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A Study on the Reduction of Inorganic Arsenic in Hijiki and Rice Using the Various Pretreatments and Inductively Coupled Plasma Mass Spectrometry

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Abstract : Several pretreatment methods have been developed to reduce the inorganic arsenic, which is known to be highly harmful to humans, among various arsenic species present in hijiki and rice. The pretreatment methods were selected and developed as methods that can be non-harmful even after treatment and easily applied. Hijiki was applied by two methods. One was soaking in water at room temperature for various durations and the other was boiling of it in water for a short period of time. Rice was soaked in water with different rice-to-water ratios for various durations. The most effective method that reduced the inorganic arsenic in hijiki was to repeat parboiling for 5 minutes twice, which led to 79% reduction of the inorganic arsenic in it. In the case of rice, soaking for 24 hours at the ratio of 1:5 (rice:water) resulted in 51% reduction of inorganic arsenic in rice.

Key words : Arsenic speciation, inorganic arsenic, ion chromatography, inductively coupled plasma-mass spectrometry

Introduction

Arsenic had been used as a raw material for pesticides and wood preservatives.[1] The International Agency for Research on Cancer (IARC) under the World Health Organization (WHO) classified arsenic and inorganic arsenic compounds into Group 1 (carcinogenic to humans).[2]

Toxicity of arsenic compounds depends on its chemical forms. Inorganic arsenics such as arsenate (As^{5+}) and arsenite (As^{3+}) are toxic. They might increase the risk of cancer and cardiovascular disease.[3-5] Dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are less toxic compared to As^{5+} and As^{3+}. Arsenobetaine (AsB), arsenocholine (AsC), and arsenosugars are almost non-toxic.[6-8] Thus, it is important to determine and reduce the inorganic arsenics in food.[9-11]

In general, As^{3+}, As^{5+}, DMA, and MMA are mainly present in rice.[12,13] Rice is known to be one of the agricultural products with high concentration of inorganic arsenics.[14-16]

Accordingly, the Joint Expert Committee on Food Additives (JECFA) recommended a maximum level for inorganic arsenics in milled and parboiled rice as 200 mg/kg.[17] The European Commission regulated the maximum levels of inorganic arsenic in rice ranging from 100 mg/kg to 300 mg/kg depending on the type of food.[18] China also set the maximum level of inorganic arsenic to 150 mg/kg.[19] Korea Food and Drug Association (KFDA) has established the maximum contaminant level of inorganic arsenic in rice to be 200 mg/kg,[20] as Europe and China.

Hijiki (*Hizikia fusiforme*) has been also known to contain a high arsenic concentration. Previously it was reported that the arsenic concentration in its dried leaves was 231.0 mg/kg and that in the dried branches was 91.2 mg/kg.[21] In particular, the arsenic species was mainly inorganic arsenic that is arsenate.[22] KFDA has established the maximum contaminant level of inorganic arsenic in hijiki-based food to be 1 mg/kg[23], while the maximum in China and Australia are 1.5 mg/kg and 1 mg/kg, respectively.[19,24]

Several methods to reduce the arsenics in hijiki and rice have been reported so far, but most of them have been done for the total arsenic and the cooking methods reducing total arsenic.[22,25-31] Few studies have been done for the reduction of inorganic arsenic.[22,27,32-35] Park, et al. reported a method to reduce total arsenic, inorganic arsenic and arsenobetaine in hijiki.[22] It showed that heating hijiki at 90°C and soaking it in 2% NaCl aqueous solution could reduce 80-90% of the inorganic arsenics in it.

In this study, several pretreatment methods applicable before cooking have been investigated to reduce inorganic

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arsenics present in hijiki and rice. The pretreatment methods not noly unharmful but also easily applicable were suggested to reduce inorganic arsenic in hijiki and rice.

Experimental

Instruments

Arsenic species in rice and hijiki were separated and measured by ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS). The IC system consists of a separator column (250 × 4.1 mm, PRP – X100 10 μm, Hamilton), a guard column (20 × 2.1 mm, PRP – X100 10 μm, Hamilton), and a pump (GS50, Dionex). For sample injection, a 6-port 2-position injection valve (Rheodyne) and a syringe (750 RN, Hamilton) were used. An ICP-MS (SPECTROMS, Spectro) was used for determination of the separated arsenic species. The operation parameters of the IC-ICP-MS are listed in Table 1.

For the sample preparation, water bath (WB-11, Daihan Scientific, Korea), ball mill (MM-440, Retsch, Germany), sonicator (Powersonic 420, HwaShin tech, Korea), centrifuge (VS-550, VISION SCIENTIFIC Co. LTD, USA), heating block (MaXtable H10, Daihan Scientific, Korea), vortex mixer (VM-10, Daihan Scientific, Korea), oven (LDO-250F, Labtech, Italy), 50 mL tube (Centrifuge tubes, JET Biofil, China) were used.

Chemicals

Standard solutions of As^{3+} (sodium metaarsenite, 98%), As^{5+} (sodium arsenate dibasic heptahydrate, ≥98%,), MMA (disodium methylarsenate hexahydrate, 98%) and DMA (sodium cacodylate, ≥98%) were purchased from Sigma-Aldrich (USA). Internal standard of AsB (RM No 626, arsenobetaine calibrant solution, 1031 mg/kg) was purchased from BCR (EU). For sample extraction, the nitric acid (HNO_3, 70%) and methanol (CH_3OH ≥99.9%) were obtained from Sigma-Aldrich (USA). The stock solutions of 100 mg/kg using As^{3+}, As^{5+}, DMA, and MMA were prepared. Deionized water (18 MΩ×cm) was prepared using the water purification system (Purelab, ELGA, UK) for preparing all samples and standards.

For the extraction of arsenic species from hijiki, the mixture of water and methanol (the ratio 1:1) was prepared. For the eluent of IC, 20 mM ammonium phosphate was prepared using ammonium phosphate dibasic (NH_4HPO_4, ≥98%, Sigma-Aldrich, USA). The pH of the eluent was adjusted to 6 using phosphoric acid (85-95%, Fluka, UK).

Table 1. Operating parameters for IC-ICP-MS.

IC	
Analytical column	PRP – X100 (10 μm, 250 × 4.1 mm)
Guard column	PRP – X100 (10 μm, 20 × 2.1 mm)
Flow rate	1 mL/min
Injection volume	200 μL
Mobile phase	20 mM ammonium phosphate (pH 6)
ICP-MS	
RF power	1450 W
Argon flow	
Coolant	12 L/min
Auxiliary	0.85 L/min
Nebulizer	2.3 L/min
Monitor *m/z*	74.9

Sample preparation

Rice

Rice was obtained from local markets (Muangun, Chonnam, South Korea). The rice samples were soaked in water for the various times (6, 12, and 24 h). The ratios of rice to water were set to 1:1, 1:2, and 1:5 at room temperature. After the pretreated samples were completely dried in the oven at 40°C, those were powdered by a ball mill. A 1.0000 g of the powdered sample was placed in the 10 mL extraction solvent of 0.28 M HNO_3 for two hours,stirred vigorously by a vortex mixer, and heated at 95°C for 30 min. Then, it was cooled down and centrifuged at 3500 rpm for 10 min. After the supernatant was filtered by 0.45 μm filter, the final volume of 9.0000 g was prepared with the addition of 800 μL H_2O_2 to oxidize As^{3+} to As^{5+} for analysis.[36]

Hijiki

Dried hijiki (Wando, South Korea) were obtained. A 50 g of the dried sample was soaked in 1 L distilled deionized water (DDW) at room temperature as a soaking method to reduce arsenic species. For a parboiling method, the sample of 50 g was parboiled in a hot water (95°C) while the temperature was constantly maintained in a thermostat. After the pretreated samples were completely dried at the 40°C oven, those were powdered by ball mill. The powdered samples of 0.4000 ± 0.0005 g were placed in the 20 mL extraction solvent, a 1:1 mixture of methanol and water, for two hours by the ultrasonicator, then it was centrifuged at a 4500 rpm for 20 min. After the supernatant was filtered by 0.2 μm filter, the solvent of methanol was removed at a thermostat (64°C) for 2.5 h. The final volume of 50 g was made by DDW (by weight) for analysis.

Results and Discussion

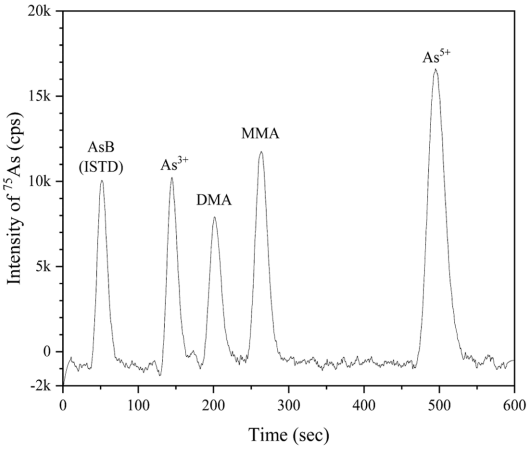
Chromatogram of arsenic species in rice and hijiki

The prepared samples were introduced into the anion exchange IC which separates arsenic species, then determined by ICP-MS. The chromatogram of 10 mg/kg standard arsenic species (As^{3+}, DMA, MMA, and As^{5+}) with the internal standard of AsB is shown in Figure 1a.

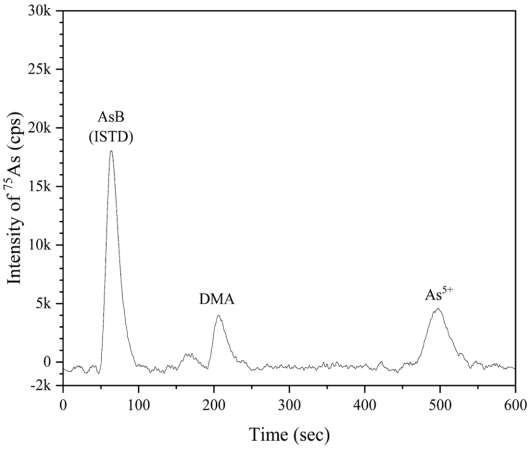
The internal standard was injected before sample injection. In this study, As^{3+} was oxidized to As^{5+} because, in most case, the sum of As^{3+} and As^{5+} is referred to as total inorganic arsenic.

The chromatogram of raw rice is shown in Figure 1b. The major species in the rice sample were found to be arsenate and DMA. The chromatogram of the raw hijiki sample was shown in Figure 1c that indicated the major species was arsenate.

a) Chromatogram of 10 μg/kg standard arsenic species (As^{3+}, DMA, MMA, As^{5+})



b) Chromatogram of arsenic species in Rice



c) Chromatogram of arsenic species in hijiki

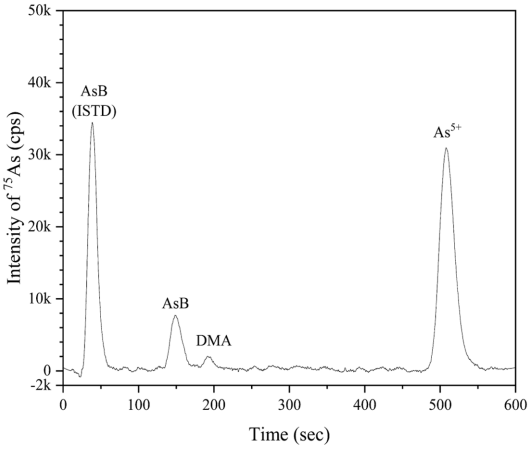


Figure 1. Chromatograms of arsenic species in hijiki and rice.

Rice

Rice was soaked in water for a while (1 – 24 hours) at room temperature. The mass ratios of rice to water were varied from 1:1 to 1:5. Actually, the soaking method is widely used before cooking in Asian countries.

The soaking time was fixed to 6 h, then the ratios of rice to water were varied by 1:1, 1:2, and 1:5. The inorganic arsenic in rice was reduced by 20-25% as shown in Figure 2. The ratios of rice to water did not significantly affect the reduction; The variation of results with the ratios was within the standard deviation.

The soaking time was fixed to 12 hours, then the ratios of rice to water were varied by 1:1, 1:2, and 1:5. The reduction of inorganic arsenic in rice by the 12 hours of soaking was much better than that that by the 6-hour soaking. As shown in Figure 2, the inorganic arsenic in rice was reduced by 19%, 40%, and 45% at 1:1, 1:2, and 1:5, respectively. It was found that the soaking time was important.

As shown in Figure 2, when the soaking time was fixed to 24 h and the ratios of rice to water were varied by 1:1, 1:2, and 1:5, the inorganic arsenic in rice was reduced by 39%, 44%, and 51% at 1:1, 1:2, and 1:5, respectively. The longer soaking time was much effective in reducing the inorganic arsenic in rice. We did not investigate further in terms of soaking time in consideration of the realistic time

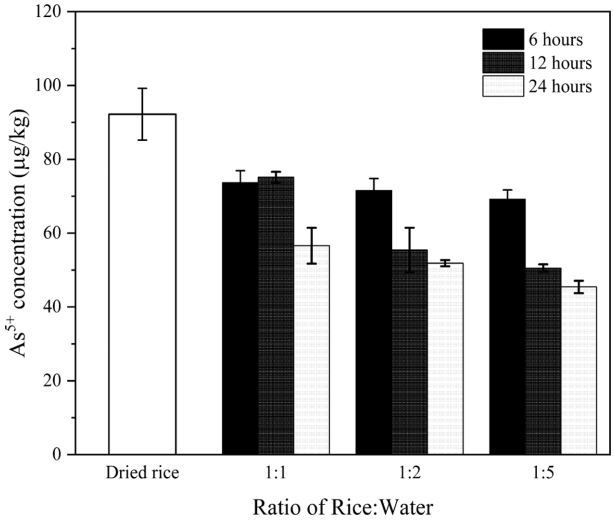


Figure 2. The concentration change of inorganic arsenic in rice by 6 to 24 h soaking method. The error bar represents standard deviation of three replicate measurements.

used in cooking. We could expect the longer soaking time and the increment of ratio of water to rice could reduce the inorganic arsenic in rice further.

Hijiki

In this study, various pretreatments methods were investigated for the purpose of reduction of inorganic arsenic in hijiki.

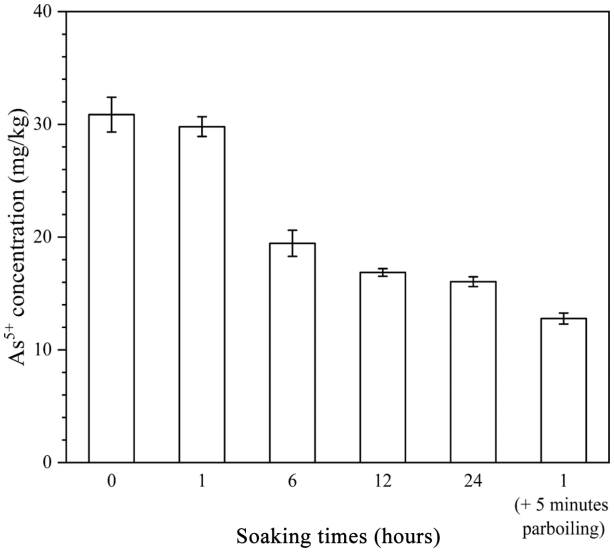


Figure 3. The concentration change of inorganic arsenic in hijiki by soaking methods with time. The error bar represents standard deviation of three replicate measurements.

Hijiki was soaked in the water for a while (1 – 24 h) at room temperature, then the inorganic arsenics in it were measured. The average concentrations of inorganic arsenic were reduced by 3.5%, 37%, 45% and 48% by 1, 6, 12, and 24 h of soaking, respectively, as shown in Figure 3. The results showed that the longer soaking could reduce the larger amount of inorganic arsenic in hijiki. Finally, the soaking (25°C) and parboiling (95°C hot water) methods were combined. It was parboiled for five minutes after it was soaked in the water for an hour. It resulted to the reduction of inorganic arsenic in hijiki by 58%. It was realized that the parboiling was very effective for the reduction of inorganic arsenic in hijiki.

Hijiki was parboiled in the hot water for a short time (5-30 min), then the inorganic arsenic in it was measured. As the results are shown in Figure 4, the parboiling for 5 min was the most effective. It reduced the inorganic arsenic by 63%. It is interesting to observe that the effect was decreased as it was parboiled for the longer time. As the results could be dependent on the instrument setting and individual capabilities, the same experiments were repeated three times. However, the same results were obtained. The result implies that the hijiki might absorb the inorganic arsenic back in water.

To investigate the parboiling effect further, hijiki samples were parboiled for 5 min twice. The hot water was refreshed for the second parboiling after the first parboiling water was discarded. It reduced the inorganic arsenic by 79%. It was the most effective and simple method, and it might be very useful for people who cook hijiki.

The performance of acetic acid for reduction of inorganic acid in hijiki was also investigated. As the results are

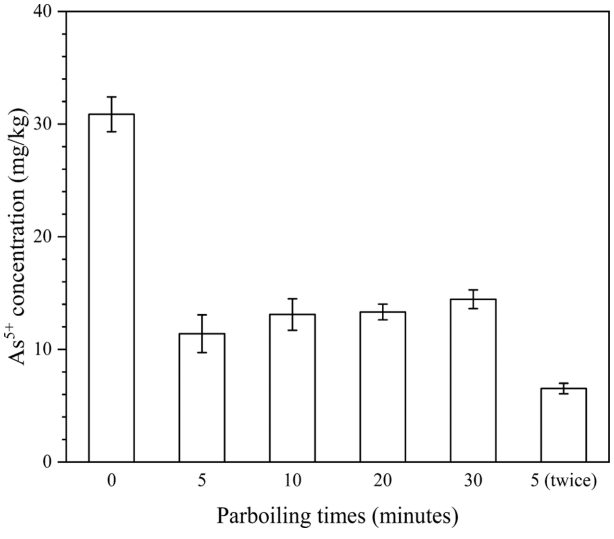


Figure 4. The concentration change of inorganic arsenic in hijiki by parboiling treatment method. The error bar represents standard deviation of three replicate measurements.

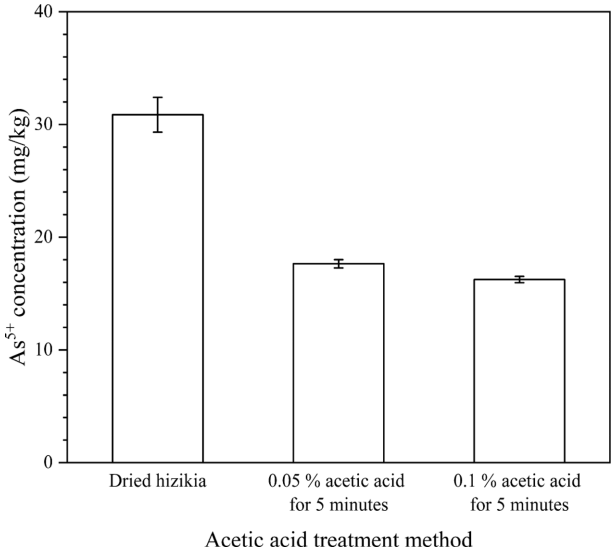


Figure 5. The concentration change of inorganic arsenic in hijiki by acetic acid treatment method. The error bar represents standard deviation of three replicate measurements.

shown in Figure 5, the 0.05 and 0.1% concentrations of acetic acid were used while hijiki was parboiled in hot water (95°C) for 5 min. As a result, the inorganic arsenics were reduced by 43% and 47% at the 0.05 and 0.1% acetic acid solution, respectively. It was found that the acetic acid was less effective than the water.

Conclusions

Several pretreatment methods were investigated for the reduction of inorganic arsenics in rice and hijiki by IC-ICP-MS. The parboiling method of hijiki for five minutes twice could reduce 79% of the inorganic arsenic in it. The soaking method with the ratio of 1:5 (rice:water) for 24 h could reduce 51% of inorganic arsenic in rice. The suggested methods are not labor-oriented and time-consuming. The intake of inorganic arsenic by rice and hijiki might be significantly reduced using these simple pretreatments before cooking.

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