Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

Heavy metals concentration in "etak" tissue at different processing stages

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Received 23 November 2018
Accepted 17 February 2019
Online 23 May 2019

Keywords:
Corbicula fluminea, "etak", heavy metals, traditional snack, Kelantan

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Abstract
Corbicula fluminea, known as "etak" in Kelantan, Malaysia is a freshwater bivalve. This clam is widely consumed as traditional snack by the locals. However, lately there are numerous reports in local newspapers that claim "etak" to cause health effects due to eating contaminated "etak". Hence, this study aims to determine the heavy metals concentration in fresh, smoked and exposed "etak" sold at the stall as a baseline study in order to develop a method for removing the heavy metals content in its tissues. This study involves sample collection in the stalls around Kelantan, sample preparations via acid digestion and heavy metal determination using Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer. The results showed the heavy metals (Cr, Zn, Mn and Cu) concentrations (μg/g) in all "etak" tissue were at different level for fresh (Cr: 1.02±0.35ppm, Zn: 74.57±2.76ppm, Mn: 40.22±9.96ppm and Cu: 15.27±1.41ppm), smoked (Cr: 0.42±0.02ppm, Zn: 54.62±17.83ppm, Mn: 50.13±2.31ppm and Cu: 20.94±8.81ppm) and exposed (Cr: 0.53±0.08ppm, Zn: 63.07±8.44ppm, Mn: 50.41±6.92ppm and Cu: 12.80±0.40ppm) samples. The results obtained were compared with the permissible limits set by Malaysian Food Regulations 1985 (Cu: 30.0ppm and Zn: 100.0ppm), FAO/WHO 1984 (Mn: 5.4ppm) and IAEA – 407 (Cr: 0.75ppm). For fresh "etak", Zn and Cu concentration were below the standards, while Mn and Cr were exceeded the permissible limits. For smoked "etak" and exposed "etak" at stall, all heavy metals concentration were within the permissible limits, except Mn contents. This study successfully determine the baseline concentration of the heavy metals in "etak".

1. INTRODUCTION

Asian clams (*Corbicula fluminea*) is the popular traditional snack among Kelantanese with the local name known as "etak". In aquatic ecosystems, "etak" can be considered as the most important species. This species also appeared as a small clam that covered with shell and slightly a triangular in shape. The colour of its shell is usually yellowish brown and black with concentric sulcation as shown in Figure 1 (Mouthon, 2001). This species are able to spread rapidly due to its rapid growth rate, high fecundity and early sexual maturity (Sousa, 2008). In North America, "etak" was portrayed as a standout amongst the most essential molluscan bother species at any point brought into the United States (Yan et al., 2009). This species will produce eggs and followed by sperm when they were reaching on their maturity stage. They produced eggs and sperms simultaneously (Ishibashi et al., 2003).

This clam is also the most requests freshwater shellfish for consumption in a few states in Malaysia for example, Kelantan, Terengganu, Perak and Pahang. In general, there is a growing demand for Asian clams in Malaysian markets especially in Kelantan. They consume smoked "etak" as a snack. The preparation of smoked "etak" will begin with marinating process followed by smoke process before it ready to be consumed.

Figure 1: Fresh "etak" (before smoked) sampled in Kelantan, Malaysia.

There are three processing stages of "etak" which may contribute to the accumulation of heavy metals. In the first stage, this clam is able to accumulate heavy metals from polluted river or water stream in its tissue due to their inherent filter feeder ability. Second stage is during the smoking stage of "etak". After "etak" harvesting, the local

eISSN Number: 2462-2389

depurate the clam in clean water. Then, a two hour marinating process with garlic, lemon grass, salt and monosodium glutamate (MSG) was carried out prior the smoking process above the firewood. "Etak" also could accumulate the heavy metals in the last stage as the smoked "etak" is sold in the morning market and along the roadside where the pollution comes from surrounding such as vehicle emission. All of the three stages are suspected in concentrating the heavy metals. Lately there are numerous reports in local newspapers that claim "etak" to cause health effects due to eating contaminated "etak" (Aweng & Mohamed, 2011). Hence, the aim of this study is to determine the concentration of selected heavy metals (Cr, Zn, Mn and Cu) in fresh, smoked and exposed "etak" sold at the stall as a baseline study in order to develop a method for removing the heavy metals content in its tissues. Thus, this study is necessary to evaluate the concentration of heavy metals contaminants that may accumulate in the "etak" tissue. The study focused on the heavy metals contaminants in fresh "etak" tissues, smoked "etak" tissues and exposed "etak" tissues at the selling point, prior to identify the source of pollutants and in order to propose a safe smoking method of "etak" in order to secure the food security and provide customer trust to the smoked "etak" sellers.

2. MATERIALS AND METHODS

2.1. Sampling of "etak"

The sampling of fresh, smoked and exposed "etak" at the selling point was performed for three months duration. A total of 200 pieces of "etak" for all three stages were collected from the "etak salai" stall at Kampung Kasar, Pasir Mas, Kelantan (N 6°2'46.78732", E 102°10'12.31856") as shown in Figure 2. The shell length and height of each clams were measured using digital caliper to ensure the constant adult size of the clam. The tissues was composed and dried in oven for 3 days at 60°C to get a consistent weight. After that, the dried samples were grinded using pestle and mortar to obtain homogeneous sample. The samples were kept in zipper bag and stored in desiccator (for storage). In order to get an accurate heavy metals concentration, the samples analysis were carried out in triplicates.

2.2. Sample preparation and heavy metals analysis

A total of 5 gram of sample was digested by using hot plate for fresh and smoked "etak" tissues. The dried samples were mixed with 5 mL of H_2SO_4 and 5 mL of HNO_3 in a beaker. Then, the beakers were set on hot plate at 60°C for 30 minutes. After the reaction was happened, the beaker was covered with watch glass. After 30 minutes, the beaker was allowed to cool and 10 mL of HNO_3 was added. The temperature was rise gradually until 150°C. After all the tissues were completely digested, all the samples were let to cool at room temperature and 1 mL of 30% H_2O_2 was added for every half an hour until the clear solution appear. After the clear solutions were obtained, the samples then were filtered through Whatman® qualitative filter paper, Grade 1. The digestion process was referred to Perkin Elmer Corporation (1996).

Then, the solutions were continued filtered by using syringe filter before transfer into 50 mL of falcon tube and follow up with dilution with deionized water. The filtrate was stored in refrigerator at 20°C before undergoing further analysis. All of the samples were analysed for Cr, Mn, Zn and Cu using a Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer (AAS). The method was suggested by previous study by Wong et al. (2017).

Figure 2: The smoking and selling of "etak". A: the smoking platform of "etak"; B and C: the selling of "etak" along the roadside in Pasir Mas, Kelantan.

3. RESULTS AND DISCUSSION

The length, height and weight of "etak" were tabulated in the Table 1. A total of 300 pieces of individual "etak" were analyzed for heavy metal in this research. The size of the "etak" ranged from 11.73 mm to 28.31 mm in length, averaging 20.04±1.84 mm. While, Figure 3 displays the heavy metals content in "etak" tissues for all three stages with permissible limits (Malaysian Food Regulation 1985, FAO/WHO 1984 and IAEA – 407) concentration border lines.

Table 1: The length, height and weight (mean ± standard deviation) of "etak".

	Length(mm)	Height(mm)	Weight(g)
Mean	20.04±1.84	21.17 ±7.18	3.78 ± 0.86

From the result obtained, all of the heavy metals concentration detected were compared to Malaysian Food Regulations 1985(Cu: 30.0ppm and Zn: 100.0ppm), FAO/WHO 1984 (Mn: 5.4ppm) and International Atomic Energy Agency (IAEA – 407) (Cr: 0.75ppm) (Wyse et al., 2003). The Cr in the fresh samples (1.02±0.35ppm) were higher than the standard value, and it reduced to the safe value after smoked (0.42±0.02ppm). The Cr value was then

eISSN Number: 2462-2389

increased after exposed in the selling site (0.53±0.08ppm) which was still within the standard value. High Cr level in the fresh samples was supported by Govind (2014) that found Cr is usually accumulated in aquatic animals from the natural habitat especially freshwater. The Cr remain in the tissue through the metal-protein bonding (Frank et al., 2008). Meanwhile, the reduction of Cr concentration in smoked samples was believed due to the effect of depuration in water at postharvest (El-Gamal, 2011). The Zn concentration in "etak" tissue had the same trend like Cr, but all the Zn concentration in three processing stages were within the standard limits. The Zn concentrations in "etak" tissues were found 74.57±2.76ppm in the fresh samples, 54.62±17.83ppm in the smoked samples and 63.07±8.44ppm in the exposed sample at the selling sites.

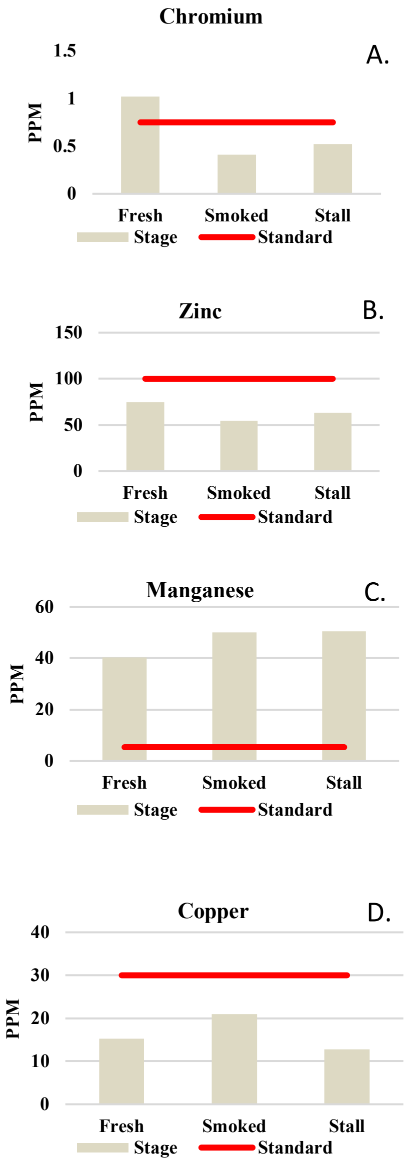

Figure 3: The concentration of heavy metal (A) Chromium; (B) Zinc; (C) Manganese; (D) Copper in the tissue of "etak" at each processing stage.

Mn concentrations in the "etak" tissue at all stages were recorded higher than the Malaysian Food Regulation 1985 and potentially affect the health of the consumers. The Mn level in fresh sample (40.22±9.96ppm) indicates that the Mn was accumulate naturally by the clam from the dissolution of sediment into the river water with low pH (Ha et al., 2011). The Mn was found higher in the smoked sample (50.13±2.31ppm) and carried over to exposed sample (50.41±6.92ppm), which can be explained by the firewood ashes that were transferred to "etak" soft tissue during the smoking process (Kalembkiewicz, 2008). On the other hand, the Cu were within the limit of the standard, but smoked "etak" (20.94±8.81ppm) showed higher Cu concentration than fresh (15.27±1.41ppm) and exposed "etak" (12.80±0.40ppm). This may be due to the contamination at the smoking stage whereby the ash from the fire attached to the "etak" tissue and remain in the flesh by the drying process cause by the heat (Maar et al., 2018).

4. CONCLUSION

The research showed that Zn and Cu concentrations (ppm) in all of the "etak" samples were safe and below the permissible limits set by Malaysian Food Regulations 1985, while concentration of Cr in fresh stage and Mn in all processing stages exceeding the permissible limits set by IAEA – 407 and FAO/WHO 1984. This study successfully determine the baseline concentration of the heavy metals content in "etak" and their possible source from the environment, thus could be a guideline for safety consumption of smoked "etak" in the future. The future research can focus on reducing the heavy metals concentration in the "etak" and cook this clam in a hygienic processing condition.

ACKNOWLEDGEMENT

We would like to express our deepest gratitude to the Malaysia Ministry of Higher Education TRGS grant coded R/TRGS/A08.00/00244A/005/2016/000389 for supporting this research. We would also like to thank Universiti Malaysia Kelantan for providing facilities and instruments for conduct this research.

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eISSN Number: 2462-2389