硝酸による酸部分分解とHPLC/ICP-MSを用いた米中の無機ヒ素定量法

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Evaluation of a Nitric Acid-based Partial-digestion Method for Selective Determination of Inorganic Arsenic in Rice

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Arsenic (As) uptake in human occurs via the food chain mainly. The Joint FAO/WHO Expert Committee on Food Additives has established the provisional tolerable weekly intake level for As as an inorganic As (iAs) value, because iAs in food is much more toxic than organic As. In this study, we studied an acid based partial-digestion method for the complete extraction of arsenicals from rice. HPLC/ICP-MS was used to determine the concentration of iAs selectively. The conditions adopted to extract arsenicals from a 0.5 g of finely ground rice sample were addition of 2 mL of 0.15 mol/L nitric acid and heating at 80°C for 2 h. The LOD and LOQ for iAs were 0.0024 and 0.0079 mg/kg dry weight, respectively. Recovery studies showed good accuracy. When the method was applied to ten short-grain brown rice samples, the iAs concentrations were 0.108–0.227 mg/kg dry weight and the total As concentrations were 0.118–0.260 mg/kg dry weight. Although dimethylarsinic acid was also detected in most samples, the percentage of iAs content in total As content was 62.2–96.3%. Thus, iAs was the principal As species in the short-grain brown rice samples tested.

Key words: arsenic; inorganic arsenic; rice; HPLC/ICP-MS; partial digestion; nitric acid

Introduction

Uptake of arsenic (As) in human mainly occurs via the food chain, and, in special cases, also via occupational exposure14,15. Since the inorganic arsenic (iAs) in food is much more toxic than the organic As4,15, the JECFA (the Joint FAO/WHO Expert Committee on Food Additives) has established a provisional tolerable weekly intake (PTWI) level as an value (0.015 mg/kg bw/week).

The major amount of ingested As comes from fish, shellfish, and seaweed, in which most of As compounds are fortunately non-toxic arsenobetaine (AB) or arsenosugars5. In contrast to the seafoods, the rice plant bio-accumulates more toxic iAs species, arsenate and arsenite5,10. Therefore, the contribution of rice to the total intake of iAs may be considerably high. For example, when a person weighing 50 kg consumes 150 g of brown rice containing As at the level of 0.16 mg/kg dry weight16 per day, the As intake is calculated to be 0.0034 mg total As/kg bw/week. If all As compounds in rice are present as iAs, this intake corresponds to 23% of the PTWI value. Consequently, the speciation of As in rice is very important from the viewpoint of risk-assessment of As for the Japanese population.

One of the requirements for assessing the risk of consuming food containing As is a quantitative or nearly quantitative method for determining arsenicals without transformation of the original chemical species. The main problems encountered have been low recovery and oxidation/reduction between As(III) and As(V)10. Quantitative extraction is also essential, because the extraction procedure may selectively extract non-toxic species from food and/or leave toxic species unextracted within the solid matrix. Arsenic speciation demands mild extraction so as to maintain species integrity. Although speciation studies have been widely performed for As in marine products, the speciation studies of As in rice are not numerous. In those studies, methanol, water, a methanol/water mixture, and trifluoroacetic acid (TFA) have been used as solvents to extract As species from rice samples10,14,15. Although quantitative extraction of As from some foods has been achieved with these solvents, it has been difficult to achieve thorough extraction from rice samples. Attempts to utilize an accelerated solvent extraction technique1,10,16,17 or enzymatic ultrasonic probe18 have been reported for rice, but As extraction from rice samples remains difficult6,19.

In our preceding report, the nitric acid-based partial-digestion method was assessed as a mean of achieving complete As extraction from hijiki samples19. In this paper, therefore, the partial acid-digestion method was
applied for rice, to completely extract iAs from rice samples. Nitric acid was used under conditions such that organic As species were not converted to iAs. This method was coupled with HPLC/ICP-MS to determine As species including iAs separately.

**Materials and Methods**

**Reagents**

Sodium arsenate (Na₂HAsO₄), sodium arsenite (NaAsO₂), dimethylarsinic acid (MMA), trimethylarsine oxide (TMAO), and trimethylarsenic (DMA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals were of reagent grade or of the highest grade available commercially. All laboratory glassware and plastic ware used were immersed in approximately 2 mol/L HNO₃ at least overnight and rinsed with ultra-pure water prepared with a Milli-Q SP Ultrapure Analytical Water System (Millipore, Bedford, MA) to avoid contamination with various ions. Water of this grade was used throughout the experiment.

**Rice**

Short-grain brown rice samples were obtained from supermarkets in the Tokyo Metropolitan area and by mail order in Japan. Before analysis, all samples were carefully ground into fine powders with a grinder (Micron Milcer all samples were separately detected within 10 min. Signal ion monitoring at m/z 75 and a sampling rate of 1 Hz were used to collect the chromatographic data.

**Determination of total As**

A finely ground powdered rice sample (0.1–1 g dry weight) was weighed, transferred to a Kjeldahl flask, and heated with nitric acid (10 mL). Then, 5 mL of sulfuric acid was added to the flask, and heating was continued until white fumes of sulfuric acid appeared. Hydrogen peroxide (2 mL, 30%) was added to the flask, and heating was continued. After cooling, 15 mL of saturated ammonium oxalate solution was added to the flask, and heating was continued. A 5 mL volume of potassium iodide solution (40%) was added, and after the solution had been allowed to stand for 30 min, a 5 mL volume of ascorbic acid solution (10%) was added. Finally, water was added to adjust the volume to 50 mL. The total amount of As was measured by hydride generation-atomic absorption spectrometry (HG-AAS) (Varian Spectra AA220 with VGA-77, Varian Japan, Tokyo).

**Extraction efficiency**

Extraction efficiency [extraction [%)] was evaluated as the ratio of extracted As content to total As content. The As concentration of each extract solution was determined by ICP-MS after dilution with 0.1 mol/L nitric acid solution, unless otherwise noted.

**Results and Discussion**

**Speciation analysis of standard arsenicals and a partially digested rice sample by HPLC/ICP-MS**

The HPLC/ICP-MS system was used to analyze six standard As compounds, i.e., arsenate, arsenite, MMA, DMA, AB, and TMAO. The concentration of each standard was 5 ng As/mL. As shown in Fig. 1, they were separately detected within 10 min.

**Fig. 1.** HPLC/ICP-MS chromatogram of six standard As compounds

Sample injection volume, 20 μL; concentration of each standard As compound, 5 ng As/mL.
Concentration of nitric acid as extraction solution and extraction time

Our preceding study on hijiki samples\(^2^0\) suggested that organic As species were not converted to iAs at 80 °C and that complete extraction of As was possible by selecting optimal extraction conditions. Accordingly, a temperature of 80 °C was also adopted in this study, and several factors were optimized as described below.

First, various acid concentrations were tested to identify the lowest nitric acid concentration that could be used without reducing the extraction efficiency. The results are shown in Table 1-1. A powdered rice sample (0.5 g dry weight) was mixed with 2 mL of 0.075, 0.10, 0.15, 0.30, and 1.0 mol/L nitric acid solutions, and 2.0 mol/L trifluoroacetic acid (TFA) in 10 mL capped HDPE centrifuge tubes, and the solutions were heated for 2 h at 80°C. The iAs concentration and extraction efficiency were almost the same at the 0.10 mol/L concentration and above. The DMA concentration did not increase at the higher nitric acid concentrations. Since the pH of the solution for analysis was 2-3 in the case of 0.15 mol/L nitric acid and adjustment of the pH was unnecessary, the 0.15 mol/L concentration was adopted.

Addition of 2.0 mol/L TFA and heating at 100°C for 6 hr had been adopted to extract As from rice\(^1^7\), whereas in our study the sample was treated with 2.0 mol/L TFA and heated at 80°C for 2 hr. Black insoluble matter remained after the TFA treatment. Moreover, compared to the nitric acid treatment, the As(III) and As(V) concentrations were higher and lower, respectively. Furthermore, the pH of the solutions for analysis was 1. Therefore, TFA was not used in this study.

Next, the extraction time was varied (Table 1-2). A milled rice powder sample (0.5 g dry weight) was mixed with 2 mL of 0.15 mol/L nitric acid solution in a 10 mL capped HDPE centrifuge tube and heated for 0.5, 1, 2, 4, 6, or 8 hr at 80°C. Since the extraction efficiency (%) of As was almost the same from 1 hr to 4 hr, the extraction time was fixed on 2 hr.

Nitric acid is generally regarded as an oxidative acid. However, As(III) was detected even in the case of 1.0 mol/L nitric acid. This might suggest the presence of reductive compound(s) in brown rice.

Based on the results described above, a 0.15 mol/L nitric acid concentration and 2 hr extraction time were adopted.

Method validation

The method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy.

| Table 1-1. Effect of nitric acid concentration on extraction efficiency |
|------------------------|----------------|----------------|----------------|--------|---|----------------|
| Extraction solution  | Concentration (mg/kg dry weight) | Extraction\(^2\) (%) |
| As(V) | As(III) | iAs (As(V)+As(III)) | MMA | DMA | Total As extracted\(^1\) |
| 0.075 mol/L HNO\(_3\) | N.D. | 0.079 | 0.079 | N.D. | 0.013 | 0.120 | 87.6 |
| 0.10 mol/L HNO\(_3\) | 0.013 | 0.106 | 0.119 | N.D. | 0.022 | 0.127 | 92.7 |
| 0.15 mol/L HNO\(_3\) | 0.026 | 0.105 | 0.120 | N.D. | 0.025 | 0.129 | 94.2 |
| 0.30 mol/L HNO\(_3\) | 0.031 | 0.091 | 0.122 | N.D. | 0.026 | 0.126 | 92.0 |
| 1.0 mol/L HNO\(_3\) | 0.019 | 0.101 | 0.120 | N.D. | 0.023 | 0.127 | 92.7 |
| 2.0 mol/L TFA | 0.009 | 0.114 | 0.123 | N.D. | 0.021 | 0.125 | 91.2 |

Data are means of duplicate analyses.
N.D.: Not detected.
1 The As concentration of each extract solution was measured by ICP-MS only after dilution with 0.1 mol/L nitric acid solution.
2 Extraction efficiency (%) was calculated as the ratio of extracted As content to the total As content. The total As concentration was determined to be 0.137 ± 0.005 mg/kg dry weight (n = 5).
iAs (as As(V) or As(III)) was quantified by using a linear calibration plot \( r=0.9998 \) that was established by using standard iAs solutions having As concentrations of 0.5, 1, 5, 10, and 20 mg/mL.

The LOD and LOQ were set at 3 times and 10 times the value of iAs concentration was determined to be 0.0435 mg/kg dry weight and 0.08 mg/kg dry weight as twice the iAs concentration of an un-spiked rice sample (iAs: 0.0435 mg/kg dry weight). Either As(V) or As(III) was added to the sample level was satisfactory. Recovery studies for MMA and DMA were also performed by spiking 0.01 mg/kg and 0.08 mg/kg dry weight. The recovery of MMA and DMA was 92.3% to 101.5%. The method was also applied to polished rice. When iAs was added to polished rice at the final concentration of 0.22 mg/kg dry weight, the recovery of iAs ranged from 101% to 105% (data not shown), suggesting that the method can be applied to both brown rice and polished rice.

Precision was evaluated by calculating the coefficient of variation (CV), which is the ratio of the standard deviation (S.D.) of the value given in Table 2. Recovery of iAs at 0.01 mg/kg and 0.08 mg/kg dry weight ranged from 82.2% to 106.1%.

Accuracy was evaluated by calculating recovery in five replicate analyses. The recovery studies were performed by spiking 0.01 mg/kg dry weight as the LOQ level and 0.08 mg/kg dry weight as twice the iAs concentration of an un-spiked rice sample (iAs: 0.0435 ± 0.0008 mg/kg dry weight, mean ± S.D. \( n=10 \)). The LOD and LOQ values of iAs were 0.0024 mg/kg dry weight and 0.0079 mg/kg dry weight, respectively.

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Application to brown rice samples

The method was applied to ten commercial brown rice samples. The concentrations of iAs detected were in the range from 0.118 to 0.260 mg/kg dry weight. Total As concentrations were in the range from 0.04 mg/kg dry weight. Since the CV obtained was 62.2-96.3%, the precision both in terms of accuracy and precision.

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