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Effectiveness of Apple Cider Vinegar Immersion with and Without Mother as a Decrease in Lead (Pb) Levels in Green Mussels (*Perna viridis*)

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Abstract: Lead is a heavy metal that often pollutes aquatic biota such as green mussels. Green mussels that contain lead if consumed in humans can pose health risks such as long-term assumptions or in high levels can cause poisoning. To reduce the lead level, we can use apple cider vinegar with and without mother which contains acetic acid and citric acid. The purpose of this study is to observe lead levels of green mussels after soaking with apple cider vinegar with and without mother, percentage decrease in green mussels lead content and the effectiveness of apple cider vinegar with and without mother in reducing lead levels. This study was a quasi-experiment by soaking green mussels using apple cider vinegar with and without mother for 5, 10, 15 minutes. The sample is prepared by drying the sample in the oven and extracting the sample using wet destruction so that a clear liquid is obtained that can be measured using an atomic absorption spectrophotometer. The results showed that the average lead levels sample soaked by apple cider vinegar with mothers were 0.219 mg/L, 0.103 mg/L, 0.088 mg/L, without mothers were 0.205 mg/L, 0.173 mg/L, 0.125 mg/L. The percentage of lead decrease was 2.6%, 49%, 51% used apple cider vinegar with mother while without mother is 8%, 15%, 30%. After the one-way ANOVA test, a p-value of > 0.05 was obtained with no effect of lead reduction. Based on research apple cider vinegar with and without mother can reduce lead levels, but statistically, the decrease is not significant.

Keywords: Lead, Green Mussels, Apple Cider Vinegar

1. INTRODUCTION

Lead is a toxic metal whose use is widespread in the industrial world such as the battery, oil, and paint industries [3]. The use of lead in the industrial field causes the spread to the human environment and poses health risks. Lead exposure in children will decrease intelligence (IQ) while in adults it will cause hypertension and immunotoxicity [10]. Lead can not only cause disturbances in humans but can cause disturbances in waters, especially the sea. The sea is a route of maritime industry activity and the final destination of waters. The activity generates residues, including ship oil spills, paint disposal, and uncontrolled disposal of lead waste [5]. The way to detect pollution can be using marine animals such as green clams.

Green mussels are biota that are susceptible to heavy metal pollution including lead because green mussels have a very high ability to accumulate lead where, in addition to filtering microscopic

particles such as plankton, green mussels absorb lead for consumption [20].In the study [12], green mussels could absorb 10 times more lead, ranging from <5 to 59-64 μg, compared to blood mussels (Anadara antiquata) and simping mussels (Amusium pleuronectes). On the other hand, green mussels are used for consumption by the public because of their delicious taste and economical price [2]. In addition, green mussels have a high nutritional content such as protein 21.9%, fat 14.5%, carbohydrates 18.5%, ash 4.3%, and water 40.8%. The high nutritional content is proportional to the high level of lead [16].

To reduce lead levels in green clams, metal-binding agents such as acetic acid and citric acid compounds are needed. The acid content is found in one of the food additives, namely apple cider vinegar. Apple Vinegar is a fermented fruit vinegar that converts sugar into alcohol and is processed into acetic acid [19]. There are 2 types of apple cider vinegar circulating on the market, namely apple cider vinegar with and without mother. Apple cider vinegar with mother does not go through the pasteurization process so it produces apple cider vinegar that is rich in probiotics and high in acid, while apple cider vinegar without mother has gone through a pasteurization process that has the potential to reduce probiotics and acid content in it [15]. The mother vinegar in apple cider vinegar is the substance resulting from the process of making apple cider vinegar itself. This substrate forms like jelly or a thin layer consisting of a group of enzymes, acetic acid bacteria and cellulose. This vinegar is without a filtering process so it is still present in it and is often considered more useful [4].

According the research [1], acetic acid and citric acid can reduce lead levels whereas blood mussels soaked with acetic acid for 1 and 3 hours can reduce lead levels by 4% and 23%. Meanwhile, soaking with citric acid for 1 and 3 hours can reduce lead by 2% and 20%. The purpose of this study is to see a decrease in lead levels using apple cider vinegar with and without mother that contains these acid compounds. This research was carried out with a variation of 5, 10, 15 minutes because in the community soaking green mussels before cooking requires a minimum of time so that the cooking process becomes efficient and does not change the taste or texture of the green clams. In previous studies, there was an efficient time of 15 and 30 minutes. So, researchers want to see a decrease in lead levels with minimal time.

2. MATERIALS AND METHODS

This study is a quasi-experimental study that analyzes the decrease in lead levels using apple cider vinegar with and without mother. This research was conducted at the Samarinda Industrial Research and Standardization Center (Baristand) from December 2023 - May 2024. The sample used was in the form of 150 grams of green clam meat where each examination was carried out in a duplo with 3 variations (5, 10, and 15 minutes) and 2 treatments (apple cider vinegar with mother and apple cider vinegar without mother). Each examination uses 5 grams of samples and then a duplicate is carried out so that 15 grams of samples are needed. The sampling technique used is purposive sampling with the criteria of green mussel samples measuring 4-6 cm in a fresh state.

2.1. Materials

The tools used in this study were the flame Atomic Absorption Spectrophotometer (SSA), measuring flask, erlenmeyer, glass funnel, porcelain cup, desiccator, oven, volume pipette, analytical scale, and stopwatch. The materials used in this study were green clam meat, apple cider vinegar with and without mother, HNO_3 65%, H_2O_2, $NH_4H_2PO_4$, and lead standard solution.

2.2. Methods

This research includes three stages, namely pre-analytical, analytical, and post-analytical. The first stage is the pre-analytical stage which includes preparation of tools and materials. Next is the analytical stage. The main standard solution has a concentration of 1000 mg/L which is diluted to 100 mg/L using aqua dest. Then a standard series solution was made with concentrations of 0, 0.25, 0.5, 0.75, and 1 mg/L from the raw solution of 100 mg/L. Then the lead level was tested using an atomic absorption spectrophotometer and the absorbance of each solution was recorded and a curve was made to determine the straightline equation. If the linear regression correlation coefficient r > 0.995 is obtained, the AAS tool is ready to use.

Sample preparation is carried out by separating the green scallop meat to be used then the sample grinding process is carried out using a blender. Then, a fine sample was followed by 3 duplications weighing 15 grams with a classification of three parts without treatment and six parts of apple cider vinegar treatment soaked with and without a parent for 5, 10, and 15 minutes. After that, the drying process is carried out in an oven at a temperature of 70-80^{o}C for 6 hours and put on a desiccant. The dried samples were subjected to a wet destruction process where 5-10 ml of HNO_3 65% and 2 ml of H_2O_2 were added and reheated using an oven at a temperature of 180^{o}C for 30 minutes until the color of the solution became clear. The solution is transferred to a 50 ml measuring flask, then a modifier matrix and aquades are added to the border.

Lead testing begins with a standard reading to see the calibration curve that aims to obtain a regression line equation of the lead standard curve. After obtaining the standard curve, the sample was tested with SSA with a wavelength of 283.3 nm so that the lead content was obtained. After the analytical stage, it is followed by the post-analytical stage where the calculation of the lead level obtained is carried out.

The calculation of lead levels is:

$$\text{Pb concentration mg/kg} = \frac{(D-E)xFpxV}{W}$$

Information:

D : Concentration of microgram samples per l from SSA

E : Concentration of microgram blanks per l of SSA

Fp : Dilution factor

V : The final volume of the sample solution (ml), must be changed to liter units

2.3. *Data Analysis:*

The results of the data obtained were analyzed descriptively and statistically. Descriptively, lead levels are presented as percentages and result in a decrease in lead levels. Furthermore, a statistical test was carried out which began with a normality test with a significance of 0.070 > 0.05 and a homogeneity test of 0.100 > 0.05 which showed normal and homogeneous data. After the data is normal and homogeneous, it is continued with the one-way ANOVA test. One-way ANOVA test and obtained a p value of 0.388 > 0.05 which means that there is no effect of reducing lead levels after soaking apple cider vinegar with and without mother.

3. RESULTS AND DISCUSSION

3.1. Average Lead (Pb) Levels in Green Mussels (Perna viridis)

Green mussels are soaked and after soaking apple cider vinegar with and without mother for 5, 10, and 15 minutes to see the lead level. The results of the lead level check can be seen in Table 1 as follows:

Table 1. Average Lead Levels in Green Mussels

		Lead Levels After Soaking	
	Initial Lead Levels (mg/L)	**Apple Vinegar with *Mother* (mg/L)**	**Apple Vinegar Without *Mother* (mg/L)**
W1 (5 minutes)	0.225	0.219	0.205
W2 (10 minutes)	0.205	0.103	0.173
W3 (15 minutes)	0.180	0.088	0.125

Based on the results of the study in Table 1 and Figure 1, the average results of green clam lead content with apple cider vinegar soaking with and without mother were obtained. Soaking apple cider vinegar with mother with the highest average lead content for 5 minutes is 0.219 mg/L and the lowest for 15 minutes is 0.088 mg/L. Soaking apple cider vinegar without mother with the highest average lead content for 5 minutes is 0.205 and the lowest for 15 minutes is 0.125 mg/L. Where the longer the soaking time, the lower the lead level, this result is in line with the study [11] that soaked green clams using tomato filtrate for 30 minutes, the highest lead content was 0.65 mg/L and the lowest for 90 minutes was 0.39 mg/L. This is because the long soaking time can make citric acid and acetic acid compounds in apple cider vinegar and citric acid in tomatoes interact with the samples. This is supported by the theory [6] that acetic acid can bind lead at a certain time, the longer the soaking time affects the lead level. This shows a reduction in lead levels either using apple cider vinegar with or without mother, according to the General Standard for Contaminants and Toxins in Food and Feed Codex Stan 193-1995 amended in 2019 the limit of lead metal contamination in fish commodities that can be consumed is 0.3 mg/L which results in lead levels after immersion below the set limit so that it is safe to consume.

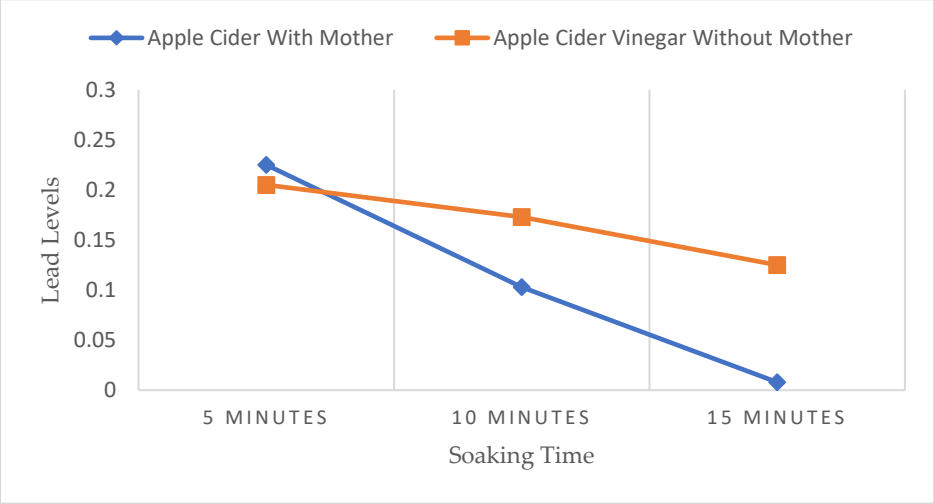

Figure 1. The Decrease in Lead (Pb) Levels

3.2. Percentage Decrease in Lead (Pb) Levels in Green Mussels (Perna viridis)

The percentage of lead reduction was obtained by reducing the lead level before treatment by the level after treatment divided by the level before treatment and multiplied by 100%. The results of the calculation of lead reduction can be seen in Table 2 as follows:

Table 2. Percentage Decrease in Lead Levels in Green Mussels

Time	Drop-down margin			
	Until		Percentage	
	With *Mother* (mg/L)	Without *Mother* (mg/L)	With *Mother* (%)	Without *Mother* (%)
5 Minutes	0.006	0.020	2.6	8
10 Minutes	0.102	0.032	49	15
15 Minutes	0.092	0.055	51	30

According to Table 2, the highest percentage of lead content reduction was soaking apple cider vinegar with the mother for 15 minutes by 51% and the lowest percentage was soaking apple cider vinegar with the mother for 5 minutes by 2.6%. The decrease in lead levels was relatively large from 2.6% to 51%, this can occur due to the use of apple cider vinegar with mother, which in addition to containing organic acids also contains more probiotics, enzymes, and organic acids than apple cider vinegar without mother. Enzymes and bacteria increase the biological and chemical activity of apple cider vinegar so that it can lower lead even more [15]. According to the time interval where from 5 minutes to 15 minutes can make a longer interaction of the content of the organizer lower the lead. According to the theory [7] where acetic acid can bind lead at a given time, the longer the immersion time affects the lead content.

These results are not in line with a previous [1] that soaking blood clam meat using acetic acid and 25% citric acid separately for 1 and 3 hours. The decrease in using acetic acid for 1 hour was 4% and for 3 hours was 23%. Meanwhile, the decrease using citric acid for 1 is 2% and for 3 hours is 2% and 20%. This result is different from the results obtained by the researcher due to the difference in the solution used as a sample soak. The research [1] uses acetic acid and citric acid separately while the researcher uses apple cider vinegar which contains 2 compounds, namely acetic acid and citric acid. According to theory [17] the decrease in metal content can be caused by the ability of chelating agents or substances that bind metals to the sample soaking solution. When the chelating agents, namely acetic acid and citric acid in apple cider vinegar, are used to lower lead, the lead level will decrease to the maximum.

Based on the theory of the content of citric acid and acetate, it can bind lead (chelator agent) so that the lead level is lower. The binding mechanism of citric acid to lead is due to the ability of carboxylate groups to bind lead. According to the theory [7] citric acid, it has a structure of 3 carboxylic acids that can form complexes with metals such as lead. The carboxyl group will release protons (H^+) and remain citrate ions (-COO-). The theory [13] states that when the proton acid is released, then the lead ion (Pb^{2+}) will be released from its complex and bind to citric ions to form citric

salts and dissolve in the citric acid solution so that the lead level decreases. In addition to seeing a decrease in lead levels, a test of the effectiveness of soaking apple cider vinegar was also carried out.

As for acetic acid (CH_3COOH) is the main constituent in apple cider vinegar which functions as an effective settling agent. Acetic acid can interact and bind lead through a chemical process, resulting in a complex that is insoluble in water. Research explains that citric acid compounds can act as heavy metal carriers [14]. When citric acid interacts with heavy metals, they form a complex bond. In this bond, atoms that have free ions will bond with heavy metals [18].

According to the theory [8], acetic acid dissolved in water will give hydrogen ions (H^+) and enlarge the hydrogen ions in the solution. This reaction will form a lead acetate compound that breaks down into separate ions. Since acetic acid is a weak acid, it will release a small amount of hydrogen ions (H^+) in the solution and make lead acetate tend to be intact without fully decomposing into separate ions. In addition to seeing a decrease in lead levels, an effectiveness test of apple cider vinegar soaking was also carried out.

3.3. Effectiveness of Apple Vinegar with and Without Mother in Reducing Lead (Pb) Levels in Green Mussels (Perna viridis)

Based on the results of the study, each shellfish that was soaked experienced a decrease. To determine the effectiveness of apple cider vinegar with and without mother in lowering lead, statistical tests were carried out. Statistical tests were carried out using the one-way ANOVA test which began with testing normality and homogeneity tests. The normality test was carried out with the Shapiro-Wilk test to see the normality of the distribution of research data. A homogeneity test was also performed, which used the Levene test to see if the data were homogeneous. The results of the normality test with a significance of 0.070 > 0.05 and the homogeneity test were met with a significance of 0.100 > 0.05 which showed normal and homogeneous data. After the data is normal and homogeneous, it is continued with the one-way ANOVA test.

The results of the one-way ANOVA test obtained a significance value of 0.388 > 0.05 which showed that Ho was accepted, namely there was no decrease in lead content by soaking apple cider vinegar with and without mother within 5, 10, and 15 minutes. The results showed that apple cider vinegar with and without mother was not significant in lowering lead levels. This is due to the limited variability of the data obtained where this study requires a larger number of samples to obtain precision so that it can detect differences in each treatment. This result is not in line with research [9] where there is a significant influence between soaking green mussles and the length of soaking using lime in lowering lead. Lime contains citric acid, which can dissolve green clam lead, as well as apple cider vinegar with and without mother. Apple cider vinegar with and without mother contains citric acid and acetic acid which can lower lead.

4. CONCLUSION

Based on the research that has been carried out, the average results of lead content before 5, 10, and 15 minutes of immersion were 0.225 mg/L, 0.205 mg/L and 0.180 mg/L respectively; apple cider vinegar treatment with mother 5, 10, 15 minutes sequentially 0.219 mg/L, 0.103 mg/L and 0.088 mg/L. The treatment of apple cider vinegar without mother for 5, 10, 15 minutes was 0.205 mg/L, 0.173 mg/L and 0.125 mg/L. Percentage decrease in lead content after apple cider vinegar treatment with mother

5, 10, 15 minutes respectively 2.6%, 49%, 51%; Motherless apple cider vinegar treatment 5, 10, 15 minutes sequentially 8%, 15%, 30%. Soaking apple cider vinegar with and without mother for 5, 10, 15 minutes can reduce lead levels but is not statistically effective in reducing green mussels lead levels.

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