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The Effect of Chitosan of Ark Clam Shells to Reduce Pb and Hg Level and Amount of Bacteria in the Blood Cockles Meatball

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The Effect of Chitosan of Ark Clam Shells to Reduce Pb and Hg Level and Amount of Bacteria in the Blood Cockles Meatball

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Abstract. Chitosan of ark clams shell is regarded as untapped waste; it has potential as a heavy metal chelating agent and anti-bacterial. The objective of this research is to examine the effect of chitosan of the ark clams shell as a chelating agent to decrease of Pb and Hg level in the blood cockles meatball. This research is also aimed at exploring the capability of chitosan of the shell in decreasing the bacteria within blood cockle meatball. This research conducted complete randomized design with the provision of five-level of chitosan concentration, i.e., 0%, 0.5%, 1%, 1.5%, 2.0%. Atomic-Absorbent Spectrophotometric (AAS) method used to examine the level of Pb and Hg within the meatball, while the total of bacteria was determined by using Total Plate Count (TPC) method. The data were analyzed by using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test. The results reveal that the administration of chitosan of ark clam on blood cockle meatballs reduced Pb levels from 0.195 ppm to 0.168 ppm, while Hg levels reduced from levels of 0.018 to 0.008 ppm, and the number of bacteria from 2.4×10^2 to 1.4×10^2 colony/g. In conclusion, the chitosan of the ark clams shell can ensure the safety of the meatball product.

Keywords. Blood cockles; chitosan; Pb; Hg; bacteria

1. Introduction

Blood cockle (*Anadara granosa*) has red-coloured pigments which contain haemoglobin. Blood cockle can be found in the muddy part of the coastal area. Blood cockle is one of the sea products which is rich in protein. This protein content made blood cockle as an alternative food to fulfil the protein need of for the community. Blood clam or cockle can be processed to make meatballs. Meatballs are an alternative meal which can generally be found across Indonesia, including in Gorontalo. Meatballs made from blood cockle is a variation of the type of meatballs as meatball is usually made from beef, fish, and chicken.

In addition to having high protein content, blood cockle also contains heavy metals from the environment where the cell is found such as plumbum (Pb) and mercury (Hg) [1,2]. Besides, blood cockle is also vulnerable to bacteria contamination. Blood cockle which contains heavy metals and bacteria can cause health problems when it is consumed in a large proportion. Therefore, to maintain the food security of the blood cockle products, there is a need to reduce the heavy metal properties or bacteria within the product, hence the product made from blood cockle is safe to be consumed.



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One of the alternatives to reduce the heavy metal content and bacteria is by utilizing the ark clam shell into chitosan that can be applied in the food processing. Chitosan is the chitin that has been rid of its acetyl compound through deacetylation process. Previous research showed that the utilization of 2% chitosan from white shrimp could reduce the effect of Pb in tofu clam [3]. Besides, chitosan also has antioxidant and anti-bacterial properties [4-6].

One of the residues from the clam shell that can be utilized as chitosan is the shell of ark clam. This utilization of ark clam shell as chitosan is also an alternative for waste management into a product of economic value. This study was aimed at evaluating the effect of chitosan derived from ark clam shell as a chelating agent for Pb and Hg, as well as reducing the number of bacteria in the meatball made from blood cockle.

2. Materials and Methods

The ingredients of meat meatball are blood cockle, spices (salt, pepper, turmeric, onion, and garlic), tapioca starch, rice flour, egg, and water. Blood cockles were taken from the Boalemo regency of Gorontalo province. The ingredients to create chitosan are ark clam shell, NaOH, HCl, distilled water, HNO₃. The ingredients to check the number of bacteria are Plate Count Agar (PCA), acetate acid 1%, alcohol 70%, *aquades*, BPW (Buffered Peptone Water 0.1%), and spiritus. Research tools consist of AAS (Atomic absorption spectrophotometer), glass equipment, microwave tube, and Nessler tube, desiccator, oven, boiling pan, stove, knife, cutting plate, lab cloth, spoon, and bowl. This research used completely randomized design with 5 types of chitosan concentrates (0%; 0.5%; 1%; 1.5%; and 2.0%) which applied into the meatball mixture. The measurement of Pb and Hg level was measured using the AAS from ZEEnit 700. The level of Hg and Pb were measured in mg/kg. Whereas, the level of microbes were calculated using the TPC. Amount of microbes are calculated in cell/mL. The data of the Pb and Hg and bacteria level were analyzed using the ANOVA and least significance difference (LSD).

3. Result and Discussion

3.1. Level of Pb in blood cockle meatball

The result of the ANOVA test showed that the level of Pb in blood cockle meatball supplemented with the chitosan from the ark clam shell was significantly reduced ($p = 0.000$). The level of Pb in meatball supplemented with chitosan from the ark clam shell with the concentrate of 0%; 0.5%; 1%; 1.5%, and 2% in sequence are 0.195 ppm, 0.182 ppm, 0.176 ppm, 0.169 ppm, 0.168 ppm (see Table 1). The LSD test showed that supplementation of 0.5% chitosan was able to reduce the level of Pb in blood cockle meatball.

Table 1. Level of Pb in blood cockle meatball supplemented with chitosan derived from ark clam

No	Formula	Level of Pb (ppm)	The maximum level of pollutant SNI 7387:2009
1	Meatball without chitosan	0.195	1.5 ppm
2	Meatball with 0.5% chitosan	0.182	
3	Meatball with 1% chitosan	0.176	
4	Meatball with 1.5% chitosan	0.169	
5	Meatball with 2% chitosan	0.168	

Indonesian National Standard (SNI) 7387:2009: on the level of Pb in fish and fisheries product including molluscs, crustacean, echinoderms, amphibian, and reptile.

Administration of chitosan made from ark clam reduced the Pb level in blood cockle meatball. Similar research showed that the concentrate and length of soaking of the chitosan from white shrimp

also significantly influenced the reduction of Pb in the meat of the tofu clams [3]. Reduction of Pb level in blood cockle meatball due to the administration of chitosan from ark clam is suspected due to the chitosan which has an amino group and hydroxyl chains, hence made chitosan to have a high chemical reaction and caused the polyelectrolyte of the cation. This cation polyelectrolyte character caused the chitosan to have a role as an ion exchanger and functioned as heavy metal (Pb) absorbent.

The amino group can bond with heavy metal Pb. The amino group as a chelating agent will bind the Pb. The Pb that binds with the amino group (NH_2) will form $\text{Pb}(\text{NH}_2)_2$. In this condition the heavy metal that is bound in amino group will stabilize the Pb, thus, the toxic property of this heavy metal Pb will be reduced. The higher the chitosan concentration administered to the mixture the higher the number of an amino group (NH_2) that will be able to bind the level of heavy metal Pb.

Pb level in meatball where chitosan from ark clam is administered range between 0.168 – 0.182 ppm. The lowest level of Pb (0.168 ppm) is obtained through the administration of highest level of chitosan, 2%. Level of Pb in meatball supplemented with chitosan from ark clam is below the level of maximum Pb pollutant standard set by SNI 7387:2009, that is 1.5 ppm (for fish and fisheries product, including molluscs, crustacean, echinoderms, amphibian, and reptile) [7]. This study showed that chitosan derived from the shell of ark clam has potential as a food security-keeper for a claims-based meal.

3.2. Level of Hg in blood cockle in meatball

The ANOVA test showed that the level of Hg for blood cockle meatball which receives chitosan from ark clam had experienced significant reduction ($p = 0,000$) of its Hg level. The average Hg for blood cockle meatball supplemented with chitosan derived from ark clam shell with the concentrate of 0%; 0,5%; 1%; 1,5%; and 2% in sequence are 0.018 ppm, 0.009 ppm, 0.008 ppm, 0.008 ppm, 0.008 ppm (Table 2).

The result of the LSD test showed that the administration of chitosan with different concentration resulted in a significant difference in mercury level in the meatball that receives no chitosan administration. However, the administration of various chitosan concentrate resulted in a similar mercury level.

Table 2. Level of Hg (ppm) in blood cockle meatball with the administration of chitosan derived from ark clam

No	Formula	Levels of Hg (ppm)	Maximum allowed level of pollutant SNI 7387:2009
1	Meatball without chitosan	0.018	1 ppm
2	Meatball with 0.5% chitosan	0.009	
3	Meatball with 1% chitosan	0.008	
4	Meatball with 1.5% chitosan	0.008	
5	Meatball with 2% chitosan	0.008	

Indonesian National Standard (SNI) 7387:2009: Level of Hg on fish and fisheries products including molluscs, crustacean, echinoderm, amphibian, and reptile.

Administration of chitosan made from the shell of ark clam on the blood cockle meatball has proven to reduce the level of Hg from 0.018 ppm to 0.008 ppm. The lowest level of mercury is obtained through the administration of 2% of chitosan. The reduction of Hg in blood cockle meatball is parallel to the increase of chitosan concentration administered to the meatball. The reduction of Hg level in the

meatball is suspected due to the group N in chitosan which reaction is by binding the pollutant metal [8].

Level of mercury in meatball supplemented with chitosan derived from ark clam is below the allowed standard of Hg set by the SNI 7387:2009, that is 1 ppm (for fish and fisheries products including molluscs, crustacean, echinoderms, amphibian, and reptile) [7]. This means that the chitosan made from the shell of the ark clam has the potential to reduce the Hg in the meatball, thus, safe to be consumed.

3.3. Quantity of bacteria in the blood cockle meatball

The results showed that administration of chitosan derived from ark clam significantly ($p = 0.000$) reduced amount of bacteria in the blood cockle meatball. The average amount of bacteria in the meatball constantly reduced along with the increase of chitosan concentration administered to the blood cockle meatball. Based on the results of TPC analysis show that a total value of bacteria in the blood cockle meatball which receive the chitosan treatment with the following concentration 0%, 0.5%, 1%, 1.5%, and 2% in sequence were: 2.4×10^2 colony/g, 1.8×10^2 colony/g, 1.8×10^2 colony/g, 1.6×10^2 colony/g, and 1.4×10^2 colony/g (see Table 3).

The LSD test showed that an amount of bacteria in meatball that receive chitosan made from ark clam shell is significantly different with a number of bacteria in blood cockle meatball with no chitosan administration. This result also showed that the 0.5% concentrate of chitosan was able to reduce the amount of bacteria in the meatball.

Table 3. Total bacteria (colony/g) in blood cockle meatball that receive ark clam chitosan.

No	Formula	Total bacteria (colony/g)	Limit TPC value (SNI No. 7388: BPOMRI 2016)
1	Meatball without chitosan	2.4×10^2	5×10^5 colony/g
2	Meatball with 0.5% chitosan	1.8×10^2	
3	Meatball with 1% chitosan	1.8×10^2	
4	Meatball with 1.5% chitosan	1.6×10^2	
5	Meatball with 2% chitosan	1.4×10^2	

Note: Indonesian National Standard (SNI) 7388:2009; Drug and food security control agency of the Republic of Indonesia (BPOMRI), 2016: maximum level of allowed pollutant bacteria in fish and fisheries products including steamed or boiled and or fried molluscs, crustacean, and echinoderm.

An average amount of bacteria decreased following the increase of chitosan concentrate. The average amount of bacteria in blood cockle meatball was 2.4×10^2 colony/g to 1.4×10^2 colony/g. The smallest amount of bacteria exists in the administration of 2% chitosan. The result from other research showed that the concentration of pure chitosan influenced the total log of the microbes, texture (mucus), fungus appearance, smell, and visual acceptance of the wet noodle [9]. Besides, the chitosan from the sampling clam of 0.01 µg/disk and 0.02 µg/disk [10] and chitosan made from the shrimp skin with the concentrate of 1.5% reduce the number of *kamaboko* bacteria in *kurisi* fish [11].

The chitosan made from the ark clam shell has the ability to reduce the number of bacteria in blood cockle meatball as it has positive polycation that is able to inhibit the growth of bacteria and fungus [12]. Chitosan has amino group (NH_2), which in its further reaction would be protonated to become NH_3^+ which will be able to bind the negative load within the membrane of the bacteria in amino group (NH_3^+) owned by the chitosan will be able to form a strong bond with the characteristics of the membrane

of the microbe that has negative load. This creates depolarization of the membrane of the microbe cell, hence disturb the integrity of the microbe cell. Thus, the membrane of the cell becomes unable to regulate the circulation of the substance to and from the cell. As a consequence, the membrane of the cell was destroyed and experienced lysis hence; the metabolism activity will be inhibited and caused death for the microbe [13,14].

The amount of bacteria in blood cockle meatball is below the maximum allowed Indonesian National Standard (SNI) for steam or boiled and or fried fish and fisheries product including molluscs, crustacean, and echinoderms that is 5×10^2 colony/g [7,15]. This study showed that the chitosan made from the shell of the ark clam has the potential to be used for food safekeeping from bacteria.

4. Conclusions

Chitosan made from the shell of the ark clam has the ability to significantly reduce the level of Pb, Hg, and bacteria in the blood cockle meatball. Therefore, administration of chitosan made from the shell of the ark clam to blood cockle meatball can produce safer to be consumed.

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