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Original Article

Effect of Temperature on the Extraction of Various Arsenic Compounds from Dried Hijiki, *Sargassum fusiforme* by Water-soaking as a Pre-cooking Process‡

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Summary

Commercial dried Hijiki, *Sargassum fusiforme*[1)], was soaked in 30 volumes of water for 20 min at various temperatures (0−75℃). The total amount of arsenic was determined by thermal neutron activation analysis and the types of the arsenic compounds released were determined by an ICP-MS instrument equipped with HPLC.

The ratios of the total arsenic amounts retained in the swollen Hijiki tissues to those released into water indicated validity of the water-soaking process as a method to diminish arsenic levels in Hijiki. The higher the temperature, the more arsenic was extracted from the Hijiki tissues within a short time. Out of the arsenic compounds extracted at 30℃, 60 % was arsenate and the rest (40 %) was an organic arsenic compound, X_1, having a chromatographically corresponding retention time to arsenobetaine. Other components were less than a few percentage of the total arsenic.

Those arsenic compounds seem to exist in a dispersed form in the tissues, because the observation under a scanning electron microscope did not show any peak of arsenic by line analysis, selected area analysis and/or particle analysis.

Keywords : Hijiki, *Sargassum fusiforme* ; *Phaeophyta* ; water-soaking as a pre-cooking process ; thermal neutron activation analysis ; HPLC-ICP-MS ; arsenate ; arsenobetaine-like compound ; scanning electron microscopy.

Introduction

Commercial products of Hijiki, *Sargassum fusiforme*[1)], which belongs to the *Phaeophyta* family, contains rather higher amounts of arsenic[2−4)] than the members of other families. Substantial amounts of arsenic accumulated in the plant tissues are removed through the drying process of the raw materials ; however the levels of retained arsenic in the dried Hijiki are at times high. These arsenic levels often hampered consumption of Hijiki[5)] as foodstuffs.

Previously, we reported the effect of water-soaking on the diminution of arsenic contents[6, 7)], retained in the commercial dried Hijiki : more than 70 % of the total arsenic could be removed from the Hijiki through the pre-cooking treatment, examined by varying the water-temperature and time-lapse of water-soaking.

In the present paper, we report the behavior of the respective arsenic compounds eluted during the water-soaking pre-cooking process for dried Hijiki.

Materials and Methods

1. Sample plants

Hijiki was harvested at the seashores of the Tsushima Archipelago, Japan. The commercial products of Hijiki were all dried mixtures of the leaves, stalks and apexes and were stored below 4℃ until use.

2. Pre-cooking conditions

One g of the dried Hijiki was sampled while being mixed

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uniformly from the bulk, cut into pieces of 0.5 to 1 cm in length, and put into a small vial placed in a constant-temperature water-bath. Thirty mL of extra pure water was added and the mixture was stirred slowly (one stroke per second) for a lapse of time. Then, the Hijiki samples were rapidly separated through a glass funnel under vacuum. The volumes of the water extracts and the weights of the separated residues were measured, and all the solid samples were lyophilized. Two accurately measured aliquots of each sample were used for thermal neutron activation analysis.

3. The water-soaking time

The Hijiki samples were soaked in extra-pure water for 20 min or 40 min.

4. The temperature range

For water-soaking, the vials containing the samples were placed in a water bath maintained at 30℃, 45℃, 60℃ and 75℃, and shaken slowly at one stroke per second.

The vials for 0℃ (actual temperature was 2℃) were placed in iced water in a refrigerator and those for 15℃ were placed in a water bath kept at that temperature by adding some ice continuously.

5. Arsenic determination[2)]

The extracts were micropipetted onto a piece of filter paper, dried, and the whole was subjected to irradiation analysis. The mean of two determinations was expressed as the amount of arsenic per unit weight of a dried sample or unit volume of a liquid sample.

The dried samples were separately packaged in small polyethylene bags. To determine arsenic concentrations in the samples, 40 of those bags were put together in a polyethylene Neuma-capsule, with 10 bags of various amounts of a standard arsenic compound; two of the standard specimens were arranged for every 8 specimens of Hijiki. The standard solution of arsenic was prepared by dissolving $Na_2HAsO_4 \cdot 7H_2O$ of the guaranteed grade reagent into extra pure water.

6. Thermal neutron activation analysis[3)]

The samples in the Neuma-capsules were irradiated in a flux of 10^{13} neutrons$\cdot cm^{-2} \cdot sec^{-1}$ for 20 min in the center position of the nuclear reactor of the Research Reactor Institute, Kyoto University. After a cooling time of 72 h, the arsenic content in the samples was determined by gamma radiation from ^{76}As using a pure Ge gamma-detector at 559.1 keV. The energy levels of ^{60}Co and ^{137}Cs were used for calibration.

7. Determination of various types of arsenic compounds[8)]

The water extracts obtained after the water-soaking were filtered through a microfilter and injected to HPLC, coupled to an ICP-MS instrument.

8. HPLC (High Performance Liquid Chromatography)[8)]

A water soluble sample, 10 μL, was injected onto a reverse-phase column, Capcell Pack C18 ODS (250×4.6 mm, Shiseido, Japan), equipped with Dionex and eluted with 0.5 % aq. methanol, containing 5 mM 1-butanesulfonic acid sodium salt and 2 mM malonic acid at the flow rate of 0.8 mL/min.

9. ICP-MS (Inductively Coupled Plasma - Mass spectrometry)[8)]

The HPLC was connected to the ICP-MS, Elan 6000 ICP-MS (Perkin Elmer, USA), which was performed with Ar nebulizer gas at the flow rate of 0.85 litter/min.

10. Scanning Electron Microscopy

A scanning electron microscope, JSM-6390LA (JEOL, Japan), equipped with an energy-dispersive X-ray analyzer was used.

Results

1. The arsenic level in Hijiki samples

Commercially prepared dried Hijiki samples were obtained in a bulk. Their arsenic contents were 89.1 ± 6.4 μg arsenic per g of dry sample in average (± standard deviation) of the present lot.

2. The eluted arsenic compounds

The eluted arsenic compounds consisted mainly of two components. The major one was arsenic acid and the other corresponded to the chromatographic retention time of arsenobataine. The latter was designated as X_1.

Table 1 Elution Pattern of arsenate

Arsenate		
Temperature (℃)	Time (min)	μg As/g dried Hijiki
2	20	11.89
15	20	29.53
30	20	36.24
45	20	50.67
60	20	47.78
75	20	61.47
30	40	39.06

Dried Hijiki was soaked in 30 volumes of water at the temperature as indicated for 20 min or 40 min. The eluted arsenate was determined by HPLC-ICP-MS insturument. The detail conditions were described in the text.

Table 2 Elution Pattern of arsenobetaine-like compound, X_1.

Arsenobetaine-like compound, X_1		
Temperature (℃)	Time (min)	μg As/g dried Hijiki
2	20	22.36
15	20	19.91
30	20	23.00
45	20	22.57
60	20	20.97
75	20	25.04
30	40	22.30

The experimental condition is as described in the legend of Table 1 and in the text.

3. The elution pattern of the soluble components after the water-soaking

The results were shown in Tables 1 and 2. The higher the temperature of the soaking water, the more arsenate was eluted. During the first 20 min, 12, 36, 51, and 62 μg arsenic/g dry weight of tissue were removed, at 2, 30, 45, and 75℃, respectively. On the other hand, the amounts of the arsenobetaine-like compound eluted were 20 to 25 μg arsenic/g dry weight of tissue at all temperatures.

4. Electron microscopic survey of the arsenic distribution in the tissues

The observation of freeze-fractured samples under the scanning electron microscope equipped with an energy dispersive X-ray analyzer under low vacuum did not show any clearer peak of arsenic by line analysis, selected area analysis and/or particle analysis, even when Hijiki samples containing highest concentrations of arsenic were used.

Discussion

After the water-soaking treatment, the water compartment in the soaked Hijiki contained two major compounds, arsenate and a compound, relative retention time on the HPLC corresponding to arsenobetaine. All the samples extracted at different temperatures, including as high as 75℃, showed the same retention time corresponding to authentic arsenobetaine, without any deformation on the chromatogram of HPLC.

As a minor component, only trace amount of dimethylarsenic acid was detected.

As the commercial dry Hijiki was a product of boiled and dried before our experiment, this arsenobetaine-like compound seems to be stable and not to be extractable during the boiling of the raw plant tissues. Thus, this chemical structure is interested from the physiological aspect and its chemical structure is under investigation by us.

Arsenic in Hijiki tissues could not be detected as particles under ultramicroscopic observation, so those arsenic compounds seem to exist in dispersed form in the tissues.

The major compounds, arsenate and arsenobetaine-like compound, showed different patterns of temperature de-

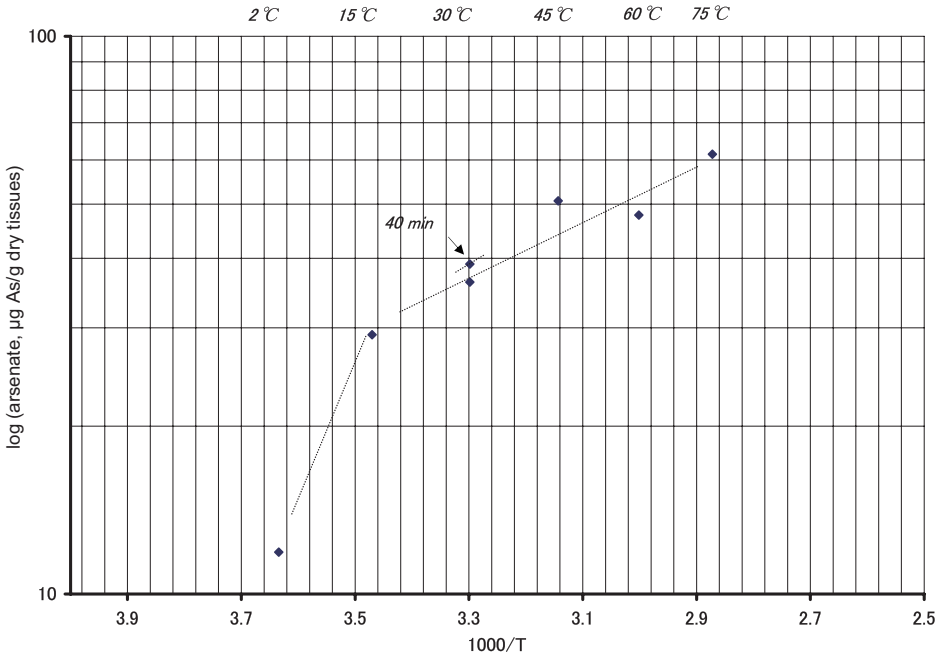

Fig. 1 Elution pattern of arsenate from the water-soaked Hijiki at various temperature.
The ordinate axis represents the log of the arsenic concentration eluted from the Hijiki, soaked in 30 volumes of water at various temperature for 20 min. The eluted compound, arsenate was identified and quantitatively determined by a HPLC-ICP-MS instrument as described in the text. The abscissas axis represents the reciprocal of the absolute temperature (T). The plot, indicated as 40 min, expresses the value of 40 min of the soaking-time.

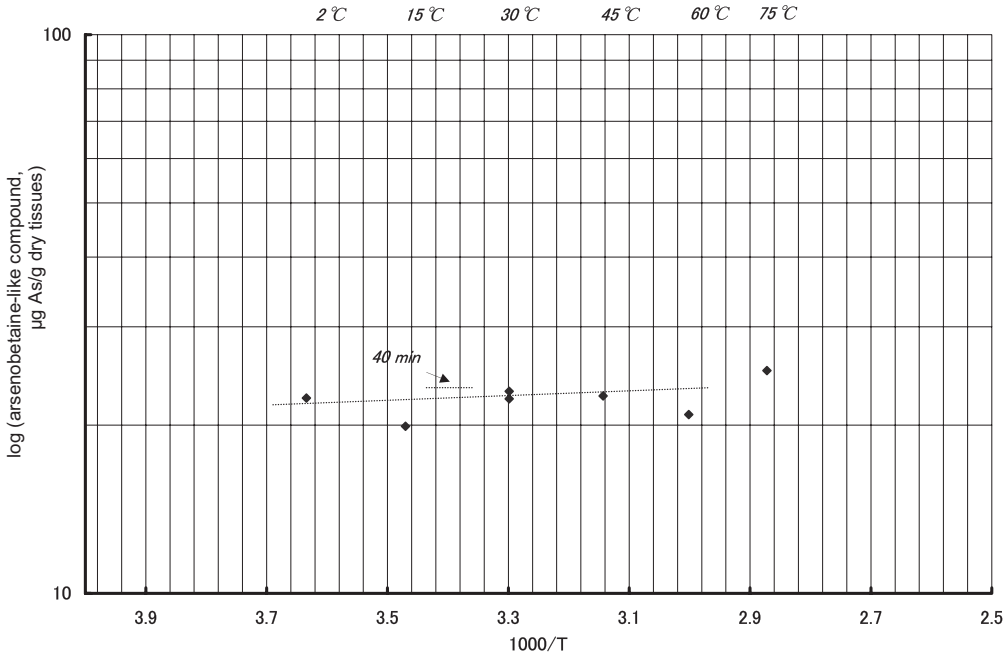

Fig. 2 Elution pattern of arsenobetaine-like compound from the water-soaked Hijiki at various temperature.
The ordinate axis represents the log of the concentration of arsenobetain-like compound eluted from the Hijiki, soaked in 30 volumes of water at various temperature for 20 min. The eluted compound, arsenobetaine-like compound was identified and quantitatively determined by a HPLC-ICP-MS instrument as described in the text. The abscissas axis represents the reciprocal of the absolute temperature (T). The plot, indicated as 40 min, expresses the value of 40 min of the soaking time.

pendency in their elution behavior as shown in Table 1 and 2.

This temperature dependency, expressed on logarithmic values of eluted amount to reciprocal temperatures, seems to be clearly different type (Fig. 1 and Fig. 2).

The arsenobetaine-like compound seems to be easily extractable with a low barrier by existing on the surface of the cells or tissues, whereas arsenate may have a greater barrier to the water extraction by existing inside the cells or tissues.

Other arsenic components existed in the fresh Hijiki plants might be loosed during the preparing process of commercial dried Hijiki.

Out of the beneficial minerals, magnesium and zinc may exist mostly in soluble form in the cells of Hijiki[9], at concentrations of 6.87 mg Mg and 13.5 μg Zn/g tissues on the dry weight basis, respectively. In contrast to them, calcium and iron, found to exist at concentrations of 18.6 mg Ca and 886 μg Fe/g tissue on the dry weight basis, were observed to exist mostly in cellular particles in Hijiki[9].

During the water-soaking process, the water compartment can be squeezed out and discarded, possibly resulting in lesser amounts of retained arsenic[7], while the losses of calcium and iron existing mostly in particles are only 50 % and 30 %, respectively, of the original[9].

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