The content of analyte is under LOQ.

As shown in Table 4, the contents of Cr (III) in all of tested nutrimental foodstuffs range from 0.12 mg/kg to 1.83 mg/kg, which are much higher than the chromium (total) contents in some selected Slovenian foodstuffs (<0.05 to 0.21 mg/kg)[33] and Hungarian winter wheat (0.01 to 0.45 mg/kg)[34]. Therefore, the selected nutrimental foodstuffs are proved to be more efficient than the traditional foods as chromium source. It is also found that the contents of Cr (VI) in the eggs, pine pollen powder capsules, high calcium brain tonic capsules, zinc capsules, white coffee and nutrient super calcium powder could not be detected. That indicated the contents of Cr (VI) in those foods were extremely low and below the limit of quantification (LOQ) (0.003 mg/kg) of the analytical method. The contents of Cr (VI) in four kinds of tibet-garlic capsules range from 0.005 to 0.010 mg/kg, which are below the MRL of Cr (VI) in foods.

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