

Heat-Assisted Aqueous Extraction of Rice Flour for Arsenic Speciation Analysis

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A versatile heat-assisted pretreatment aqueous extraction method for the analysis of arsenic species in rice was developed. Rice flour certified reference materials NIST SRM1568a and NMIJ CRM 7503-a and a flour made from polished rice were used as samples, and HPLC–ICP-MS was employed for the determination of arsenic species. Arsenite [As(III)], arsenate [As(V)], monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) were detected in NIST SRM, and As(III), As(V) and DMAA were found in NMIJ CRM and the prepared polished rice flour. The sums of the concentrations of all species in each rice flour sample were 97–102% of the total arsenic concentration in each sample.

KEYWORDS: Arsenic species; speciation; HPLC–ICP-MS; rice flour, unpolished; rice flour, polished; water extraction method

INTRODUCTION

Toxic elements in the human diet pose an obvious health risk (1). The accurate assessment of this hazard and the associated safety evaluation are necessary for the maintenance of public health. In addition, food analysis can provide a measure of the degree to which the agricultural production system might be polluted.

It is well established that arsenic is toxic. The Food and Agriculture Organisation/World Health Organisation (FAO/WHO) have recommended a provisional tolerable weekly intake (PTWI) of not more than 15 μg of inorganic As/kg of body weight (2). However, there are several naturally occurring chemical forms of arsenic, and the toxicity varies greatly from form to form. Hence, it is important to determine which chemical forms are present in foodstuffs and in the environment as well as analyzing the total concentration of the element (3, 4).

Rice is a staple of the diet in Asia and contributes more arsenic to the Asian diet than all other agricultural products. There has been, however, little published information on the chemical forms of arsenic in rice. Rice is also produced and eaten in the USA and Europe, and is an increasingly important component of the diet in those areas. To ensure that rice can be safely consumed, it is thus necessary to know and to monitor potentially harmful constituents.

At present, the food sanitation law in Japan and the Codex Alimentarius Commission (CAC) do not regulate for the arsenic concentration in rice, although the CAC has stated that it was necessary to set standards for inorganic arsenic compounds in foodstuffs (5). However, an accurate technique for the speciation analysis of arsenic in rice samples is lacking. Extraction methods involving heating with acids such as nitric

or trifluoroacetic acid (TFA), the use of enzymes, and some physical techniques such as ultrasonication and accelerated solvent extraction have been studied as methods for extracting rice and other samples prior to arsenic speciation analysis (3, 6–11). However, the ratios of inorganic arsenic species are sometime changed during extraction using these techniques, and it is difficult, therefore, to know their concentrations in the original samples. Hence, the final result is often expressed as total inorganic arsenic even though As(III) and As(V) have been determined independently by inductively coupled plasma mass spectrometry (ICP-MS) or another technique after separation by high performance liquid chromatography (HPLC). In addition, when acid is used as the extractant, the acidity of the extract is high, resulting in rapid deterioration of the HPLC column. Moreover, few published accounts have included SI traceability and accuracy evaluation is difficult.

We developed a microwave-assisted extraction technique using water as the solvent for arsenic speciation analysis of rice samples (12). We examined a range of extractants and extraction techniques and found that microwave-assisted extraction with water was the most suitable technique for rice flour samples. However, the microwave extraction system has low versatility and skill is necessary to achieve consistently accurate analysis. The work reported here was undertaken to develop a universally applicable technique for the extraction of arsenic species in rice samples prior to their analysis by HPLC–ICP-MS.

MATERIALS AND METHODS

Arsenic Determination. An ICP-MS (Agilent 7500c, Agilent, Tokyo, Japan) equipped with a micromist nebulizer (100 μL type) and a Scott spray chamber (2 $^{\circ}\text{C}$) was used. The usual operating parameters for the ICP-MS were as follows: incident rf power was 1500 W, outer Ar gas flow rate 15 L min^{-1} , intermediate Ar gas flow rate 0.9 L min^{-1} , carrier Ar gas flow rate 0.8 L min^{-1} and makeup Ar gas flow rate 0.4 mL min^{-1} . The ICP-MS was operated with He as the collision cell gas (3 mL min^{-1}) to

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reduce $^{40}\text{Ar}^{35}\text{Cl}^+$ interference with ^{75}As . The ion count was monitored at $m/z = 75$. Arsenic species were separated by HPLC (L-6000 pump, Hitachi High Technologies Co. Ltd., Tokyo, Japan) with an automatic sample injector (Nanospece SI-2, Shiseido Co. Ltd., Tokyo, Japan) and direct introduction into the ICP-MS. A C_{18} ODS L-column (150 mm \times ID 4.6 mm, Chemicals Evaluation and Research Institute, Tokyo, Japan) and a CAPCELL PAK C_{18} MG column (150 mm \times ID 4.6 mm, Shiseido Ltd., Tokyo, Japan) were used with the mobile phase containing 10 mM sodium 1-butanedisulfonate/4 mM malonic acid/4 mM tetramethylammonium hydroxide/0.05% methanol (pH 3.0) at a flow rate of 0.5 mL min^{-1} . The exit of the HPLC column was connected to the nebulizer of the ICP-MS with PEEK tubing.

Calibration Standards. The Japan Calibration Service System (JCSS) arsenic standard solution (1000 mg L^{-1} As, made from high purity As_2O_3) was used as the As(III) source standard solution (Kanto Chemical Industries Ltd., Tokyo, Japan).

As(V) certified reference material (NMIJ CRM 7912-a), dimethylarsinic acid (DMAA) certified reference material (NMIJ CRM 7913-a) and arsenobetaine (AsB) certified reference material (NMIJ CRM 7901-a), supplied by the National Metrology Institute of Japan/National Institute of Advanced Industrial Science and Technology (NMIJ/AIST, Ibaraki, Japan), were used as the source standard solutions. All are SI traceable.

Monomethylarsonic acid (MMAA) was prepared from the commercially available reagent after a purity evaluation had been carried out. It was dissolved in water to prepare the stock standard solution containing 1000 mg As kg^{-1} .

Working mixed standard solutions (0.5–30 ng As/g) were prepared daily by mixing the stock solutions and diluting with water. AsB was used as the internal standard during the analyses.

Certified Reference Materials (CRMs). The rice flour CRMs analyzed were NIST SRM 1568a rice flour (National Institute of Standards and Technology, NIST, USA) and NMIJ CRM 7504-a white rice flour. The certified values of total arsenic in NIST SRM 1568a and NMIJ CRM 7405-a are 0.29 ± 0.03 mg kg^{-1} and 0.098 ± 0.007 mg kg^{-1} , respectively. The certified values of As(III), As(V) and DMAA in NMIJ CRM 7405-a are 0.077 ± 0.0029 mg kg^{-1} , 0.0130 ± 0.0009 mg kg^{-1} , 0.0133 ± 0.0009 mg kg^{-1} , respectively.

Sample Preparation of a Polished White Rice Flour. A quantity of polished white rice flour was pulverized in a food processor, and the resulting powder was separated using neuron mesh. Particle size samples of < 150 μm , 150–250 μm , 250–500 μm and > 500 μm were collected. These samples were used to investigate the influence of particle size on extraction efficiency.

The total arsenic concentration in the original polished white rice flour was determined by graphite furnace atomic absorption spectrometry (AAAnalyst800, Perkin-Elmer, Sciex, Thornhill, ON Canada) following microwave-assisted digestion (MLS 1200-mega, Milestone MLS, Leutkirch, Germany) with HNO_3 – HClO_4 –HF mixed acid, and the concentration of total arsenic was determined to be 0.59₅ mg kg^{-1} .

Arsenic Extraction Procedure. The standard conditions for extraction were a liquid/solid (L/S) ratio of 10 (extraction solvent 10 g/sample 1 g). A portion of the rice flour was placed in a 50 mL polypropylene centrifuge tube, and 10 g of water containing 10 ng g^{-1} of AsB was added. The capped tube was placed into a heating block (Digi PREP, SCP Science Inc., Canada) system at 90 °C for 3 h. The rice flour–water suspension was centrifuged at 4000 rpm for 5 min, and the supernatant was passed through a 0.45 μm syringe-type PVDF membrane filter and the filtrate analyzed.

A gravimetric method was employed in all preparations in this study. Blank tests were performed to investigate possible arsenic contamination; none was detected.

Factors for converting between dry mass and wet mass were obtained by measuring the mass loss after drying portions of the samples at 85 °C in an oven (drying oven DV 400, Yamato Co. Ltd., Japan) for 15 h.

RESULTS AND DISCUSSION

Technique for Accuracy Determination. HPLC–ICP-MS was employed for arsenic speciation, and chromatograms obtained with the standard solutions are shown in Figure 1. When an ODS column was used for the analysis, As(V), As(III), MMAA,

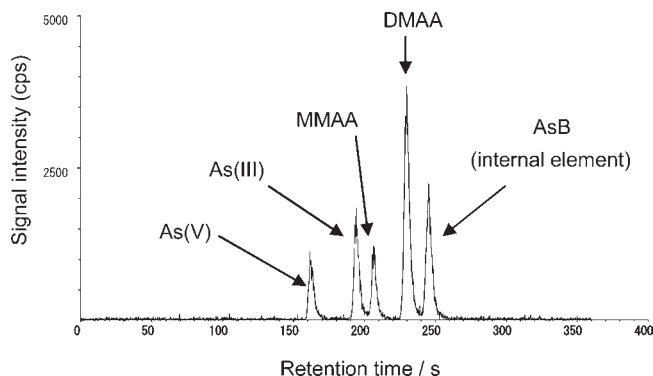


Figure 1. Chromatograms of arsenic species by HPLC–ICP-MS: sample standard solution, As(V) 4 ng g^{-1} , As(III) 6 ng g^{-1} , MMAA 4 ng g^{-1} , DMAA 15 ng g^{-1} , AsB 9 ng g^{-1} ; column, ODS C_{18} MG; injection volume, 10 μL ; flow rate, 0.5 mL min^{-1} .

DMAA and AsB were well separated by the system. The limits of detection (LOD, $\sigma = 3$) of As(III) and As(V) were 0.2 ng g^{-1} and of MMAA and DMAA were 0.1 ng g^{-1} .

An extract of rice flour contains large amounts of starch, and therefore degradation of the HPLC column occurred when measurement time was long and/or the replication number was large, and chromatogram peaks became poorly resolved. In particular, degradation of the column was faster when polished white rice flour was analyzed because it contains larger amounts of starch than comparable amounts of unpolished rice flour. A guard column did not increase chromatographic efficiency, and therefore, no guard column was used in this study.

Repeat analysis ($n = 30$) revealed a tendency for peak width to increase and peak height to decrease with increasing number of replications. Also, small measurement errors occurred because the viscosity of the rice flour extract was high. Repeat analysis ($n = 30$) was carried out with an extract of rice flour containing approximately 1 ng g^{-1} each of As(V), MMAA and DMAA and 5 ng g^{-1} of As(III). Repeatability for As(III) and As(V) was approximately 6%, and for MMAA and DMAA was approximately 5%. AsB was used as an internal standard to correct for measurement error. The repeatability of each species was then reduced to less than 2.5%. Hence, internal standard method using AsB was applied in this work.

Effect of Extraction Time and Other Parameters on Unpolished Rice Flour Extraction. In our previous work, we developed a microwave-assisted extraction (MarsX, CEM, Matthews, NC) technique with water prior to arsenic speciation analysis of rice flour (12). The extraction temperature was investigated with this technique, and it was possible to achieve complete extraction of arsenic species in the rice flour sample in 30 min at 80 °C. The arsenic species were not changed by the measurement procedure, and no problems were encountered by standard addition and recovery tests. The effect of changing the solvent was also investigated by using water, methanol and mixtures of the two. It was found that water was the best solvent for extracting arsenic species from rice flour, giving consistent results and good stability from a simple procedure.

With microwave-assisted extraction, pressure and the external force of the microwaves act on the sample. Gelatinization of the sample was encountered when conditions were enough to achieve 100% extraction. On the other hand, the rice flour was ashed at temperatures of more than 100 °C, and the extraction amount decreased. Therefore, a simple extraction technique using an oven or a heating block was used to achieve same efficiency as microwave-assisted extraction.

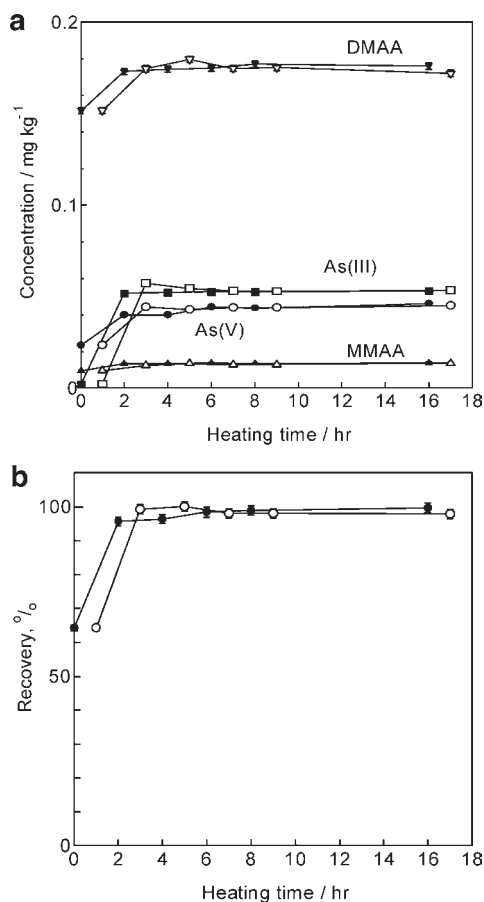


Figure 2. (a) Effect of heating time on extraction of arsenic species from unpolished rice flour: heating temperature, 90 °C; rice flour, NIST SRM 1568a; ●, ○, As(V); □, ■, As(III); ▲, △, MMAA; ▼, ▽, DMAA; ●, ■, ▲, ▼, heating only; ○, □, △, ▽, ultrasonication 1 h after heating. (b) Effect of heating time on extraction of arsenic species from unpolished rice flour: heating temperature, 90 °C; rice flour, NIST SRM 1568a; ●, ○, total arsenic (sum of each species); ●, heating only; ○, ultrasonication 1 h after heating.

To promote gelatinization of the rice flour, the extraction temperature was set at 90 °C to maximize the extraction of the arsenic species, and the duration of heating was then investigated in this work. Furthermore, extraction behavior was examined with and without ultrasonication (VS-100III, As one Co. Ltd., Japan) after heating. Results for the NIST SRM sample (unpolished rice) are shown in **Figure 2**, and the chromatograms obtained by HPLC–ICP–MS are shown in **Figure 3** (see the Supporting Information).

For a heating time of 0 h, the sample was shaken by hand for 1 min, and then the mixture was left for 12 h at room temperature. In addition, a sample was ultrasonicated for 1 h at room temperature. The extracted As(V), MMAA and DMAA were approximately 60%, 70% and 85%, respectively, when compared with the amounts extracted by techniques employing heating, but less than 5% of the As(III) was extracted. Therefore, the total arsenic extracted (the sum of all arsenic species) was only 64% of the total arsenic present.

When the sample was heated, the amount extracted was approximately 100% if the heat was applied for 2 h or more. Ultrasonication had no effect. The maximum heating time tested was 16 h; however, the amount of each species extracted remained constant over 2 to 16 h (no change to the concentration of each species, nor their relative proportion). Thus water was able to

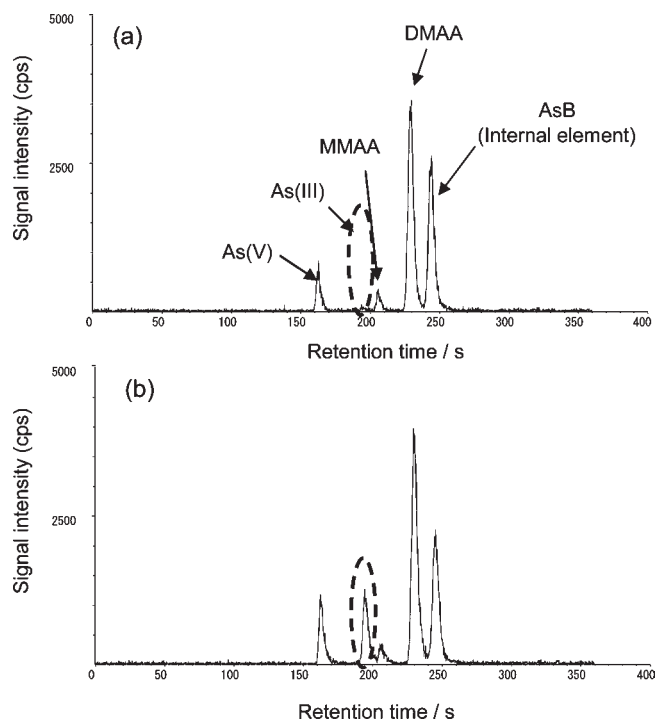


Figure 3. Chromatograms of extracted arsenic species by HPLC–ICP–MS. Sample: NIST SRM 1568a; (a) nonheating extraction; (b) heat-assisted extraction.

extract approximately 100% of arsenic at a temperature of 90 °C, and arsenic speciation analysis of the extract was possible. Results of the proposed extraction by heating with water and by microwave-assisted extraction are shown in **Table 1**. The results of the proposed technique were good in agreement with the results of microwave-assisted extraction, and the sum of the recoveries of all species was 99% of the total arsenic present.

In addition, the effects of the heating temperature within the range 80–100 °C and the heating method (oven or heating block system) on extraction of NIST SRM were investigated, and no differences were observed; the results were the same in each case.

Effect of Experimental Conditions on the Extraction of Polished Rice Flour. Ultrasonication after heating had no effect on the extraction of arsenic species from NIST SRM unpolished rice flour. The flour made from polished rice was then examined. Extraction with water using the heating block system (90 °C, 3 h), ultrasonication (1 h, without heating), and steeping in water (without heating) for 12 h were investigated for the NMIJ CRM polished rice flour. Results are shown in **Table 2**.

When the sample was not heated, the recoveries of As(V), As(III) and DMAA were 80%, less than 10% and 50%, respectively. The results were similar to those for the NIST SRM unpolished rice flour; the amount of As(III) extracted was notably low when heating was not applied. When the sample was heated at 90 °C for 3 h, the amount of As(III) extracted increased, and the recoveries of all species were in the range 96–108% when the proposed technique was employed. However, there was a tendency for As(V) to register slightly higher values and As(III) slightly lower values than expected from the certified values. The ratio of As(III) to As(V) was constant at the same heating temperature, and the proposed heating technique is simple to use. We consider the heating technique is appropriate for the monitoring of arsenic species in rice flour samples.

Effect of the Sample Mass and Heating Method. The sample mass and the amount of extract influence thermal efficiency in the

Table 1. Results of Arsenic Speciation of NIST SRM^a

	As(V)	As(III)	MMAA	DMAA	total
proposed method	0.045 ± 0.001 16% ^b	0.053 ± 0.001 19% ^b	0.013 ± 0.001 4% ^b	0.175 ± 0.002 61% ^b	0.286 ± 0.002 (99%) ^c
MW extraction ^d	0.044 ± 0.002 15% ^b	0.052 ± 0.001 18% ^b	0.012 ± 0.001 4% ^b	0.173 ± 0.002 60% ^b	0.281 ± 0.002 (97%) ^c
certified value					0.29 ± 0.03

^a Unit: mg kg⁻¹. Mean ± SD. ^b Component ratio. ^c Recovery in parentheses. ^d Reference 12.

Table 2. Results of Arsenic Speciation of NMIJ CRM^a

	As(V)	As(III)	DMAA	total
proposed method	0.0140 ± 0.0005 (108%) ^b	0.0740 ± 0.0023 (96%)	0.0134 ± 0.0001 (101%)	0.101 ± 0.005 (104%)
ultrasonication	0.0103 ± 0.0004 (79%)	0.0071 ± 0.0002 (9%)	0.0062 ± 0.0001 (47%)	0.022 ± 0.001 (23%)
steeping	0.0104 ± 0.0004 (80%)	0.0044 ± 0.0001 (6%)	0.0062 ± 0.0001 (47%)	0.021 ± 0.001 (22%)
certified value	0.0130 ± 0.0009	0.0770 ± 0.0029	0.0133 ± 0.0009	0.098 ± 0.007

^a Unit: mg kg⁻¹. Mean ± SD. ^b Recovery in parentheses.

Table 3. Effect of Sampling Mass and Heating Temperature with Heating Block System

wt/g								
sample	extract	L/S ratio	extraction temp/°C	As(V)	As(III)	i-As	DMAA	total
1.00	10.00	10.0	90	0.0135 (104%) ^a	0.0729 (95%)	0.0864 (96%)	0.0140 (106%)	0.100 (102%)
0.50	10.00	20.0	90	0.0140 (108%)	0.0739 (96%)	0.0880 (98%)	0.0136 (102%)	0.102 (104%)
0.50	5.00	10.0	90	0.0140 (107%)	0.0733 (95%)	0.0872 (97%)	0.0138 (104%)	0.101 (103%)
0.25	5.00	20.0	90	0.0140 (108%)	0.0755 (98%)	0.0894 (99%)	0.0136 (103%)	0.103 (105%)
0.50	5.00	10.0	100	0.0178 (137%)	0.0692 (90%)	0.0871 (97%)	0.0137 (103%)	0.101 (103%)
0.50	5.00	10.0	95	0.0143 (110%)	0.0735 (95%)	0.0878 (98%)	0.0134 (101%)	0.101 (103%)
0.50	5.00	10.00	85	0.0136 (105%)	0.0732 (95%)	0.0868 (96%)	0.0137 (103%)	0.101 (103%)
0.50	5.00	10.0	80	0.0137 (105%)	0.0736 (96%)	0.0873 (97%)	0.0134 (101%)	0.101 (103%)
certified value (mg/kg)				0.0130 ± 0.0009	0.0770 ± 0.0029		0.0133 ± 0.0009	0.098 ± 0.007

^a Recovery in parentheses.

Table 4. Effect of Sampling Mass with Oven Heating

wt/g							
sample	extract	L/S ratio	As(V)	As(III)	i-As	DMAA	sum
1.00	10.00	10.0	0.0225 (173%) ^a	0.0688 (89%)	0.0914 (102%)	0.0140 (106%)	0.105 (108%)
0.50	10.00	20.0	0.0260 (200%)	0.0639 (83%)	0.0900 (100%)	0.0136 (102%)	0.104 (106%)
0.50	5.00	10.0	0.0230 (177%)	0.0633 (82%)	0.0862 (96%)	0.0138 (104%)	0.100 (102%)
0.25	5.00	20.0	0.0270 (208%)	0.0615 (80%)	0.0885 (98%)	0.0132 (99%)	0.102 (104%)

^a Recovery in parentheses.

heating process. Therefore, the effect of the liquid/solid ratio was studied. A heating block system and an oven were tested to determine the most efficient heating method. Results obtained with the heating block system and the oven are shown in **Tables 3** and **4**, respectively. As the results show, changing the liquid/solid ratio did not change either the recoveries of the various species or the total recovery. However, there was a tendency for As(V) to

increase with oven heating under the same thermal conditions as the heating block. With the heating block, thermal energy is transmitted directly to the container in the oven, thermal transmission is through air (space), and it appears that this difference is significant. Moreover, the relative proportions of the inorganic arsenic species and the amounts extracted were different for the polished rice depending on the temperature and the heating method, although these differences were not observed for the NIST SRM unpolished rice flour.

Thus the range of conditions for extracting the polished rice flour is narrower than that for unpolished rice flour. Optimal conditions were a liquid/solid ratio of 10, a heating temperature of 90 °C, and use of the heating block system.

Effect of Particle Size. Investigation of the extraction technique employing heat and water was carried out using CRMs. The particle size of the CRM powders was very small, and they had been sterilized using ⁶⁰C γ radiation. Thus, the sample properties were different from those of natural rice flour, and therefore, the effect of particle size on the extraction of arsenic species was investigated using an actual white rice flour sample. Results are shown in **Figure 4**.

To facilitate the investigation of extraction efficiencies, arsenic speciation analysis with the proposed technique and determination of moisture content were carried out for each powder of different particle size. The moisture content was used to correct the concentration data. Because we considered that moisture in the rice flour sample would be inside the particles, we anticipated moisture would increase with decreasing surface area of the powder, but the results showed that moisture increased with increasing surface area. Results of arsenic speciation analysis following the heat extraction technique are also shown in **Figure 4**. The quantities of the arsenic species extracted decreased with increasing particle size of the sample.

The total concentration of arsenic species in the sample with particle size less than 150 μm agreed with the total arsenic concentration obtained by GFAAS after microwave-assisted digestion. For the samples with larger particle size, complete

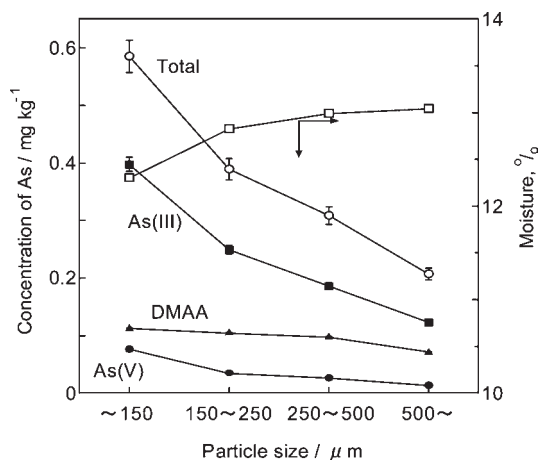


Figure 4. Effect of particle size on extraction of arsenic species from polished rice flour: heating temperature, 90 $^{\circ}\text{C}$; rice flour prepared, polished rice flour; \circ , total; \bullet , As(V); \blacksquare , As(III); \blacktriangle , DMAA; \square , moisture.

extraction was difficult, in particular, the recovery and extraction efficiency of As(III) decreased. Recovery measured against the total arsenic concentration was greatly decreased.

Particle size, therefore, has a large effect on extraction efficiency with complete extraction being achieved with particle size less than 150 μm . Hence, rice samples should be milled to small particle sizes when arsenic speciation analysis is to be carried out.

Observation of Sample Structure by Scanning Electron Microscopy (SEM). Without heating, the extraction efficiency of As(III) in the rice samples was markedly lower than for other arsenic species, and as a consequence, the total amount of arsenic extracted was very low. However, it is important to know the concentrations of As(III) and As(V) because inorganic arsenic has higher toxicity than the organic species. Also, more efficient extraction efficiency was achieved with the unpolished rice sample and analysis was easier than for polished rice. It was considered that the polished rice used in this study contained larger amounts of starch and protein than the same mass of unpolished rice. Possibly the lower extraction rate for As(III) without using heat resulted from its binding to protein and being enclosed inside the starch granules of the rice. Therefore it is probable that extraction efficiencies for As(III) increase as the starch structure is broken down by heating. Moreover, it is likely that smaller particle size promotes effective heat transfer and facilitates the destruction of the starch structure.

The surface and the structure of NMIJ CRM polished rice were observed using a scanning electron microscope (SEM SM-300, TOPCON Ltd. Co.). The original powder made from polished rice, a sample after steeping in water without heating, a sample ultrasonicated for 1 h without heating and a sample after heat-assisted extraction were examined, and the results are shown in **Figure 5**. Large starch particles were observed in the raw material, and no changes to the structure or particle size were brought about by steeping in water or by ultrasonication. On the other hand, the structure of the starch granules was broken by heating, and the surface of the sample become smooth. Thus, if As(III) is located inside the starch granules, it would be difficult to efficiently extract it without breaking the structure of the granules.

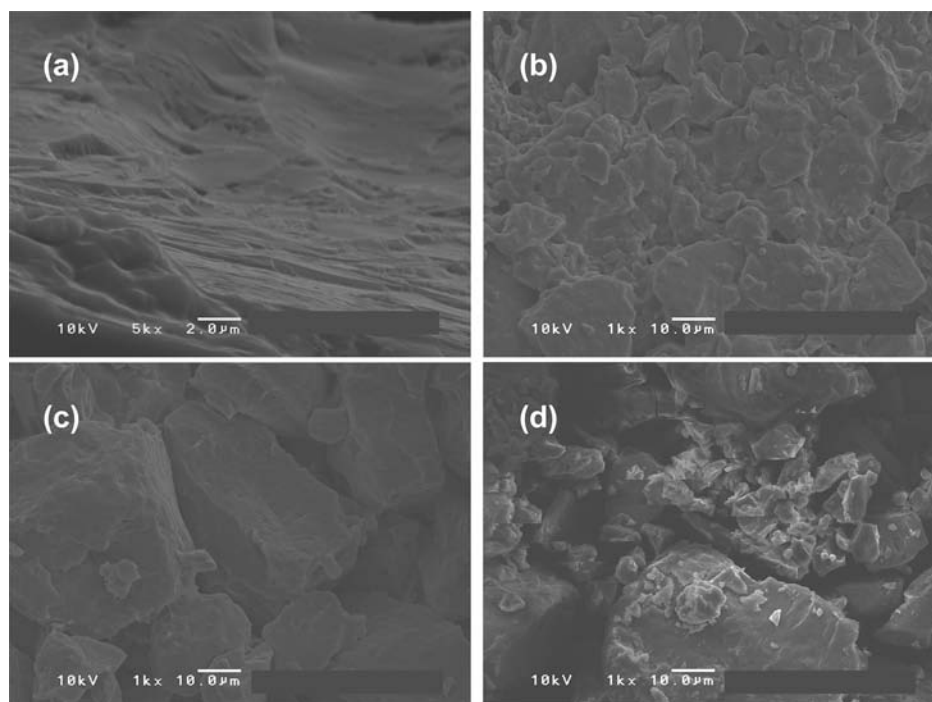


Figure 5. Results of SEM observation of surface of rice flour samples. Rice flour sample NMIJ 7503-a: (a) heating at 90 $^{\circ}\text{C}$; (b) ultrasonication without heating; (c) steeping in water; (d) original polished rice powder.

Table 5. Results of Arsenic Species in Real Rice Samples Determined Using Heat-Assisted Extraction with Water^a

sample ID	As(V)	As(III)	MMAA	DMAA	sum	total ^b
unpolished type A	0.060 ± 0.001	0.094 ± 0.002	0.003 ± 0.0005	0.016 ± 0.001	0.173	0.17 ± 0.01
unpolished type B	0.041 ± 0.001	0.067 ± 0.002	0.003 ± 0.0003	0.006 ± 0.0006	0.117	0.11 ± 0.02
unpolished type C	0.040 ± 0.001	0.098 ± 0.002	0.005 ± 0.0004	0.016 ± 0.001	0.159	0.15 ± 0.01
polished type D	0.023 ± 0.002	0.091 ± 0.001	nd ^c	nd	0.114	0.11 ± 0.01
polished type E	0.027 ± 0.002	0.063 ± 0.001	nd	0.001 ± 0.0003	0.091	0.09 ± 0.01
polished type F	0.038 ± 0.001	0.115 ± 0.002	nd	0.025 ± 0.001	0.178	0.18 ± 0.01

^a Unit: mg kg⁻¹. Mean ± SD. ^b Result of GFAAS or ICP-MS after microwave-assisted digestion. ^c Not detected.

Application to Real Samples. Heat-assisted extraction was applied to real samples to determine the arsenic species. Six kinds of rice flour samples (3 unpolished rice flour and 3 polished rice flour) were used for this study. The obtained results are shown in **Table 5**. The sums of each species in all 6 samples were in good agreement with the total arsenic concentration that was determined by GFAAS and/or ICP-MS after microwave-assisted digestion with HNO₃–HClO₄–HF.

A simple extraction pretreatment was developed for the analysis of arsenic species in rice flour. The method involved heat-assisted extraction with water. Complete extraction of all arsenic species was possible by aqueous treatment of rice flour samples at 90 °C for 3 h. Extraction of rice flour consisting of particles with diameters of less 150 μm is desirable.

Extraction of As(III) from rice flour was difficult because As(III) is present inside starch granules. Therefore, the efficiency of As(III) extraction increases if the starch is broken down by acidic or enzymatic hydrolysis. However, the relative proportions of the arsenic species present may be changed when acids are used in the extraction.

When the proposed heat-assisted aqueous extraction method was used, there was a tendency for slightly higher As(V) and lower As(III) values to be recorded for polished rice flour than for unpolished rice flour. Also, exact comparison of arsenic speciation analysis of polished and unpolished rice was difficult because only a narrow temperature range (85–90 °C) in the extraction process was suitable for polished rice and was best delivered by a heating block. Flour from unpolished rice gave consistent results for arsenic speciation analysis for a temperature range of 80–100 °C for either heating method (block or oven), a range of heating times (2–16 h) and a liquid/solid ratio range of 10:1–10:0.5. Therefore, unpolished rice provides a better matrix on which to perform universal testing. Microwave-assisted extraction with water is the best technique to ensure accurate arsenic speciation analysis of both of unpolished and polished rice flour because it completely extracts all arsenic species facilitating an analysis of higher precision, and the relative proportions of the two inorganic arsenic species are not changed. However, the extraction method involving heating with water proposed here is valuable for environmental monitoring and for the risk evaluation of food because the technique is simple and easy to apply.

Supporting Information Available: Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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