Speciation Analysis of Arsenics in Commercial Hijiki by High Performance Liquid Chromatography-tandem-mass Spectrometry and High Performance Liquid Chromatography-inductively Coupled Plasma Mass Spectrometry

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Edible brown alga (hijiki in Japanese, *Hijikia fusiforme*) contains not only a high content of inorganic arsenic (iAs) but also various arsenosugars (AsSugs) which are metabolized to dimethylarsinic acid (DMA) in mammals. Since DMA is considered to be carcinogenic in rodents, it is necessary to accurately measure the contents of AsSugs as well as iAs for the risk assessment of seaweed consumption. Seven commercially available dried-hijiki products and two raw hijiki products were analyzed. Total-As was measured by inductively coupled plasma mass spectrometry (ICP-MS) with the Dynamic Reaction Cell (DRC) mode after acid-digestion. After water-extraction, AsSugs were detected by HPLC-MS/MS with multiple reaction monitoring in the positive ion mode and speciation analysis of arsenics was performed by HPLC-ICP-MS. The ranges of total-As obtained by acid digestion (A-TAs) in nine hijiki samples were 37.1–118.6 μg As/g dry weight (dw), and those of water extracted total-As (W-TAs) were 18.4–81.0 μg As/g dw. The ratios of water extracted iAs (W-iAs) to A-TAs ranged from 24.5 to 60.1%. The major compound detected was arsenate in all samples (8.9–70.5 μg As/g dw). Dimethylarsenosugar sulfate, AsSug 408, showed the highest peak among AsSugs detected. The content ratio of water extracted AsSugs (W-AsS) to A-TAs was estimated to be from 3.7 to 27.6%. The contents of A-TAs, W-iAs and W-AsS varied depending on the hijiki product. HPLC-MS/MS detected AsSugs more sensitively than HPLC-ICP-MS. Since iAs could not be detected by HPLC-MS/MS, combined analysis consisting of HPLC-MS/MS and HPLC-ICP-MS is necessary for accurate determination of arsenic species in seaweed products and also for the toxicological evaluation of AsSugs.

Key words —— hijiki, arsenosugar, HPLC-MS/MS, inorganic arsenic, dimethylarsinic acid

INTRODUCTION

The consumption of seaweed as a diet food has been increasing in recent years in Western countries.[1–3] The edible brown alga *Hijikia fusiforme*, called hijiki in Japanese, has a high content of inorganic arsenic (iAs). Therefore, in 2004, the Food Standards Agency (FSA) of the United Kingdom[4] advised consumers to avoid eating hijiki.[5] The iAs concentration in hijiki depends on the producing district and manufacturing method.[6,7] Five oxo-arsenosugars (oxo-AsSugs) have been detected in hijiki as well as other seaweeds.[8–10] In humans, oxo-AsSug is metabolized and excreted into urine as dimethylarsinic acid (DMA) and several oxo- or thio-dimethylarsenic compounds.[11,12] In 2004, the International Agency for Research on Cancer (IARC)[13] declared that there is sufficient evidence indicating that DMA, a major metabolite of iAs in mammals, is carcinogenic in animal experimental models. Therefore, in order to assess the risk from

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hijiki ingestion, both AsSugs and iAs should be determined. Arsenic speciation analysis in biological samples is generally performed using HPLC inductively coupled plasma mass spectrometry (HPLC-ICP-MS).[14–16] Recent publications have reported the usefulness of HPLC with tandem mass spectrometry (HPLC-MS/MS) for the identification and determination of organoarsenic compounds in biological samples.[17–19] These analytical apparatuses have made it possible to detect known arsenic compounds in samples without standard compounds and to estimate As concentration using appropriate standards.[20, 21]

In this study, we determined total arsenic and water-extracted iAs, and estimated the AsSugs contents of 7 commercially available dried-hijiki products and two raw hijiki products using HPLC-ICP-MS and HPLC-MS/MS for the speciation analysis of arsenics in hijiki products from various regions of Japan and elsewhere in east Asia produced using several different production systems.

MATERIALS AND METHODS

Chemicals —— Sodium arsenite (AsIII), sodium arsenate (AsV), methanearsonic acid (MMA), and arsenobetaine (AsBe) were purchased from Wako Pure Chemical (Osaka, Japan). DMA, trimethylarsine oxide (TMAO), and arsenocholine (AsCho) were obtained from Tri Chemical Laboratory (Yamanashi, Japan). Germanium standard solution (Kanto Chemical, Tokyo, Japan) was used as an internal standard for HPLC-ICP-MS analysis. Nitric acid (HNO_3, TAMAPURE AA-100, 68%, Tama Chemicals, Kawasaki, Japan) and H_2SO_4 [for Ultratrace Analysis (Ultratrace Anal.), 97%, Wako Pure Chemical] were used for sample treatment. Ammonium bicarbonate (NH_4HCO_3, Wako Pure Chemical) was used for the HPLC mobile phase. Ultrapure water was purified by Milli-Q Element A-10 with Quantum ICP cartridges (Millipore, Tokyo, Japan). A certified reference material, CRM No. 18 (human urine), obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan, was used to validate the procedure used for analysis.

Samples of Commercial Dried-hijiki and Raw-hijiki —— As shown in Table 1, nine hijiki samples derived from six different producing districts were collected from six stores in each producing district. Two of the commercial samples were imported from China (Hong Kong) and South Korea and packed by a Japanese provider. Two raw hijiki samples were harvested on the seashores of Amatsu and Kominato, respectively, which are located on the Boso peninsula of Chiba prefecture. Each raw sample was immediately washed with fresh water, sealed in an airtight plastic bag, and then stored by freezing at −80°C until analysis. The manufacturing methods for commercial dried-hijiki can be broadly divided into two systems, one that involves a boiling system in a large vessel, and the other a closed or open steam-system.[22] In Japan, major manufacturing methods for commercial dried-hijiki basically use a steam system.[22] A system involving boiling has been used traditionally in South Korea and China.[22] Moreover, there are two types of commercial dried-hijiki, naga-hijiki, which consists of the entire seaweed plant, and me-hijiki, which is the sprouts of the seaweed.[22]

Preparation of the Hijiki Samples —— The commercial dried-hijiki samples and the lyophilized raw hijiki samples were finely ground to powder using a food processor. For total-As analysis, twenty milligrams of the powdered hijiki samples was digested by adding 0.5 ml of HNO_3 and 0.5 ml of H_2SO_4 and then kept at 330°C for 1 hr using a dry block bath. After cooling, the solutions were adjusted to 50 ml with 0.1 M HNO_3 and their total arsenic concentrations were determined by ICP-MS using the Dynamic Reaction Cell (DRC) mode.

For speciation analysis, powdered hijiki samples (0.5 g) swollen by the addition of 20 ml of ultrapure water were treated with an ultrasonic cell disruptor (Misonix, Inc., Farmingdale, NY, U.S.A.) for 1 min, and centrifuged at 3000 rpm for 15 min. The supernatant collected was adjusted to 50 ml by adding ultra-pure water. The supernatant was applied to speciation analysis of arsenic compounds by HPLC-ICP-MS and by HPLC-MS/MS.

Determination of Total Arsenic Concentration —— The acid digested solution was diluted to 50 times with buffer containing 0.5% aqueous ammonia, 2 mM EDTA, and 0.8 mM TritonX-100. The determination was performed by the standard addition method. The diluted solution was introduced into an Elan DRCII ICP-MS (PerkinElmer SCIEX, Concord, Ontario, Canada) using the DRC mode. The instrument settings were as follows: radio-frequency (RF) power 1300 W, argon plasma gas flow 15 l/min, auxiliary flow 1.2 l/min, and nebulizer flow 1.0 l/min. A coaxial-type nebulizer was used; skimmer and sample cones were platinum, and elemental As was measured at m/z

Table 1. Hijiki Producing Areas and Production Systems Used for Their Dried-products

Sample	Producing area	Production system
1 me-hijiki (sprouts of weed)	China (Hong Kong)	unknown
2 me-hijiki (sprouts of weed)	South Korea	boiling system[a)]
3 me-hijiki (sprouts of weed)	Mie prefecture (Japan)	boiling system[a)]
4 naga-hijiki (whole of weed)	Ohita prefecture (Japan)	closed-steam system
5 me-hijiki (sprouts of weed)	Ohita prefecture (Japan)	closed-steam system
6 naga-hijiki (whole of weed)	Amatsu (Japan)	open-steam system
7 naga-hijiki (whole of weed)	Kominato (Japan)	open-steam system
8 raw hijiki (whole of weed)	Amatsu (Japan)	
9 raw hijiki (whole of weed)	Kominato (Japan)	

a) The production systems used as printed on their packages.

Table 2. Fragmentation Parameters of MRM for Arsenic Compounds[24)]

compounds	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
DMA	138.9	90.8	30	24
MMA	140.9	90.8	30	30
AsBe	179.0	119.9	30	24
AsSug 254	255.0	96.9	25	30
AsSug 328	329.0	96.9	25	32
AsSug 391	392.0	96.9	25	30
AsSug 392	393.0	96.9	25	32
AsSug 408	409.0	96.9	20	38
AsSug 482	483.0	96.9	30	36

of 75 and 77. The DRC mode operation settings were as follows: ammonia (NH_3, > 99.999%) was used as reaction gas, and the rejection parameter q (RPq) of the DRC, the axial field voltage, and the flow rate of NH_3 were optimized and set at 0.5, 275 V, and 0.3 ml/min, respectively. No interference from argon-chloride at $m/z = 75$ and 77 was detected with NaCl solutions ranging from 10 mM to 500 mM. The instrument limit of detection (LOD) of As aqueous standard solution and the method limit of quantitation (LOQ) were calculated according to the definition stipulated by Japanese Industrial Standards (JIS).[23)] The counts per second (CPS) of a blank solution at m/z 75 and 77 were almost equal to the background levels of 13–17 CPS and 3–7 CPS of ICP-MS, respectively. The LOD and the LOQ were calculated as 0.2 μg/l and 1.1 μg/l, respectively. The total As concentration in the reference material, NIES CRM No.18 urine, was determined to be 137.5 ± 4.2 μg/l ($n = 5$) and the value was within the range for the certified value of 137 ± 11 μg/l.

Detection of AsSugs in Hijiki Samples —— Detection of AsSugs was performed using an HPLC system (Alliance 2695, Waters, Milford, MA, U.S.A.) connected to a Quattro micro API tandem mass spectrometer (Waters) with electrospray ionization (ESI) positive ion mode by setting the capillary voltage to 0.5 kV, ion source temperature to 120°C, desolvation nitrogen gas temperature to 400°C, desolvation gas flow to 600 l/h, and cone gas flow to 50 l/h. Collision induced dissociation (CID) was performed with argon gas introduced into the collision cell placed between the quadropoles. Multiple reaction monitoring (MRM) was optimized to detect CID fragmentations (mass transition) of selected AsSugs, AsBe, DMA, and MMA. The fragmentation parameters of MRM[24)] are shown in Table 2. The molecular structures of the selected AsSugs are shown in Fig. 1. The divert valve was set to introduce only the HPLC effluent from 2 to 50 min to the mass analyzer. An anion exchange column PRP-X100 (250 × 2.0 mm i.d., Hamilton, Reno, NV, U.S.A.) was used under the following conditions: mobile phase of 20 mM NH_4HCO_3 (pH 8.0), flow rate of 0.2 ml/min, and column temperature of 40°C. Ten microliters of supernatant extracted from hijiki was injected into the HPLC.

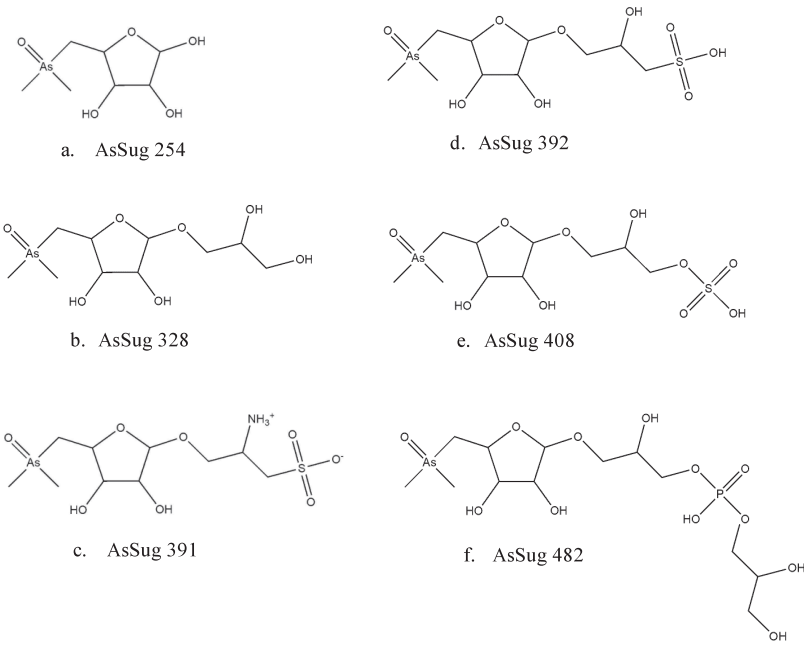

Fig. 1. Chemical Structures of Six AsSugs

For the assignment of ^{75}As, an HPLC system (GL Science, Tokyo, Japan) connected to an Elan DRCII ICP-MS was used under the same conditions except a thicker diameter column [PRP-X100, 250 × 4.6 mm inner diameter (i.d.)] at a flow rate of 1.0 ml/min was used. The injection volume of the supernatants was 50 μl.

Determination of Arsenic Compounds in Hijiki by HPLC-ICP-MS —— For the estimation of AsSug content**,** the same HPLC-ICP-MS system and analytical conditions as in "Analysis of AsSugs in hijiki samples" was used. Since we do not have AsSug standards, we used a DMA standard calibration curve to estimate the As concentration in each peak of AsSug. For AsIII, AsV, MMA, and DMA determination, an HPLC-ICP-MS system (consisting of a Model HP1200 and a Model HP7500cx, Agilent, Santa Clara, CA, U.S.A.) was used. The same column (PRP-X100, 250 × 4.6 mm i.d.) was used under the following conditions: mobile phase 20 mM NH_4HCO_3 (pH 9.5), flow rate 1.2 ml/min, temperature 40°C, and injection volume 50 μl. Stock standard solutions of AsIII, AsV, MMA, DMA, and AsBe were prepared by dissolving each compound in ultra-pure water at a concentration of 100 mgAs/l. The final diluted aqueous standard solutions (5 μg/l and 50 μg/l) were prepared from stock standard solution before use. To obtain precise measurements, 100 μg/l of germanium solution was used as the internal standard for ICP-MS by a post-column addition method. The ICP-MS detection mass was set to m/z 75 ($^{75}As^+$), m/z 72 ($^{72}Ge^+$), and m/z 35 (^{35}Cl) to monitor the chloride ion source of m/z 75 of ArCl. The instrument settings were as follows: RF power 1500 W, argon gas flows of plasma 15 l/min, and carrier gas flow 1.1 l/min. A coaxial-type nebulizer, nickel sample, and skimmer cones were used. LOD for AsIII, AsV, MMA, DMA, and AsBe were calculated according to the definition given by Gibbons.[25] The LOD of AsIII, AsV, MMA, DMA, and AsBe measured by HPLC-ICP-MS were calculated as 0.8, 1.1, 0.2, 0.3, and 0.5 μg As/l, respectively. When a measurement was below the LOD, it was calculated to be half the value of these limits. The AsBe and DMA concentrations in the reference material of CRM No.18 urine were determined to be 75.3 ± 0.7 μg/l and 30.4 ± 0.4 μg/l (n = 5) and the values were within the ranges for the certified values of 69 ± 12 μg/l and 36 ± 9 μg/l, respectively.

RESULTS

Detection of AsSugs in Hijiki Samples by HPLC-MS/MS

Using MRM techniques, we detected 9 organoarsenics in water extracts of hijiki samples (Table 2). The chromatograms of ^{75}As detection by HPLC-ICP-MS and MRM by HPLC-MS/MS of a raw hijiki sample harvested in Kominato (sample

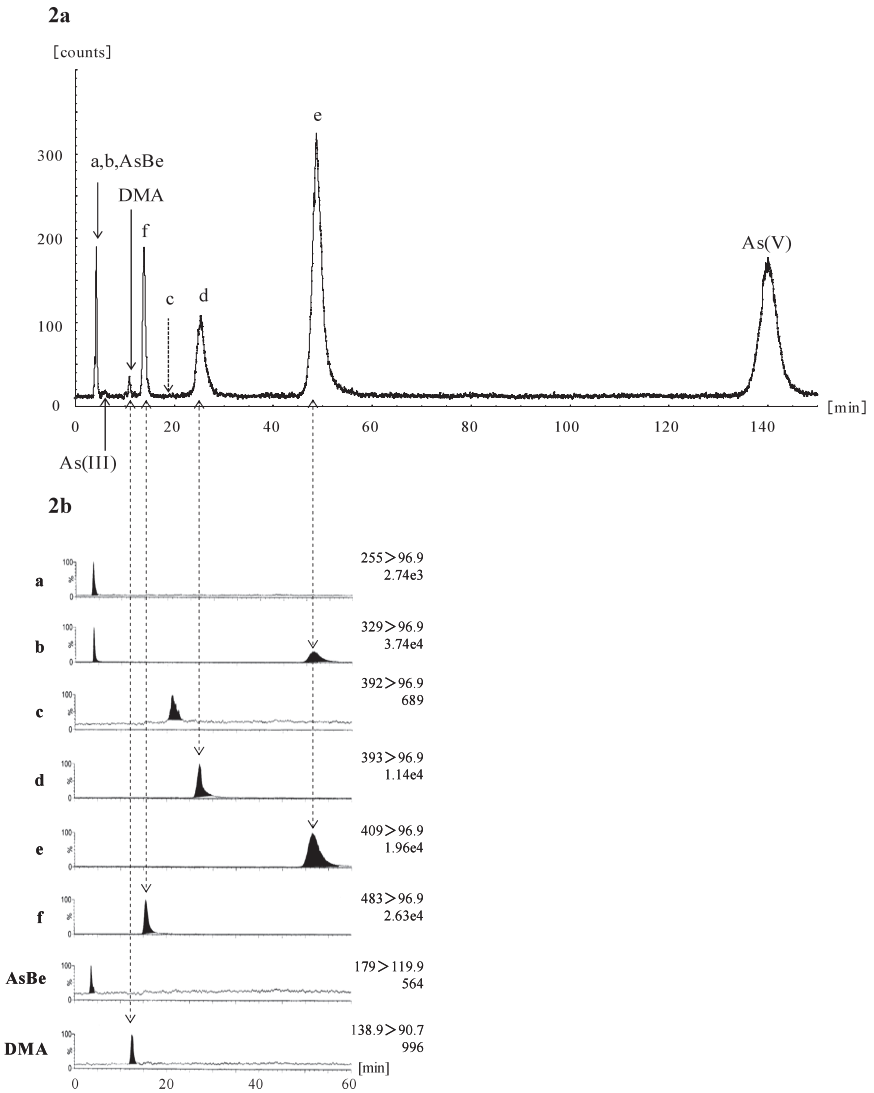

Fig. 2. HPLC-ICP-MS Chromatogram (2a), and HPLC-MS/MS (MRM) Chromatograms (2b) of a Raw Hijiki Sample Harvested in Kominato (Sample #9) with Mobile Phase at pH 8.0

Analytical conditions are described in text. The ordinates were normalized to 100% for the base peak of each MRM chromatogram. (a), AsSug 254; (b), AsSug 328; (c), AsSug 391; (d), AsSug 392; (e), AsSug 408; (f), AsSug 482.

#9) are shown in Fig. 2a and 2b, respectively. Seven peaks of arsenic compounds including two peaks of inorganic arsenic compounds (AsIII and AsV) were detected by HPLC-ICP-MS (Fig. 2a). However, with the exception of MMA, eight of the nine organoarsenics monitored were detected by HPLC-MS/MS conducted simultaneously (Fig. 2b). In the HPLC-ICP-MS chromatograms, compounds with molecular ion $[M+H]^+$, and m/z of 255 (AsSug 254), 329 (AsSug 328), and 179 (AsBe) were detected as unresolved peaks because of the close retention times, while that with $[M+H]^+$ and m/z of 392 (AsSug 391) was not detected because of the low concentration. In HPLC-MS/MS analysis, a molecular ion peak of m/z 141 (MMA) was not detected. Low sensitivity for MMA in analysis using MS/MS has been reported.[20] The MS/MS spectra of four major AsSugs, 328, 392, 408, and 482, are shown in Fig. 3. AsSug 408 had a daughter ion with m/z of 328.6, and further fragmentation of the m/z 328.6 ion gave rise to an ion with m/z of 96.8 (Fig. 3e). The other three AsSugs (328, 392, and 482) showed fragmentations to their product ion with m/z of 96.9 (Fig. 3b, 3d, and 3f). The MS/MS spectra of AsSug 254, AsSug 391, and AsBe were not obtained because of their low intensity; however, AsBe was confirmed by spiking standard compounds. AsSug 391 and AsBe were not detected in samples #1 and #6, respectively.

Table 3. Concentrations (μg As/g dw) of Water-extracted Arsenic Compounds in Hijiki Samples by Speciation Analysis Using HPLC-ICP-MS

Sample	As(III)	DMA	As(V)	AsSugs					
				328[a)]	391	392	408	482	Total
1	0.10	2.61	27.61	0.95	ND[b)]	0.12	1.79	0.08	2.96
2	0.44	2.71	55.33	1.48	ND	0.29	4.77	0.09	6.64
3	0.08	1.21	51.10	1.25	ND	0.22	3.85	0.05	5.39
4	0.13	2.72	50.93	0.85	ND	0.46	2.99	0.06	4.36
5	0.79	1.88	70.52	1.50	ND	0.48	5.75	0.05	7.79
6	0.26	0.74	8.94	0.32	ND	0.27	7.77	0.04	8.41
7	0.09	0.85	13.55	0.42	ND	0.27	10.15	0.05	10.90
8	0.25	0.16	17.15	1.44	ND	0.50	12.81	ND	14.78
9	0.16	0.25	17.21	1.39	ND	0.56	18.10	0.04	20.09

Values represent means of concentration of arsenic compounds as As (n = 3). *a*) The values are estimated from a peak of ArSug 328 containing AsBe and AsSug 254. *b*) ND; not detected by HPLC-ICP-MS.

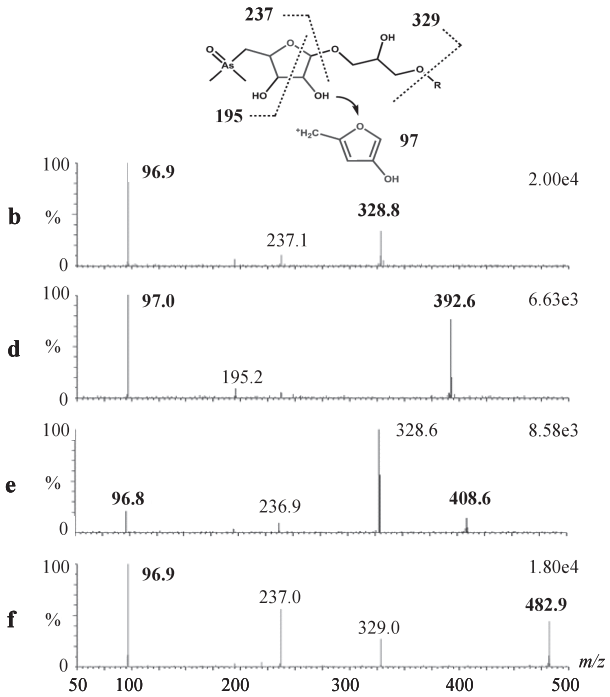

Fig. 3. The MS/MS Ion Spectra
(b) AsSug 328, (d) AsSug 392, (e) AsSug 408, and (f) AsSug 482.

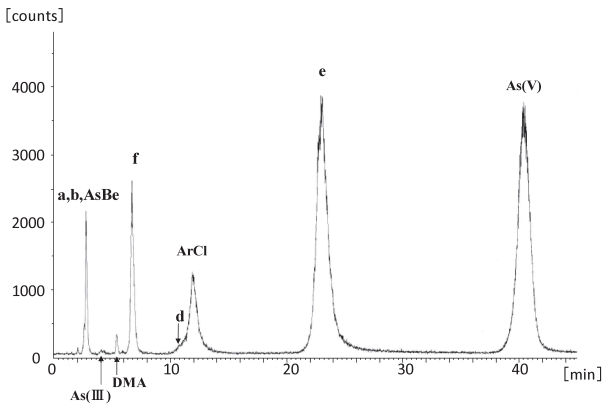

Fig. 4. HPLC-ICP-MS Chromatogram of a Raw Hijiki Sample Produced in Kominato (Sample #9) with Mobile Phase at pH 9.5
(a), AsSug 254; (b), AsSug 328; (d), AsSug 392; (e), AsSug 408; (f), AsSug 482; AsBe.

Determination of Arsenic Compounds in Hijiki Samples by HPCL-ICP-MS

For the speciation analysis, a mobile phase with a pH of 9.5 was used to improve the detection limit of AsV. The HPLC-ICP-MS chromatogram of the speciation analysis of arsenic compounds in a water extract of a raw hijiki sample from Kominato (sample #9) is shown in Fig. 4. In all nine hijiki samples, AsSug 391 was not detected, and AsSug 328, AsSug 254, and AsBe were unresolved and detected as one peak, therefore, their As concentration was estimated as a mixture.

The arsenic concentrations in water extracts of the nine hijiki samples are shown in Table 3. Arsenic concentrations of peaks of unresolved and AsSugs were estimated using the nearest-neighbor standard arsenic species (DMA). In all nine samples, the major compound detected was AsV. The concentrations of the AsV produced by the open steam system (samples #6 and #7) were much lower than other manufacturing methods (samples #1–5). The DMA concentrations in raw hijiki (samples #8 and #9) were very low, and the DMA concentrations in the open-steam system products (samples #6 and #7) were 3–4 times higher than those in raw samples. The major AsSug in water extracts was AsSug 408. The concentrations of AsSug 408 in hijiki samples harvested in the Boso area (samples #6–9) were higher than in those produced in other districts. The concentrations of AsSug 392 in raw hijiki samples

Table 4. Total As and Water-extracted iAs, AsSugs, and Total As in Hijiki Samples and Their Ratios to Total As

Sample Number (#)	Acid-digested Total-As (μg As/g dw) (A-TAs)	Water-extracted As (μg As/g dw)			W-TAs /A-TAs (%)	W-iAs /A-TAs (%)	W-AsS /A-TAs (%)
		Total-As (W-TAs)[a)]	Total-iAs (W-iAs)[b)]	Total-AsSugs (W-AsS)[c)]			
1	80.9 ± 9.4	33.4 ± 2.0	27.7 ± 1.7	3.0 ± 0.0	41.3	34.3	3.7
2	112.6 ± 1.5	65.1 ± 2.7	55.8 ± 2.0	6.6 ± 0.6	57.8	49.5	5.9
3	86.7 ± 6.7	59.0 ± 3.3	51.2 ± 2.9	5.4 ± 0.3	68.0	59.0	5.9
4	86.3 ± 5.7	58.1 ± 1.8	51.1 ± 1.5	4.4 ± 0.3	67.4	59.2	5.1
5	118.6 ± 11.3	81.0 ± 2.6	71.3 ± 2.0	7.8 ± 0.7	68.3	60.1	6.6
6	37.1 ± 1.2	18.4 ± 0.7	9.2 ± 0.4	8.4 ± 0.4	49.5	24.8	22.7
7	48.7 ± 2.4	25.4 ± 1.3	13.6 ± 0.5	10.9 ± 0.7	52.1	28.0	22.4
8	59.3 ± 8.4	32.4 ± 1.2	17.4 ± 0.1	14.8 ± 1.2	54.6	29.3	24.9
9	72.8 ± 1.8	37.7 ± 1.7	17.4 ± 0.3	20.1 ± 1.5	51.8	23.9	27.6

Each sample solution was measured in triplicate. Each value represents the mean ± S.D. (n = 3). *a*) Sum of the concentrations of water-extracted inorganic As, arsenosugars and DMA indicated in Table 3. *b*) Sum of the concentrations of inorganic arsenic compounds As(III) and As(V) indicated in Table 3. *c*) Sum of the concentrations of arsenosugars indicated in Table 3.

(#8 and #9) were higher than those in other samples. AsSug 482 was not detected in sample #8.

The concentrations of total As and water-extracted As in hijiki samples are summarized in Table 4. Total-As concentrations obtained by acid-digestion of nine hijiki samples ranged from 37 to 119 μg As/g dw. The highest level of total As was found in the Ohita product (sample #5), which was almost three-fold higher than that of the lowest of the Amatsu product (sample #6). The concentration of total water-extracted arsenic compounds obtained by speciation analysis ranged from 18.4 to 81.0 μg As/g dw and their content ratios to the total As were about 60%. The lowest concentration of iAs was observed in the hijiki produced in Amatsu (sample #6). The ratios of iAs to total As ranged from 25 to 60%. Hijiki products produced by the Boso-method (samples #6 and #7) showed relatively low iAs levels compared with the other methods.

DISCUSSION

The results of the present study suggest the existence of six oxo-AsSugs in commercial and raw hijiki analyzed by MRM of HPLC-MS/MS. The four major AsSugs in the present study, 328, 392, 408 and 482, were identified by a high-resolution MS of HPLC-Quadrupole time-of-flight (QTOF)-MS (unpublished data). However, we could not clearly identify AsSug 254 and 391 or AsBe with the MS/MS ion spectra because of the overlapping peaks and the very low concentrations. The existence of AsSug 391 in *Hijiki fusiforme* was reported by Edmonds *et al.*[10)] Formation of AsSug 254 from AsSugs 328, 392, 408 and 482 in acidic environments has been reported.[26)] Even though AsBe was recently detected in six kinds of marine algae,[27)] its contamination from epifauna should be examined more carefully. Therefore, further study is necessary to confirm the existence of AsSug 254 and AsBe in hijiki seaweed.

It has been pointed that ICP-MS response for arsenic is essentially uniform for all (known) arsenic species allows this detector, in most cases, to quantify the various arsenicals by comparison with simple standards such as As(V) or arsnobetaine.[21)] Since we could not obtained AsSug standards, we estimated the quantity of AsSugs using DMA having near-retention time to AsSugs in the present study. We, however, had paid much attention to matrix effects *etc*. because the result will always be subject to them. Among the AsSugs determined, the content of AsSug 408 was the highest and it corresponded to 5–48% of the total water-extracted As. The speciation analysis of arsenic in seaweeds has made progress recently,[17,28)] and consequently some AsSugs (dimethylarsinoyl ribose derivatives) have been identified as major organic arsenics in seaweeds.[29–31)] Shibata *et al.*[29)] reported that AsSug 408 was the major, AsSug 392 was the minor, and AsSug 482 and 391 were the trace constituents in *Hijiki fusiforme*. AsSug 482 was not determined in our samples of #8. Almela[8)] and Raab *et al.*[9)] reported the concentrations of AsSug 328, 392, 408 and 482 in water extracts of hijiki products. Schmeisser *et al.*[30)] reported the detection of the four AsSugs in hijiki and that the content of As-

Sug 408 was the highest at 11.3 mg As/kg. This value is very similar to our samples (samples #6–9) produced in the Boso area. Castlehouse *et al.*[31] reported that the four AsSug were detected in both *Laminaria digitata* and *Fucus vesculosus*, with the major species being AsSug 392, which was found in only slight amounts in our hjiki samples.

In our HPLC-ICP-MS analysis of raw hijiki samples (samples #8 and #9), the early co-eluted As peaks at the positions of AsSug 254, AsSug 328 and AsBe were four-fold of those in processed samples (samples #6 and #7). Gamble *et al.*[32] reported that AsSug 408 and AsSug 482 were degraded to DMA via AsSug 328 in a basic environment. In acidic conditions, the formation of AsSug 254[26] and an increase in DMA with heating[33] were also reported. Both of our Boso hijiki products (#6 and #7) contained DMA at the concentration of 0.8 μg/kg dw hijiki, while the DMA levels in two raw hijiki samples (#8 and #9) were much lower (0.2 μg/kg dw hijiki), and these changes in DMA amount may be enhanced by heating.

Our results confirmed the previous findings that the total-As concentrations obtained by acid digestion in hijiki products depend on the producing district.[6,7] Previous studies on arsenic in seaweed have shown that the majority of arsenic can easily be extracted by water or water/methanol extraction.[34] Our values of the total-As obtained by water extraction using ultrasonication are consistent with the values already reported.[8,9,15,31]

The present study indicates that AsSugs in hijiki account for 3.7–27.6% of total-As (Table 4). Taking into consideration that the major metabolite of AsSugs is DMA, which is carcinogenic in rodents,[13] for the safe consumption of hijiki by humans, the levels of not only total-As and/or inorganic arsenic but also those of each AsSug species should be determined. Six oxo-AsSug were detected in this study by HPLC-MS/MS, which provides more information about the chemical structure than HPLC-ICP-MS, although HPLC-MS/MS cannot detect inorganic arsenics. Therefore, analysis performed using combined HPLC-MS/MS and HPLC-ICP-MS is necessary for the estimation of arsenic species in seafood as well as for the toxicological evaluation of AsSugs.

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