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EFFECT OF COOKING METHODS ON THE REDUCTION OF LEAD (Pb) CONTENT IN KEPAH (*Polymesoda erosa*) SHELLS

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ABSTRACT

Kepah shells have important economic value and are widely used as food ingredients because they contain high nutritional content. Heavy metals that enter the waters will generally be accumulated in sediments and absorbed by biota. As filter feeder, Kepah accumulate heavy metals from the environment that toxic to humans. This study aimed to determine the effect of cooking methods on the concentration of lead (Pb) in Kepah shell. The measurement of Pb content was conducted according to SNI 2345.5-2011 by Atomic Absorption Spectrophotometer (AAS). Result showed that different cooking methods influenced the Pb content in Kepah shells. Frying and boiling processes reduced the Pb concentration of 46.34% and 31.48%, respectively.

Keywords: Kepah; Polymesoda erosa; frying; boiling; lead (Pb)

INTRODUCTION

Seafood contains nutrients such as omega-3 fatty acids, essential amino acids, minerals, vitamins, and low cholesterol (Ikem and Egibor, 2005; Sioen *et al.*, 2007; Ganjavi *et al*., 2010). Shellfish products are widely used as food ingredients because they contain high nutritional content such as protein, vitamin B12, essential amino acids, and other important mineral such as Ca, Fe, Zn, Cu (Baboli and Velayatzadeh, 2013; Nurjanah *et al*., 2014). As one of the fisheries product, Clam shells (known Kepah) have important economic value. In addition, this organisms also contain high nutrients, including protein (7.06-16.87%), fat (0.40-2.47%), carbohydrates (2.36-4.95%), and provide energy of 69-88 kcal/100g of meat (Amin, 2009).

Heavy metal pollution is a serious problem that needs to be considered (Haq-Nawaz *et al*., 2011). This is due to the characteristics of heavy metals which are toxic and biologically it can not be degraded (Lata *et al.*, 2013). Heavy metals that enter the waters will generally be accumulated in sediments and absorbed by biota, therefore benthic organisms that live permanently in these habitats have the potential to be contaminated (Tabari *et al*., 2010; Mendil *et al*., 2010; Vieira *et al*., 2011). Heavy metal contamination can be transferred to the human body through the food chain which has the potential to cause health problems (Ismahene and El-Hadi, 2012).

Previous study showed that shellfish as a filter feeder can accumulate heavy metals from the environment (Nguyen *et al*., 2013; Lata *et al*., 2013; Alyani *et al*., 2017; Dharmadewi, 2020) more than other types of aquatic biota (Darmono, 2001). Therefore, shellfish are widely used as aquatic bioindicators (Chen *et al.*, 2004; Jean-Christophe *et al*., 2004; Jeena and Abbas, 2010; Monica *et al*., 2016). The estimation of heavy metal content in clam body tissue can provide information about pollution conditions and monitoring the potential risk of contamination in humans (Guerra-Garcia *et al*., 2010).

Aquatic organisms, including shellfish are considered a major source of heavy metal contaminants for humans (Dural *et al*., 2006; Turkmen *et al*., 2006) through direct consumption of contaminated organisms. These metals are stored in the body and will be transferred to the highest levels in the food chain (Norouzi *et al*., 2012; Palaniappan and Karthikeyan, 2009; Al-Yousuf *et al*., 2000). One of the heavy metals that are toxic and harmful to human health is lead (Pb) (Djedjibegovic *et al*., 2012; Sari *et al*., 2014; Lomolino *et al*., 2016). Lead (Pb) is bioaccumulative in the body of organisms (Saputra, 2018). The toxicity of Pb lead anemia and growth retardation in children (Rashed, 2001), tissue damage, disorders of intelligence, reproductive disorders (Wibisono, 2005; BSN, 2009), and disorders of the central nervous system (Islam *et al*., 2015; Castro-González and Mendez-Armenta, 2008).

Many coastal community, especially in West Kalimantan consume seafood including shellfish. The presence of heavy metals accumulated in the body of aquatic organisms encourages various efforts to reduce the levels of these metals. There are various methods of shellfish processing before consumption (Kananke *et al*., 2015), such as boiling, steaming, frying, and roasting (Inobeme *et al*., 2020). In general, cooking or processing improves the taste, aroma, and delicacy. Several studies reported that cooking methods can reduce the concentration of lead in shellfish (Satrya *et al*., 2019), shrimp, lobster (Gheisari *et al*., 2016), and fish (Mehdipour *et al*., 2018). This study aims to evaluate the effect of different cooking methods on the reduction of lead (Pb) content in Kepah shells (*polymesoda erosa*).

RESEARCH METHODS

Samples Collection and Analysis

Kepah samples were obtained from the seller in Peniti Village, Mempawah Regency, West Kalimantan. The cooking process was carried out at the Marine Science Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. Analysis of water content, ash content, and determination of the concentration of lead (Pb) metal was carried out in the laboratory of PT. Sucofindo (Persero) Pontianak, West Kalimantan.

Preparation of $Pb(NO_3)_2$ Solution

Put as much as 3.312 g of $Pb(NO_3)_2$ into a volumetric flask, then added with distilled water, shaken until completely dissolved, then adjusted to final volume of 100 mL.

Samples Preparation

Sample preparation was carried out by separating the meat and shells of Kepah. Then, meat was cleaned using aquadest then drained. Samples then cut into small pieces and put into a pan. Furthermore, added ± 50 mL of 100 ppm $Pb(NO_3)_2$ metal solution stock, stired, and was left for 2 hours in closed condition. A hundred ppm of $Pb(NO_3)_2$ is an initial heavy metal concentration before a reduction in processing. After that, sample was weighed of ±75.6 g for each control treatment, boiling, and frying.

For boiling process, heat the water until it reached at 100 ºC, then sample was put into the pan for 45 minutes (Sari *et al*., 2014). Furthermore, sample was drained, cooled to room temperature, then put in a zip plastic. Whereas the frying process, heat the oil then sample was put into a frying pan until it was completely submerged and cooked for 25 minutes. Then, the sample was drained, cooled to room temperature, and put in a zip plastic.

Determination of Water and Ash Content

The determination of water content was carried according to the AOAC method (2005) while the determination of ash content was held according to the AOAC method (2007). The water and ash contents were calculated following the formula :

$$\text{water content } (\%) = \frac{B - C}{B - A} \times 100\ \% \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where A is weight of empty porcelain crucible (g), B is weight of porcelain crucible + initial sample (g), and C is weight of porcelain crucible + dry sample (g).

$$\text{ash content } (\%) = \frac{W1 - W2}{W} \times 100\ \% \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where W is weight of sample before ashing (g), W_1 is weight sample + of porcelain crucible after ashing (g), and W_2 is weight of empty porcelain crucible (g).

Dry Ashing

The dry ashing was conducted according to the SNI 2354.5:2011 method (2011). Each sample that had been dried and refined was weighed of 0.5g in a porcelain cup. Furthermore, positive control was prepared by making spiked Pb as much as 0.5 mL of a standard solution of Pb 1 mg/L, then added to the samples before being put into the ashing furnace. Spiked was evaporated on a hotplate at a temperature of 100 ºC until dry. Samples and blanks were put into the ashing furnace and the temperature was increased to 100 ºC step by step every 30 minutes until it reached 450 ºC and maintained for 18 hours.

Samples and blanks were removed from the ashing furnace and cooled to room temperature. After cooling, added 1 mL of HNO_3 65%, shaken until all the ash was dissolved in the acid, then evaporated on a hotplate at 100 ºC until dry. After that, samples and blanks were put into the ashing furnace. Temperature was gradually increased by 100 ºC every 30 minutes until it reached 450 ºC and maintained for 3 hours. Hereinafter, samples and blanks were cooled at room temperature. As much as 5 mL of 6 M HCl was added to each sample, then shaken until all the ash was dissolved in the acid. Furthermore, evaporated on a hotplate at a temperature of 100ºC, then added 10 mL of 0.1 M HNO_3 and the solution was transferred to a 50 mL volumetric flask and adjusted with 0.1 M HNO_3. The Pb contents was calculated following the formula :

$$Pb\ (\mu g/g) = \frac{(D - E) \times Fp \times V}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where D is sample concentration (µg/L), E is concentration of blank solution (µg/L), Fp is dilution factor, V is final volume of sample solution (mL), and W is sample weight (g).

RESULT AND DISCUSSION

Characteristics of Kepah Shells

According to the morphological observations, samples of kepah have dark brown shell, hard, bilateral symmetry, flat at the edges and convex in the middle. In addition, morphometric measurements were also carried out, including length, width, thickness of the shell, and total weight.

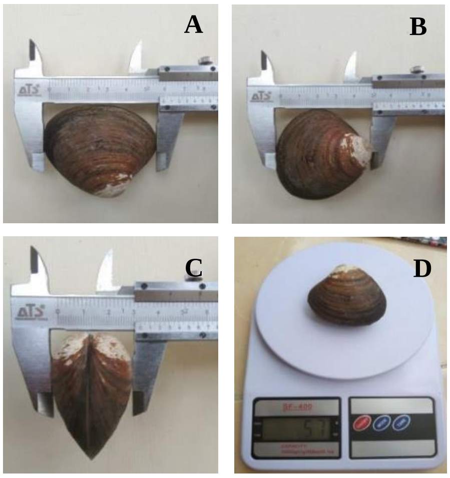

Figure 1. Morphometric characteristics of Kepah (A) length (B) width (C) thickness (D) total weight

A twenty of Kepah were collected in this study. The result showed that the average value of the Kepah shell length was 50.7 mm, width was 4.68 mm, thickness was 2.59 mm, and the total weight was 41.5 g. Previous studies exhibited that Kepah found in the mangrove vegetation of *Avicennia marina* in Tanah Laut Regency waters, South Kalimantan, had an average shell length of 41.3 mm and width of 34.7 mm. While Kepah found in *Apiculata* have length of 41.31 mm and width of 34.46 mm (Kadarsah and Susilawati, 2019).

Table 1. Morphometric Measurements of Kepah

Morphometric parameter	Average
Length (mm)	50.7
Width (mm)	46.8
Thickness (mm)	25.9
Weight (g)	41.5

The environmental parameter conditions greatly affect the growth of shellfish, such as temperature, salinity, pH, and characteristic of substrate. Kepah grows optimally in tidal zones with muddy substrate and high organic matter content (Yunitawati, 2012), and these habitats are mostly found in estuary (Kelana *et al*., 2015). West Kalimantan has many estuary flows that support the growth and development of shellfish, so they have a relatively large morphometric size. Furthermore, estuaries are one of the most productive waters with nutrient input from both land and ocean (Rangkuti *et al*., 2017).

Concentration of Lead (Pb) in Kepah Shells

We observed the effect of cooking/processing methods on the concentration of lead (Pb) in Kepah shells. The result showed that the concentration of Pb for frying and boiling process had a lower value than the control. This indicates that cooking process can reduce the concentration of heavy metals contained in Kepah tissue. Frying method decreased the concentration of Pb of 53.66 ppm or about 46.34% (Table 2). Several factors that affect the decrease in heavy metal concentrations include temperature, time, and type of media (Morshey *et al*., 2015; Adebote and Eke, 2018) used during the processing, as well as the addition of acid solutions (Yusbarina and Marlianis, 2013; Sari *et al*., 2014; Mahardhika *et al*., 2016). Frying method at high temperatures can accelerate protein degradation (Ganjavi *et al*., 2010). These conditions resulted in the release of bonds between proteins and various types of metals including lead (Pb). The Pb ions dissolve into the oil and will evaporate along with the evaporation of the oil as a frying medium.

Table 2. Concentration of Lead (Pb) in Kepah Shells

Treatment	Replicat 1 (ppm)	Replicat 2 (ppm)	Average (ppm)	Reduction (%)
Control	100	100	100	0
Frying	54.21	53.11	53.66	46.34
Boiling	68.01	69.02	68.52	31.48

Several previous studies observed that frying can reduce Pb content in fish (Diaconescu *et al*., 2013; Ibrahim *et al*., 2018; Inobeme *et al*., 2020), and shrimp (Atia *et al*., 2018). Furthermore, Atia *et al*. (2018) reported that frying can reduce as much as 60% of Pb concentration. The decrease was due to Pb dissolved into the oil during the cooking (Gokoglu *et al*., 2004). In many cases, fish meat showed a significant decrease in heavy metal concentrations after processing. Heat treatment of frying method triggers a decrease heavy metals concentration in the tissues of all fish species (Mitra *et al*., 2011; Devi and Sarojnaline, 2012).

In this study, frying method was carried out using palm oil. It is known that the boiling point of palm oil is between 173-227 ºC (Japir *et al*., 2016). In addition, frying using vegetable oil (sunflower) has also been reported to reduce Hg metal content from fish fat (Panichev and Panicheva, 2016). In Indonesia, many people consume shellfish because they contain high nutritional value such as essential and non-essential amino acids (Abdullah *et al*., 2013; Abdullah *et al*., 2017), omega-3 and omega-6 fatty acids, as well as various types of minerals (Nurjanah *et al*., 2013). Shellfish are processed using various cooking methods to increase added value. Frying was conducted using oil as heating medium (Muchtadi, 2008). The frying method has several advantages, such as relatively short processing time and heat food ingredients below 100 ºC (Ghidurus *et al*., 2010).

The Pb concentration decreased to 68.52 ppm (31.48%) after boiling process with temperature of 100 ºC. The decreasing value was lower than frying method. This is due to the temperature during the boiling process was not high enough, even though boiling uses water media which allows heavy metals to dissolve. Several previous studies have stated that boiling can reduce the metal content of Pb in lobster and shrimp (Gheisari *et al*., 2016), fish (Ersoy, 2011; Mehdipour *et al*., 2018), and shellfish (Nujanah *et al*., 2015; Atia *et al*., 2018). Nurjanah *et al*. (2014) also showed a decrease in the metal content of Pb in Kupang Merah shellfish (*Musculista senhausia*) after the boiling process. Fresh *M. senhausia* samples taken from Kenjeran Beach Surabaya, East Java contained Pb of 0.66 ppm and decreased to 0.13 ppm after boiling.

Another factor leading the heavy metal reduction is processing time. Atia *et al*. (2018) reported that boiling for 30 minutes can reduce the heavy metal content in shrimp. This decrease due to the loss of these metals during processing as free salts, or may be related to dissolved amino acids and non-coagulated proteins that bind to metals (Ersoy *et al*., 2006; Ganbi, 2010; Hajeb *et al*., 2014). Heating during cooking process accelerate protein degradation (Ganjavi *et al*., 2010) which affect the heavy metal content in food. High temperatures also cause changes in the chemical form of the metal or separate the metal from the proteins found in shellfish (Bala *et al*., 2017). Furthermore, Sari *et al*. (2014) also reported that boiling the meat of *Anadara granosa* for 45 minutes showed the highest reduction in Pb levels (32.33%) compared to boiling for 15 minutes (24.8%) and 30 minutes (31.86%).

Moreover, the addition of acid was also reported to reduce the concentration of heavy metals. Mahardhika *et al.* (2018) stated that soaking green mussels with 100% tomato fruit solution for 90 minutes without processing reduced Pb concentration very significantly (59.79%), compared to soaking for 30 minutes (32.98%) and 60 minutes (39. 17%). Another study described that soaking treatment using lime can reduce Pb concentration from 102.02 g/L to 56.85 g/L or 45.59% after boiling (Alpatih *et al*., 2010).

Common way to process shellfish for consumption is done by boiling method. Boiling using water as media will increase the solubility of the substances (Winarno, 2008).

Therefore, the estimated heavy metal residue in food, especially shellfish, is an important step to protect consumers from animal protein sources. In addition, it is an effective strategy in reducing heavy metal content. Cooking process plays an important role in changing the texture, color, food taste, increasing the solubility and digestibility of nutrients, as well as destructing and reducing the number of microbes (Estiasih and Ahmadi 2009). Processing is also important to make a better flavor of food and a safe product for consumption (Gerber *et al*., 2009). In addition, the cooking method also affect the concentration of heavy metals (García-Arias *et al*., 2003; Bayen *et al*., 2005; Oyekunle *et al*., 2020), and the level of metal toxicity in food (Eboh *et al*., 2006; Ahmed *et al*., 2011; Ajani *et al*., 2013).

Moisture Content and Ash Content in Kepah Shells

Kepah shells which had been added with 100 ppm of Pb solution and previously dried showed the water content of 36.12% per dry weight. Previous studies reported that water content in several shellfish such as *Anadara granosa* fresh meat was 74.37% (Nurjanah *et al*., 2005), *Solen* sp. (82.31%) (Nurjanah *et al*., 2013), and *Musculista senhausi* (81.57%) (Nurjanah *et al*., 2014). Other studies also measured the water content of *Meretrix metrix* (79.98%), *Pholas dactylus* (83.78%), *Babylonia spirata* (78.44%) (Abdullah *et al*., 2017), and fresh *P. erosa* (83.28%) (Sukina *et al*., 2020).

The water content is closely related to the body size of the biota, the greater the size and weight of the body, the greater the water content (Abdullah *et al*., 2017). In addition, the water content is also influenced by species, age of biota, different environmental conditions, and the level of freshness of the organism (Ayas and Ozugul, 2011). High water content in fishery products cause rapid damage if not accompanied by a good handling process.

Table 3. Moisture Content and Ash Content in Kepah Shells

Parameter	Value (%)
Moisture content	36.12
Ash content	8.12

The value of ash content in Kepah shells was 8.12%. Exposure to Pb increased the mineral content in shellfish. Based on the results of previous studies, the ash content in fresh meat of *P. erosa* was 1.53% (Sukina *et al*., 2020). The value was lower because there was no addition of metals or minerals. Abdullah *et al*. (2017) stated that the ash content in *M. meretrix* was 1.37%, *P. dactylus* (1.19%), and *B. spirata* (1.20%). Other studies also observed the ash content of *M. senhausi* (1.86%) (Nurjanah *et al*., 2014), *Solen* (1.53%) (Nurjanah *et al*., 2013), and *A. granosa* (2.24%) (Nurjanah *et al*., 2005). Ash is an inorganic substance residual from the combustion of an organic material. Ash content plays an important role as the main indicator to see the mineral content in the organism's body (Abdullah *et al*., 2017). The mineral content of fishery and aquatic products is highly dependent on the ash content (Nurimala *et al*., 2015).

CONCLUSION

Different cooking methods influenced the Pb content in Kepah shells. Frying and boiling processes reduced the Pb concentration of 46.34% and 31.48%, respectively.

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