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Influence of Preparation and Isolation Methods on Calcium Content Measurements in the Sea Water Shrimp (*Metapenaeus sp.*)

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Abstract. Ebi are small, dried shrimp consumed as a whole. Although they are assumed to have high calcium content, the actual calcium content of this genus has not been determined. This study aimed to determine the calcium content in *Metapenaeus sp.* and to investigate the effects of preparation (oven and non-oven) and isolation (dilution, acid digestion, and dry ashing) methods on ion selective electrode and atomic absorption spectrometry measurements. Our results showed that the highest calcium content [6.769 ppm (oven) and 7.785 ppm (non-oven)] was obtained in samples treated with acid digestion. The calcium content differed according to the preparation and isolation methods, indicating that both methods influence the calcium content measurements.

1. Introduction

Indonesia is a primary producer of aquatic resources, including fish, shrimp, and seaweed [1,2]. Shrimp is a high-availability, high-demand commodity, with 600,000 tons produced in 2012 [3]. Shrimp can be consumed fresh or dried to prevent it from decomposing. In Indonesia, dried shrimp is called ebi [4]. Ebi is commonly made from small shrimp because the entire organism, including the body and head, can be processed and consumed as a whole.

The most common types of shrimp used to make ebi in Indonesia are krosok shrimp, red shrimp, and yellow shrimp, all of which are salt water shrimp of the genus *Metapenaeus* [4]. This genus was identified by Wood-Mason and Alcock in 1891 and is thus occasionally called *Metapenaeus* Wood-Mason; species of this genus live in waters with a salinity level of 32,613–36,410 and temperature of 19°C–28°C [5,6].

Studies investigating the nutritional composition of ebi have reported that ebi contain many nutrients and minerals, including calcium. Data reported in 2009 indicate that the consumable portions of ebi have a calcium content of 7.600 ppm [7], whereas a study by Maharani and Julshamn in 1977 has reported a calcium content of 5.080 ppm [8]. Both studies have shown that ebi has a high calcium content (5.000–7.000 ppm), but only the body of shrimp, without the head, was studied. Thus, we performed quantitative measurements of the calcium content of whole ebi (the body and the head), which is assumed to have a high calcium content.

Calcium is an important mineral for humans, necessary for the optimal development of bone and teeth, maintaining fluid and hormone homeostasis, neurotransmission, heart regulation, muscle contraction, and blood clotting [9]. Calcium is the main mineral that forms the enamel and dentin in the



form of hydroxyapatite. The calcium ions in saliva serve to remineralize enamel and decrease the risk of caries in cases of anticipated pH drops that cause demineralization [10]. Calcium also prevents bone resorption, maintains alveolar bone density, and prevents bacterial adhesion to biofilms that lead to dental caries [11].

Because calcium is supplied to the body through dietary intake, intake of foods containing high calcium content is needed. Salt water Ebi (*Metapenaeus sp.*) are an excellent source of calcium that are easy to obtain, can be stored for a long time, are easy to process, and are affordable. Using standard laboratory procedures, the measurement of calcium content in foods requires tissue preparation and the isolation of pure calcium. The methods used for these procedures have not been compared with respect to their effects on calcium content measurements. Such comparisons are needed to ascertain that the quantitative data obtained are accurate.

Quantitative calcium levels can be determined as a measure of free calcium ion (Ca^{2+}) concentration using a potentiometer sensor with an ion selective electrode (ISE) [12,13]. In addition, atomic absorption of free calcium atoms can be determined using atomic absorption spectrometry (AAS) [14]. A combination of these two methods yields the calcium concentration in parts per million (ppm). This reliable, reproducible method allows for the comparison of calcium content between substances.

This study aimed to use this method to accurately determine the calcium content of salt water ebi, determine whether the preparation and isolation methods affect the calcium measurements, and compare measurement methods using ISE and AAS.

2. Methods

Pure, unaltered ebi was collected to be used as samples. Certification that the organisms were of the *Metapenaeus* genus was obtained from the Marine Science and Technology of Institut Pertanian Bogor. The samples were divided into two groups: the oven group (80°C for 20 min) and non-oven group (not heated). The samples from each group were ground in a blender (Braun) until reaching a sand-like consistency. Each sample was then divided into three for different preparation methods: the dilution method, acid digestion, and dry ashing.

2.1. Dilution

The sample (0.5 gram) was poured into a volumetric flask, and aquadem was added. The solution was then filtered and poured into another volumetric flask using a glass funnel and filter paper. To this solution was added 4 M ISAB KCl (1 mL) and aquadem to a final volume of 50 mL. The solution was shaken until it became homogenous.

2.2. Acid digestion

The sample (0.5 gram) was poured into a digestive tube, and 68% dense nitric acid (HNO_3) (8 mL) and 30% hydrogen peroxide (H_2O_2) (1 mL) were added. The tube was then closed, placed in the cartridge, and inserted into a digestive microwave (Sineo, Shanghai Xinyi Wavelet Chemistry Technology Co., Shanghai, China). Samples were incubated at 130°C for 10 min, 150°C for 5 min, and 180°C for 15 min. The solution was then filtered into a volumetric flask using a glass funnel and filter paper. Subsequently, 4 M KCl (1 mL) was added to the solution, followed by aquadem to a final volume of 50 mL. The solution was then shaken until it became homogenous.

2.3. Dry ashing

Tests show that 2 g of ebi produces 0.5 g of ash. The ebi sample (2 g) was placed in a porcelain mug in an oven, and the temperature gradually increased. The starting temperature of 26°C was raised to 450°C and held for 15 min. The temperature was then raised in 50°C increments to a final temperature of 550°C and held for 5 h. Successfully heated ashes were white in color. The mug was then removed from the oven and allowed to cool at room temperature. The mass of ashes was then measured (approximately 0.5 g). The sample was placed in a glass beaker, 65% HNO_3 (1 mL) was added, and the sample was placed on an electric hotplate, with stirring until dry. Further, 37% HCl (1 mL) was added to the beaker,

and the solution was heated to boiling with stirring. Deionized water was then added, and the solution allowed to cool at room temperature. Mineral deposition on the bottom of the glass indicated that the dry ashing was successful. The solution was then filtered into a volumetric flask using a glass funnel and filter paper. Subsequently, 4 M KCl (1 mL) was added to the solution, followed by aquadem to a final volume of 50 mL. The solution was then shaken until it became homogenous.

A standard solution was made using CaCl_2 1000 ppm diluted to concentrations of 50, 100, 150, 200, and 250 ppm. Subsequently, 4 M KCl (1 mL) was added to the solutions, followed by aquadem to a final volume of 50 mL. The potential difference was then measured using ISE or absorbance with AAS as the reference value for the sample solution. From the potential difference (on ISE), the concentration ($[M]$) and log value $\log[M]$ were calculated. A linear regression equation was determined by plotting $\log[M]$ vs potential difference. The potential difference value was then inserted into the equation to obtain the $\log[M]$ value, which was then converted into ppm mass/mass (mg/kg).

Using AAS, a linear regression equation was determined by plotting the concentration (standard ppm) vs. absorbance. The absorbance value of the samples was then inserted into the equation to obtain the ppm mass/mass (mg/kg) value.

3. Results

Figure 1 shows the standard curve for the potential difference vs. $\log[\text{Ca}^{2+}]$, indicating a linear relationship with a slope of 10. The concentration of each sample was determined from the potential difference using the standard curve (Table 1 and Figure 2). In addition to the statistically significant difference in concentrations, there was a strong and positive correlation between oven and non-oven samples using the Pearson correlation with significance set to 0.0001 and a correlation coefficient (r^2) of 0.995.

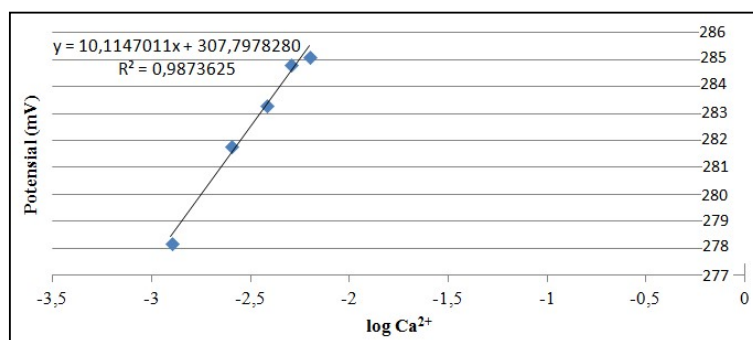


Figure 1. Linear regression graph of ISE standard solutions

Table 1. Potential difference and $[\text{Ca}^{2+}]$ of Ebi (*Metapenaeus sp.*)

Isolation method	Preparation Method			
	Non-oven		Oven	
	Potential difference (mV)	$[\text{Ca}^{2+}]$ (ppm m/m) mg/kg	Potential difference (mV)	$[\text{Ca}^{2+}]$ (ppm m/m) mg/kg
Dilution	299.57	606.0056 (SD, ± 48.1365)	301.6	963.7018 (SD, ± 21.937)
Acid Digestion	231.27	0.107 (SD, ± 0.005)	245.3	2.603 (SD, ± 0.005)
Dry ashing	278.53	1.2812 (SD, ± 148)	292.13	28.3841 (SD, ± 3.5718)

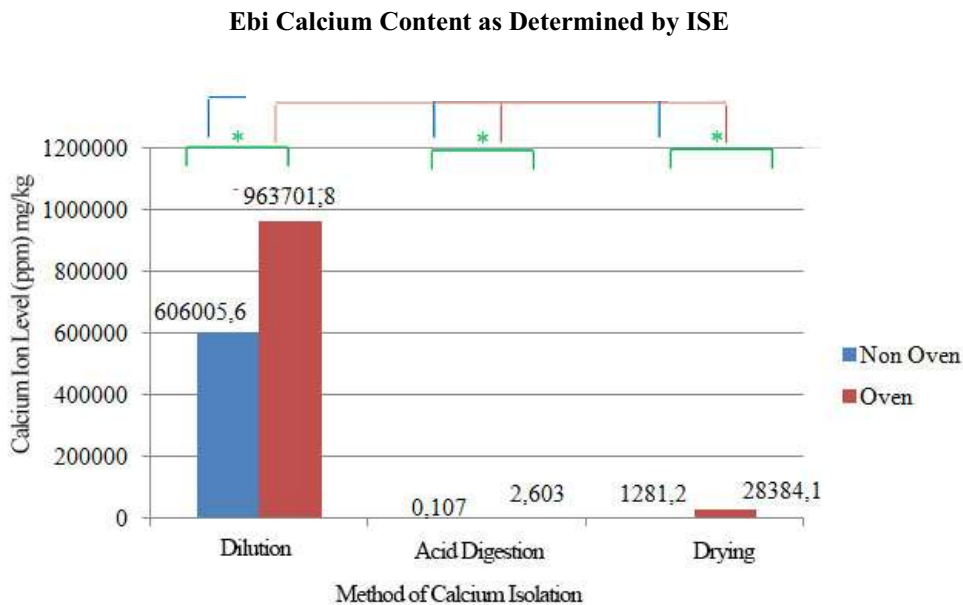


Figure 2. Calcium content of ebi (*Metapenaeus sp.*) as determined by ISE

- *P<0.05 between oven and non-oven preparation method (*t-test*) on each method of isolation
- * P<0.05 between non-oven samples for each method of isolation (ANOVA)
- * P<0.05 between oven samples for each method of isolation (ANOVA)

Figure 3 shows the standard curve of absorbance vs. [Ca]. From the equation, the concentration of samples was determined based on the measured absorbance (Table 2; Figure 4). Further, non-oven and oven samples differed significantly and showed a strong, positive correlation using the Pearson correlation with significance set at 0.0001 and a correlation coefficient (r^2) of 0.947.

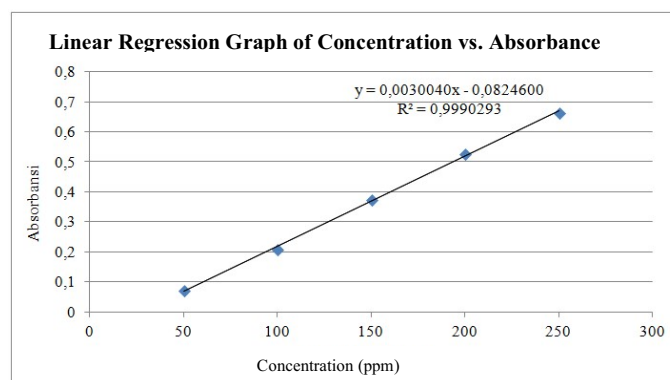
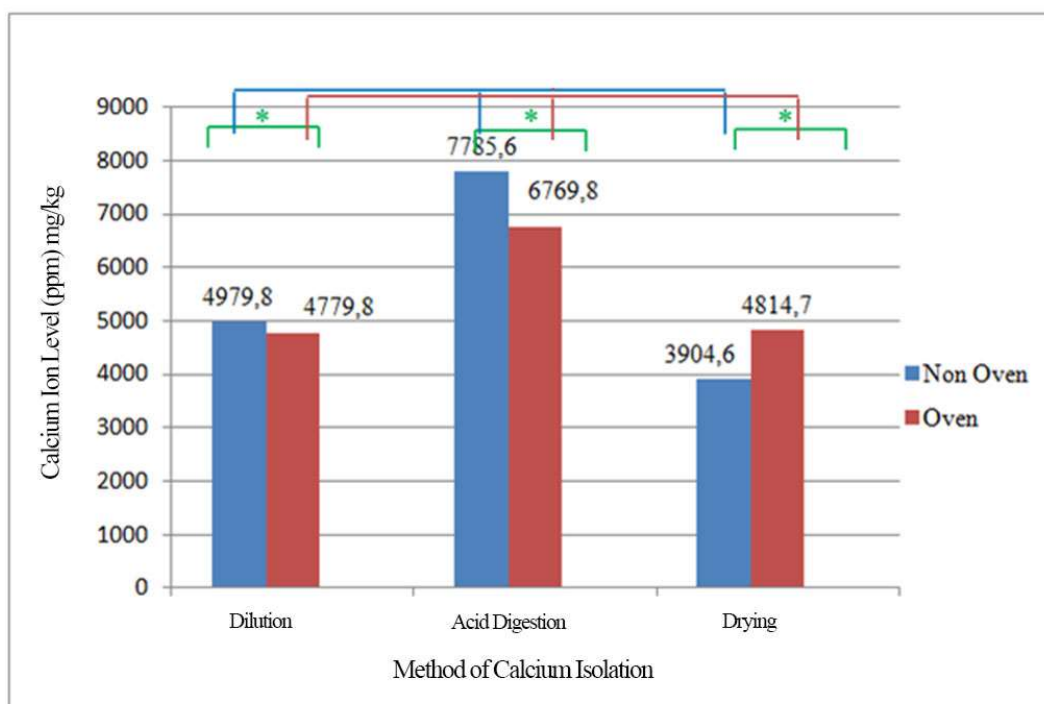


Figure 3. Linear regression graph of ISE standard solutions

Table 2. Absorbance and $[Ca^{2+}]$ of Ebi (*Metapenaeus sp.*)

Isolation Method	Preparation method			
	Non-Oven		Oven	
	Absorbance	$[Ca^{2+}]$ (ppm m/m) mg/kg	Absorbance	$[Ca^{2+}]$ (ppm m/m) mg/kg
Dilution	0.0695	4.9798 (SD, ± 110)	0.0629	4.7798 (SD, $\pm 321,5$)
Acid digestion	0.154	7.7856 (SD, $\pm 85,5$)	0.1246	6.7698 (SD, $\pm 65,4$)
Dry ashing	0.388	3.9046 (SD, $\pm 41,9$)	0.4968	4.8147 (SD, ± 160)

[Ca] in Ebi as Determined by AAS**Figure 4.** $[Ca^{2+}]$ in Ebi as determined by AAS

- *Statistically significant difference between oven and non-oven preparation method (*t-test*) according to isolation method
- *Statistically significant difference between non-oven samples according to isolation method (ANOVA)
- *Statistically significant difference between oven samples according to isolation method (ANOVA)

4. Discussion

The oven group samples showed the highest calcium content in every type of isolation method, as measured using ISE. Heating affects ionization energy, thereby increasing the concentration of Ca^{2+} ions. Because ISE detects free ions, heating results in an increase in the measured $[Ca^{2+}]$ [14]. Among the isolation methods used, dilution resulted in no interference but showed the highest kinetic energy

value, indicating that dilution is a method that releases the highest free calcium ion content; however, the facts are inconsistent. Dilution is the most simple method of isolating an element, affecting only the analyte concentration and main matrix [15]. This method uses a neutral pH solution with no stirring to reduce the risk of contamination and the loss of pure analyte and has a fast processing time. However, dilution does not eliminate all organic materials and does not entirely disrupt ionic bonds [15]. This result happens because of the interferences in measurement. The differing effects of each isolation method results in differences in the measured calcium content of ebi using ISE compared with those previously reported [7,8]. Thus, ISE measurements tend to be biased.

Evidence of bias in ISE measurements can be observed by the slope of 10.115 from the equation $y = 10.1147011x + 307.7978280$ compared with the average slope of the Ca^{2+} ion, which is 26, with 8% error per potential difference. This difference suggested that the ISE tools were contaminated or expired, thereby decreasing the accuracy and precision. Despite the expectation that ISE should give more accurate measurements because it measures free ions created during preparation and isolation, the ISE measurements in this study were of low accuracy due to limitations, such as interference and measurement errors.

Limitations of the ISE electrode include interference by other ions in the sample, selectivity coefficient, low accuracy in measuring high ionic strength solutions, and memory effects [16]. Steps were taken to prevent interference, such as adding ISAB and washing the electrode with deionized water between measurements. Isolation steps were conducted to better purify the calcium ions, but the dilution method still yielded the highest concentrations, indicating that ion interference still occurred.

The highest calcium content was obtained from the dilution method in both the oven and non-oven groups. However, these data were not used in this study because the ISE measurements gave a slope that was not in accordance with the reference data.

Using AAS, the calcium content in non-oven dilution and acid digestion samples was higher than that in oven samples. For the dry ashing method, the calcium content was higher in the oven samples than in non-oven samples. This result can be attributed to several factors. Using the dilution method, the calcium content was higher in non-oven samples because AAS measures the atoms at their lowest energy level [14]. In non-oven samples, kinetic energy is only added through dilution, whereas oven samples also obtain thermal energy during heating [15]. Heating activates the atoms to an excited state; thus, when measured with AAS, they are not at the lowest energy level. In addition, heating can cause ionization interference by newly formed ions, thus reducing the absorbance [17]. The same phenomenon occurs with the acid digestion isolation method, where higher calcium levels are observed in non-oven samples because the digestion process releases more free calcium atoms. Compared with the oven samples, in which ionization interference occurs, the number of atoms at the lowest energy level is lower due to the rapidly formed atomic vapor and the release of bonds and reactions resulting from the influence of strong acids [17]. However, acid digestion proved to be better than dilution for isolating calcium atoms. This study observed that higher calcium levels were detected in samples treated by acid digestion, which uses strong acids to break bonds, and in those treated by microwave digestion, which prevents element loss and keeps the reaction atmosphere isolated so that it is contaminant free.

The measured calcium concentration was higher in dry ashing samples treated in the oven than in non-oven samples. Non-oven dry ashing samples retain more organic materials than samples that undergo the heating process. The resulting effect is a process called “double combustion,” in which external combustion occurs due to the high temperature of the dry ashing process plus internal combustion of the organic material, which burns the analyte and decreases the element concentration [15]. The organic material in dry ashing samples burned perfectly because the preceding heating process prevented double combustion.

The highest calcium levels measured by AAS were in acid-digested non-oven samples (7.7856 ppm). Acid digestion is considered to isolate calcium atoms well at low energy levels and free from ionization interference due to heating during preparation. However, under actual conditions in cultures in which ebi is generally consumed after cooking, the calcium content was higher in acid-digested oven samples than in those prepared using other methods. In ebi, the highest calcium content (7.785612 ppm) was

observed in acid-digested non-oven samples, the calcium content of acid-digested oven samples (6.7698 ppm) was close to that of the reference data for the calcium content of ebi. Our statistical analysis indicated that acid digestion is the best isolation method for obtaining accurate calcium content, with non-oven samples giving higher concentrations than oven samples.

5. Conclusions

This study revealed that the measured calcium content of salt water ebi differs between ebi prepared with and without oven heating. Measured calcium content also differs between samples treated with different isolation methods, including the dilution method, acid digestion, and dry ashing. A correlation was observed between the measured calcium content and the preparation and isolation methods used. The acid digestion isolation method leads to higher calcium content than dry ashing or dilution when measured by AAS. Calcium content measurement using ISE did not yield valid results.

6. References

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