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Decrease of arsenic in edible brown algae *Hijikia fusiforme* by the cooking process†

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A type of edible sea brown algae, *Hijikia fusiforme*, contains a high concentration of inorganic arsenic. In July 2004, the British Food Standard Agency (FSA) advised people not to eat a type of seaweed called Hijiki because it contained high levels of arsenic. We examined the removal of inorganic arsenic compounds in *H. fusiforme* by performing a soaking procedure with pure water, and the excretion of arsenic contained in Hijiki was investigated in mice. The total arsenic was measured by hydride generation–atomic absorption spectrometry (HG-AAS), and the speciation analysis of arsenic was monitored by high-performance liquid chromatograph coupled with inductively coupled plasma mass spectrometry (HPLC/ICP-MS). It was observed that 28.2–58.8% (w/w) of the total arsenic in edible alga *H. fusiforme* was eluted with water, and 49.3–60.5% (w/w) of arsenic in the residue of Hijiki was dissolved by cooking. Thus, 88.7–91.5% (w/w) of arsenic in Hijiki is removable by the cooking process. When Hijiki was given to mice, dimethylarsinic acid (DMAA) was mainly metabolized in urine. It became evident that soaking with water and cooking are effective for removing arsenic in edible brown algae. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: *Hijikia fusiforme*; arsenic; HPLC/ICP-MS; cooking process; marine food; seaweed; arsenosugar

INTRODUCTION

Marine organisms frequently contain high amounts of arsenic and differ from terrestrial organisms with regard to arsenic concentration and its various chemical forms. The arsenic concentration in a terrestrial organism rarely exceeds 1 μg/g (dry weight). However, the arsenic concentration in a marine organism ranges from 1 to 100 μg/g. A marine animal mainly contains organic arsenic compounds such as arsenobetaine and inorganic arsenic content is as low as 2% of the total arsenic content. The arsenic content in a sea algae is mostly organic and some edible seaweeds contain a dimethylarsenic compound such as arsenosuger. It was reported that the inorganic arsenic content of marine algae is less than 10%, although it is more than the arsenic content of marine animals.[1] However, there are exceptions and some seaweeds contain high ratios of inorganic arsenic. In particular, *Hijikia fusiforme* has a high inorganic arsenic content of approximately 50%. In July 2004, the British Food Standard Agency (FSA) advised people not to eat a type of seaweed called Hijiki because it contained high levels of inorganic arsenic that could act as a carcinogen. Moreover, in Canada, the same advice was given and administrative guidance was provided by the authorities in October 2001. It is important to confirm the safety of marine products for human consumption because many Japanese consume a wide variety of marine products.

In this report, we examined the removal of arsenic compounds by performing a soaking procedure with water, and the excretion of arsenic contained in *H. fusiforme* was investigated in mice.

† This paper is based on work presented at the 12th Symposium of the Japanese Arsenic Scientists' Society (JASS) held 5–6 November 2005 in Takizawa, Iwate Prefecture, Japan.

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MATERIALS AND METHODS

Instruments

The total amount of arsenic was measured in an absorption spectrophotometer Spectr AA220 (Varian) operated at

193.7 nm and equipped with a heated quartz tube, after the arsenic was reduced to arsine by using an arsine generation system VGA77 (Varian).

Speciation analysis of arsenic compounds was performed by HPLC/ICP-MS. The HPLC system consists of a carrier reservoir CR670, a pump PU611 (GL Sciences), and an auto sampler with a column oven MIDAS (Spark Holland). The column was an Inertsil AS (2.1 × 150 mm, GL Sciences) and the cartridge guard column was an Inertsil AS (1.5 × 10 mm, GL Sciences). The ICP-MS system was an ELAN DRC-*e* (PerkinElmer). Each sample was transferred to a polyethylene vial (GL Sciences) at the determination step. The peak retention times and areas were determined with a TotalChrom Workstation version 6.2.0 (PerkinElmer). The analytical conditions of the HPLC/ICP-MS are shown in Table 1.[2,3]

Table 1. Analytical conditions of HPLC-ICP/MS

HPLC	
Column	Inertsil As (2.1 × 150 mm)
Column temperature	40 °C
Flow rate	0.20 mL/min
Injection volume	5 μL
Mobile phase	10 mM sodium 1-butanesulfonate 4 mM tetramethylammonium hydroxide 4 mM malonic acid 0.5% Methanol
ICP-MS	
RF power	1.5 kW
Plasma gas flow	18 L/min
Nebulizer gas flow	0.91 L/min
m/z	75 (As)

Chemicals

Standard compounds of arsenate (Na_2HAsO_4), arsenite ($NaAsO_2$), methylarsonic acid (MAA), dimethylarsinic acid (DMAA), arsenobetaine (AB), trimethylarsine oxide (TMAO), tetramethylarsonium (TetMA) and arsenocholine (AC) were purchased from Trichemical Laboratory (Yamanashi, Japan). (*R*)-(2′, 3′-dihydroxypropyl)-5-deoxy-5-dimethylarsinoyl-β-D-riboside (arsenosugar) was synthesized from 1-*O*-acetyl-tri-*O*-benzoil-β-D-ribofuranose, (*S*)-1,2-*O*-isopropylidene glycerol, and dimethylarsinous iodide by the modification method of McAdam and Stick.[4–9] The chemical structures of the arsenic standard in this work are shown in Fig. 1. Other reagents used in the present experiment were analytical reagent grade. Nitric acid, sulfuric acid, hydrogen peroxide, diammonium hydrogen citrate and methanol were purchased from Kanto Chemicals (Tokyo, Japan); sodium 1-butanesulfonate and tetramethylammonium hydroxide pentahydrate, Tokyo Chemical Industry Co. (Tokyo, Japan); and malonic acid, Wako Pure Chemicals Co. (Osaka, Japan). Pure water obtained using a Milli-Q water system (Nihon Millipore Kogyo, Tokyo, Japan) was used for the preparation of reagents and standards.

Samples

The edible brown algae, *Hijikia fusiforme*, was supplied by the Hijiki Cooperative Society Japan (Ise, Mie, Japan). These

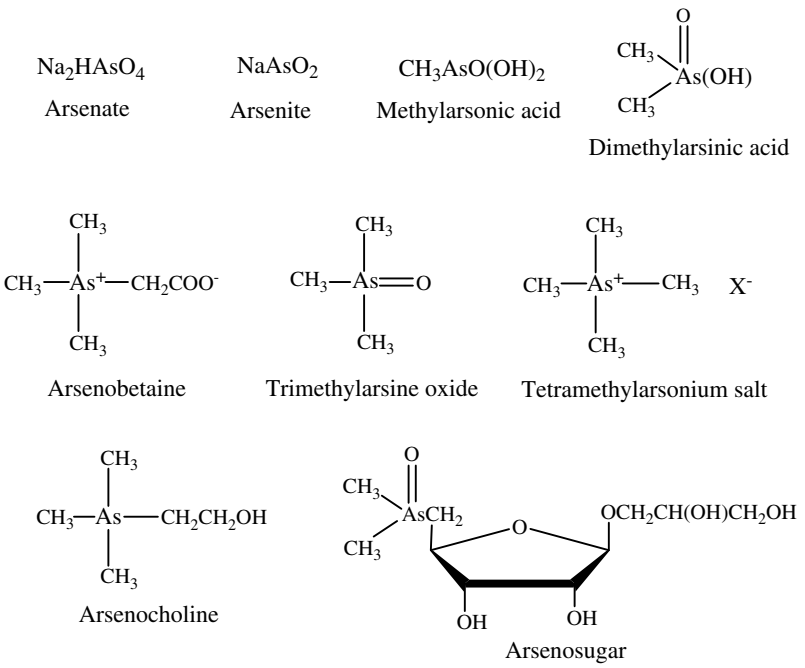

Figure 1. Chemical forms of arsenic standard.

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Hijiki Samples, a long portion and a sprout, were gathered from Mie (Japan), South Cholla Province (South Korea) and Zejiang (China).

Water soaking for dried Hijiki

The dried Hijiki (5 g) was soaked in 20 mL pure water for 30 min at room temperature. The soaked Hijiki was separated from the extract by filtration. The solution was filtrated by a 0.2 μm filter and the speciation analysis of arsenic was determined by the HPLC/ICP-MS.

Cooking method for Hijiki

The soaked Hijiki (5 g) was boiled in 30 mL of pure water for 20 min at 90 °C as a cooking process. The cooked Hijiki was separated from the extract by filtration. The arsenic speciation analysis of the extracts was determined by the HPLC/ICP-MS after filtration by a 0.2 μm filter. The cooked Hijiki was used to determine the residual amount of arsenic after digestion by the analysis method for the total amount of arsenic.

Total arsenic analysis

The sample of uniform Hijiki powder was weighed and transferred into a digestive vessel. Nitric acid (5 mL, 60%) and hydrogen peroxide (2 mL, 30%) were added to the vessel and samples were digested by using a microwave digestion system (O. I. Analytical). After the samples were turned into ashes, the degraded acid solution was transferred into a beaker to which sulfuric acid (1.5 mL, 96%) was added, and the beaker was covered with a glass dish.[10] Digestion was performed on a hotplate below 230 °C until dense fumes of sulfur trioxide appeared. After the digestion returned to room temperature, 0.1 mL of 25% (w/w) ammonium hydrogencitrate was added. The digested solution was neutralized with ammonium hydroxide. Hydrochloric acid (4 mL, 25%, w/w), ascorbic acid (2 mL of 20%, w/w) and potassium iodide (2 mL of 20%, w/w) were then added to the sample solutions; water was then added in order to bring the solution volume to 100 mL. The total amount of arsenic was measured by hydride generation–atomic absorption spectrometry (HG-AAS).

Speciation analysis of arsenic compounds in Hijiki

The dried Hijiki (5 g) was soaked in 20 mL of pure water for 3 h at room temperature. The soaked Hijiki was separated from the extract by filtration. The separated Hijiki were mashed by mortar and homogenized. The arsenic compounds were dissolved into solution of pure water with ultrasonic extraction for 15 min, and the solution was centrifuged. The solution was filtrated and the speciation analysis of arsenic performed by HPLC/ICP-MS.

Treatment of mice

The male mice were fed an arsenic free diet for 1 week and starved for one day before the samples were administered. The liquid samples, water used for soaking Hijiki, arsenate, DMAA and arsenosugar were used for oral administration, and for the solid sample, cooked Hijiki was made freely accessible in the cages.

Analysis of arsenic metabolites in urine and feces

The urine samples were collected each time the mouse urinated up to 12 h, and collected every 3 h after the initial 12 h. The collected urine samples were filtered using a 0.2 μm filter and the speciation analysis of arsenic was performed by HPLC/ICP-MS. The feces samples were collected every 6 h, and arsenic compounds were extracted with a mixture of methanol : water (1 : 1, v/v) at 60 °C. The solution was centrifuged and the supernatant was obtained. The supernatant was evaporated and dissolved in water. The solution was filtrated and the speciation analysis of arsenic was performed using HPLC/ICP-MS.

RESULTS AND DISCUSSION

Analysis of arsenic standard compounds by HPLC/ICP-MS

The arsenic standard compounds were analyzed by the HPLC/ICP-MS system. Nine arsenic standard compounds of arsenate, arsenite, methylarsonic acid (MMA), dimethylarsinic acid (DMAA), arsenobetaine (AB), trimethylarsine oxide (TMAO), tetramethylarsonium (TetMA), arsenocholine (AC) and arsenosuger were separated from each other within 410 s as shown in Fig. 2. The concentration of arsenic standards, excluding the arsenosugar, was found to be 10 μgAs/g.

Total arsenic of *Hijikia fusiforme*

The results of the total amount of arsenic in *H. fusiforme* are shown in Table 2. The total arsenic concentrations in the Hijiki gathered from the shore of Japan, South Korea, and China were 41.7–46.7 μgAs/g, 65.6–79.8 μgAs/g and

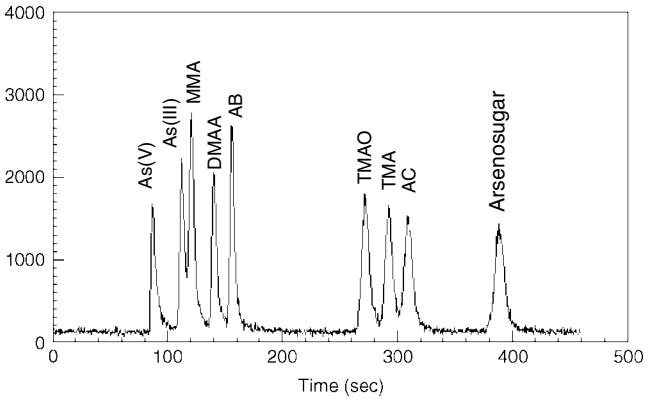

Figure 2. HPLC/ICP-MS chromatogram of nine arsenic-standard compounds. Sample injection volume: 5 μL. Arsenic standard compounds: 10 μgAs/g.

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Table 2. Total arsenic contents of *Hijikia fusiforme* (μgAs/g, dry weight)

Source	Type	Concentration (μgAs/g) Sample A	Sample B
Japan	Sprout Hijiki	41.7	44.4
	Long Hijiki	45.8	46.7
South Korea	Sprout Hijiki	71.5	65.6
	Long Hijiki	79.5	79.8
China	Sprout Hijiki	48.6	36.0
	Long Hijiki	37.5	42.4

36.0–48.6 μgAs/g, respectively. There are several reports of arsenic content in seaweeds; our results did not reveal a high concentration of arsenic in the seaweed samples.[11–15]

Speciation analysis of arsenic compounds in *Hijikia fusiforme*

Inorganic arsenate was mainly contained in Hijiki, and the ratio of inorganic to organic arsenic was 55.4–88.1%; the minor components of arsenic were inorganic arsenite (0–28.1%), dimethylarsinic acid (0.6–4.8%), and arsenosuger (0.9–3.1%) (Table 3 and Fig. 3). These results show that arsenic was present substantially in the form of arsenate in *H. fusiforme*.

Decrease of arsenic in *Hijikia fusiforme* by cooking process

The decrease of arsenic in *H. fusiforme* by soaking in water and cooking procedure is shown in Table 4 and Fig. 3, respectively. It was observed that 28.2–58.8% of the total arsenic in algae was removed in the soaking water, and 49.3–60.5% of the total arsenic was eluted by cooking; thus, 88.7–91.5% of the total arsenic is removable by the cooking

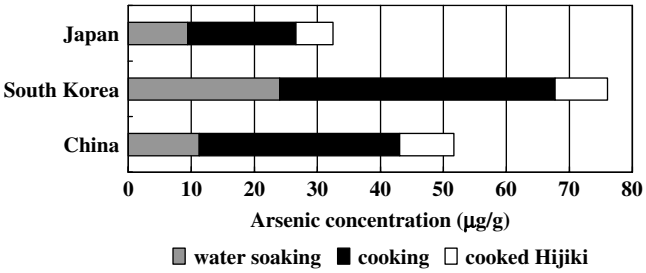

Figure 3. Decrease of arsenic in *Hijikia fusiforme*.

Table 3. Concentration of arsenic species in *Hijikia fusiforme* (μgAs/g, dry weight)

Source	Type		Concentration (μgAs/g seaweed, dry weight) As(V)	As(III)	DMAA	Arsenosugar	Remnant	Total
Japan	Sprout Hijiki	A	32.0 (76.6)	1.5 (3.7)	0.4 (1.0)	0.5 (1.2)	7.3 (17.5)	41.7
		B	29.0 (65.3)	10.3 (23.3)	1.0 (2.2)	1.4 (3.1)	2.7 (6.1)	44.4
	Long Hijiki	A	36.8 (80.3)	0.7 (1.6)	1.0 (2.1)	0.5 (1.2)	6.8 (14.9)	45.8
		B	25.0 (53.5)	12.6 (27.1)	0.9 (1.8)	1.0 (2.2)	7.2 (15.4)	46.7
South Korea	Sprout Hijiki	A	60.5 (84.6)	1.3 (1.9)	2.9 (4.1)	1.2 (1.7)	5.6 (7.8)	71.5
		B	51.2 (78.0)	4.0 (6.2)	3.1 (4.7)	0.8 (1.2)	6.5 (9.9)	65.6
	Long Hijiki	A	66.8 (84.0)	N.D.[a]	2.0 (2.5)	0.9 (1.1)	9.8 (12.3)	79.5
		B	69.0 (86.5)	N.D.[a]	1.3 (1.7)	0.7 (0.9)	8.7 (10.9)	79.8
China	Sprout Hijiki	A	38.8 (79.7)	N.D.[a]	1.8 (3.8)	0.4 (0.8)	7.6 (15.6)	48.6
		B	30.7 (85.2)	N.D.[a]	0.2 (0.6)	0.6 (1.7)	4.5 (12.5)	36.0
	Long Hijiki	A	32.1 (85.5)	N.D.[a]	1.3 (3.5)	0.5 (1.4)	3.6 (9.6)	37.5
		B	32.4 (76.4)	N.D.[a]	0.8 (1.9)	0.4 (0.9)	8.8 (20.8)	42.4

[a] N.D.: not detected, (): ratio of the retained As in the soaked plants (%).

Table 4. Concentration of arsenic species during cooking process (μgAs/g, dry weight)

Source	Water soaking As(V)	As(III)	DMAA	Arsenosugar	Cooking procedure As(V)	As(III)	DMAA	Arsenosugar	Remnant	Total
Japan	8.6 (26.6)	0.2 (0.5)	0.4 (1.3)	0.2 (0.6)	15.7 (48.4)	0.4 (1.2)	0.8 (2.5)	0.2 (0.7)	5.9 (18.2)	32.5
South Korea	21.4 (28.2)	0.4 (0.5)	1.2 (1.6)	1.0 (1.3)	40.5 (53.2)	0.6 (0.8)	2.1 (2.8)	0.5 (0.7)	8.3 (10.9)	76.0
China	9.7 (18.8)	ND[a]	1.1 (2.0)	0.5 (0.9)	29.6 (57.2)	ND[a]	2.0 (3.8)	0.3 (0.5)	8.6 (16.6)	51.7

[a] ND, not detected; () ratio of the retained As in the soaked plants (%).

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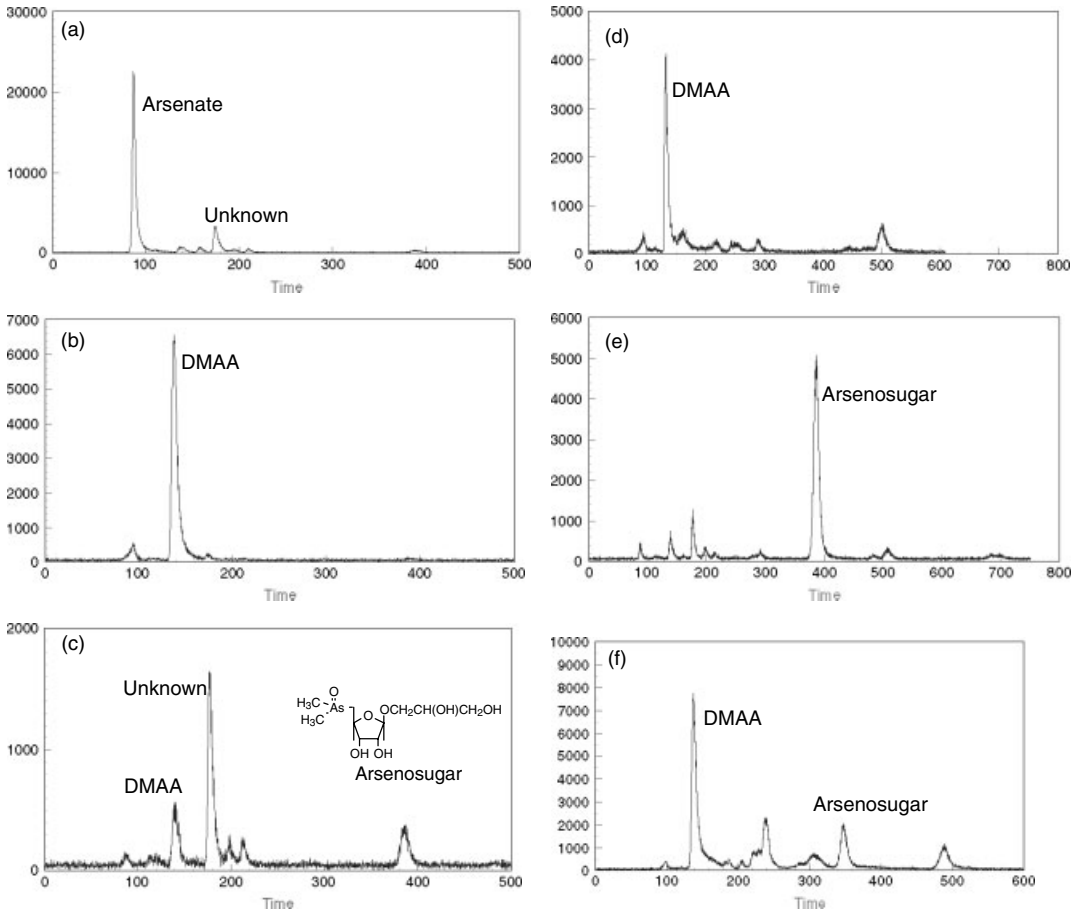

Figure 4. HPLC/ICP-MS chromatograms of arsenic metabolites in urine and feces: (a) soaking water; (b) urine with administered soaking water; (c) feces with administered soaking water; (d) urine with administered cooked Hijiki; (e) feces with administered cooked Hijiki; (f) urine with administered arsenosugar.

process. 75.0–81.4% of the eluted arsenic was arsenate (Table 4). It became evident that the soaking in water and cooking are effective in removing arsenic from the edible brown algae.

Speciation analysis of arsenic excreted in urine and feces

The chromatograms of speciation analysis of the arsenic excreted in urine and feces are shown in Fig. 4. Arsenate was metabolized to DMAA in urine after the administration of the soaked water [Fig. 4(b)]. It was suggested that the absorption of arsenosugar is difficult when compared with other arsenic compounds because more arsenosugar and an unknown compound were found in feces than urine. The suggestion was supported by the result of the administration of the cooked Hijiki [Fig. 4(d, e)]. We confirmed that arsenate was excreted in urine after metabolism to DMAA, and DMAA was excreted in urine without further transformation. The chromatograms of the administered arsenosugar are shown in Fig. 4(f). The arsenosugar was metabolized to DMAA with five types of unknown compounds in the urine.

CONCLUSION

The British FSA announced that Hijiki is dangerous for consumption, because it contains a large amount of arsenate. In this study, the inorganic arsenic compound in Hijiki was removed by the Japanese cooking method, that involves soaking Hijiki in water, and cooking. The speciation analysis of arsenic was performed by HPLC/ICP-MS system because determination of arsenate is very important. The arsenic standard compounds were separated from each other, and our method was found to be reliable. We confirmed that approximately 90% of arsenic was removed by cooking and more than 30 MgAs/g (dry weight) of arsenate was contained in Hijiki; however, most of the arsenate was eliminated by the cooking process. The result of Hijiki administration showed that a large amount of arsenic was metabolized to DMAA and excreted in urine. This inorganic arsenic was metabolized quickly, demonstrating the safety of Hijiki; it was necessary to consider the metabolism rate of arsenate as a safety factor. These results suggest that the Japanese cooking method is effective in removing arsenic in Hijiki.

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