Original Article

Effects of boiling treatment upon the distributions of selenium and mercury in short-necked clam

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Abstract
In this paper, the effect of boiling treatment upon the distribution of selenium and mercury in the edible tissues of the short-necked clam *Ruditapes philippinarum* was investigated in relation with each elution profile of both elements to the cooking liquid (generally called "*Kaijiru*" in Japan). During the boiling treatments (5 min, 10 min, 20 min and 30 min at about 90 ℃), the variation of both levels were not apparently observed in the edible tissues, however, the level of dissolved selenium in the cooking liquid tended to increase slightly with time. The chemical species of the dissolved selenium was mainly organic. On the other hand, dissolved mercury was not detected from the fact that the mercury level was extremely low in the edible tissues. This suggested that a trace amount of Hg-Se complex as a final detoxified substance was not excreted out of body by means of the boiling treatment. The Se/Hg value (the molar ratio of selenium to mercury) in the edible tissues of the short-necked clam as an indicator of the safety of marine products was almost constant (about 100 ≧ 1) through all boiling treatments, and then the safety of mercury in the edible tissue was confirmed against the boiling treatments.

Key words
selenium, mercury, distribution, short-necked clam, boiling treatment

1. Introduction

In recent years, several findings on human health have been obtained concerning marine foods, from the chemical point of micro-nutrients. Especially, it has been reported that the extracts from small-sized bivalves such as the short-necked clam *Ruditapes philippinarum* are very healthy (Baba, 1955; Yoneda, 2011). However, the behavior of trace elements is little known during the processing of shellfish.

Kaijiru is one of the traditional processed foods in Japan, and therefore it will be noteworthy to investigate the distributions of selenium and mercury as trace bio-related elements in *Kaijiru* as one of the simply processed sea foods.

In this present paper, firstly the effects of boiling treatment upon the distribution of selenium and mercury in the edible tissues of the short-necked clam as a material of *Kaijiru* are reported, in relation with each elution profile of both elements to the cooking liquid.

2. Materials and methods

2.1 Materials

2.1.1 Edible tissue samples

Ten specimens of the short-necked clam *Ruditapes philippinarum* from Yamaguchi Prefecture were submitted in the present study. The ranges of shell length and edible weight were 2.8 to 3.2 cm and 1.32 to 2.01 g on the basis of wet weight, respectively. There was little difference of the degree of growth in each specimen. The whole edible tissues were removed from these shell bodies, and stored in a freezer at -30 ℃ until analyzed. Those sample's concentrations are shown on the basis of the dry weight as about 85 % of water content

2.1.2 Edible tissue and cooking liquid samples treated by boiling

Each 1.5 kg of whole weight of short-necked clams containing about 35 wt% of edible tissues were boiled in a pan filled with 2.0 L of distilled water for 5, 10, 20 and 30 min at about 90 ℃, respectively. Each whole edible tissue was sampled from 50 specimens of each portion, and stored in a freezer at –30 ℃ until analyzed. Those sample's concentrations are shown on the basis of the dry weight as about 85 % of water content. Each filtrate was used as a cooking liquid.

2.2 Methods

2.2.1 Determination of selenium in edible tissue samples

The oxidation number of selenium exists as –2, +4, and +6 in aquatic organisms. The minus divalent selenium exists as an organic form, and this form is the selenide species assigned to the selenohydryl groups (-SeH or SeHg and SeCd) substituting for sulfur of the thiol group or bonding to heavy metals such as Hg and Cd. The chemical forms of the plus tetravalent and hexavalent seleniums are the selenite and selenate species joined to two neighboring thiol groups in the protein, respectively (Gasiewicz and Smith, 1978; Cappon and Smith, 1981; Iwata et al., 1982).

The total selenium concentration and the concentration of the low oxidation states of selenium (selenide and selenite species) (abbreviated as T-Se and [Org.Se+Se(IV)], respective-

ly) in each specimen were measured using gas chromatography with an electron capture detector (Toei and Shimoishi, 1981). The concentration of the selenate species was estimated by the difference between T-Se and [Org.Se+Se(IV)], and abbreviated as Se(VI).

2.2.2 Determination of dissolved selenium in cooking liquids

Each filtrate described in 2.2.1 was obtained with the double step filtrations using a glass filter (mesh size: 17G3) and a membrane filter (core size: 0.45 μm). Each filtrate was diluted to 1/100 and then measured using gas chromatography with an electron capture detector (Shimoishi, 1973). In the determination of dissolved selenium, Org.Se and Se(IV) was separately determined and abbreviated as Org.Se and Se(IV), respectively.

The total selenium concentration and the concentration of the selenite species was also abbreviated as T-Se and Se(VI), as the case of the abbreviation in tissues of the short-necked clam, respectively.

2.2.3 Determination of mercury

The total mercury concentration in each specimen was measured by a flow injection analysis system using cold vapor atomic absorption spectrometry (FIAS-CV-AAS) preceded by a wet digestion in a microwave oven, and abbreviated as T-Hg (Aduna de Paz et al., 1997). Those sample's concentrations are also shown on the basis of the dry weight as about 85 % of water content only in the case of edible tissue samples.

3. Results and discussion

3.1 Selenium and mercury distributions in edible tissues

The ranges of [Org.Se+Se(IV)], Se(VI) and T-Se were 1.22_6 to 2.28_0, 0.32_0 to 1.11_3 and 1.54_6 to 3.32_0 μg/g ($1.98_0 \pm 0.10_0$, $0.63_3 \pm 0.08_7$ and $2.61_3 \pm 0.16_7$ μg/g as each mean concentration), respectively.

The range of T-Hg was 0.04_7 to 0.07_3 ($0.06_7 \pm 0.00_7$ μg/g as a mean concentration), respectively.

3.2 Selenium and mercury distributions in edible tissues treated by boiling

Each mean concentration of [Org.Se+Se(IV)], Se(VI) and T-Se in edible tissues boiled for 5, 10, 20 and 30 min at about 90 °C were $2.13_3 \pm 0.19_1$, $0.711 \pm 0.15_0$ and $2.84_4 \pm 0.22_1$ μg/g, $2.19_9 \pm 0.20_0$, $0.82_8 \pm 0.11_5$ and $3.02_7 \pm 0.32_1$ μg/g, $2.23_8 \pm 0.15_0$, $0.47_1 \pm 0.01_2$ and $2.70_9 \pm 0.18_0$ μg/g and $2.55_2 \pm 0.15_0$, $0.25_8 \pm 0.11_0$ and $2.81_0 \pm 0.15_1$ μg/g, respectively.

The mean concentration of T-Hg in edible tissues boiled for 5, 10, 20 and 30 min at about 90 °C was $0.07_8 \pm 0.00_7$, $0.07_3 \pm 0.00_7$, $0.07_2 \pm 0.00_7$ and $0.06_5 \pm 0.00_7$ μg/g, respectively.

Both element data described above are shown with boiling times in Figure 1, and the variation of the mean molar fraction of selenium is shown in Figure 2.

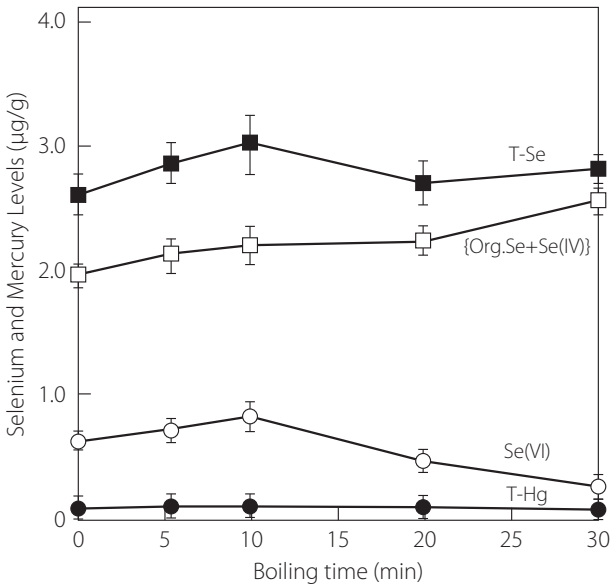

Figure 1: The distribution profiles of selenium and mercury in edible tissues of short-necked clams with boiling time

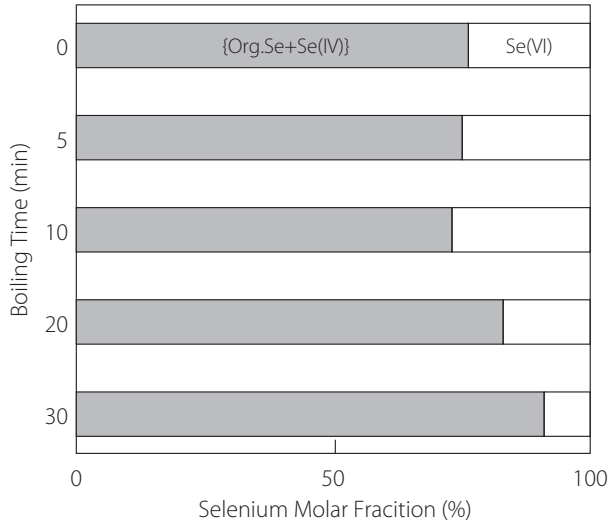

Figure 2: The variation of selenium molar fraction in edible tissues of short-necked clams with boiling time

3.3 Selenium and mercury distributions in cooking liquids treated by boiling

Each mean dissolved concentration of Org.Se, Se(IV), Se(VI) and T-Se in cooking liquids boiled for 5, 10, 20 and 30 min at about 90 °C were $11.90_5 \pm 1.05_1$, $0.72_0 \pm 0.05_4$, $2.39_0 \pm 0.20_1$ and $15.01_5 \pm 1.07_1$ μg/L, $15.96_0 \pm 1.02_0$, $0.53_0 \pm 0.03_4$, $5.16_5 \pm 0.53_1$ and $21.65_5 \pm 1.38_1$ μg/L, $21.34_0 \pm 1.34_5$, $0.68_5 \pm 0.04_4$, $1.28_0 \pm 0.10_1$ and $23.30_5 \pm 1.49_0$ μg/L and $24.73_5 \pm 1.58_3$, $0.44_5 \pm 0.02_8$, $3.37_0 \pm 0.21_6$ and $28.55_0 \pm 1.82_1$ μg/L, respectively.

The dissolved mercury was not detectable in a pan during

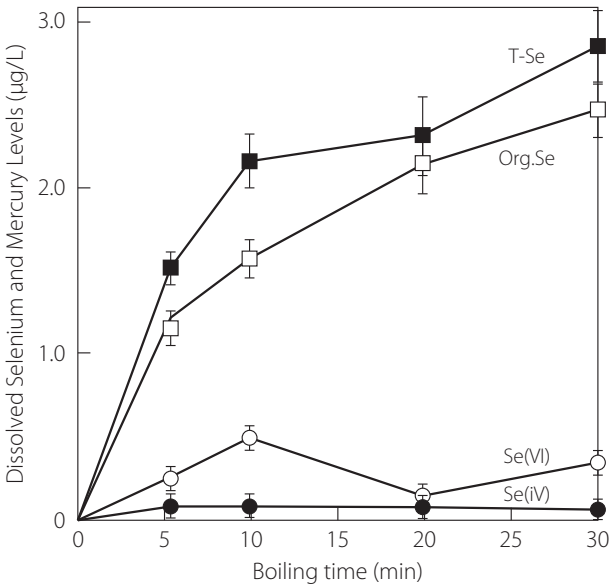

Figure 3: The distribution profiles of selenium and mercury in cooking liquids with boiling time

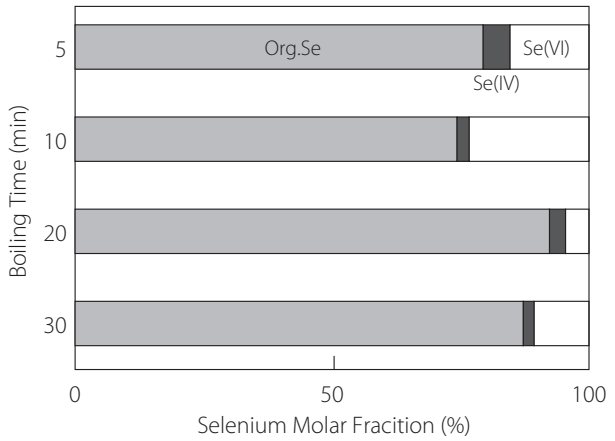

Figure 4: The variation of selenium molar fraction in cooking liquids with boiling time

10, 20 and 30 min of boiling treatments at about 90 ℃.

Therefore, only the dissolved selenium data described above is shown with boiling times in Figure 3, and the variation of the mean molar fraction of dissolved selenium is shown in Figure 4.

4. Conclusion

From the profiles of selenium and mercury distribution shown in Figure 1, it is apparent that there was little change in both element levels by taking into account slight variations such as each experimental values, whole weight or water content of samples in each pan. However, Figure 3 shows that the total dissolved selenium, especially the organic selenium in each cooking liquid, tends to increase with boiling time. Here, the corresponding level is ppb, while both element levels in edible tissues are ppm, suggesting that the distribution ranges of dissolved selenium concentration in Figure 3 are included in the variations of corresponding selenium concentration described in Figure 1.

From the mean molar fraction of selenium in edible tissues shown in Figure 2, the increase of low oxidation states of selenium species is also observed with the decrease of hexavalent selenium species. This may suggest that the selenium is reduced from hexavalent to tetravalent or organic (minus divalent) state during boiling treatments.

On the other hand, from the mean molar fraction of selenium in cooking liquids shown in Figure 4, the significant increase of low oxidation states of selenium species, especially organic selenium, was observed with boiling time. This may be due to the elution of organic selenium as one of final metabolites produced by boiling, in addition to amino acid such as SeCys or SeMet with free seleno-hydryl group without mercury. Here, each elution of hexa- and tetra-valent selenium species were also observed. These may be due to each elution of unmetabolized hexavalent species (SeO^{4-}) and a water-soluble amino acid such as selenotaurin containing tetravalent selenium.

Moreover, a trace amount of selenium-mercury complex as a final detoxified substance may not be excreted from the shellfish body by means of the boiling treatment because dissolved mercury was not detectable.

Concerning the present short-necked clam, each molar ratio of T-Hg to T-Se in the edible tissues during boiling was also calculated as an indicator of safety against toxicity due to the accumulation of mercury (Kai et al., 2013; 2014). The range of those ratios was 92.63 to 109.82 and the mean ratio was 100.63 ± 20.05 ($\geqq 1$). The profile is shown with boiling times in Figure 5 and the ratio was almost constant during boiling, suggesting the safety of those sampled short-necked

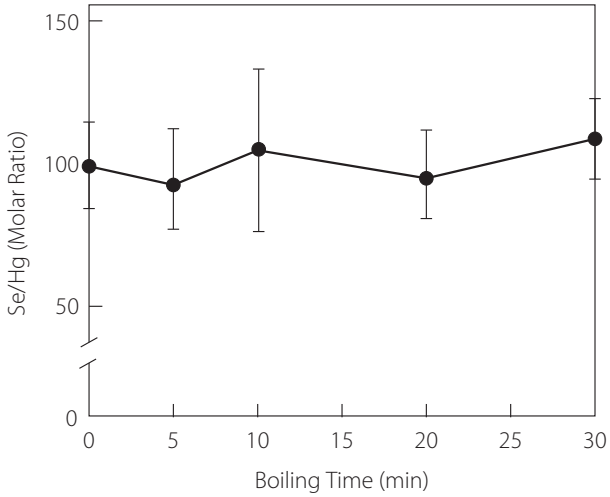

Figure 5: The profile of Se/Hg (molar ratio) in edible tissues of short-necked clams with boiling time

clams treated by boiling.

Further studies on the boiling treatment using several species of fish, shell fish, crustaceans or seaweeds etc., should be made in order to clarify the overall behavior of selenium and mercury in marine products.

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