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Analysis of inorganic arsenic in foods by hydride generation-cold trap-atomic absorption spectrophotometry

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SUMMARY

The JECFA (the Joint FAO/WHO Expert Committee on Food Additives) has set a PTWI (provisional tolerable weekly intake) value of arsenic at a quantity of more toxic inorganic arsenic, since the toxicity of arsenic in foods differs greatly between inorganic arsenic and organic arsenic. To determine the inorganic arsenic contents in food samples such as seaweed, rice and water samples, a speciation analysis method by hydride generation-cold trap-atomic absorption spectrometry was applied. To extract inorganic arsenic efficiently, arsenic in foods was extracted with mixed acids (nitric acid and perchloric acid). When some water samples containing germanium as organic germanium compound at the high concentrations were applied to this system, the peak of germane was obviously detected at an earlier retention time than that of arsine in spite of the use of lamp for arsenic detection. Thus, arsine could be detected separately from germane by hydride generation-cold trap-atomic absorption spectrometry, even if both arsenic and germanium were present.

INTRODUCTION

Since the toxicity of arsenic in foods differs greatly between inorganic arsenic and organic arsenic, the JECFA (the Joint FAO/WHO Expert Committee on Food Additives) has established a PTWI (provisional tolerable weekly intake) value of arsenic at a quantity consistent with more toxic inorganic arsenic. As a part of the project for estimating the intake of inorganic arsenic through foods and water, a speciation analysis method by hydride generation-cold trap (HG-CT) -atomic absorption spectrometry was applied to determine the inorganic arsenic contents selectively in several foods and water samples.

MATERIALS AND METHODS

Reagents

Standard mixed solution of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide was purchased from Wako Pure Chemical Industries, Ltd. Inc (Osaka, Japan). Nitric acid (68%) and perchloric acid (70%) of ultrapure analytical grade (TAMAPURE-AA-100) were purchased from Tama Chemical Industry (Kanagawa, Japan). Other chemicals were of reagent grade or of the highest grade available commercially.

Equipment

Shimadzu ASA-2sp (Kyoto, Japan) was used as arsenic speciation pretreatment system for HG-CT process. Measurement principle is based on that arsenic compounds are separated accor-

ding to the boiling points of the respective arsenic hydrides. Thermo Elemental Solaar M5 (Kanagawa, Japan) was used as an atomic absorption spectrophotometer (AAS).

Food and water samples

Food samples including infant formulae and baby foods and water samples were obtained in the Tokyo Metropolitan area and by mail order in Japan.

Sample preparation

Foods were first heated with nitric acid, and then perchloric acid was added. Heating was continued until white fume of perchloric acid appeared to remove nitric acid. After heating, water was added to prepare the solution for analysis. Water samples were analysed without heating after filtration with 0.45-μm filter.

Measurements

The acidic solution was applied to the ASA-2sp. Arsenic species in the solution were reduced to the respective hydrides with sodium boronhydride solution, introduced by a carrier gas (helium) to the U-tube filled with quartz wool cooled with liquid nitrogen, and then collected. Next, the U-tube was pulled out of the liquid nitrogen. Arsine, monomethylarsine, dimethylarsine and trimethylarsine were vaporized in turn, depending on their boiling points, and were introduced to the AAS for monitoring.

The optimal conditions for obtaining high sensitivity were examined for the combined ASA-2sp (Shimadzu) and AAS (Thermo Elemental) system. Several types of foods and some water samples were analyzed under the optimized conditions.

RESULTS AND DISCUSSION

To apply this speciation method to a solid food, food containing arsenic must be converted to a solution. Moreover, to be detected, all arsenic compounds must be present as species that can be transformed into hydrides. Whether or not the organic arsenic compounds were degraded into inorganic arsenic on heating was examined using commercially available reagents, namely, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide and arsenobetaine. These organic arsenic compounds were not converted into inorganic species at the temperature below 110°C. Thus, the conditions under which organic arsenic compounds in foods did not change into inorganic species were determined.

Since the original conditions of the ASA-2sp pretreatment system were set to be optimal for the atomic absorption spectrophotometer of said corporation, the optimum conditions with the Solaar M5 spectrophotometer were studied. First, with the quartz cell of the Solaar M5 system heated electrothermally, helium gas flow rate was optimized. However, atomic absorbance was only one-third of that with the Shimadzu spectrophotometer. Moreover, the resolution of dimethylarsine and trimethylarsine was insufficient. Furthermore, the background level was not steady even when the flow rate of helium gas was changed. Since quartz cells supplied by the two companies differ in shape, the quartz cell from the Shimadzu Company could not be fitted to the Solaar M5 system.

Next, therefore, another heating method, in which a quartz cell is heated with a flame, was investigated. For this purpose, a new quartz cell suited to the Solaar M5 system was prepared. The optimum conditions for various factors were selected. Under the optimum conditions selected, the detection limit obtained with standard arsenite solution was 0.022 ppb. When this method was applied to a dry hijiki sample, the coefficient of variation for arsine was 2.6%, a satisfactory value. This method was also applied to pulverized rice, baby foods, infant formulae and water samples.

When some water samples containing germanium as organic germanium compound at the high concentrations were applied to this system, a tiny peak of germane was obviously detected at an earlier retention time than that of arsine, in spite of the use of lamp for arsenic detection. Germanium is known to have a spectral line at 193.7 nm, which is also the wavelength of dominant spectral line of arsenic. Therefore, this method, hydride generation-cold trap-atomic absorption spectrophotometry, may be considered to be a useful technique to detect arsine and germane separately from foods and water samples containing both inorganic arsenic and germanium.

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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

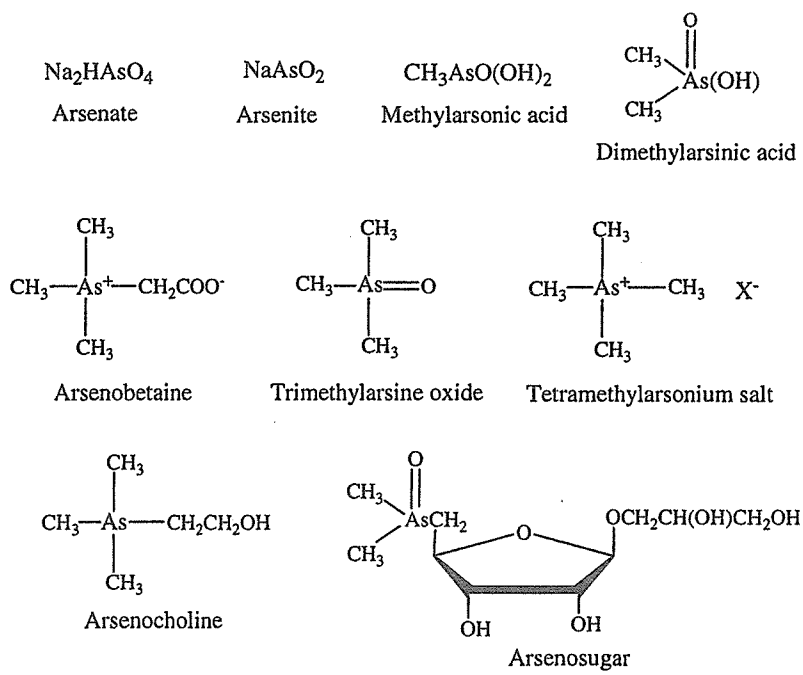

Figure 1. Chemical forms of arsenic standard.

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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

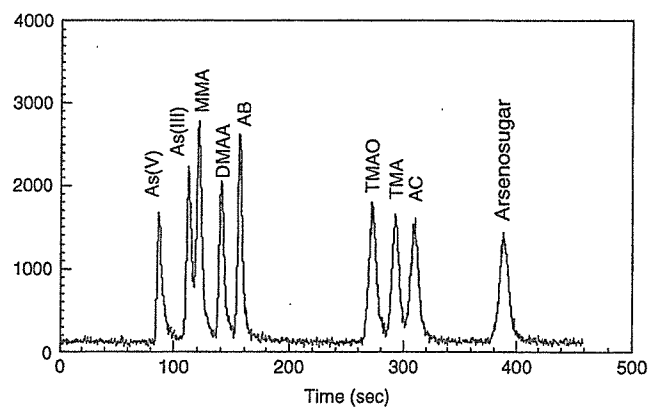

Figure 2. HPLC/ICP-MS chromatogram of nine arsenic-standard compounds. Sample injection volume: 5 μL. Arsenic standard compounds: 10 μgAs/g.

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

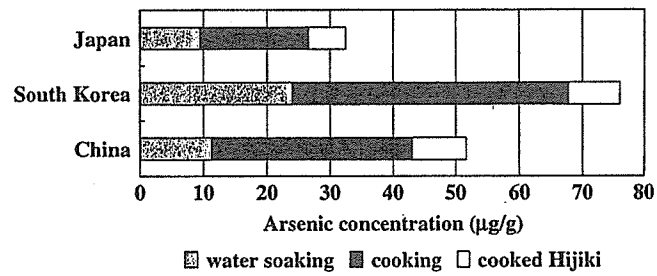

Figure 3. Decrease of arsenic in *Hijikia fusiforme*.

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 (%).

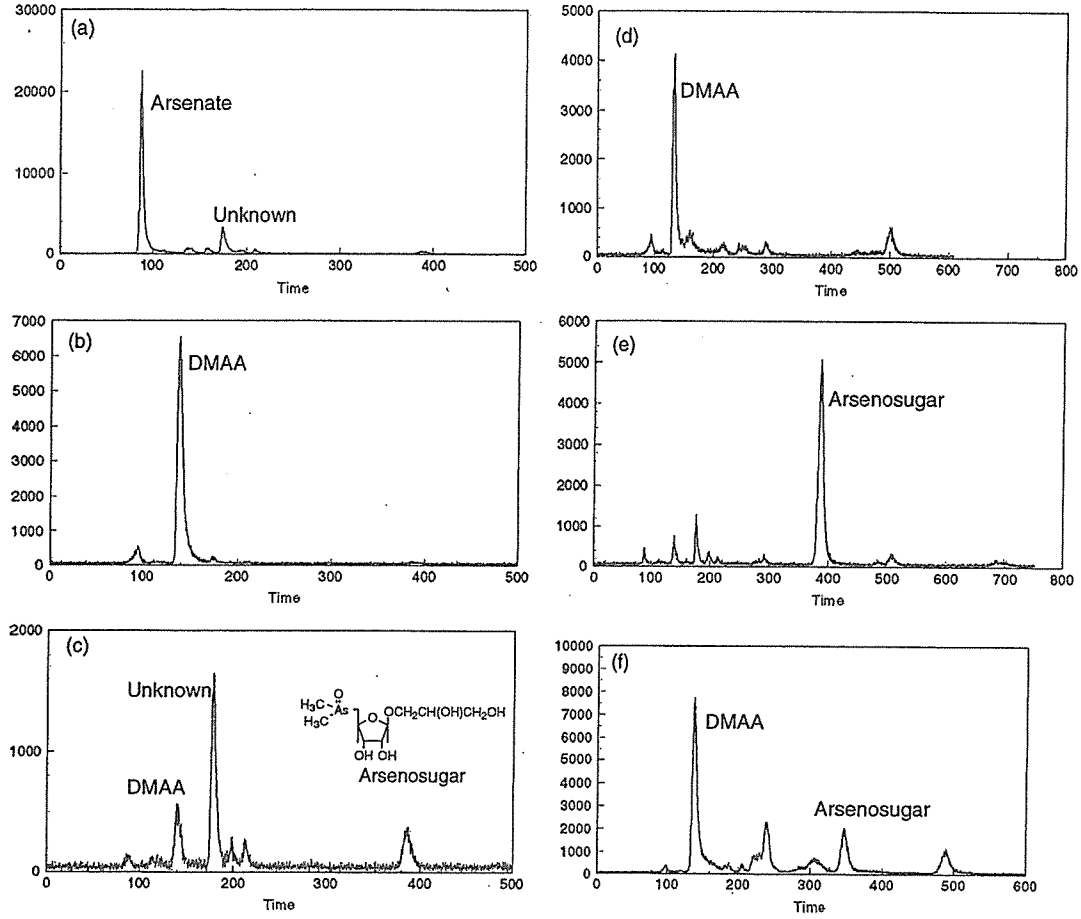

Figure 4. HPLC/ICP-MS chromatograms of arsenic metabolites in urine and feces: (a) soaking water; (b) urine with administered soaking water; (c) feces with administered soaking water; (d) urine with administered cooked Hijiki; (e) feces with administered cooked Hijiki; (f) urine with administered arsenosugar.

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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マウスを用いたヒジキ中ヒ素化合物の代謝

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Intake and Excretion of Arsenic Compounds in Edible Brown Algae in Mice

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Summary

A kind of edible sea brown algae, *Hijikia fusiforme*, contains high amount of inorganic arsenic. British Food Standard Agency (FSA) advised people not to eat a type of seaweed called Hijiki, on July 2004, because of the high levels of arsenic that it contains. In this study, we examined the removal of arsenic compounds by soaking procedure with water, and the excretion of arsenic contained in *H. fusiforme* was investigated in mice. The arsenic compounds were determined by hydride generation-atomic absorption spectrometry (HG-AAS), and the speciation analysis of arsenic was used by high performance liquid chromatograph coupled with inductively coupled plasma mass spectrometer (HPLC/ICP-MS). It was made clear that the 28.2～58.8% of the total arsenic in alga were removed with water, 49.3～60.5% of arsenic eluted by heated cooking procedure, thus 88.7～91.5% of total arsenic is removable with cooking process. Hijiki was given to the mice, dimethylarsinic acid (DMAA) was mainly metabolized in urine. It became clear that soak with water and heated cooking procedure are effective in removal of arsenic from edible brown algae.

Key words: *Hijikia fusiforme*, arsenic, HPLC/ICP-MS, cooking process, marine food, seaweed, arsenosugar

海洋生物には多量のヒ素が含まれており，陸棲と海棲動物ではそのヒ素濃度及び化学形態に大きな違いが見られる。陸棲動物のヒ素濃度は1 μg/g (乾重量) 以上になることはほとんどないが，海棲動物では数μg/gから100 μg/gにおよぶことがある。これらには有機ヒ素化合物が主として存在し，無機ヒ素は全ヒ素の2%以下と低い。海藻など海洋植物に含まれるヒ素の大部分も有機体であり，藻類ではarsenosugarのようなジメチルヒ素化合物である。また，無機ヒ素は海棲動物と比べて多いものの10%未満である[1)]。しかし，ヒジキには無機ヒ素が多く，50%前後含まれている。このことから2004年7月，イギリス食品規格庁は自国民に対し「ヒジキを食べるべきでない」との勧告を出した。また，カナダでは2001年10月に同様の勧告が出されており，行政指導がとられている。海産食品を多食する日本人にとってこれ

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ら海産物摂取による食生活の安全性を確保することは重要である。

今回我々はヒジキを通して摂取されるヒ素量を把握する事を目的として，ヒジキ中ヒ素化合物の調理過程における除去ならびにヒジキに含まれるヒ素化合物のマウスにおける体内動態について検討した。

実験方法

1. 試薬・実験材料

Arsenate (Na_2HAsO_4), arsenite ($NaAsO_2$), methylarsonic acid (MAA), dimethylarsinic acid (DMAA), arsenobetaine (AB), trimethylarsine oxide (TMAO), tetramethylarsonium (TMA), arsenocholine (AC) はトリケミカル (Yamanashi, Japan) より購入した。(*R*)-(2',3'-dihydroxypropyl)-5-deoxy-5-dimethylarsinoyl-β-D-riboside (arsenosugar) はMcAdamらの方法を用い1-*O*-acetyl-tri-*O*-benzoil-β-D-ribofuranose, (*S*)-1,2-*O*-isopropylidene glycerol, dimethylarsinous iodideより合成した[2-7]。本研究で使用した標準ヒ素化合物の構造式をFig. 1に示した。

また，実験材料として用いたヒジキは，長ヒジキ及び芽ヒジキを国内産，韓国産，中国産それぞれ2検体ずつ用いた。

2. 検出方法

総ヒ素量の測定にはバリアン社製水素化物発生装置VGA-77，原子吸光光度計SpectrAA-220を用いた。マイクロウェーブ分解装置はO. I. Analytical社製Microwave Digestion Systemを使用した。形態別ヒ素化合物の測定にはHPLC/ICP-MSを用いた。HPLCはジーエルサイエンス社製であり，ヒ素の検出器であるICP-MSはパーキンエルマー社製ELAN DRC-*e*を用いた。HPLC/ICP-MSの測定条件をTable 1に，標準品 (10 ppb, 5 μL) のクロマトグラムをFig. 2に示した[8,9]。

3. 水戻しによるヒジキ中ヒ素の溶出の検討

細切したヒジキ0.5 gに純水を20 mL加え，30分間浸漬した。この溶液を一定量にメスアップした後，ろ過を行い，HPLC/ICP-MSでヒ素を形態別に測定した。

4. 加熱調理によるヒジキ中ヒ素の溶出の検討

水戻ししたヒジキに純水を30 mL加え，20分間90℃で加熱した。放冷後，溶出液は一定量にメスアップした後，ろ過を行い，HPLC/ICP-MSでヒ素を形態別に測定した。なお，残渣は総ヒ素量の分析と同様の方法で残存ヒ素量を測定した。

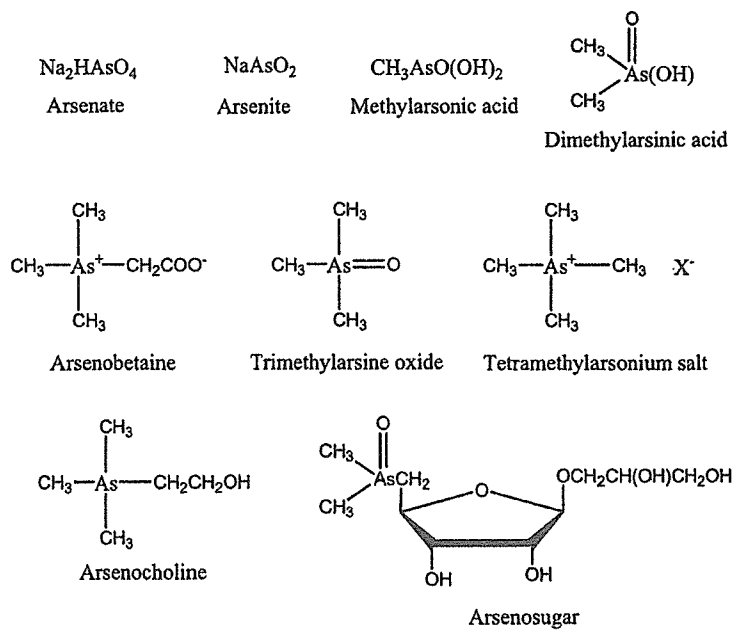

Fig. 1 Chemical forms of arsenic standard.

Table 1 Analytical conditions of HPLC/ICP-MS

HPLC	
Column	Inertsil As (2.1 × 150 mm)
Column temp.	40℃
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)

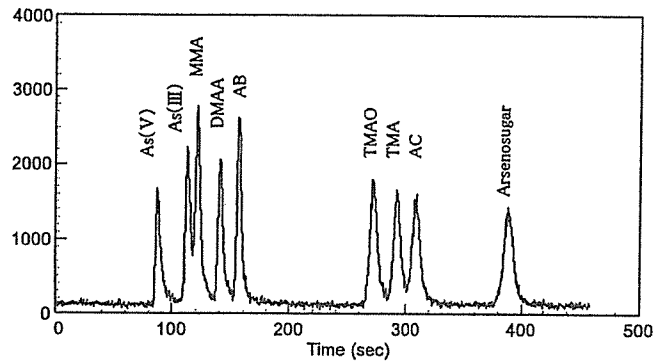

Fig. 2 HPLC/ICP-MS chromatogram of arsenic standard compounds and arsenosugar. Sample injection volume: 5 μL. Arsenic standard compounds: 10 μgAs/L.

5. ヒジキ中の総ヒ素量分析

破砕器により粉末にし，均一化したヒジキ粉末試料を正確に0.5 g量りとり，硝酸5 mL，過酸化水素水2 mLを加え，マイクロウェーブ分解装置で分解した。分解液をビーカーに移し，硫酸を加えてホットプレート上で加熱し，硝酸を除去した。放冷後，クエン酸水素二アンモニウム水溶液を加え，アンモニア水を用いて中和し後，塩酸，20%ヨウ化カリウム水溶液，20%アスコルビン酸水溶液を加えて還元を行った。この溶液を適宜希釈し，水素化物発生装置に導入して原子吸光光度計で測定した。尚，検量線作成はあらかじめ原子吸光用ヒ素標準試薬を用いてヒ素として5 ppb，10 ppb，15 ppbとなるようにした試料に塩酸，20%ヨウ化カリウム水溶液，20%アスコルビン酸水溶液を加えた後，純水を用いて100 mLとしたものを測定し，吸光度より求めた。

6. ヒジキ中ヒ素化合物の形態別分析法

破砕器により粉末にし，均一化したヒジキ粉末試料0.1 gに硝酸を1 mL加え，80℃で1時間加熱した。放冷後，アンモニア水で中和し，純水で50 mLに定容した。測定サンプルはろ過後，HPLC/ICP-MSでヒ素を形態別に測定した。

7. ヒジキ飼料調製法

調理後のヒジキを再現するため以下の処理を行った。すなわち芽ヒジキ0.5 gを20 mLの純水を用いて水戻し後，ヒジキをろ取した。更に水戻したヒジキに30 mLの純水を加えて30分間加熱処理し，調理ヒジキとした。調理ヒジキを凍結乾燥した後，破砕しマウス用ヒジキ飼料とした。

8. マウス尿・糞試料前処理法

マウス尿はゲージ内のろ紙に吸収させ，1日ごとに採尿した。ろ紙を50%メタノールで抽出し，抽出液を減圧乾固後，純水で再溶解させ測定試料とした。

マウス糞は1日ごとに採取し，50%メタノールで抽出した。遠心分離後，上清を分取しODSカラムを用いて粗精製した。通過液を減圧乾固し，純水で再溶解させ測定試料とした。

結果及び考察

日本，韓国，中国産のヒジキを分析した結果，総ヒ素は日本産41.7～46.7 μg/g，韓国産65.6～79.8 μg/g，中国産36.0～48.6 μg/gであった (Table 2)。これらは，これまで報告されているヒジキ中のヒ素量12.0～182.6 μg/gの値の範囲内であり，特に高濃度のヒ素を含んでいるものではなく，産地別の違いも見られなかった[10-14]。

水戻し液及び加熱調理液を測定した結果，水戻しにより総ヒ素の28.2～58.8%，また加熱調理により49.3～60.5%のヒ素が溶出し，水戻しと加熱調理により総ヒ素の88.7～91.5%のヒ素を除去できる事が確認された (Fig. 3)。調理過程により溶出したヒ素のうち88.7～91.5%がarsenateであったため，調理過程は無機ヒ素の溶出に有効であると考えられる。

乾燥ヒジキ及び調理ヒジキのヒ素を形態別に測定した結果のクロマトグラムをFig. 4に示した。調理過程により9割のヒ素が溶出したものの我々が食する調理ヒジキにもまだヒ素が残存していることがわかる。そこで，マウスを用いて調理ヒジキ中ヒ素化合物の体内動態について検討を行った。

調理ヒジキをマウスに10日間連続投与した結果，投与したヒ素量の63.0～84.6%が尿及び糞中に主としてジメチルアルシン酸として排泄された。排泄量の66.5～87.3%は尿中に排泄された。このことより，体内に摂取されたヒ素は比較的速やかに排泄されることが推定された。

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

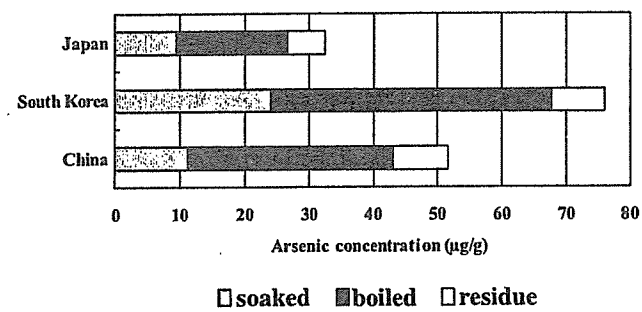

Fig. 3 Decrease of arsenic in *Hijikia fusiforme*.

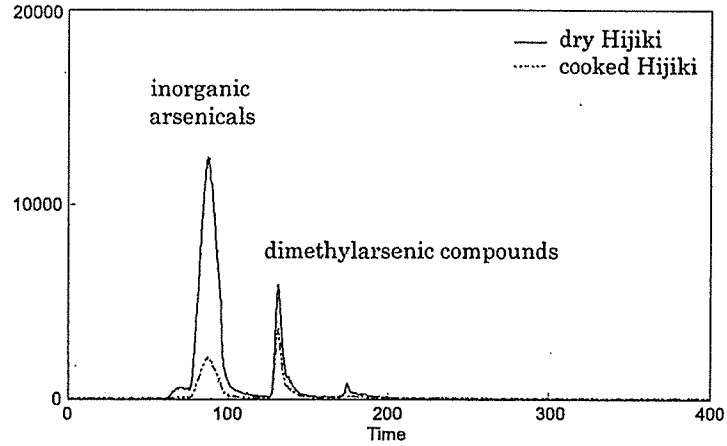

Fig. 4 HPLC/ICP-MS chromatogram of dry Hijiki and cooked Hijiki.

本研究の結果，ヒジキ中のヒ素は調理過程により約9割が除去されること，調理後のヒジキをマウスに投与した結果，約8割が速やかに排泄されることが明らかとなった。これらの結果はヒジキが安全な食品であることを示していると考えられる。しかし，高濃度投与ではあるものの，ジメチルアルシン酸の発癌性を示唆する実験結果も存在することから，今後はジメチルアルシン酸をはじめとするジメチルヒ素化合物の生体影響について検討を行う予定である。

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