Removal of heavy metals from green mussels (*Perna viridis*) using pineapple (*Ananas comosus*) solution as a source of citric acid

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Abstract. Green mussel is a type of shellfish that is widely consumed by humans. Excessive consumption of green mussels can be harmful to health because they are prone to contain heavy metals. One of the methods to reduce the heavy metal content of green mussels is to use citric acid in the pineapple solution. The Green mussel was soaked in pineapple solution for 30 min, 60 min and 90 min. The content of heavy metals such as Pb, Cd, and As was analyzed by ICP-MS. The results showed that after soaking green mussels in pineapple solution for 30 minutes, they reduced 85% Pb, 80,95% Cd, and 53,23% As. The duration of soaking green mussels in pineapple solution for 30 minutes overall had a better quality than soaking for 60 and 90 minutes, with a moisture content of 74,43 ± 0,39 (%), protein content 15,21 ± 0,35 (%), pH level 6,74 ± 0,10, organoleptic with a confidence interval of 7,48<μ< 7,54.

1. Introduction
Green mussel (*Perna viridis*) are biota that are commonly found in Indonesian waters. Green mussels are favoured by the public because of their delicious and savory taste. The value of mussel production increases every year. The increase in production of mussels is estimated to be 12% per year, which is about 87 thousand tons in 2020 to 137 thousand tons in 2024. The yield of green mussels per hectare per year can reach 200-300 tons of the whole mussels or around 60-100 tons of mussel meat [1]. The nutritional content of green mussels is quite high and has economic value, but green mussels are susceptible to contaminants such as heavy metals due to the ability of the filter feeder owned by mussels to absorb substances from the waters of their habitat. Green mussels are found in polluted coastal environments. These shells can be used as an indicator of pollution by heavy metals in the waters [2].

Heavy metals such as Pb, Cd, and As that accumulate in green shells can cause harmful effects to humans when consumed. One of the methods to reduce levels of heavy metals is to use citric acid which is a metal binding (chelating agent). Citric acid is one of the organic acids that is commonly used as a chelating and neutralizing agent of heavy metals. Citric acid forms complexes with heavy metals through
the bonds of citrate anions with cations [3]. Natural sources of citric acid are found in fruits such as pineapples. The citrate ion contained in pineapple can bind metals so that it can remove metal ions that accumulate in green mussels as citrate complexes. The organic acid content in pineapple solution is dominated by citric acid, which is 0.439-1.151 g/100 mL, while malic acid is 0.073-0.391 g/100 mL and isocitric acid is 80-265 mg/L [4].

This study aims to determine the effect of different durations of soaking green meat in pineapple solution on the reduction of lead, cadmium, arsenic levels, and to determine its effect on moisture content, protein content, pH, and organoleptic values.

2. Materials and Methods

The materials used in this study were green mussels (Perna viridis) from Kedung Jepara waters, Central Java – Indonesia, with an average length of 7-8 cm, pineapples weighing 0.8-1 kg, and aquades. The tools used in this research are ICP-MS (Inductive Coupled Plasma – Mass Spectrometry), oven, analytical balance, Kjeldaldester, desiccator and pH meter.

2.1. Sample preparation

The green mussels were then washed to remove impurities and boiled for a short time to remove the shell and flesh. The mussel meat was then soaked in the pineapple solution for 30 min, 60 min, and 90 min. Pineapples were cut into pieces and weighed as much as 1000 grams, then blended and filtered using a cloth filter [5].

2.2. Heavy metals analysis

The heavy metals content analysis was carried out using the [6] method with modifications and using the ICP-MS instrument. The sample was mashed with a blender and then 1.5 g was weighed in the vessel. The sample in the vessel was added with 10 mL of concentrated HNO₃. The sample was put into a microwave digester to be destroyed using a gradual temperature of up to 150°C for 10 minutes and continued at 150°C (hold) for 15 minutes. The digested sample solution was cooled and put into a 50 mL volumetric flask. The vessel was rinsed with distilled water and combined the results of the rinses with the results of destruction in a 50 mL volumetric flask. The sample solution in a 50 mL volumetric flask was added with 0.4 mL of an internal standard. The sample solution in a 50 mL volumetric flask was diluted with distilled water to the mark and then homogenized. The sample solution was measured in intensity in the ICP MS system.

2.3. Moisture content analysis

Analysis of moisture content was using a method based on [7]. The procedure for analyzing the moisture content is that the porcelain cup to be used is coded according to the sample code and is dried in an oven at a temperature of 100 – 105°C for 15 minutes. The porcelain cup was taken and cooled in a desiccator for 15 minutes and then weighed. A sample of 2 g was weighed in a porcelain dish and then weighed again and dried in an oven for 16 hours at 100°C. Then the sample was cooled in a desiccator and weighed until it is constant. The water content is calculated by the formula:

\[
\text{Moisture content (\%)} = \frac{B - C}{B - A} \times 100\%
\]

Where:

- A = Weight of the cup (g)
- B = Weight (cup + sample) before drying (g)
- C = Weight of sample (cup + sample) after drying (g)

2.4. Protein content analysis

The sample was weighed as much as 1 g and then put in a 300 ml Kjeldahl flask. The Kjeldahl flask containing the sample was added to a mixture of 1 g of selenium and 12 ml of H₂SO₄. Sample is destructed at a temperature of 420°C for approximately 1 hour until a clear solution is then cooled. After
cooling, 50 ml of distilled water and 40% NaOH were added to the Kjeldahl flask. The sample was then distilled at a distillation temperature of 100°C. The distillation result was accommodated in a 250 ml Erlenmeyer flask containing a mixture of 25 ml of boric acid (H3BO3 4%). After the distillate volume was 40 ml and the color was bluish green, the distillation process was stopped. Then distillate with 0.2 N HCl until a pink color change occurs. The titrant volume is read and recorded. The blank solution was analyzed as an example. Protein content can be calculated using the following formula:

\[
\text{Protein content (\%)} = \frac{(V_p - V_b) \times N \times HCl \times 14,007 \times Fk}{\text{Sample weight (g)}}
\]

Where:
- \(V_p\) = HCL volume required for sample titration (ml)
- \(V_b\) = HCL volume required for blank titration (ml)
- \(N\) = HCl solution normality
- \(Fk\) = conversion factor (6.25 for fishery products)

2.5. pH value
The pH measurement is done with a digital pH meter based on the method [9], namely by using a pH meter that has previously been calibrated using a buffer solution. Measurements were carried out directly by dipping the pH eye into the that had been dissolved in distilled water until the pH value indicated on the pH meter screen was stable.

2.6. Organoleptic test
The organoleptic test was carried out according to method [10] using an organoleptic scoresheet with 30 panelists. Parameters observed in organoleptic test were appearance, aroma, taste, and texture.

2.7. Statistical analysis
The design of this study used a completely randomized design. The parametric data were processed using one-way analysis of variance (ANOVA), then using Tukey mean test and non-parametric data were analyzed by Kruskal Wallis test using SPSS version 16.0 for windows (SPSS Inc., Chicago, USA) with three replications.

3. Results and Discussion

3.1. Heavy Metals Content
The percentage reduction in heavy metal content of green mussels with differences in soaking time using pineapple solution are presented in Figure 1.
In general, the decrease in lead, cadmium, and arsenic levels at 30 minutes of soaking resulted in higher heavy metals reduction. The soaking time of 90 minutes in this study actually showed a lower reduction in heavy metal content. The percentage that occurs varies because it depends on the type of metal that has different abilities when bonded with other compounds. According to [11], there is no general order of efficiency for organic acids in reducing heavy metal levels. The order of citric acid in heavy metal extraction efficiency (in descending order) is Zn > Pb > Cd. Research by [12], removal of heavy metals in T. thynnoides using sodium acetate (SAT) and trisodium citrate (TSC) for one hour had successfully removed heavy metals Cd, Cu and Ni. SAT had successfully removed 73.04% of Cd, 50.46% of Cu and 80.94% of Ni. TSC removed each metal by 66.58%; 49.63%; and 19.26%. The different percentages in removal of heavy metals is due to the factors such as the type of heavy metal and the high stability of the structured complex produced from chelation.

3.1.1. Lead (Pb) content. The results of analysis of the Lead content of green mussels with differences in soaking time using pineapple solution are presented in Figure 2. The lead content of green mussels when soaking for 30 minutes decreased from 0.20 ppm to 0.03 ppm (85%). The lowest decrease in lead levels occurred in green mussels with a long soaking time for the pineapple solution of 90 minutes.

Note: The data is average yield of three replications ± standard deviation; different superscripts show significantly different (p<0.05)

Figure 2. Effect of soaking time with pineapple solution on Pb content of green mussels.

The effective soaking time for the green mussels using the pineapple solution based on Figure 1 is 30 minutes. Green mussels with a duration of soaking for 90 minutes also decreased lead levels compared to controls, but the levels were higher when compared to soaking for 30 minutes and 60 minutes. The lead content in green mussels with a soaking time of 90 minutes was higher, presumably because the pineapple solution was saturated with metal ions so that the metal absorption process by the pineapple solution had reached equilibrium. According to [13] the highest lead removal occurred at an immersion time of 45 minutes, which was as much as 80%. The immersion time using a high concentration of pineapple extract is able to eliminate the lead content of juario fish, but if it is used for a long duration it would cause the bond between the metal and citric acid to break again.

The saturated pineapple solution has a lower ability to interact with metal ions so that the bonds between the functional groups of the pineapple solution and lead are easily released. These results are different from the results of research [14], that the soaking time of green mussels using acetic acid of up to 90 minutes can reduce heavy metal levels, but still exceeds the allowable limit. The lead content of green mussels before soaking with acid was 2.879 ppm, decreased to 1.5 ppm after soaking for 90 minutes.
3.1.2. Cadmium (Cd) content. The results of analysis the Cadmium content of green mussels with differences in soaking time using pineapple solution are presented in Figure 3. The removal of cadmium levels that occurred during the soaking time for 30 minutes was decreased from 0.21 ppm to 0.04 ppm (80.95%). The soaking time in 60 minutes decreased by 61.90% and 90 minutes by 66.67%, but the longer the soaking time resulted in a higher decrease in the nutritional content of shellfish. So, the most effective time to reduce cadmium of green shells using pineapple solution is 30 minutes. According to [15] the duration of soaking of green mussel meat with different lengths of time 0, 15, 30 and 45 minutes can have a significant effect on cadmium levels. The most effective soaking time to reduce the content of cadmium levels was at a treatment time of 30 minutes.

![Figure 3. Effect of soaking time with pineapple solution on Cd content of green mussels.](image)

The removal of heavy metal levels in green mussels was caused by the bond between heavy metals and the citric acid functional group in the pineapple solution. The soaking time will affect the bond strength of citric acid with heavy metals because when the pineapple solution has reached the saturation point to form complex bonds, the metal ions that have been bound can be released again. The removal of cadmium levels is also caused by the nature of the cadmium ion itself. The nature of the cadmium ion will affect the ability of the adsorbent to bind. Cadmium is known to have a lower ionic radius than the ion in lead. According to [16], adsorbents are better able to absorb Pb<sup>2+</sup> than Cd<sup>2+</sup> in an aqueous solution. This is most likely due to the difference in the ionic radius of the Pb<sup>2+</sup> and Cd<sup>2+</sup> cations. Larger ionic radius reduces the electrostatic properties of metal ions and favour covalent interactions between metal ions and the functional groups of the adsorbent surface.

3.1.3. Arsenic (As) content. The results of Arsenic content analysis green mussels with differences in soaking time using pineapple solution are presented in Figure 4. The highest removal in arsenic levels occurred at the soaking time of 30 and 60 minutes, which showed no significant difference. Soaking green mussels for 30 and 60 minutes can reduce arsenic levels to 0.94 ppm (53.23%) and 0.96 ppm (52.23%). Arsenic levels with the soaking time of 30 and 60 minutes had reached the quality standard for arsenic levels, while the levels at soaking for 90 minutes still exceeded the quality standard for arsenic, that is 1 ppm. The decrease in arsenic levels was caused by the interaction of citric acid in pineapple solution with arsenic levels in green mussels. According to [17] in the body of shellfish, heavy metals are bound to proteins or peptides to form metallothionein compounds, in the presence of citric acid are released and then metal ions bind to OH- and COOH-.

The removal in arsenic levels in green mussels after the soaking in pineapple solution is smaller than the lead and cadmium levels. This can be caused by factors such as the nature of arsenic which is more difficult to decompose when compared to the heavy metals lead and cadmium. Another factor that caused it was thought that the levels of arsenic in the control green mussels was higher than the levels of lead and cadmium so that the bond between metal ions that occurred in green mussels and citric acid
was more easily decomposed. Research by [18], that the removal of arsenic levels was the lowest compared to lead and cadmium in green mussels soaked in three types of chelating agents for 1 hour. Trisodium citrate reduces As content: 38.13%, Pb: 68.90%, Cd: 70.49%, sodium acetate reduces As content: 56.50%, Pb: 88.57%, Cd: 68.01%, and disodium oxalate reduces As 46.89%, Pb: 85.46%, Cd: 68.01%.

**Figure 4.** Effect of soaking time with pineapple solution on As content of green mussels.

### 3.2. Moisture content

The levels of water content are presented in Figure 5. Soaking green mussels in a pineapple solution causes a decrease in moisture content. The moisture content of green mussel after soaked in pineapple solution for 30 minutes, 60 minutes, and 90 minutes did not show significant difference. The duration of soaking of green mussels in pineapple solution which causes a decrease in moisture content is thought to be due to the ability of mussel meat to absorb water from the environment has reached its maximum condition. There was no change in H\(^+\) ions due to the combination of pineapple fruit extract, so the moisture content in the treatment did not make any difference, and it was suspected that under these conditions the ability of the meat to absorb water from the environment was saturated [19].

**Figure 5.** Effect of soaking time with pineapple solution on moisture content of green mussels.

The decrease in moisture content is thought to be due to the ability of mussel meat to absorb water from the environment has reached its maximum condition and is caused by the boiling process of mussels during sample preparation. According to [20], nutrition changes that occur during the cooking process are influenced by several factors, namely cooking temperature, cooking time, surface area, and
type of material. This is confirmed by [21] during the cooking process there was a decrease in water and fat content due to heating.

3.3. **Protein content**

The levels of protein content are presented in Figure 6. The protein content of green mussels treated with pineapple solution at varying soaking times showed a significant difference with the control sample, but between each treatment was not significantly different although there was a decrease in the percentage between soaking for 30 minutes, 60 minutes, and 90 minutes. The soaking time using pineapple solution showed that the decrease in protein content was proportional to the decrease in heavy metal content in green mussels. According to [22], green mussel protein after soaking using tomato solution decreased. Data analysis on the immersion treatment showed lowercase letters on the same line. The protein content of mussels with soaking time of 30, 60 and 90 minutes, respectively, was decreased from 10.41% to 10.05%; 10.01%; and 8.72% of each treatments.

![Figure 6. Effect of soaking time with pineapple solution on protein content of green mussels.](image-url)

The longer the soaking time using pineapple solution will cause a higher decrease in protein in green mussels. This is because the protein in green mussels is involved in the bioaccumulation of heavy metals, so that when the protein metal complex bonds are released, the metal ions will be released from the shell. This is confirmed by [23] the interaction of metal ion complexes with proteins occurs by metalloenzymes and metal proteins. Metalloenzymes are proteins that bind metals to form highly stable bonds. Metal proteins are proteins that bind to metals and the metal ions themselves are easily exchanged with other proteins, also according to [24] metallothionein is a group of thiol proteins (containing large amounts of -SH) or proteins that physiologically bind Zn and Cu in cells, but also absorb heavy metals such as Cd and Hg.

3.4. **pH value**

The levels of pH value are presented in Figure 7. The results of the pH value of green mussels showed that the longer the soaking time the lower pH value. The decreased pH value of green mussels was due to the interaction with the acid contained in the pineapple fruit solution. The pH of the pineapple solution will affect the value of heavy metal reduction in green mussels. According to [25], heavy metal adsorption depends on different pH for each metal. The zinc removal is about 70% at pH 6 and increases to 95% at pH 8. For lead, 90% is removed at pH 4.5 and increases to 99% at pH 6. Other metals such as cadmium, copper, and arsenic can be reduced at pH 6. pH 4-8.

The decrease in pH value can also be caused by the presence of the bromelain enzyme in pineapple which is acidic. According to [26], there was an increase in bromelain concentration, the pH of the meat sample decreased so that it was more acidic. In fact, bromelain extract can cause muscle hydrolysis to release amino acids that can lower the pH of meat.
3.5. Organoleptic

The results of organoleptic testing of green mussels using pineapple solution with different lengths of soaking time are presented in table 1.

Table 1. Effect of soaking time with pineapple solution on organoleptic of green mussels.

| No | Soaking time (min) | Appearance | Odor | Taste | Texture | Average |
|----|--------------------|------------|------|-------|---------|---------|
| 1  | 0                  | 8.26±0.98<sup>a</sup> | 8.40±0.93<sup>a</sup> | 7.53±0.89<sup>a</sup> | 7.93±1.01<sup>a</sup> | 7.99 < µ < 8.06 |
| 2  | 30                 | 7.86±1.00<sup>b</sup> | 7.46±0.86<sup>bc</sup> | 6.93±0.63<sup>bc</sup> | 7.80±0.99<sup>b</sup> | 7.48 < µ < 7.54 |
| 3  | 60                 | 8.13±1.00<sup>b</sup> | 7.60±0.93<sup>c</sup> | 6.60±0.81<sup>cd</sup> | 6.60±0.81<sup>bc</sup> | 7.19 < µ < 7.26 |
| 4  | 90                 | 7.40±0.81<sup>b</sup> | 6.46±0.89<sup>d</sup> | 6.33±0.95<sup>d</sup> | 6.26±1.11<sup>c</sup> | 6.58 < µ < 6.65 |

3.5.1. Appearance. The difference in the appearance of green mussels was caused by soaking using a solution of pineapple fruit that has a yellow color. The length of soaking time also affects the appearance of the green mussels. The longer soaking time will cause the green shells to become more yellowish. The most abundant pigments in pineapple are carotene and xanthophyll. The content of carotene in pineapple is greater than that of xanthophyll. These two pigments play a role in giving the pineapple its distinctive color, which is yellowish [27].

The changing appearance of green mussels meat can also be caused by the presence of the bromelain enzyme in the pineapple solution which causes the breakdown of the muscle fibers of the mussel meat, so that the appearance of the mussel meat changes. According to [28] meat soaked in pineapple solution at a concentration of 10% has the highest appearance value compared to concentrations of 20% and 40%.

3.5.2. Odor. The results showed that the green mussels which were soaked for 90 minutes had a strong pineapple smell so that the green clams lost their specific odor. The longer the soaking time in the pineapple solution, the stronger the pineapple smell on the green mussels, presumably due to the reduced mineral and metal content of the shellfish due to the addition of an acidic pineapple solution. When the mineral acid atmosphere is bound to fat, it will decrease in proportion to the destruction of fat, because in an acidic environment these minerals will combine with organic substances that are lost from the material. The main cause of food quality decline, especially meat, is due to changes in fat components through fat oxidation processes or hydrolytic reactions originating from enzymes [29]. Research by [30], showed that the duration of soaking time using pineapple solution for 5 minute, 10 minute and 15 minute has different odor value. The longer time of duration has the lower green mussel’s specific odor, the decreased of each treatment by 8.33±0.95; 7.80±0.99; and 7.60±0.93.
3.5.3. 
Taste. The low organoleptic value of the green mussels was caused by the length of soaking using the pineapple solution. The longer the process of soaking the shellfish causes the taste of the shells is sourer due to the sour taste of the pineapple. The soaking time for 30 minutes was still acceptable to the panelists because it had a mean above the value of 7. The soaking for 60 minutes and 90 minutes had an average value below 7 because the taste of pineapple was more dominant than the specific types of shellfish flavor. According to [31], pineapple is an exotic fruit that has high economic value because of its aroma, taste, and freshness. Its physicochemical composition and nutritional value are considered useful in the manufacture of value-added compounds such as antioxidants, organic acids, bromelain, and phenolic compounds. Research by [32], soaking green mussel using orange solution changed the specific taste. The highest taste value on green mussel meat that is in the control by 8.67±0.75, while after soaking it decreased by 7.60±0.93. This can be occurred due to the acid content in the orange solution is sufficient high meat, resulting in the taste of green mussels obtained is too acidic.

3.5.4. 
Texture. The highest value of organoleptic texture of green mussels was obtained in control of green mussel at 7.93±1.01, while the lowest value of texture was shown by the soaking treatment for 90 minutes, namely 6.26±1.11. Soaking for 60 minutes and 90 minutes causes the green mussel meat to become softer, inelastic, and easily crushed. The texture value of green mussel meat decreases when it is soaked in pineapple solution for a longer time. The muscle fibers of the green mussel meat texture are decomposed due to hydrolysis by the bromelain enzyme so that the tissue becomes soft. The reduction of meat cohesiveness is the result of proteolytic enzymes in myofibrillar proteins [33]. According to [34], it showed that the provision of pineapple juice had a very significant effect (P<0.5) on meat tenderness. The meat given pineapple juice 3% produce tender meat parameters with a score of (2.3) when compared to meat that is not given pineapple juice with tough with a score (3.78). This difference is caused by the provision of pineapple fruit juice which contains the proteolytic enzyme bromelain so that it can break down protein molecules into smaller amino acid molecules and also damage chemical bonds in the meat so that it makes the meat soft.

4. Conclusion

The conclusions that can be drawn from the research on the effect of soaking green using solution of pineapple at different times are that the duration of soaking green mussels showed significantly different results. Citric acid content in pineapple solution can reduce the level of heavy metals in green mussels. Soaking time using pineapple solution for 30 minutes can reduce lead levels to 0.03 ± 0.03 ppm (85%), cadmium 0.04 ± 0.03 ppm (80.95%) and arsenic to 0.94 ± 0, 13 ppm (53.23%). Soaking time of 60 minutes caused a decrease in lead to 0.06 ± 0.04 ppm (70%), cadmium 0.08 ± 0.04 ppm (61.90%) and arsenic 0.96 ± 0.06 ppm (52.23 %). Soaking time for 90 minutes reduced lead levels to 0.14 ± 0.06 ppm (30%), 0.07 ± 0.04 ppm cadmium (66.67%), and 1.20 ± 0.04 ppm arsenic (40,29%). The duration of soaking green mussels using a pineapple solution which is effective against the characteristics of green mussels (Perna viridis) is 30 minutes.

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