

# Speciation and Monitoring Test for Inorganic Arsenic in White Rice Flour

Tomohiro Narukawa,\* Akiharu Hioki, and Koichi Chiba

Inorganic Analytical Chemistry Division, National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 3, 1-1-1 Umezono, Tsukuba, Ibaraki, 305-8563, Japan

**ABSTRACT:** A monitoring test for arsenic species in white rice flour was developed and applied to flours made from 20 samples of polished rice collected from locations all over Japan. The arsenic species in white rice flour made from five samples each of four types of rice were analyzed by HPLC-ICP-MS after a heat-assisted aqueous extraction. The total arsenic and major and minor element concentrations in the white rice flours were measured by ICP-MS and ICP-OES after microwave-assisted digestion.  $91 \pm 1\%$  of the arsenic in the flours was extractable. Concentrations of arsenite [As(III)], arsenate [As(V)], and dimethylarsinic acid (DMAA) were closely positively correlated with the total arsenic concentrations. The total arsenic concentration in flours made from rice collected around Japan was  $0.15 \pm 0.07 \text{ mg kg}^{-1}$  (highest,  $0.32 \text{ mg kg}^{-1}$ ), which is very low. It was thus confirmed that the white rice flour samples collected in this experiment were not suffered from noticeable As contamination.

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

## ■ INTRODUCTION

Ensuring the safety of the human diet by evaluating the risk of certain chemical components of foodstuffs is essential for the maintenance of human health. In addition, analysis of some foodstuffs can indicate possible environmental pollution of soil and water.

Arsenic (As) is widely distributed in the biosphere and exists as a wide range of chemical species. Both oxidation states of inorganic As, As(III) and As(V), and various organic As compounds, such as simple methylated compounds and arsenosugars, have been shown to occur naturally in organisms. The naturally occurring organoarsenic is nontoxic or of low toxicity to humans, but As(III) is highly toxic.<sup>1,2</sup> Therefore, it is important to know which As species are present in the organisms that contribute to human diet as well as knowing the total concentration of As.

The Codex Alimentarius Commission (CAC) does not regulate for the As concentration in rice, although the CAC has stated that it was necessary to set standards for inorganic As compounds in foodstuffs. However, the Food and Agriculture Organisation/World Health Organization (FAO/WHO) has recommended a provisional tolerable weekly intake (PTWI) of not more than  $15 \mu\text{g}$  of inorganic As/kg of body weight.<sup>3</sup>

Japanese people ingest about 80% of their total As intake from seafood, but rice is the main contributor of As intake among agricultural products. In general, there is little information on As in agricultural products. Also, the food sanitation law of Japan does not regulate for the As concentration in rice. However, rice flour is an important matrix for analysis, and it is an important food throughout Asia and other parts of the world. Unsurprisingly then, many studies of As speciation in rice have been reported.<sup>4–7</sup> Different extraction procedures and extractants have already been evaluated and have demonstrated that arsenic is difficult to extract from the rice matrix.<sup>8–19</sup> D'Amato et al. showed that four 2 h cycles at  $50 \text{ }^\circ\text{C}$  were required to achieve quantitative extraction of arsenic species from SRM 1568a using a 1:1 water–methanol mixture and ultrasonication.<sup>20</sup>

It was thus obvious that shaking and ultrasonic extraction for 1 or 2 h at room temperature would yield unsatisfactory results. One report has been published<sup>21</sup> on As species in rice other than Japanese rice, but there has been no information on rice cultivated in Japan. An international proficiency test on the determination of total and inorganic As in rice has been carried out.<sup>22</sup>

Microwave extraction was not evaluated in previous work until we investigated a microwave-assisted extraction for rice samples and reported on some techniques to determine As species they contain.<sup>23,24</sup>

The total amount of As in rice flour depends on the soil in which the rice is grown. Each area in Japan has its own type of rice, and this must be taken into account when any risk assessment of As in rice is carried out.

We have investigated As speciation in rice samples, and some extraction conditions and determination techniques have been reported. In the current study, the proposed technique was used for monitoring inorganic As in white rice flour samples. White rice flour was collected from four types of rice from each of five production areas. Thus, the proposed technique was applied to 20 white rice flour samples as a monitoring test for inorganic As.

## ■ MATERIALS AND METHODS

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

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gas (3 mL min<sup>-1</sup>) to reduce <sup>40</sup>Ar<sup>35</sup>Cl<sup>+</sup> interference with <sup>75</sup>As. The ion count was monitored at *m/z* = 75. <sup>128</sup>Te was used as internal standard element for total As determination.

An ICP-OES (Optima 5300 DV, Perkin-Elmer, Kanagawa, Japan) equipped with a concentric nebulizer and a cyclonic spray chamber was used for the determination of major and minor elements. The typical operating parameters for the ICP-OES were as follows: incident rf power was 1400 W, outer Ar gas flow rate 15 L min<sup>-1</sup>, intermediate Ar gas flow rate 0.2 L min<sup>-1</sup>, and carrier Ar gas flow rate 0.8 L min<sup>-1</sup>. Y was used as internal standard element.

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 CAPCELL PAK C<sub>18</sub> MG column (250 mm × i.d. 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 0.75 mL min<sup>-1</sup>. The exit of the HPLC column was connected to the nebulizer of the ICP-MS with PEEK tubing. Arsenobetaine was used as an internal standard chemical species.

A STRAT D (Milestone MLS, Leutkirch, Germany) was used for microwave-assisted digestion to decompose the samples.

Total nitrogen (N) was measured by Kjeldahl analysis, and the obtained values were converted to protein using a factor of 5.95.

**Calibration Standards.** The Japan Calibration Service System (JCSS) arsenic standard solution (1000 mg L<sup>-1</sup> As, made from high purity As<sub>2</sub>O<sub>3</sub>) 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 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<sup>-1</sup>.

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.

Custom Assurance Standard (XSTC-622B) was used as major and minor element standard solution (SPEX CertiPrep, Inc., NJ).

**Certified Reference Materials (CRMs).** The rice flour CRMs analyzed were NIST SRM 1568a Rice Flour (National Institute of Standards and Technology, NIST, U.S.) and NMIJ CRM 7503-a white rice flour. The certified values of total As in NIST SRM 1568a and NMIJ CRM 7503-a are 0.29 ± 0.03 and 0.098 ± 0.007 mg kg<sup>-1</sup>, respectively. The certified values of As(III), As(V), and DMAA in NMIJ CRM 7503-a are 0.077 ± 0.0029, 0.0130 ± 0.0009, and 0.0133 ± 0.0009 mg kg<sup>-1</sup>, respectively.

**Sample Collection and Preparation of Polished White Rice Flour.** Five samples each of four types of polished white rice flour were collected. Rice grown in Japan includes both location-specific and more generally grown types. The type and area of collection of 20 white rice flours are summarized in Table 1. Different types 1 and 2 were collected in the same area. Type 3 grows in a wide area of Japan and was collected from widely different locations. Four samples of type 4 were collected in same area, and one sample of type 4 was collected from a different location. Therefore, 20 white rice flour samples consisted of four types from eight different areas. They were collected in 2007–2010.

Samples were prepared by pulverizing a quantity of polished white rice flour in a food processor and separating the resulting powder using 250 μm nylon mesh. The fraction consisting of particles less than 250 μm in diameter was used as a sample.

**Digestion and Extraction Procedure.** Analysis of Total Concentrations of Arsenic and Major and Minor Elements. White rice powder (0.25 g) was weighed precisely and transferred to a PFA

**Table 1. Summary of Rice Types Giving the Flours Used in This Study, Their Production Areas, and Years of Collection**

sample ID	rice type	production area	FY
1-1	type 1	M	2007
1-2	type 1	M	2007
1-3	type 1	M	2007
1-4	type 1	M	2007
1-5	type 1	M	2007
2-1	type 2	M	2007
2-2	type 2	M	2007
2-3	type 2	M	2007
2-4	type 2	M	2007
2-5	type 2	M	2010
3-1	type 3	O	2007
3-2	type 3	T	2007
3-3	type 3	N	2007
3-4	type 3	K	2007
3-5	type 3	C	2007
4-1	type 4	A	2009
4-2	type 4	A	2009
4-3	type 4	A	2010
4-4	type 4	A	2007
4-5	type 4	N	2010

vessel, to which 5 g of HNO<sub>3</sub> and 1 g of H<sub>2</sub>O<sub>2</sub> were added. The operating program of the microwave system was as follows: the samples were heated at 200 W for 5 min in the first step, 300 W for 5 min in the second step, 500 W for 10 min in the third step, and 600 W for 5 min in the fourth step, and then cooled to room temperature. Sample was then made up to 50.0 g with water. Sample solutions were used for the determination of total concentrations of arsenic, and major and minor elements. Blank tests for the procedure were also performed.

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 for 24 h. The correction factors were also used in the As speciation analyses.

**Arsenic Speciation.** Our previous work was relevant in investigating the extraction conditions for the arsenic species in white rice flour.<sup>23,24</sup> Complete extraction of arsenic species from rice flour is difficult to accomplish without changing the arsenic species. The external forces of microwaves and pressure act on the sample during microwave-assisted extraction. Gelatinization of the sample was encountered when conditions were enough to achieve 100% extraction. It is a useful technique for extracting arsenic species, but it is difficult to treat a lot of samples at one time. On the other hand, heat-assisted extraction is useful for dealing with many samples at one time, and it is simple to apply. However, the extraction ratios of the arsenic species are sometimes a little lower than for the microwave-assisted technique. There is also a problem that some of the As(III) present may be oxidized to As(V) during the extraction process. As one of the aims of this study is developing a monitoring test for white rice flour, we employed the heat-assisted technique for extracting inorganic arsenic.

The standard conditions for extraction were as follows: 1 g of the rice flour was placed in a 50 mL polypropylene centrifuge tube, and 10 g of water was added. The capped tube was placed into an oven at 90 °C for 3 h. The rice flour–water suspension was centrifuged at 4000 rpm for 5 min, the supernatant was passed through a 0.45 μm syringe-type PVDF membrane filter, and the filtrate was analyzed by HPLC-ICP-MS.

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

## RESULTS AND DISCUSSION

**Concentrations of Total Arsenic and of Major and Minor Elements.** The total As, major, and minor element

Table 2. Concentrations of Total As, Major, and Minor Elements in White Flours

sample ID	mg/kg								
	As	P	Na	K	Mg	Ca	Fe	Mn	Zn
1-1	0.204	1090	<20	744	366	49	3.2	8.2	15.3
1-2	0.131	920	<20	698	261	46	2.6	7.7	14.8
1-3	0.142	910	<20	687	268	43	2.0	8.4	15.4
1-4	0.091	1150	<20	866	372	45	2.5	8.5	17.3
1-5	0.140	1100	<20	821	361	47	2.7	8.4	15.0
2-1	0.196	1110	<20	836	349	49	2.2	9.5	12.3
2-2	0.135	1020	<20	731	320	49	2.2	9.7	12.4
2-3	0.097	1080	<20	819	330	45	2.2	10.9	12.7
2-4	0.137	690	<20	455	160	40	<2	8.4	11.8
2-5	0.176	1020	<20	789	331	45	<2	7.8	16.5
3-1	0.322	1060	<20	935	353	50	2.3	7.6	16.6
3-2	0.083	620	<20	474	109	38	<2	7.5	16.1
3-3	0.110	940	<20	704	254	40	<2	8.9	15.6
3-4	0.156	1150	<20	968	340	43	2.4	9.4	20.8
3-5	0.304	1270	<20	947	481	53	3.4	6.5	17.2
4-1	0.092	1020	<20	881	281	39	2.1	13.1	15.5
4-2	0.150	930	<20	857	258	47	2.1	8.7	14.1
4-3	0.120	620	<20	555	122	40	<2	8.2	18.2
4-4	0.101	1150	<20	970	310	45	2.5	14.6	18.9
4-5	0.088	1000	<20	847	291	44	2.6	8.5	19.0

concentrations in white rice flour were determined by ICP-MS and ICP-OES following microwave-assisted digestion of the rice samples with  $\text{HNO}_3\text{-H}_2\text{O}_2$  mixed acid. To validate the method for total As, the NIST SRM 1568a rice flour and NMIJ CRM 7503-a white rice flour were analyzed. Results ( $n = 6$ ) for total As, major, and minor elements in the rice samples are given in Tables 2 and 3. Results for total As in the two certified

Table 3. Total As in the CRMs

	certified value (mg/kg)	expanded uncertainty $k = 2$ (mg/kg)	result (mg/kg)
NMIJ CRM 7503-a	0.098	0.007	$0.0972 \pm 0.0013$
NIST SRM 1568a	0.29	0.03	$0.310 \pm 0.003$

reference materials were in good agreement with their certified values.

The concentrations of total As ranged from  $0.322 \text{ mg kg}^{-1}$  (sample 3-1) to  $0.083 \text{ mg kg}^{-1}$  (sample 3-2). The precision of replicate analysis ( $n = 3$ ) for each sample was less than 1%, and the analytical precision of the measurement showed no correlation with the concentrations of major and minor elements. Possibly, the concentrations of these elements in rice samples are dependent primarily upon the environment of the production area.

An analysis of variance (ANOVA) based on the Japanese food guideline was carried out to evaluate the precision of total As analysis with one sample.<sup>25</sup> Repeatability (RSD%) was 0.6, and reproducibility (within-laboratory) (RSD%) was 1.2. The recommended values of repeatability (RSD%) and reproducibility (RSD%) in the guidelines are less than 15 and less than 20, respectively; therefore, the results were satisfactory.

**Evaluation of Analytical Precision and Validation of Arsenic Speciation.** Extraction conditions and validation for As species in rice samples has been previously reported by us. Evaluation of validation with ANOVA based on the guidelines for As species was therefore carried out on a single sample. Results are shown in Table 4. No problems were encountered except for As(V). The results of reproducibility (within-laboratory) for

Table 4. Results of Repeatability and Reproducibility (Within Laboratory) Tests

	repeatability (RSD %)	reproducibility (RSD %)
As(III)	1.6	2.1
As(V)	9.0	24.5
i-As <sup>a</sup>	1.7	2.3
DMAA	2.6	5.7

<sup>a</sup>i-As: Sum of As(III) and As(V).

As(V) exceeded 20% because the concentration of As(V) in the sample was very low. However, the analytical precision of inorganic As treated as a single species presented no problems. Therefore, we considered the results can be acceptable for a monitoring test.

#### Arsenic Speciation in White Rice Flour Samples.

Results of As speciation for 20 white rice flours are shown in Table 5. Several small peaks resulting from unknown compounds (UK-X) were observed on the chromatograms. When the unknown peaks were compared to eight 8 As species standards (As(III), As(V), MMAA, DMAA, TMAO, TeMA, AsB, and AsC) using HPLC-ICP-MS, three of the unknown peaks, UK-1, UK-2, and UK-3, were observed close to the peaks of MMAA, TMAO, and TeMA, respectively. However, the retention times of the unknown peaks were slightly different from those of the standards, and they were therefore treated as unknowns.

The extraction ratios of the As species, calculated from the sum of the concentrations of As species and the concentration of total As, were approximately 90% for each samples. Repeat analysis ( $n = 10$ ) was carried out on one sample to determine the precision of the extraction procedure. The extraction ratio was  $92 \pm 2\%$ , with a relative standard deviation (RSD) of less than 2%. Therefore, we considered the proposed procedure was appropriate for use as a monitoring test, and the data precise enough to allow comparisons to be made.

**Distribution of Arsenic Species.** The concentrations of both i-As and DMAA increased with increasing total As concentration.

Table 5. Concentrations of As Species in the Rice Flours and Their Contribution to Total Arsenic<sup>a</sup>

ID	mg/kg								ext. ratio (%)
	As(III)	As(V)	i-As	DMAA	UK-1	UK-2	UK-3	sum	
1-1	0.120	0.005	0.125	0.054	0.004	0.000	0.003	0.186	91.1
1-2	0.086	0.004	0.091	0.025	0.001	0.001	0.002	0.120	91.6
1-3	0.095	0.004	0.099	0.028	0.000	0.001	0.002	0.130	91.2
1-4	0.065	0.005	0.070	0.012	0.000	0.000	0.001	0.083	90.2
1-5	0.089	0.003	0.092	0.031	0.001	0.001	0.001	0.127	90.5
2-1	0.119	0.005	0.125	0.050	0.006	0.001	0.003	0.185	94.3
2-2	0.098	0.003	0.101	0.020	0.001	0.001	0.001	0.123	90.9
2-3	0.071	0.003	0.074	0.014	0.000	0.000	0.000	0.088	90.9
2-4	0.086	0.005	0.091	0.029	0.001	0.001	0.002	0.124	90.7
2-5	0.131	0.005	0.136	0.020	0.000	0.000	0.003	0.159	90.4
3-1	0.165	0.022	0.187	0.100	0.004	0.001	0.005	0.297	92.4
3-2	0.066	0.003	0.069	0.008	0.000	0.000	0.000	0.077	92.8
3-3	0.085	0.003	0.088	0.011	0.001	0.000	0.000	0.101	91.2
3-4	0.113	0.006	0.119	0.024	0.002	0.001	0.001	0.146	93.2
3-5	0.188	0.008	0.197	0.075	0.007	0.000	0.005	0.284	93.3
4-1	0.054	0.002	0.056	0.025	0.000	0.001	0.002	0.085	92.5
4-2	0.088	0.004	0.092	0.037	0.002	0.000	0.005	0.136	90.4
4-3	0.085	0.004	0.089	0.014	0.001	0.001	0.003	0.109	90.3
4-4	0.074	0.004	0.078	0.015	0.000	0.000	0.000	0.093	92.4
4-5	0.063	0.004	0.067	0.011	0.000	0.001	0.001	0.080	90.9

<sup>a</sup>i-As, sum of As(III) and As(V); UK, unknown peak; sum, sum of all As species; extraction ratio = sum of As species/total As.

As(III) and i-As both showed good correlations with total As; the correlation factors ( $R^2$ ) were 0.936 and 0.958, respectively (Figure 1). The correlation factor ( $R^2$ ) for As(V) and total As

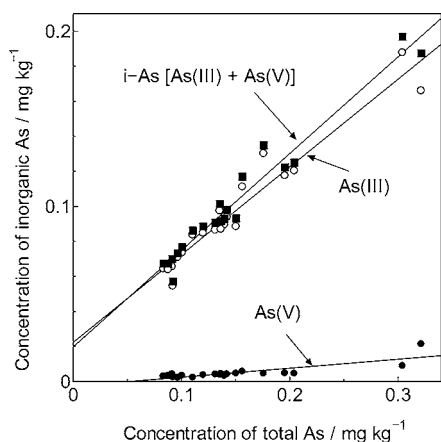


Figure 1. Correlation of the concentration of i-As with total As.

was 0.671, the low value resulting from the low As(V) concentration. However, when i-As as a whole was considered, it accounted for  $70 \pm 7\%$  of total As for the 20 samples collected from a wide range of locations in Japan. i-As accounted for approximately 33% of total As in NIST SRM 1568a. This SRM was produced in the U.S., and the raw material was unpolished rice flour, and therefore it is difficult to compare with white rice flour. However, the relative standard deviation of the percentage of i-As in the Japanese white rice flours was less than 10%, and thus the relative amount of i-As was remarkably constant as samples were collected from all over Japan.

The correlation factor ( $R^2$ ) of DMAA with total As was 0.908 (Figure 2). The percentage that DMAA contributed to total As in the 20 samples was  $18 \pm 6\%$  (RSD 35%). When the close correlation of percentage DMAA with total As and the small

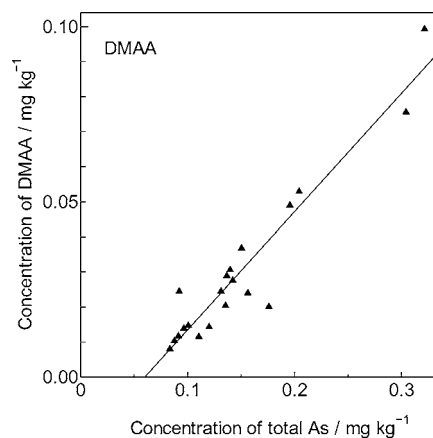


Figure 2. Correlation of the concentration of DMAA with total As.

RSD of percentage i-As are considered, it appears that the distribution error factor for DMAA (an RSD of 35%) is surprisingly large.

The distribution error for DMAA for flours made from type 1 and type 2 rice samples, which were collected from the same location, was  $19 \pm 5\%$  (RSD 27%), and that from type 3 rice, which was collected from over a wide area, was  $18 \pm 9\%$  (RSD 51%). There is a possibility that the concentration of DMAA might sometimes depend on some man-made influence as well as on natural causes because DMAA had been used in the past as a component of weed killers. Therefore, we suppose that the concentrations of DMAA might be influenced by historical background and associated regulations.

The highest percentage that DMAA contributed to total As was 3.2 that of the lowest. The minimum amounts of total As and DMAA were in the "T" area; the minimum amounts of i-As were in the "A" area.

Phosphorus (P) is chemically similar to As, and sometimes it can be of interest to examine them together. The concentrations

Table 6. Concentrations of Total As and As Species in Rice with Different Degrees of Polishing<sup>a</sup>

DP%	mg/kg						total As	ext. ratio %
	As(III)	As(V)	i-As	DMAA	sum			
100	0.145 ± 0.001	0.011 ± 0.002	0.156 ± 0.002	0.006 ± 0.001	0.161 ± 0.002	0.173 ± 0.001	93.3	
90	0.089 ± 0.001	0.003 ± 0.001	0.092 ± 0.001	0.005 ± 0.001	0.097 ± 0.001	0.107 ± 0.000 <sub>4</sub>	90.0	
70	0.064 ± 0.001	0.002 ± 0.000 <sub>2</sub>	0.066 ± 0.001	0.005 ± 0.001	0.071 ± 0.000 <sub>4</sub>	0.079 ± 0.000 <sub>4</sub>	89.5	
50	0.051 ± 0.001	0.001 ± 0.000 <sub>1</sub>	0.052 ± 0.001	0.004 ± 0.001	0.056 ± 0.000 <sub>1</sub>	0.063 ± 0.001	89.6	
bran						0.725 ± 0.012		

<sup>a</sup>DP%, degree of polishing; 100, 0% polishing; 90, 10% polishing; 70, 30% polishing; 50, 50% polishing; bran, residue of 90% of degree of polishing; i-As: sum of As(III) and As(V); sum, sum of all As species; total As, total As concentration following microwave-assisted digestion; ext. ratio, extraction ratio sum/total As × 100; extraction of As species, heat-assisted extraction with water.

of P in the white rice flours were very much higher than the concentrations of total As, and the P concentrations in the 20 samples showed little variation. However, there was a weak correlation between total P and total As ( $R^2 = 0.151$ ) (Figure 3).

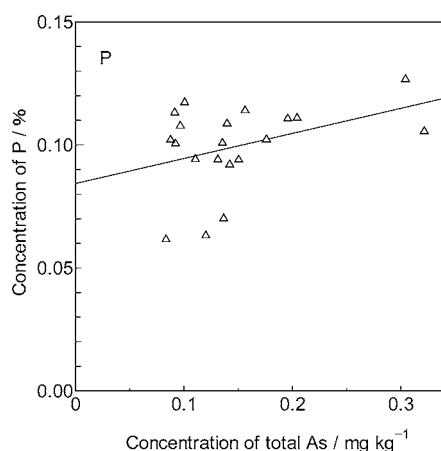


Figure 3. Correlation of the concentration of total P with total As.

#### Influence of Protein Level on Extraction Efficiency.

When white rice flour is heated, the structure of the starch granules is destroyed and the flour assumes a high density. As a consequence, the extraction efficiency is greatly decreased. Protein in the white rice flour samples was determined by measuring the total N and applying a factor of 5.95. Each sample was analyzed twice, and the precision of replication analysis was better than 1.5%.

The relationship between amounts of protein and extraction efficiency is shown in Figure 4. Unexpectedly, there was a slight

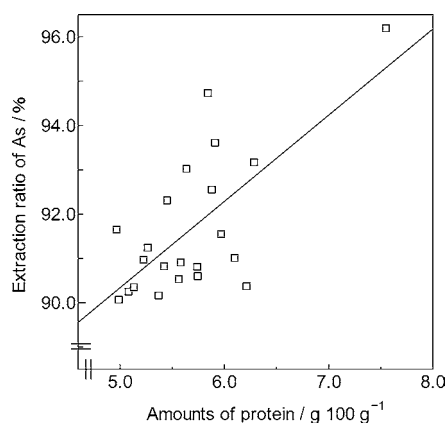


Figure 4. The relationship between the amounts of protein and the extraction efficiency of As.

tendency for As extraction efficiency to increase with increasing amounts of protein.

The major components of food are carbohydrate, protein, and fat, and as rice contains little fat, when protein levels increase, the carbohydrate (starch) level tends to decrease. It was possible, therefore, that extraction efficiency was higher with higher amounts of protein and lower amounts of starch.

#### Influence of Degree of Polishing of Rice on Arsenic

**Extraction.** In this study, white rice flour made from polished rice was used. Generally, the degree of polishing of white rice is 90–92%; that is, 8–10% of the outer bran layers are milled off. Changing the degree of polishing will change the relative amount of protein in the white rice flour, and thus may also change the efficiency of extracting As species. Therefore, we prepared as a starting material a rice flour made from brown unpolished rice. Flours were prepared from materials in the following series, 100% brown rice, 90% (90% of the grain remaining, 10% of the outer layers milled off), 70% (30% milled off), and 50% (50% milled off). The total As concentration was determined in each flour following microwave-assisted digestion, and As speciation analysis was carried out following extraction. Results are shown in Table 6. The highest total As concentration was in unpolished brown rice, and the total As concentrations in the other three samples decreased with the increasing degree of polishing. This showed that higher concentrations of As are present in the bran layer and/or surface of the rice grains. The concentration of inorganic As followed the same trend. However, the relative amounts of each As species in the samples were not changed.

The extraction efficiency of As species was highest, at 93%, from flour from unpolished rice, but there was no trend discernible for the flours made from polished rice. Polished white rice contains larger amounts of starch than does the same mass of unpolished rice because unpolished rice contains bran and other components, and, therefore, unpolished rice could be extracted more efficiently.

The concentration of total As in the bran that contributed the outer 10% milled from brown rice was much higher than in the rice sample as a whole. This confirmed the higher concentration of As in the rice bran.

The extraction and analytical procedure proposed here is useful for monitoring inorganic As in rice flour. The method is simple and can analyze many samples at one time. However, the extraction efficiencies for the various As species strongly depend on several factors such as particle size of the flour, degree of polishing of the rice, and sugar and starch containing levels. Thus, it is very most important to formulate strict guidelines for sample preparation for the monitoring if it can be universally applied.

## AUTHOR INFORMATION

### Corresponding Author

\*Tel./fax: +81-29-861-6889. E-mail: tomohiro-narukawa@aist.go.jp.

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