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Temperature-dependent extraction of trace elements in edible brown alga hijiki, *Hizikia fusiforme*

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Abstract

Trace elements were extracted from natural hijiki by heating with water (25–95 °C) or commercial hijiki by soaking, washing, and rinsing with water at room temperature. Eleven elements (As, B, Cd, Ce, Cu, Fe, Li, Sn, Tl, Y and Zn) were determined with ICP-MS in the acid-digested and diluted solutions of commercial/natural hijiki or in the water extracts of natural hijiki. Extractions were effective in removing metals in the range 55–65 °C. The traditional procedure of soaking and washing commercial hijiki was inadequate to sufficiently lower trace elements. The iron concentration in commercial hijiki was high, indicating a possible contribution from the iron cauldron used in producing commercial hijiki. Results showed unsuitability of the commercial production process for reducing trace elements sufficiently. To lower trace elements, a procedure involving two steps was proposed for preparing consumable hijiki. Hijiki may be recommended as a supplement for deficiencies of B, Fe, Cu and Zn.

Keywords: Arsenic; Trace elements; Hijiki; *Hizikia fusiforme*; Hot water extraction

1. Introduction

The edible brown alga hijiki, *Hizikia fusiforme*, is widely consumed in Asia, and in particular Japan. Hijiki is also consumed in western countries in soups, salads, and vegetable dishes, particularly in Asian restaurants. The literature reports regarding anti-tumor activity, have further initiated interest in the consumption of hijiki as a health food (Van Netten, Hoption Cann, Morley, & Van Netten, 2000). However, the high concentrations of inorganic arsenic in hijiki have raised serious toxicological concerns among consumers during the past two decades. For example, based on survey results that showed high levels of inorganic arsenic, the Canada Food Inspection Agency (2001) and the UK Food Standards Agency (2004) have issued press releases advising citizens not to consume hijiki. Similarly, hijiki has been added to New Zealand's high risk foods list (2004). The accumulation of inorganic arsenic (Yasui, Tsutsumi, & Toda, 1978) in hijiki, at high concentrations, has initiated a number of studies focussing on chemical forms of arsenic in hijiki (Adachi et al., 1980; Watanabe, Hirayama, Takahashi, Kokubo, & Ikeda, 1979; Yamauchi & Yamamura, 1979). An extensive subsequent study (Edmonds, Morita, & Shibata, 1987), which involved isolation and structural identification, mainly based on NMR spectroscopy, showed occurrence of about 50% of the total arsenic load in hijiki in the form of arsenic-containing ribofuranosides. Kuehnelt, Irgolic, and Goessler (2001) have studied the extractive removal and subsequent analysis by HPLC-ICP-MS of eight arsenic compounds from hijiki. Recently, Hanaoka et al. (2001) confirmed substantial removal of arsenic during the

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traditional soaking and washing procedure employed before cooking hijiki. In response to the growing needs to correctly evaluate the chemical forms of arsenic in hijiki, the National Institute for Environmental Studies (Japan) has prepared a reference material for arsenic speciation in hijiki (Okamoto, Yoshinaga, & Morita, 1996). In general, these findings have somewhat eased worries about possible poisoning from arsenic by consuming hijiki.

Limited reports, that reveal considerable accumulation by hijiki of major, minor, and/or trace elements in addition to arsenic, have appeared on the literature. Demura, Tsukada, and Yamamoto (1985) have developed a microwave digestion method for the rapid determination of five trace metals in hijiki. The distribution and average contents of seven heavy metals were determined in vegetable foods, including hijiki (Tanaka, Ikebe, Tanaka, & Kunita, 1977). Ishikawa, Izawa, Omori, and Yoshihara (1987) have studied the quantitative annual variation of 12 elements in hijiki and classified the patterns in elemental concentrations. Their table of trace element contents in Japanese foodstuffs provides an extensive list of 28 trace elements in hijiki (Suzuki, 1993).

Most of the literature citations dealing with the determinations of elemental levels in hijiki have generally focussed on accurate evaluation of a wider range of elemental concentrations, in addition to arsenic. Indeed, little is known about the effect of commercial production and the traditional washing and soaking procedures on the dietary safety of hijiki in terms of concentrations of a broad spectrum of trace elements. Commercial hijiki is produced by boiling the successively harvested alga in water. The water, stored in an iron cauldron, is used over and over, resulting in a solution concentrated with the hijiki extract (stock solution). Essentially, the production of commercial hijiki is carried out in this concentrated and repeatedly used stock solution to give the product a beautiful black colour. Recently (Hanaoka et al., 2001), a study was carried out to determine the efficiency of hot water extraction (0–60 °C), which is analogous to the commercial process of production in removing arsenic in natural, as well as commercial, hijiki. The study demonstrated inappropriateness of the commercial production scheme for lowering arsenic concentration sufficiently. Consequently, to reduce the level of arsenic, a hot-water soaking and washing procedure was recommended before cooking commercial hijiki at home.

In the present work, it was considered desirable to undertake a more elaborate study, particularly focussing on the commercial, as well as the traditional, processing steps, to ensure the dietary safety of hijiki in terms of elemental concentrations. For this purpose, the procedure developed to evaluate arsenic levels in natural and commercial hijiki (Hanaoka et al., 2001) was extended to the assessment of the concentrations of 11 trace elements (Li, B, Fe, Cu, Zn, As, Cd, Y, Sn, Ce and Tl) in hijiki. These elements occur in natural hijiki in varying concentrations expressed in μg element/g (Demura et al., 1985; Kikuchi, Nomiyama, Kumagai, Uemura, & Umae, 2002; Sakao, Ogawa, & Uchida, 1997; Tanaka et al., 1977): As 25–126; Cd 0.3–2.8; Cu 5.1–350; Mn 13–44; Pb 1.7–5.3; Zn 10.7–1170. Some of these elements are essential to man (B, Fe, Cu, Zn) and some others are considered to be extremely toxic (As, Cd, Sn), although toxicity depends on chemical nature.

2. Materials and methods

2.1. Reagents and standard solutions

Ultra pure nitric acid (70% HNO_3) was purchased from Wako Pure Chemicals Industries (Japan). High purity standard solutions containing 1000 mg element/l were purchased from the following suppliers: cerium, thallium, and yttrium from Kishida Chemicals Company (Japan); zinc, copper and iron from Wako Pure Chemicals Industries (Japan); arsenic, boron, cadmium, lithium and tin from Katayama Chemical Company. A 10.0 ml intermediate standard solution, containing 1.0 μg element/l (Li, Y, Cd, Sn, Ce, Tl), 5.0 μg element/l (Cu, Zn), 10 μg As/l and 50 μg element/l (B, Fe) was prepared from the concentrated standard solutions by diluting with HPLC grade distilled water (Wako Pure Chemicals Industries).

2.2. Collection, extraction and digestion of hijiki

2.2.1. Collection of hijiki

Fresh hijiki samples were collected from the shore of Yoshimi about 2 km east of the National Fisheries University, Shimonoseki, Japan. The alga attaching to the rocky seabed was directly hand-picked during low tide and wrapped in polyethylene bags. After transporting to the laboratory, the freshly collected samples were treated immediately, as described below. Commercial hijiki, supplied in an airtight plastic bag (D. Company, Japan), was purchased from a local supermarket.

2.2.2. Preparation of freshly collected natural hijiki

After cutting off the roots with a fine ceramic knife, the freshly collected alga was briefly rinsed with tap water, just to remove the seawater and adsorbed particles, such as rock fragments and microorganisms. The excess water was trickled through a plastic sieve. After hand-sorting the sample into Me-hijiki (leaves) and Naga-hijiki (branches), the water adhering to the surface was dried with a laboratory tissue paper and further dried at room temperature for about 5 h over a plastic tray. The wet mass of hijiki obtained after such preliminary air-drying step was determined for subsequent analysis.

2.2.3. Extraction of trace elements in natural hijiki

Wet hijiki, weighing about 10 g, and tap water (100 ml) were placed in a 400 ml Erlenmeyer flask. The flask containing hijiki–water mixture was covered with a glass funnel and immersed in a water bath that was preset to 25,

35, 45, 55, 65, 75, 85 or 95 °C. For extractions at boiling point, the flask containing hijiki–water mixture was placed on wire gauze over a low burner flame. Extractions at all temperatures were carried out for 20 min with three intermittent swirlings of the contents. The resulting extracts were passed through quantitative filter paper (Advantec, Japan) into screw-capped glass vials. The extracts collected in the glass vials were cooled to room temperature and stored at −20 °C prior to analysis. The extracted branches or leaves were collected in polyethylene bags and dried, in a freeze-drying unit, to constant mass. The freeze-dried hijiki samples were powdered in a mill (SIBATA, SCM-40A, Japan) and stored at room temperature.

2.2.4. Soaking, washing, and drying of commercial hijiki

About 10 g of dry commercial Me-hijiki, placed in a 400 ml beaker, was soaked in 200 ml of tap water for 20 min. After draining the water, the soaked hijiki was washed with 200 ml of tap water and then rinsed with 200 ml of a fresh portion of tap water. The unwashed or the soaked, washed and rinsed commercial hijiki was dried in a freeze-drying unit to constant mass.

2.2.5. Microwave digestion

When ~250 mg of dried and powdered hijiki were prepared and analyzed by ICP-MS, concentrations of metals in commercial hijiki were about 1.6 times higher than that of natural hijiki. Consequently, for microwave digestion of samples, ~240 mg dry mass of unextracted natural hijiki or ~150 mg of commercial hijiki were chosen to provide metal concentrations that were within the calibration range. A powdered sample, equivalent to ~240 mg dry mass of un-extracted natural hijiki or ~150 mg of commercial hijiki, was digested with 3 ml of 70% HNO_3 in six-high pressure quartz vessels (MultiWave microwave digestion unit, PAAR) using the heating programme developed in-house (digestions at 100 W for 5 min and at 800 W for 15 min followed by a cooling step of 15 min). Each sample was digested in triplicate. The resulting clear digests were diluted to 40 ml or to 800 ml with HPLC grade distilled water for determination of trace elements by ICP-MS.

2.3. Total element determination

For the determination of total element concentrations, appropriate volumes of the digests or aliquots (4 ml) of the extracts of hijiki, obtained at various temperatures, were diluted to 40 ml after addition of 3 ml of 70% HNO_3. The diluted digests or diluted extracts were divided into four test tubes, three of which contained exactly 10 ml portions each, for subsequent standard addition. No standard was added to the fourth test tube. Into each of the three 10 ml portions of the diluted digests or diluted extracts, 10, 50 or 100 μl of the intermediate standard solution, consisting of 11 elements, was added. The calibration solutions prepared by addition of standards, as described above, had the following concentrations (ng element/g): 0, 1, 5, or 10 (Cd, Ce, Li, Sn, Tl, Y); 0, 5, 25 or 50 (Cu, Zn); 0, 10, 50 or 100 (As); 0, 50, 250 or 500 (B, Fe). The ion currents for m/z for ^{7}Li, ^{11}B, ^{57}Fe, ^{63}Cu, ^{66}Zn, ^{75}As, ^{89}Y, ^{111}Cd, ^{118}Sn, ^{140}Ce and ^{205}Tl were simultaneously monitored by ICP-MS (Hewlett–Packard HP 4500 Series, Yokogawa Analytical Systems) and concentrations were calculated using calibration curves obtained by employing the standard addition technique. The degree of ^{40}Ar,^{35}Cl interference on ^{75}As was checked by monitoring signals for ^{75}As, ^{77}Se and ^{82}Se and applying a mathematical correction procedure (Van den Broeck, Vandecasteele, & Geuns, 1997). Such an approach showed no significant interference at high concentrations of arsenic determined in the hijiki samples. Consequently, all concentrations of arsenic are reported without introducing such corrections. The operating conditions for HP 4500 Series ICP-MS were as follows: RF generator power 1.2 kW; RF reflected power 0 W; cooling gas flow rate 15.0 l/min; nebulizer gas flow rate 1.07 l/min; interface pressure 13 Pa; vacuum pressure 3.3×10^{-4} Pa; nebulizer Babington, sampler cone platinum (1.00 mm dia), Skimmer cone Nickel (0.4 mm dia).

3. Results and discussion

3.1. Effect of sample pretreatment on microwave digestion of freshly collected and dried hijiki

Microwave digestion of samples which were not subjected to the pretreatment procedure yielded non-homogeneous digests, possibly containing silicates that were accompanied by formation of scale on vessel walls. The undigested particles were sufficiently large in size and were effective in blocking the sampling tube of the ICP-MS. Cutting of the roots of all freshly collected hijiki samples with a ceramic knife, followed by a brief washing procedure, helped eliminate extraneous materials such as microorganisms and rock fragments adhering to the surface and roots of hijiki and produced clear solutions.

3.2. Effect of extraction temperature on dry mass determination

Ten grams of wet and unextracted Me-hijiki (leaves), when freeze-dried to constant mass, revealed 86.8% (m/m) original moisture content, whereas the same mass of Me-hijiki that was extracted at 100 °C contained 91.7% moisture. A similar study done on a 10 g sample of unextracted Naga-hijiki (branches) or that extracted at 100 °C, gave moisture contents of 77.9% and 85.5%, respectively. These results showed that branches of hijiki contained less moisture than did leaves and that moisture contents increased with temperature of extraction. Mass of hijiki lost adhering to the surface of filter paper while filtering the extracts did not exceed $4.15 \pm 0.25\%$ (m/m) of total dry mass.

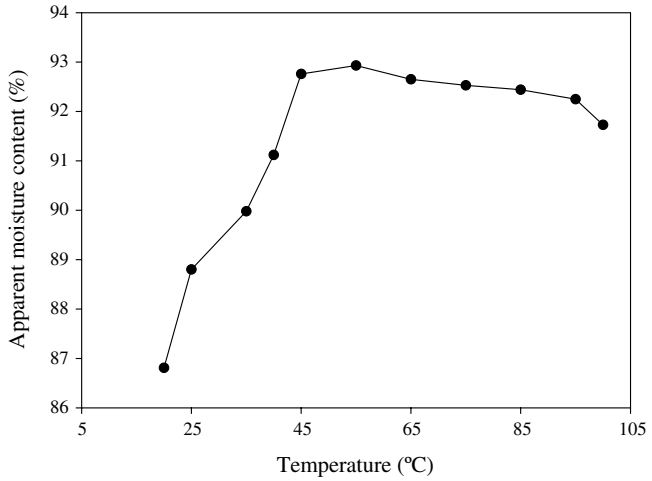

Fig. 1. Variation of moisture content in natural Me-hijiki (~10 g wet mass) determined after freeze-drying the unextracted sample at 20 °C or that was extracted with 100 ml of tap water in the temperature range 25–100 °C.

To account for this apparent variation of moisture content with conditions of extraction, the dependence of the dry mass of Me-hijiki on the temperature of extraction was determined. The results of such a study generally showed a gradual increase in moisture content of extracted hijiki with increasing temperature of extraction up to 55 °C, followed by a modest decrease in moisture content above 75 °C (Fig. 1). No further study was carried out on the contents of the extracted solution, as the purpose was to determine a factor that would express the metal concentrations in both wet and dry mass bases.

The decrease in mass of hijiki in the range 25–55 °C could be ascribed to solubilization and subsequent transfer into the aqueous phase of the algal tissue. On the other hand, the gradual increase in mass in the range 75–100 °C may be attributed to concerted effects of evaporative loss of extracting water and hardening or toughening of the algal cell wall. Both of these processes could result in decreased solubility of the tissue with consequent increase in mass or decrease in moisture content of hijiki. The subject of algal tissue toughening or cell wall hardening, which was observed in the range 75–100 °C for hijiki may require a thorough study. While grinding of unextracted hijiki, or one extracted up to 65 °C, was much easier, samples extracted in the range 75–100 °C successively required longer times to achieve powders of desirable fineness. From this observation, we concluded that, when hijiki is subjected to heating within a certain temperature range, its solubility decreases as a result of tissue toughening. Change in the properties of hijiki in the temperature range studied could possibly cause differences among moisture contents determined at different extraction temperatures. In view of the variation of mass with temperature of extraction, dry masses, equivalent to about 240 mg of not extracted hijiki, were used in all digestions and all concentrations were evaluated on such a dry mass basis.

3.3. Determination of trace elements in natural Me-hijiki

The concentrations of trace elements in natural Me-hijiki and in the corresponding aqueous extracts were determined with ICP-MS, exclusively employing the standard addition method to effectively minimize sensitivity differences caused by matrix components. When analyte ions were monitored in digests that were diluted to 40 ml, relatively poor regression coefficients, ranging from 0.88 to 0.99, were obtained for Li, B, Fe, Cu, Zn and As. This loss of linearity in calibration curves was overcome by a 20-fold further dilution of the original 40 ml solutions (or digests directly diluted to 800 ml; dilution factor ~3400). In these more dilute solutions, linear calibration curves with regression coefficients of 0.9989–0.9999 were obtained for the elements listed above. Experiments in such further diluted solutions unveiled ion count suppression ranging from 40% to 70% for Li, B, Fe, Cu, Zn and As. The signal suppressions observed on Li, B, Fe, Cu, Zn and As were probably caused by the presence of easily ionizable concomitant elements in hijiki, as observed by Gregoire (1987). This assumption is consistent with the occurrence, at several milligrammes per gramme level, of the easily ionizable K as the main component in hijiki (Ishikawa et al., 1987). The analytes with higher atomic mass (Y, Cd, Sn, Ce, Tl) did not suffer from such effects. Furthermore, the linear regression coefficients for these elements, in the un-diluted solutions, ranged from 0.9990 to 1.0000. Consequently, the determinations of Y, Cd, Sn, Ce and Tl in hijiki were carried out on the original 40 ml solutions. Concentrations of trace elements, including ions monitored, precision of replicate measurements, and limit of method determination could be calculated according to the following relationship, and are summarized in Table 1:

$$\mathrm{MDL} = 10 \times \sigma_{\mathrm{blank}} \times \text{concentration of standard solution (ng/g)} \times \text{dilution factor}/(S - B)$$

where MDL is the method determination limits, σ_{blank} is standard deviation of the blank solution, consisting of 3 ml of 70% HNO_3 similarly digested as sample and diluted to appropriate volumes, S is counts per second of a 10 or 50 ng element/g standard solution, and B is counts per second of the blank.

Of the 11 elements assayed in leaves of natural hijiki, Tl was below MDL and Y, Cd, Sn and Ce occurred at very low concentrations (130–600 ng element/g), whereas, B, Fe and As were found in the highest concentrations.

3.4. Temperature-dependent extraction of trace elements in hijiki

Freshly harvested hijiki is naturally brown. Preparation for consumption involves boiling of the brownish, freshly harvested hijiki in water. The boiling water quickly acquires a brown colour and its intensity increases with heating time and temperature, indicating solubility of the algal tissue.

Table 1
Concentrations of trace elements in digested samples of natural unboiled, natural boiled, and commercial Me-hijiki (leaves) determined by ICP-MS ($n = 3$)

Isotope	Concentration (μg element/g dry mass) in Me-hijiki (leaves)			MDL (ng/g)
	Fresh not boiled	Fresh boiled	Commercial	
^{7}Li	1.02 ± 0.08	0.35 ± 0.01	0.12 ± 0.01	1.06
^{11}B	468 ± 12	208 ± 15	330 ± 23	494
^{57}Fe	256 ± 25	173 ± 11	1224 ± 98	349
^{63}Cu	6.74 ± 0.38	4.69 ± 0.26	11.2 ± 0.6	6.37
^{66}Zn	14.3 ± 0.81	10.5 ± 0.5	20.1 ± 1.4	16.1
^{77}As	99.6 ± 0.16	63.2 ± 1.8	142 ± 1	7.18
^{89}Y	0.13 ± 0.01	0.07 ± 0.01	0.39 ± 0.09	0.93
^{111}Cd	0.62 ± 0.03	0.47 ± 0.05	1.62 ± 0.08	0.22
^{118}Sn	0.36 ± 0.03	0.23 ± 0.02	0.31 ± 0.03	4.17
^{140}Ce	0.14 ± 0.01	0.08 ± 0.01	1.38 ± 0.06	0.84
^{205}Tl[a]	< 0.82	< 0.82	< 0.82	0.82

The method determination limits (MDL) are calculated for six replicate analyses.
MDL-method determination limit.
[a] Concentration of Tl in ng/g.

In imitation of the commercial, as well as the traditional, processing practices used before cooking hijiki, extraction of trace elements in hijiki was carried out with tap water in open vessels. The procedure involved a series of batch extractions, with increasingly higher temperatures of extracting water, to characterize solubility of trace elements. Trace elements were determined in extracted and freeze-dried hijiki and in the corresponding aqueous extracts obtained at each temperature of extraction. Ion count suppression was overcome by diluting the digests of extracted hijiki, as described above. Such a problem was not encountered when determining trace elements in the 10-fold diluted extracts.

Results generally showed an increase in concentration of trace elements in the extracts with increasing temperature of extraction up to 55 °C, followed by a sharp increase at boiling point. The volume of extracting water at boiling point substantially decreased, which could possibly give rise to a sharp increase in the concentration of trace elements that were initially transferred into the aqueous phase. As described earlier, sample heating was carried out in open beakers, which can lead to irreproducible changes in the volume of the extracting water. Corrections had not been made for volume changes. To establish mass balance, the residual concentrations of trace elements in the extracted hijiki and that transferred into the aqueous phase during extraction were compared with the concentrations in the unextracted hijiki. Fig. 2 shows the efficacy of temperature-dependent extraction in removing selected elements (B, Fe, Cu, Zn, As) in the leaves of natural hijiki. With the exception of Tl, which was below MDL, similar trends were observed for the other elements considered in this study. The residual concentrations of trace elements in the extracted leaves generally appear to be the lowest, for all elements, at extraction temperatures in the range 55–65 °C.

The efficiency of boiling temperature in removing trace elements was compared with that at 55 °C (Fig. 3). A single batch extraction carried out for 20 min at 55 °C, removed 36% of Cd, 40–50% of Fe, Cu, Zn, Ce, 50–60% of As, B, Y, Sn and 73% of Li. A similar extraction, performed at boiling point, revealed the extractive removal of 20–30% of Cu, Zn, Cd, 30–40% of Fe, As, Sn, Ce, 45% of Y, 56% B, and 70% of Li. Thallium was below MDL at both temperatures. The solubility of trace elements was marginally lower at boiling point than at 55 °C. No detailed study was done to reveal the underlying reasons for the decrease in solubility of trace elements in hijiki with extraction temperature. However, the marginal decrease in solubility of trace elements exhibited at the boiling temperature of extraction could probably be related to gradual loss of extracting water through evaporation and/or toughening of the tissue of hijiki.

3.5. Traditional soaking and washing treatment of commercial hijiki

The commercial preparation of hijiki involves repeated use of the water which acquires a brown colour during the first boiling step of freshly harvested hijiki. This concentrated boiling solution is never discarded; it is repeatedly used for boiling more batches of freshly harvested hijiki, thereby imparting strongly brown coloration to the commercial product. Consequently, commercial hijiki, which is dried and packed in plastic bags, is dark brown. This pronounced colour intensity is attributable to the adsorption/absorption of colour from the repeatedly used concentrated boiling solution.

Commercial hijiki is consumed after subjecting the product to a traditional soaking and washing pretreatment. A typical traditional procedure, employed before cooking, involves soaking an amount of commercial hijiki in ample water for 15–20 min, washing with ample water, followed by a final rinsing of the once-soaked and washed hijiki with ample water. "Ample water" used to soak, wash and rinse hijiki in the traditional pretreatment procedure is qualitatively understood as an amount that fully covers the hijiki in a bowl. Recently, the effectiveness and also the necessity

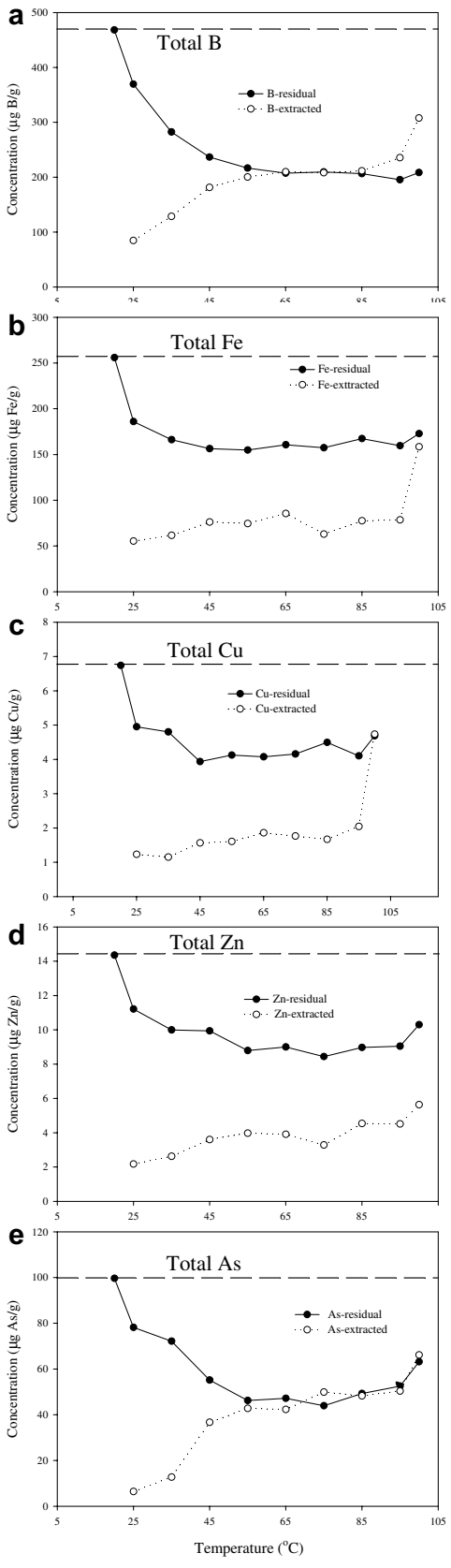

Fig. 2. Temperature-dependent extraction of trace elements in natural Me-hijiki (~10 g wet mass) with 100 ml of tap water: comparison among total, residual, and removed concentrations (on dry mass basis) of B, Fe, Cu, Zn and As.

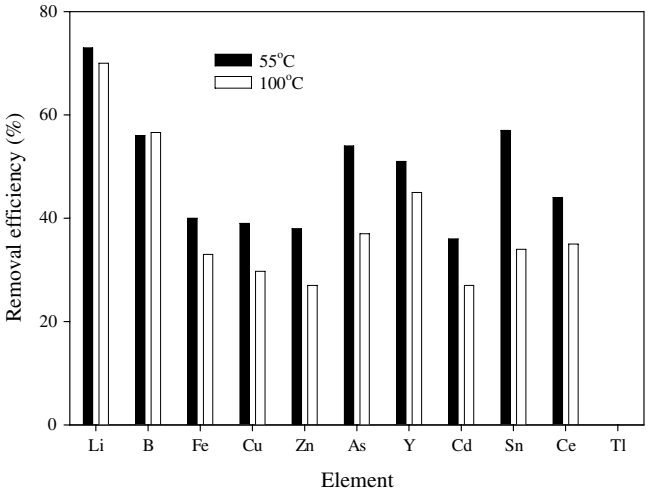

Fig. 3. Comparison of the solubility in the extracting solution of trace elements (Li, B, Fe, Cu, Zn, As, Y, Cd and Sn) in natural Me-hijiki (~10 g wet mass) between extractions carried out with 100 ml of tap water at 55 °C and 100 °C. Comparison is based on dry mass basis.

of this traditional soaking and washing treatment to minimize toxic forms of arsenic was demonstrated (Hanaoka et al., 2001).

In order to forward general recommendations about the effectiveness of such washing treatment, analysis of a broad spectrum of trace elements in washed and unwashed commercial hijiki appears desirable. The traditional procedure that involves soaking, washing and rinsing of hijiki with unquantified amounts of water was modified in this study. Instead of "ample water" recommended by the hijiki industry, we used 200 ml of tap water in each of the soaking, washing, or rinsing steps as this amount completely covered about 10 g of dried commercial hijiki placed in a beaker. Aliquots (~150 mg) of soaked, washed, dried, and powdered commercial hijiki or dried and powdered but not washed commercial hijiki were microwave-digested and trace elements were determined in diluted digests. In Fig. 4 (dotted and hatched bar graphs), a logarithmic decrease in concentration is shown for each trace element studied under the described conditions. The traditional soaking and washing procedure effectively removed 11% of Y, 15% of Zn, 25% of Cd, 30–40% of (Li, B, Fe, Cu, As, Ce), and 59% Sn. Thallium was below MDL.

With the objective of evaluating the commercial production process, a triplet comparison, involving freshly collected and boiled hijiki (white bars), not washed (dotted bars) and washed (hatched bars) commercial hijiki is shown in Fig. 4. For at least two main reasons, such comparison among the contents of trace elements in the commercial (not washed and washed) and freshly collected and boiled hijiki may not reflect actual differences that may exist among these samples. First, the initial sample collection and preparation procedures for commercial and natural hijiki were evidently different. The importance of removing all forms of extraneous materials from freshly

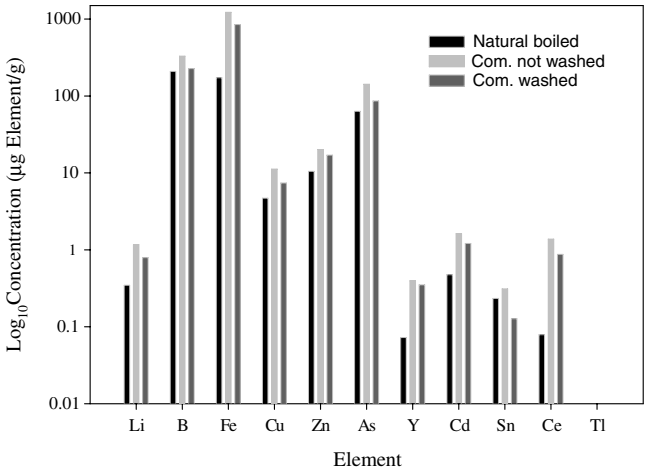

Fig. 4. Efficiency of the traditional soaking and washing procedure (with 200 ml fresh portions of tap water) in removing trace elements in commercial hijiki (~10 g wet mass).

collected hijiki before analysis was emphasized above. While fresh hijiki, collected and prepared as described in the materials and methods section in this study, was completely soluble in 3 ml of 70% HNO_3, the same mass of commercial hijiki contained a noticeable quantity of undigested materials. On the other hand, the soaked and washed commercial hijiki contained relatively no or fewer particles that were recalcitrant to the action of HNO_3 digestion. It is very likely that the introduction of extraneous materials could bias the final analytical results and possibly jeopardize comparability. The observations suggest the need for similar sample preparation procedures for making direct comparisons. Secondly, the boiling treatment caused loss of mass due to solubilization of the tissue of natural hijiki, as demonstrated by temperature-dependent extraction experiments. For the freshly collected and boiled hijiki, such losses were determined and appropriate corrections were introduced. After such revising, elements such as As, B, Cu, Fe, Li or Zn were determined in more dilute solutions (dilution factors ~3400). However, it was not possible to account for mass losses in commercial hijiki. Consequently, an apparent dilution factor of about 5500 was used in calculating concentrations of As, B, Cu, Fe, Li or Zn, which could have exaggerated the reported results for commercial hijiki.

Despite the differences recounted above, a few general observations could be noted: (1) the traditional soaking and washing procedure is effective in lowering the concentrations of trace elements considered in this study, but was insufficient to lower trace elements to the level contained in freshly collected and boiled hijiki (Fig. 4); (2) elements As, B and Fe occur in major proportions in both commercial (washed and not washed) and in the freshly collected and boiled hijiki; (3) both the temperature-dependent extraction and the traditional soaking and washing experiments do not remove appreciable quantities of Zn in hijiki; (4) arsenic, which becomes the main concern of consumers, is present in the washed product at ~8.6 µg per gramme of wet hijiki. This result indicates the inadequacy of soaking and washing commercial hijiki at room temperature for lowering arsenic. Use of soaking and washing temperatures ranging from 50–60 °C may considerably reduce concentration of arsenic in hijiki (Hanaoka et al., 2001); (5) trace elements occur at apparently higher concentrations in commercial hijiki (Fig. 4, dotted bars) than in freshly collected and boiled hijiki (Fig. 4, white bars), clearly demonstrating the likely contribution by the concentrated stock solution used during the commercial production of hijiki; (6) the concentration of Fe in commercial hijiki is considerably higher than that in boiled natural hijiki (Table 1, Fig. 4). These findings suggest possible addition of Fe from the iron cauldron used for boiling hijiki during the commercial production stage. Traditional maize and sorghum beverages brewed in cast iron vessels exhibit remarkable increase in iron concentration (Gordeuk, Bacon, & Brittenham, 1987). Computations based on wet mass of hijiki showed good agreement between the iron concentration in the washed commercial hijiki (84.9 µg Fe/g) and that in the traditional beverage of Bantu people in South Africa (~82 µg Fe/g). It is important to note that iron in the traditional beverage is present in solution form, although solubility may not necessarily imply bioavailability.

It appears essential to reflect on the health effects of the residual As in traditionally prepared hijiki. The total concentration of As in the unwashed commercial hijiki was 142 µg/g (Table 1), which dropped to ~85 µg/g upon washing the product by the traditional procedure, i.e., washing, soaking, and rinsing with "ample water" (Fig. 4). Although widely accepted allowable levels of arsenic in foodstuffs are not available, JECFA (1983) has provisionally recommended a maximum allowable level of 0.002 mg/kg body weight per day for intakes of inorganic arsenic. This equals 0.13 µg/person/day for a 65 kg body weight. Disregarding other sources of arsenic and assuming a daily hijiki intake of 1 g, the daily intake of As from prepared commercial hijiki (Table 1) would be ~85 µg/person/day. This amount is higher than the maximum allowable limit for inorganic arsenic, though an earlier report confirmed the efficiency of the traditional soaking and washing procedure to remove it. Though the actual chemical nature of residual arsenic present in the traditionally prepared hijiki necessitates further investigation, the consumption of such hijiki would certainly increase daily dietary exposure to total arsenic.

3.6. Distribution of trace elements in leaves and in branches

Two products of hijiki are supplied commercially: Me-hijiki and Naga-hijiki. Because of the relative softness, consumers tend to prefer leaves to branches of the two commercially supplied products. To estimate noticeable imbalance in elemental levels consumed as a result of such preferences, the relative distribution of trace elements was compared between leaves and branches of unboiled and boiled natural hijiki (Fig. 5). Percentage preferential accu-

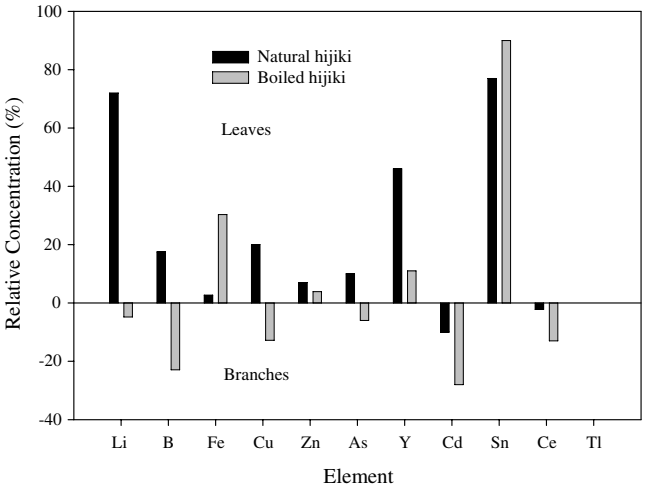

Fig. 5. Relative distribution of trace elements between leaves and branches of unboiled and boiled natural hijiki (considering concentration in leaves as reference; positive values represent predominant accumulation in leaves, negative values in branches).

mulation was calculated by considering the concentration of trace elements in leaves as reference. The positive *Y*-axis is used to designate preferential accumulation of trace elements in leaves and the negative *Y*-axis in branches of hijiki. The relative distribution patterns displayed by Fe, Zn and Ce indicate less important (<7%) preferential accumulation in leaves of unboiled hijiki. Such slight differences in accumulation patterns seem to be within experimental error. Thus, Fe, Zn and Ce may have been equally distributed between leaves and branches. The elements Li, Y and Sn are predominantly present in leaves (46–77%), whereas B, Cu and As, are more concentrated in branches than in leaves (10–20%) of unboiled natural hijiki. On the other hand, the less abundant element Cd is shown to occur more in branches than in leaves (10%) of unboiled hijiki. The shaded bars in Fig. 5 compare the distribution of trace elements between leaves and branches of boiled hijiki. Most of the trace elements (Li, B, Cu, As, Cd, Ce) that are mainly concentrated in leaves of un-boiled hijiki exhibited preferential accumulation in branches of boiled hijiki. Upon boiling, most of the trace elements in leaves are more easily solubilized than are those in branches of natural hijiki. Only Fe and Sn, however, showed strong binding to leaves than to branches of boiled hijiki. Thallium was below MDL both in leaves and in branches. Except for Li and Y (unboiled hijiki), Sn (both in boiled and unboiled hijiki), and Fe (boiled hijiki), the results generally indicate absence of any remarkable preferential accumulation for elements considered in the present work. Considerable variation in distribution could probably be related to intrinsic physiological requirements or tolerance of the parts of hijiki.

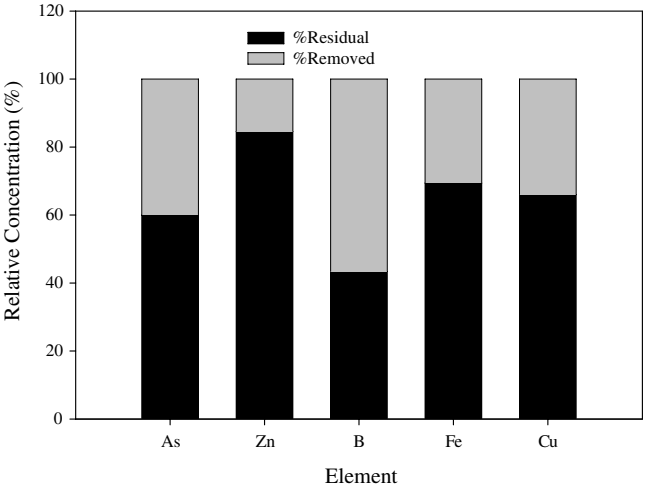

Fig. 6. Comparison between the residual and removed concentrations of trace elements occurring in major concentrations in commercial hijiki, obtained after subjecting the sample to traditional soaking and washing procedure.

4. Conclusions

To lower the concentration of trace elements in hijiki, batch extractions at temperatures ranging from 55–65 °C appear to be optimal. On the contrary, the dietary requirements impose the need for more cooking at elevated temperatures in order to achieve the most desirable texture of hijiki. Furthermore, commercial requirements demand colouring of the final product with the brown pigment resulting from the extraction process through heating hijiki in a boiling mixture. Our results showed the extractive removal of the brown pigment in hijiki at temperatures up to 65 °C. As a compromise for these contradictory but concurrent needs, we suggest the need for two batch extractions, one up to 55 °C and a second with a fresh portion of water up to boiling point. In this case, the hijiki industry may find it necessary to devise a mechanism for recovering the brown colouring pigment from the first batch of extract that is rich in trace elements.

In Fig. 6, the efficiency of traditional soaking and washing procedure in removing the main elements in commercial hijiki is shown. The elements As, B, Fe and Cu were substantially removed by soaking and washing hijiki at room temperature. However, the residual concentrations of B, Fe and Cu were considerable. Zinc is shown to occur in a less soluble form in hijiki. Whether the low solubility displayed by Zn in hijiki is related to bio-unavailability is the subject of an independent study. To maintain good health, the human body requires optimal concentrations of major, minor and trace elements. Without concerns of bioavailability, traditionally cooked hijiki may be recommended as a supplement for deficiency of B, Fe, Cu and Zn.

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