Effectiveness Tamarind to reduction of Pb content in red mussels

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Abstract. Red Mussels is a food ingredient that is quite popular in East Java. However, the content of heavy metal (Pb) red mussels is quite high above the threshold that is permitted for human consumption, which is below 1.5 ppm. Therefore, the presence of high Pb levels must be reduction according to the specified threshold. The research was conducted in two steps. The first step, Red mussel’s extraction using Tamarind solution. Tamarind contains organic acids, one of which is citric acid which acts as a metal binding agent so as to reduce the metal content. The second stage of extracting red mussels uses pure citric acid, then the results are compared. The optimum results obtained at the time of extraction and concentration using tamarind was 60 minutes and 25%, the effectiveness of citric acid in tamarind was 98.89% (initial Pb 17.19 ppm to 0.25 ppm) while pure citric acid was 99.97% (Initial Pb 17.199 ppm to 0.004 ppm).

Keyword : red mussel, tamarind, citric acid, Pb, gauss-seidel

1. Introduction

Red Mussels is one of the most popular water products that can be consumed, especially in East Java. Because it has high protein and amino acids [1], these aquatic products are also known to be cheap. But unfortunately, many cases occur when eating processed mussel consumers experience diarrheal, nausea and bloating. This is caused by the content in mussel. The results of previous studies on heavy metal content in marine organisms have been carried out [2], among others, that in Kenjeran, Surabaya, the Pb content in mussel is 28,842 ppm. In Sidoarjo white mussel Pb levels 2.4795 ppm, red mussels 5.6794 ppm. Pb content in white musels and red mussels in Pasuruan is 1.5543 ppm, and 2.9031 ppm, respectively [3]. The study shows that pollution in Sidoarjo waters is the highest compared to Surabaya and Pasuruan. The results of this study indicate that the levels of heavy metals in mussel have exceeded the threshold of heavy metal content recommended by FAO / WHO wherein the maximum number of Pb 1.5 ppm can be consumed by marine animals. According to the provisions of SNI the permissible Pb content is a maximum of 1.5 ppm while mussel is more than 1.5 ppm. Therefore, the presence of high Pb levels must be lowered according to the specified threshold.
In general, it is safe for consumption to be boiled before being processed, this can reduce the Pb content but on the other hand it will reduce the fatty acid content [4], [5]. And also amino acids, which will affect the protein content [6], [7], [8]. For this reason, it is necessary to reduce the levels of Pb in red mussels with the best conditions without reducing the nutritional content. One of these efforts is to use a metal binder (chelating agent) in the form of an acid namely citric acid in tamarind [9]. In this study using tamarind because tamarind contains organic acids, one of which is citric acid which can form complex compounds with metals [10]. And also, tamarind as a metal binding agent so that it can reduce the metal content. After that, we can know whether or not it is effective by comparing citric acid to tamarind using pure citric acid.

The purpose of this study was to reduce the Pb metal content according to the standard, then assess the effectiveness of tamarind to decrease the Pb metal in red mussels and find out the optimum concentration and time in decreasing Pb.

2. Literature Review

Mussel is a type of shellfish, a source of animal protein in the complete protein category, because it’s essential amino acid levels are high, which is around 85% - 95% of total protein. It is also a source of fat- and water-soluble vitamins. Fat soluble vitamins are A, D, E, and K, while water soluble is mainly B-complex such as B-1, B-2, B-6 (pyridoxin), B-12, and Niacin. Shellfish is also the main source of minerals needed by the body, such as iodine (I), iron (Fe), zinc (Zn), selenium (Se), calcium (Ca), phosphorus (P), potassium (K), flour (F), etc. Minerals from seafood are more easily absorbed by the body than those from beans and cereals [11].

The content of amino acids and fatty acids in mussel is very good for fulfilling the body’s nutrients. But mussel contains heavy metals because mussel has a sedentary nature, is slow to avoid pollution and has a high tolerance for pollutants. Mussel, which contains heavy metals, is a dangerous threshold if consumed because it can accumulate metals in the body and have an impact on health. Among the heavy metals that are harmful to health in mussels are Pb (lead / lead). These results indicate that the levels of heavy metals in mussel have exceeded the threshold of heavy metal content recommended by FAO / WHO wherein maximum marine animals may be consumed for Cd of 0.1 ppm and Pb of 1 ppm. According to the provisions of SNI the maximum allowable Pb content is 1.5 ppm and Cd is 1 ppm.

The Pb content in fresh red mussel is 0.66 ppm and decreases by 0.13 ppm after boiling. These results indicate that boiling can reduce Pb levels. Red Mussel is safe from Pb heavy metals because according to BPOM RI (2009) [12] and BSN (2009) on SNI 7387: 2009 the maximum Pb limit in food is 1.5 ppm [13]. The decline in the content of heavy metals in mussels has been widely studied. One effort to reduce Pb metal content is using a chelating agent, namely citric acid. Citric acid is a tricarboxylic acid that is very effective as a metal chelating agent. Citric acid is a tribasic acid that can form complexes with metals [14]. The results of the analysis of Pb levels in mussels carried out in the laboratory In UPN “Veteran” East Java showed significant results, namely before extraction of red mussel Pb levels was 17.19 ppm and after extraction the levels of Pb in red mussel were 8.77 ppm [15].

2.1 Tamarind

Tamarind is a type of fruit that tastes sour; commonly used as a spice mixture in many Indonesian dishes as flavorings or acid enhancers in food, for example in tamarind vegetables or sometimes pempek broth. Acids are also used to mix traditional herbal medicine, namely herbal medicine. Tamarind produced by a tree that is scientifically named Tamarindus Indica, belongs to the tribe of Fabaceae (Leguminosae). This species is the only member of the Tamarinds clan. Other names for tamarind are tamarind (Mly.), Asem (Jw., Sd.), Acem (Md.), Asang jawa, asang jawi (various languages in Sulawesi) and others. Also sampalok, kalamagi (Tagalog), magyee (Burma),
ma-kham (Thai), khaam (Laos), khoua me (Cambodia), me, trai me (Vietnam), and tamarind (Ingg.). The old, very ripe and dried fruit is usually called asem kawak.

Nutrients contained in tamarind include acid apple, citric acid, grape acid, tartaric acid, succinic acid, pectin and invert sugar. Tamarind contains 15% citric acid. Citric acid is an organic acid that is soluble in water. In addition to citric acid in tamarind also contains tartrate, malic acid, citric acid and succinic acid. The following are the contents of tamarind as follows: Citric acid 12.147 %; Malic Acid 9.65 %; Succinic Acid 9.40; Tartrate Acid 10.18%; Calcium Bitartrate 4.82 %; Pectin 3.453 %; Invert Sugar 3.543 %; Tannin 0.37%; Phosphor 21.34 % (Laboratorium of Gizi Unair, 2018)

In daily life citric acid is widely used in the food industry. Chemically citric acid can form complex compounds with metals because it has functional groups COOH and -OH. Therefore, citric acid contained in tamarind can be used to reduce levels of lead heavy metals [16].

2.2 Citric Acid

Judging from the perspective of metal release, citric acid is more aggressive than acetic acid compared to acetic acid, mainly because of its higher complex capacity [17]. Citric acid is also called tricarboxylic acid where each molecule contains three carboxyl groups and one hydroxyl group that is bound to carbon atoms. Citric acid is hydroxy-2 acid, 1,2,3, propane tricarboxylic or tricarboxylic hydroxy acid. Chemical formula for citric acid anhydrous: C₆H₈O₇, and as its monohydrate: C₆H₈O₇·H₂O. Citric acid in the form of crystal, colorless or white powder, odorless, sour taste, in moist air rather hygroscopic, in dry and hot air is rather fragile. Citric acid can be either anhydrous citric acid or citric monohydrate acid, odorless, colorless, clear or white crystalline powder. Citric monohydrate acid at a temperature of 75 °C begins to release the crystal water, and turns into anhydrous at a temperature of 135 °C. Anhydrous citric acid melts at 153°C. The molecular weight of anhydrous citric acid 192.12 and citric acid monohydrate 210.14 [18]. Citric acid in water is dissociated and has dissociation constants I = 8.2 x 10⁻⁴ at 18 °C, dissociation constants II and III are 1.77 x 10⁻⁵ and 3.9 x 10⁻⁷, respectively [14]. One part of citric acid can dissolve in: less than one part of water, one half part of alcohol, two parts of glycerol, 30 parts of ether, less than one part of methanol [13]. According to Anwar [16] citric acid is a food additive that is metal binding (chelating agent) so that it can free food from metal contamination. Citric acid can play a role in forming complexes with metals. As a chelating agent, citric acid can bind metal ions so that it can increase the efficiency of antioxidants.

The toxic properties of heavy metals are bound in the -OH group in the sistenyl, histidyl, hydroxyl, and phosphatic groups of proteins and purines. This causes toxicity and lethal metals. Heavy metals in the body of marine biota can be removed with citric acid because metals can bind to atoms that have free ions, while citric acid has four ions free of carboxylic groups so they can form complex ions.

3. Material and Method

The materials used in this study include: red mussels obtained from the District of Balong Dowo, Sidoarjo Regency, Peel Tamarind obtained from Rungkut Soponyono Market, Aquadest (H₂O), Nitric Acid (HNO₃) and Peroxide Acid (H₂O₂).

In extracting Pb included in leaching because it aims to take Pb solids by using acids in tamarind. [15]

The research steps are as follows:

Step I
- The Research starts by making 100 ml of tamarind solution in various concentrations, namely: 5%; 10%; 15%; 20%; 25% (in% weight). Then each was analyzed to determine the citric acid level. Furthermore, a solution of 100 ml of pure citric acid is made at various concentrations from the results of acidic analysis of Tamarind.
- 160 grams of red Mussel that has been dried and mashed is extracted using a 100 ml Tamarind solution at various concentrations above. While the extraction time taken is: 20;
30; 40; 50; 60 (minutes) At 100 °C. On the other hand the red mussel of the same weight is also extracted using a pure citric acid solution with the same conditions as above.
- The results of extraction using tamarind and pure citric acid are analyzed and compared, the optimum results obtained by optimization using the gauss-seidel method.

4. Discussion and Result

Discussion

The analysis results of testing the levels of lead in red mussel meat using the AAS analysis method showed results of 17.19 ppm. These levels still exceed the very high threshold. From the analysis result of raw materials, show different values from the literature. This can occur due to differences in habitat in Mussel. Red Mussel habitat at the edge of the beach or rather to the center (approximately 80 m from the beach) whose waves are small or rather large and into the water at low tide 1-2 m [15]. Because the red mussel habitat also affects the food intake obtained by Mussel, thus affecting the accumulation of Pb levels.

4.1 Tamarind

The variables used in extraction are 5%, 10%, 15%, 20%, and 25% by weight. Each of these variables will be analyzed for the content of citric acid. Concentration of citric acid in 100 ml Tamarind solution as follow:

**Table 1. Concentration of Citric Acid in 100 ml Tamarind Solution**

| Concentration | 5 % | 10 % | 15 % | 20% | 25 % |
|---------------|-----|------|------|-----|------|
| Tamarind.     |     |      |      |     |      |
| Citric Acid (%) | 2.756 | 6,825 | 9.338 | 10.576 | 12.147 |

In this study, red mussels were used 160 grams and extracted with tamarind solution with variations in concentrations of 5%, 10%, 15%, 20% and 25% with time variants of 20 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes with a temperature of 32°C.

**Figure 1. Effect of tamarind concentration on reduction Pb content**

Figure 1 describe the relationship between concentration and Pb metal reduction can be seen that at a variable time of 20 minutes the concentration of 5% has an effect on the extraction result, the greater concentration of Pb decrease seen in the upward and constant graphs at 25%, because the acid has saturated so it cannot absorb metal. In the variable time of 30 minutes the results obtained at a concentration of 5% were 98.31% then at a concentration of 10% it decreased to 97.91%. This happens because of saturation of the acid solution. At 40 minutes extraction time, it can be seen that
the graph tends to go up and down at a concentration of 20% which means that at a concentration of 20% and extraction time of 40 minutes the acid solution is saturated, while at 50 minutes it decreases almost 40 minutes at concentration of 20%. Similarly, at a variable time of 60 minutes the Pb yield decreased at a concentration of 20%, and it can be concluded that at a concentration of 20% the acid was saturated.

It was optimized using Matlab with the gauss seidel method that there were differences in the decrease in lead heavy metal in red mussels before and after extraction using tamarind acid filtrate. Optimization using the gauss seidel method, obtained values of x and y which have the smallest percentage error value, namely $x = -9.1170$ with $y = 0.0073$. Then substitute the equations of each variable. The results of substitution on each variable at a concentration of 25% at 60 minutes is the biggest with a value of 86.31. It was obtained that the optimum concentration for reducing Pb levels in red mussels was at a concentration of 25%. It is possible at a concentration of 25% that all carboxylic groups in citric acid are deprotonized optimally so that it is estimated to have reached saturation. The COOH-deprotonized functional group becomes COO- which is used to provide Pb metal. [6].

4.2 Effectiveness

Treatment was obtained by comparing the tamarind solution with pH 2.25 and pure citric acid with a pH of 1.73. The best treatment was found in Tamarind solution for the lowest Pb level of 0.25 ppm with a concentration of 25% with a decrease of 98.89%. While the best treatment in pure citric acid solution for the lowest Pb concentration is 0.004 ppm with a concentration of 25% with a decrease of 99.97%. The pH of citric acid is smaller because the value of the acid determination is greater than the value of the Tamarind determination.

From these results it is known that citric acid can reduce Pb metal more than citric acid in Tamarind, which can occur because the pH of pure citric acid is lower. According to Indasah [7] pH has an important role in the absorption of metals. This is because pH can affect the solubility of metal ions in solution, the ability of other metal ions to bind to the surface of the biomass. This is also because the Tamarind has ingredients other than citric acid, namely malic acid, succinic acid, tartrate acid, potassium bitartrate, pectin, invert sugar, tannin and phosphorus. Compared with tamarind, pure citric acid has more chance to reduce concentration of Pb in red mussels, according to the Ministry of domestic Affairs 2001 R & D effectiveness ratio, 25% acidic java solution and 12.147% citric acid is classified as very effective in reducing Pb metal in red mussels.

4.3 Characteristic FTIR Test

Optimization result of Pb extraction with citric acid and Pb extraction using tamarind are only a few of the characteristics tested using IR Spectrophotometry (FTIR) to determine organic and inorganic functional groups. The results of the analysis of spectra show that there are several functional group compounds identified such as alcohol functional groups (-OH), carboxyl functional groups (-COOH), carbonyl functional groups (-CH2-, -CH3-, -CH-) Aldehydes (R-CHO)
Figure 2. The results of FTIR analysis of citric acid before and after the extraction process

Figure 2 shows that the graph of the wavelength of the citric acid solvent and extraction filtrate using citric acid. In the picture there are known differences in the peak point. Citric acid solvents are known to have group absorption (\(-\text{OH}\)) at 3316.33 cm\(^{-1}\), gory alkaline (\(\text{C} = \text{C}\)) at 2154.32 carboxylic groups (\(-\text{COOH}\)) at 1224.03 cm\(^{-1}\) whereas in the extracted filtrate there are more groups of absorption The OH bond at 3351.01 cm\(^{-1}\) then the alkane bond at 2921.56 cm\(^{-1}\) and 2851.56 cm\(^{-1}\) carboxylic bond at 1731.46 cm\(^{-1}\) at wavelength 1637.14 cm\(^{-1}\) with alkane and at wavelength 1145 cm\(^{-1}\) there is this amine group indicates that there is a solvent reaction with solute in the form of red mussels.

Figure 3. The results of FTIR analysis of Tamarind (Java acid) before and after the extraction process

Figure 3 states the graph between solvents in the form Tamarind solution and extraction filtrate in Figure 2 is not much different from Figure 3. It can be observed that the alcohol group was absorbed at a wavelength of 3263.09 cm\(^{-1}\), the wavelength of 1035 contained a carboxylic acid. In the extraction results of the absorbance spectra of alcohol and carboxylates at wavelengths of 3257.50 cm\(^{-1}\) and 1612.18 cm\(^{-1}\) there is a double bond at a wavelength of 2100.35 cm\(^{-1}\). At a wavelength of 1581.63 cm\(^{-1}\) there is an aromatic ring whereas at 1327.81 cm\(^{-1}\) there is a nitro bond then at a wavelength of 1280.88 cm\(^{-1}\) 1232.72 cm\(^{-1}\) and 1145.28 cm\(^{-1}\) there is a carboxylic acid while
at 954.09 cm⁻¹ and 811.61 cm⁻¹ alkene. The results of the adsorption on tamarind acid filtrate were more than the citric acid filtrate because tamarind acid contained more organic components.

**Result**

1. The effectiveness of citric acid in Tamarind is 98.89%
2. The effectiveness of citric acid in pure citric acid is 99.97%
3. The optimum condition of red mussel extraction with Javanese acid solvent is a concentration of 25% for 60 minutes

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