Note

Determination Method for Total Arsenic and Partial-digestion Method with Nitric Acid for Inorganic Arsenic Speciation in Several Varieties of Rice

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The determination method of total arsenic (As) and the speciation method of inorganic As for non-glutinous rice reported in our preceding paper were applied to several varieties of rice under optimized experimental conditions. In the determination of total As with ICP-MS, acetic acid was added to increase the sensitivity and an internal reference method with germanium was adopted to increase the precision. The extraction temperature in the partial-digestion method with nitric acid to speciate inorganic As was raised to 100°C, because extraction efficiency over 90% was obtained from glutinous rice and colored rice at this temperature. In the investigation of polished and unpolished forms of non-glutinous, glutinous, and colored rice, the amounts of total and inorganic As were 0.04–0.54 mg/kg and 0.02–0.41 mg/kg, respectively. The color of rice was not related to total or inorganic As content. These results indicate that the inorganic As content in commercial rice should be carefully monitored.

Key words: rice; glutinous rice, arsenic; inorganic arsenic; speciation; partial digestion; HPLC-ICP/MS

Introduction

Arsenic (As) is an element that may pose a health risk to humans¹⁾. Inorganic As is known to be an initiator of carcinogenesis²⁾; therefore it is important to estimate the content of inorganic As in the diet. The Food and Agriculture Organization/World Health Organization (FAO/WHO) recommended a provisional tolerable weekly intake (PTWI) of 15 µg of inorganic As/kg body weight. Although As consumption is mainly from seafood^{3), 4)}, rice contains inorganic As, and is consumed around the world, especially in Asia as a staple food. Therefore, As in rice might be associated with a risk to human health.

Many varieties of rice are cultivated around the world. In Asia, glutinous rice (MOCHIGOME) varieties cooked by steaming for rice-cake, as well as non-glutinous rice cooked by boiling, are generally consumed. Glutinous rice is stickier than non-glutinous rice, because its starch consists mainly of amylopectin, while non-glutinous rice contains amylose. Rice varieties with colored endosperm kernels such as red,

violet-black, and green rice, considered to be of the species *Oryza sativa*⁵⁾, are also consumed in Asia. Their kernels contain tannin, anthocyanin, and chlorophyll pigments, as well as polyphenols. Colored rice is not only consumed as a staple food in some countries but also used as a pigment and a nutritional supplementation to white rice in some Asian countries. Therefore, in the evaluation of inorganic As in rice, it is important to evaluate glutinous rice and colored rice as well as non-glutinous rice.

The determination methods of total As and inorganic As in several varieties of rice were examined in this study. It was reported that higher intensities of As were obtained in ICP-MS with the addition of carbon sources, such as methanol^{6), 7)}. In this paper, acetic acid was used to increase the sensitivity of As.

For the accurate determination of inorganic As, a method with high extraction efficiency is required. Several extraction methods have been reported; amylolysis with enzyme, hydrolysis with 2 mol/L trifluoroacetic acid (TFA), and microwave extraction with water^{8)–10)}. As we have reported previously, partial digestion with nitric acid is also effective to extract inorganic As from non-glutinous rice¹¹⁾. In the previous paper, the extrac-

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tion temperature was set at the same level (80°C) as for hijiki¹²⁾ so that hijiki and rice could be treated simultaneously.

In this paper, the effect of extraction temperature was further studied with the aim of applying the method to various types of glutinous rice and colored rice, and the total and inorganic As levels in several varieties of rice were determined by use of the established method.

Materials and Methods

Chemicals

Arsenite solution (100 mg/L as As (III)), germanium (Ge) solution (1,000 mg/L), certified by Japan Calibration Service System (JCSS) for atomic absorption analysis, and nitric acid (Ultrapur-100) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were purchased from Tri Chemical Laboratories Inc. (Yamanashi, Japan). Other chemicals used were of trace element analysis grade.

Sample preparation

All of the brown or polished rice samples were purchased from a commercial retailer or an internet shop in Japan. Some brown rice samples were polished in a rice polishing machine (KG-11, Panasonic, Osaka, Japan) 2 or 3 times, until no further bran could be removed. Polished rice was ground in a food mill (Labo Milser LM-PLUS, Iwatani Corporation, Tokyo, Japan) to a particle size of less than 0.5 mm.

Digestion for total As determination

Approximately 0.5 g of each sample was weighed precisely into a PTFE digestion vessel, then 5 mL of nitric acid and 1 mL of hydrogen peroxide were added, and the mixture was left to stand in a ventilated enclosure until the initial reaction subsided. The vessels were closed with PTFE covers and digestion was performed with a microwave digestion system (ETHOS, Milestone, CT, U.S.A.). The microwave digestion program was as follows: the first step was 2 min to 70°C at 1,000 W; the second step was 5 min at 50°C to 0 W; the third step was 20 min to 200°C at 1,000 W; the fourth step was 30 min at 200°C at 1,000 W. The vessel was opened after cooling, and water was added to the digested solution. Subsequently 1 mL of acetic acid and 1 µg of Ge, as an internal standard solution, were added. The solution was transferred to a PP tube and made up to 50 mL with water.

Total As determination

Total As in the digested solution was determined using an ICP-MS machine (Agilent 7500ce, Agilent Technologies, CA, U.S.A.) in the helium collision mode to decompose ArCl interfering ion at m/z 75. The analytical conditions were: RF power, 1.6 kW; plasma gas, 15 L/min; carrier gas, 0.70 L/min; auxiliary gas, 0.90 L/min; makeup gas, 0.43 L/min. The peak intensities at m/z 75 and 72 for As and Ge, respectively,

were measured, and As concentration was calculated from the intensity ratio of As to Ge in each solution by the internal reference method.

Extraction of inorganic As from rice

Approximately 0.5 g of each sample was weighed precisely into a digestion tube, and 2 mL of 0.15 mol/L nitric acid was added. A glass cover was put in place to avoid evaporation of the solvent, and heating was carried out at 100°C for 2 hours in an aluminum heating block. The extract was made up to 10 mL with water and centrifuged at 3,500 rpm. The supernatant was transferred to a PP tube as the sample solution.

Speciation of As

To determine inorganic As, speciation analyses of As(III), As(V), MMA, and DMA were carried out with an HPLC apparatus (Agilent 1200 Series, Agilent Technologies, CA, U.S.A.) coupled with an ICP-MS machine (Agilent 7500ce, Agilent Technologies, CA, U.S.A.). The analytical conditions were reported previously¹¹⁾. The As concentration was determined from the As peak area.

Results and Discussion

Determination of total As

In evaluating a method for the determination of inorganic As in rice, the accuracy of determination of the total As content is also important, because extraction efficiency is evaluated as the ratio of extracted As to total As. To determine total As by ICP-MS, the internal reference method or the standard addition method can be used¹³⁾. The standard addition method is able to correct interference from the matrix; however, the number of measurements is greater. The internal reference method may be employed if an element similar to the internal standard is not included in the sample. Although ICP-MS is a highly sensitive method, the intensity depends on the mass number due to space-charge effects¹⁴⁾. Therefore, in this study, Ge was used as the internal reference element in the determination of total As, because its mass number is close to that of As. To increase the sensitivity, acetic acid was added.

The ICP-MS intensity of As with the addition of acetic acid was approximately 2.8-fold higher than that without acetic acid (Table 1). The limit of detection

Table 1. Effects of addition of acetic acid on the peak intensity of As (cps)

Concentration of As solution (µg/L)	Acetic acid	
	Added	Not added
0	4	1
0.2	211	76
0.5	493	178
1.0	905	341
2.5	2,530	891
5.0	4,604	1,631
10.0	9,134	3,296

Table 2. Comparison of extraction efficiency between white and colored rice

Grain color	Type	Total As (mg/kg)	Total As in extract (mg/kg)		Extraction efficiency (%)*	
			80°C	100°C	80°C	100°C
White (unpolished)	Glutinous	0.54±0.024	0.40±0.003	0.49±0.013	74	91
White (polished)	Glutinous	0.29±0.007	0.24±0.002	0.26±0.002	83	90
White (polished)	Jasmine	0.10±0.001	0.08±0.005	0.10±0.007	80	100
Violet-black (unpolished)	Glutinous	0.42±0.008	0.33±0.017	0.39±0.016	79	93
NIST1568a [#] (brown flour)	Not described	0.32±0.011	0.30±0.007	0.31±0.004	94	97

Mean±SD (3 determinations)

* Extracted total As concentration/total As concentration

[#] Certified value is 0.29±0.03 mg/kg**Table 3.** Comparison of speciation at two extraction temperatures

Sample	As(III)		As(V)		Inorganic As (As(III)+As(V))		MMA		DMA	
	80°C	100°C	80°C	100°C	80°C	100°C	80°C	100°C	80°C	100°C
Brown rice	0.306±0.002	0.397±0.003	0.019±0.003	0.015±0.002	0.325±0.005	0.412±0.003	<0.008	<0.008	0.079±0.002	0.088±0.005
NIST 1568a	0.057±0.004	0.069±0.001	0.027±0.003	0.022±0.001	0.084±0.005	0.091±0.006	0.010±0.001	0.009±0.002	0.154±0.004	0.171±0.008

Mean±SD (5 determinations)

(mg/kg)

Table 4. Speciation of As in several varieties of rice

Color	Grain type	Starch type*, Process**	Origin	As(III) (mg/kg)	As(V) (mg/kg)	MMA (mg/kg)	DMA (mg/kg)	Inorganic As [#] (mg/kg)	Total As (mg/kg)	Inorganic As/Total As (%)
White	Short	NG, P	Italy	0.052±0.003	<0.008	<0.008	0.026±0.001	0.05±0.003	0.09±0.003	56
White	Short	NG, P	Spain	0.041±0.004	<0.008	<0.008	0.009±0.001	0.04±0.004	0.05±0.004	80
White	Short	NG, P	China	0.078±0.001	<0.008	<0.008	0.019±0.001	0.08±0.006	0.10±0.004	80
White	Short	NG, P	Japan	0.191±0.019	<0.008	<0.008	0.098±0.006	0.20±0.016	0.34±0.010	59
White	Short	NG, P	Japan	0.120±0.004	<0.008	<0.008	0.012±0.001	0.12±0.004	0.10±0.010	120
White	Short	NG, NP	Japan	0.251±0.022	0.009±0.003	<0.008	0.043±0.002	0.26±0.018	0.32±0.006	81
White	Short	NG, NP	Japan	0.123±0.002	<0.008	<0.008	<0.008	0.12±0.002	0.11±0.005	109
White	Short	NG, NP	Japan	0.107±0.003	<0.008	<0.008	<0.008	0.11±0.003	0.14±0.005	79
White	Short	NG, NP	Japan	0.257±0.007	<0.008	<0.008	0.023±0.003	0.26±0.007	0.27±0.005	96
White	Short	G, NP	Japan	0.397±0.003	0.015±0.002	<0.008	0.088±0.005	0.41±0.003	0.54±0.024	76
White	Short	G, NP	Japan	0.114±0.003	<0.008	<0.008	<0.008	0.11±0.003	0.11±0.004	100
White	Short	G, P	Japan	0.078±0.003	<0.008	<0.008	0.023±0.002	0.08±0.003	0.10±0.004	80
White	Short	G, P	Japan	0.088±0.002	<0.008	<0.008	0.021±0.002	0.09±0.002	0.10±0.001	90
White	Short	G, P	U.S.A.	0.073±0.003	<0.008	<0.008	0.012±0.001	0.07±0.003	0.07±0.002	100
White	Medium	NG, P	U.S.A.	0.058±0.002	<0.008	<0.008	0.028±0.001	0.06±0.002	0.09±0.004	67
White	Medium	NG, P	U.S.A.	0.062±0.003	<0.008	<0.008	0.029±0.002	0.06±0.003	0.10±0.001	60
White	Long	NG, P	Thailand	0.066±0.004	<0.008	<0.008	0.021±0.002	0.07±0.004	0.09±0.006	78
White	Long	NG, P	Thailand	0.063±0.002	<0.008	<0.008	0.029±0.001	0.06±0.002	0.10±0.001	60
White	Long	G, P	Thailand	0.070±0.003	<0.008	<0.008	0.027±0.002	0.07±0.003	0.09±0.004	78
White	Long	NG, P	Bangladesh	0.024±0.003	<0.008	<0.008	0.015±0.001	0.02±0.003	0.04±0.001	50
White	Long (karijira)	NG, P	Bangladesh	0.023±0.001	<0.008	<0.008	0.014±0.001	0.02±0.001	0.05±0.003	40
White	Long (basmati)	NG, P	Pakistan	0.029±0.003	<0.008	<0.008	0.035±0.003	0.03±0.003	0.08±0.003	38
Violet-black	Short	G, NP	Japan	0.146±0.006	<0.008	<0.008	0.012±0.001	0.15±0.006	0.14±0.005	107
Violet-black	Short	G, NP	Japan	0.275±0.011	0.027±0.002	<0.008	0.049±0.002	0.30±0.013	0.42±0.008	71
Violet-black	Short	G, NP	Japan	0.105±0.004	<0.008	<0.008	<0.008	0.11±0.004	0.13±0.010	84
Violet-black	Short	G, NP	Japan	0.120±0.003	<0.008	<0.008	0.011±0.001	0.12±0.003	0.14±0.008	86
Red	Short	G, NP	Japan	0.166±0.005	0.010±0.002	<0.008	0.016±0.002	0.18±0.006	0.16±0.005	113
Red	Short	G, NP	Japan	0.118±0.005	0.008±0.002	<0.008	0.010±0.000	0.13±0.004	0.14±0.009	93
Green	Short	G, NP	Japan	0.086±0.001	<0.008	<0.008	0.009±0.001	0.09±0.001	0.11±0.002	82

Mean±SD (3 determinations)

* G: glutinous, NG: non-glutinous

** NP: not polished (brown) rice, P: polished rice

[#] Inorganic As was calculated as the sum of As(III) and As(V).

(LOD) and the limit of quantification (LOQ) were 0.012 mg/kg and 0.04 mg/kg, calculated as 3 times and 10 times the standard deviation of blank tests, respectively.

Extraction and speciation of As

As we have already reported, partial digestion with diluted nitric acid is a simple extraction method with sufficient efficiency for As speciation. In this study, extraction efficiency was compared at 80°C as used in the previous studies^{11), 12)} and at 100°C. White glutinous rice rather than non-glutinous rice as used in the preceding paper was investigated as both polished and unpolished rice. The extraction efficiency from violet-black glutinous rice (unpolished) and jasmine rice (polished) was also investigated.

Concentrations of total As in glutinous rice and the certified reference material (NIST1568a) are given in Table 2. Extraction efficiency from the certified reference material (NIST1568a) was 94% and 97% at 80°C and 100°C, respectively, and these values were considered to be approximately equal. The extraction efficiency from polished glutinous rice was 83% and 90% at 80°C and 100°C, respectively, so the difference was small. However, the difference in the efficiency between the two temperatures was large (14–20%) for unpolished glutinous rice and jasmine rice. Thus, the extraction efficiency at 80°C depended on the variety of rice and/or the type of rice (polished or unpolished). At 100°C, on the other hand, the extraction efficiency was over 90% for all samples tested, which was sufficient.

The results of As speciation in glutinous brown rice and NIST1568a are shown in Table 3. The concentrations of inorganic As obtained in NIST1568a were 0.084 and 0.091 mg/kg at 80°C and 100°C, respectively. Interestingly, As(V) was decreased at 100°C, though As(III) was increased. The difference in total inorganic As concentration was less than 10%. In the case of glutinous rice, on the other hand, extracted inorganic As at 100°C was significantly higher than that at 80°C. Based on the results, it was considered that extraction at 100°C could be applicable to many types of rice samples. The LOD and the LOQ of each species in this method were 0.002 mg/kg and 0.008 mg/kg, respectively, calculated as 3 times and 10 times the standard deviation of 10 determinations.

Determination of total and inorganic As in several varieties of rice

Total and inorganic As were determined for several varieties of rice. The results are given in Table 4. Total and inorganic As concentrations were 0.04–0.54 mg/kg and 0.02–0.41 mg/kg, respectively, and content ratios of inorganic As to total As were more than 56% and 60% in short and medium grain white rice, respectively. In long grain white rice, except basmati and karijira, the content ratio was 60–78%, being higher than that found in a previous study⁸⁾, but similar to that of rice in Thailand¹⁵⁾. In colored short grain rice, the concentration of inorganic As was 0.09–0.30 mg/kg, and the con-

tent ratio of inorganic As to total As was 71–113%. Total and inorganic As were not related to the color of rice. The content ratio of inorganic As in rice was much higher than that in fish and seaweed. Consequently, the inorganic As content in commercial rice represents a potential health risk. In the As speciation of rice samples, partial digestion with nitric acid at 100°C seems to be applicable to many varieties of rice.

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