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Original Article

Effects of digestive enzymes on the retained arsenic in dried Hijiki, *Sargassum fusiforme*

Yohko SUGAWA-KATAYAMA[‡1)], Masayuki KATAYAMA[1)], Hiroko IMANISHI[2)] and Kei TOMITA[2)]

[1)] *Department of Health and Nutrition, Osaka Aoyama University**,
[2)] *Department of Health and Nutrition, Gifu Women's University***

Summary

Water-swollen samples of Hijiki, *Sargassum fusiforme*, were treated with two digestive enzymes, pepsin from porcine stomach mucosa and pancreatin from hog pancreas, at 37℃ for 3, 6, 15 and 24 hr. Two fractions of arsenic, one dissolved and the other retained in the Hijiki residues were determined by thermal neutron activation analysis.

The concentration of the retained arsenic in the Hijiki residues was 27 μg As/g dry residue weight after single treatment with pepsin, 25 μg As/g dry residue weight after single treatment with pancreatin and 24 μg As/g dry residue weight after successive treatment with pepsin followed by pancreatin.

Keywords: Hijiki, *Sargassum fusiforme*; digestive enzymes (pepsin and pancreatin); arsenic concentration; thermal neutron activation analysis.

Introduction

Arsenic levels in commercial dried Hijiki products could be effectively diminished by soaking them in water in a pre-cooking process[1－3)]. However, the existing state or behavior of the retained arsenic, amounting to 20% or more of the total, was obscure. To re-evaluate the usefulness of Hijiki[4)], it is important to know the behavior of the retained arsenic in the Hijiki residues. In the digestive tract, the morphological structure of Hijiki may be altered to affect the state of the retained arsenic. To study the possible events of ingested Hijiki in the digestive tract, the authors used two digestive enzymes, pepsin and pancreatin in a model experiment.

After the digestive-enzyme treatment, more than 70% of the retained arsenic in Hijiki became soluble, and thus the amount of un-dissolved arsenic could be excreted.

Materials and Methods

1. Hijiki samples

The commercial products of Hijiki used were dried mixtures of leaves, stalks and apexes, and they were stored below 4℃ until use. The dried Hijiki samples were generously donated from the Tsushima Archipelago-Third Sectional Hijiki Processing Company, Nagasaki Pref. The average arsenic content of these samples was 89.1 ± 6.4 μg/g dry weight[2)]. The samples were cut into pieces of 0.5 to 1 cm in length, put into small vials along with incubating solutions, and placed in a constant-temperature water-bath.

2. Incubation with digestive enzymes[5)]

The dried Hijiki was swollen with 30 volumes of water at 30℃ for 20 min, and to this mixture was added more H_2O containing the digestive enzymes, pepsin and/or pancreatin. The mixtures were incubated at 37℃ for 3 hr, 6 hr, 15 hr and 24 hr.

1) Single treatment with pepsin

The Hijiki samples, suspended in 200 volumes of H_2O, were adjusted to pH 2 with 1 N HCl, and then mixed with 20 volumes of 1% pepsin solution. They were incubated at 37℃. After various time intervals, an aliquot of the mixture was neutralized with 1 N NaOH to pH 7, cooled down to ice-cold temperature and terminated the enzyme reaction with 20 volumes of 0.3 N trichloroacetic acid. The solution and the residues were separated[2)] and stored at −40℃.

‡ E-mail: katayama@osaka-aoyama.ac.jp
* Address : 2-11-1 Niina, Minoo City, Osaka 562-8580, Japan
** Address : 80 Taromaru, Gifu City, Gifu 501-3592, Japan

2) Single treatment with pancreatin

The swollen samples suspended in 200 volumes of water were adjusted to pH 7 with 1 N NaOH, and mixed with 20 volumes of 1% pancreatin solution. After incubation at 37℃ for various time intervals, an aliquot of the mixture was cooled down to ice-cold temperature, and terminated the enzyme reaction with 20 volumes of 0.3N trichloroacetic acid. The soluble fraction and the residues were separated[2] and stored at −40℃.

3) Successive treatment with pepsin and pancreatin

The swollen samples in 200 volumes of H_2O were adjusted to pH 2 with 1 N HCl, and mixed with 20 volumes of 1% pepsin solution. After 3 hr of the pepsin treatment at 37℃, the mixtures were neutralized to pH 7 with 1N NaOH, and mixed with 20 volumes of 1% pancreatin solution. After various time intervals of incubation at 37℃, the mixture was cooled down to ice-cold temperature, and terminated the enzyme reaction with 20 volumes of 0.3N trichloroacetic acid. The soluble fraction and the residues were separated[2] and stored at −40℃.

3. Arsenic determination[6, 7]

A measured volume of the soluble fraction and a weighed amount of the residues were lyophilized, and their arsenic concentrations were determined by thermal neutron activation analysis using a research reactor of Kyoto Research Reactor Institute, Kyoto University. The thermal neutrons were irradiated at 10^{13} neutrons $cm^{-2}\cdot sec^{-1}$ for 20 min. After 72 hr of cooling time, arsenic contents were determined by a gamma detector equipped with pure Ge.

4. Reagents

Reagents were Special Grade Reagents of JIS or its equivalent grade.

Pepsin, prepared from porcine stomach mucosa, was the product of the grade, 1 : 100 activity, supplied by Wako Co., Ltd.

Pancreatin, prepared from Hog pancreas, was supplied by Wako Co., Ltd. This preparation had protease activity of more than 28,000 units/g at pH 8.0, lipase activity of more than 960 units/g, and amylase activity of more than 2,800 units/g.

Results

1. Time course changes of arsenic concentrations retained in the Hijiki residues without digestive enzymes

Without digestive enzymes in the soaking water, the releasing process of arsenic in the dried Hijiki into water was expressed as the amount of retained arsenic concentrations in the residues as shown in Fig. 1. The values at 37℃ of arsenic retained in the Hijiki residues were interpolated from the values[2] at 30℃ and those at 45℃. After 1 hr, arsenic release no longer occurred.

2. Arsenic dissolved by pepsin

The time course of changes in the retained arsenic is shown in the top section of Fig. 2, indicating 69.8% of the total arsenic was dissolved by the single treatment with pepsin. The retained arsenic concentrations of the residues were similar after treatment for 3 to 24 hr, the average being 26.9 μg per g of dry residues (Table 1).

3. Arsenic dissolved by pancreatin

The time course of changes in the retained arsenic shown in the middle section of Fig. 2, indicates that 72.2% of the total arsenic was dissolved by the single treatment with pancreatin. The dissolved ratio did not change after the incubation period of 3 to 24 hr. The arsenic concentration retained in the Hijiki residues was similar after treat-

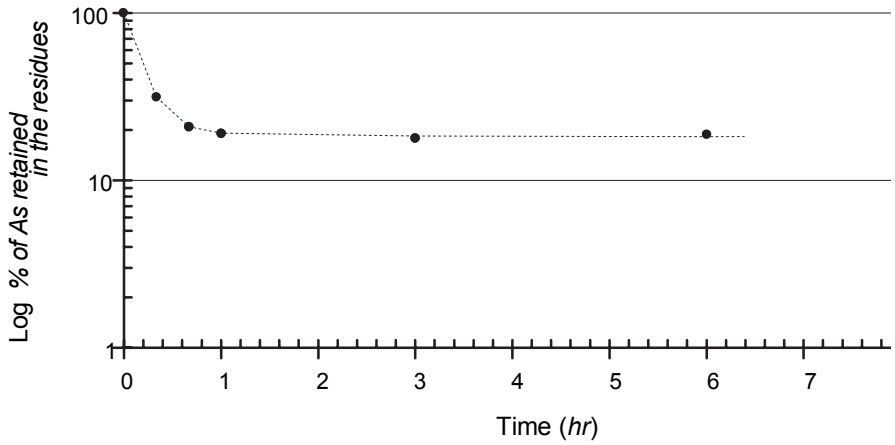

Fig. 1 The time course of changes in the retained arsenic concentration during water-soaking without the digestive enzymes at 37℃. The values were interpolated from the values[2] at 30℃ and those at 45℃.

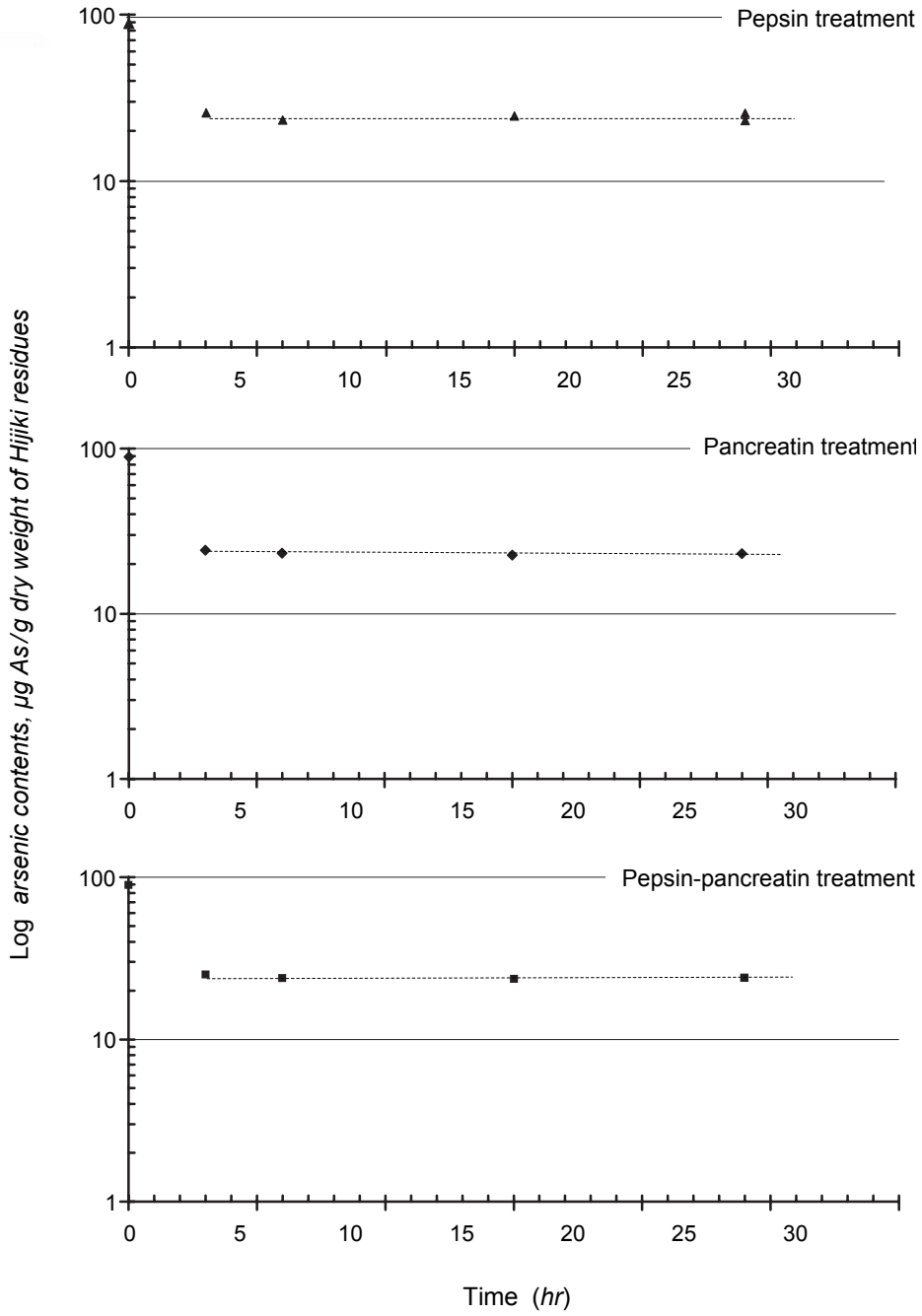

Fig. 2 Time courses of changes in the arsenic cocentration in the Hijiki residues during incubation with the digestive enzymes, pepsin and/or pancreatin, at 37℃, for 3, 6, 15 and 24 hr. The experimental conditions were as describd in the text and in Table 1.

Table 1 Arsenic concentrations in the Hijiki residues after treatment with digestive enzymes

Treatment with	μg As/g dry weight of Hijiki Residues
Pepsin	26.90 ± 2.19
Pancreatin	24.84 ± 3.67
Pepsin and pancreatin	23.48 ± 0.59

Hijiki samples, swollen in water, were treated with pepsin and/or pancreatin at 37℃ for 3, 6, 15 and 24 hr. The experimental conditions were as described in the text.
The data in duplicate or quadruple measurements were expressed in means ± SD of the concentrations at all the time points.

ment for 3 to 24 hr, the average being 24.8 μg As/g of dried Hijiki residues (Table 1).

4. Arsenic dissolved by successive treatment with pepsin followed by pancreatin

By the successive treatment with pepsin followed by pancreatin, the retained arsenic slightly diminished. The time course of changes in the retained arsenic is shown in the bottom section of Fig. 2. The dissolved arsenic was 73.6%, resulting in the mean arsenic concentration 23.5 μg As/g of dried Hijiki residues (Table 1). These values indicate that the effects of pepsin and pancreatin were not additive.

Discussion

In the previous report[2], the amount of retained arsenic was estimated by assuming that the water compartment in the residues was uniform with respect to the arsenic concentration. Comparison of the previous values[2] (Fig. 1) with the present data (Table 1) suggests lack of uniformity in the water compartment of the residues. Even if the discrepancy was fully compensated, the effect of the digestive enzymes may be less than 10%, indicating their resistance to digestion by the enzymes.

During the digestive enzyme treatment, the time course of changes in the retained arsenic concentration hardly changed (Fig. 2); the reaction of the digestive enzymes might have been finished within 3 hr. Otherwise, major components fixing arsenic in Hijiki, *Phaeophyceae* family, could not be affected by the digestive enzymes. In relation to this, big components of Mekabu, a sporophyll of Wakame, was hardly affected by digestive enzymes[5]. Wakame, *Undaria pinnatifida* is also a family of *Phaeophyceae*.

The arsenic contained in Hijiki residues after the water-soaking may be mostly excreted without being absorbed, as the retained arsenic in the tissues after the action of the digestive enzymes was nearly identical in its content before and after the enzyme treatment.

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