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POTENTIAL CHITOSAN OF WASTE SHELL MANTIS SHRIMP (*Harpiosquilla raphidea*) AS ANTIBACTERIAL

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ABSTRACT

Mantis shrimp carapace waste can be processed into chitin and chitosan as antibacterial. This study aims to determine the characteristics, and heating time of chitosan on the potential of chitosan as an antibacterial, and to obtain the best chitosan in increasing the durability of catfish meatballs. Experimental research method with the manufacture of chitin and continued with deacetylation with a long heating time to obtain chitosan. The analytical parameters consisted of chemical characteristics (moisture content, ash, and degree of deacetylation), the inhibitory power of chitosan against *Escherichia coli* and *Staphylococcus aureus* bacteria, and the effectiveness of chitosan on the quality of catfish meatballs. The results showed that the chemical characteristics of chitosan were water content (K1 3.72%, K2 4.65%, K3 5.24%), ash (K1 2.38%, K2 2.53%, K3 3.69%), and the degree of deacetylation (K1 54.31%, K2 78.04%, K3 79.51%). The results of the inhibition test of chitosan against *Escherichia coli* and *Staphylococcus aureus* bacteria using the well method showed an inhibition zone with values (K1 10.11 mm and 10.18 mm, K2 11.52 mm and 11.48 mm, K3 12.15 mm and 11. 70 mm, positive control 14.64 mm and 14.02 mm, negative control 9.25 mm and 7.35 mm). This value indicates that the activity of chitosan as an antibacterial is quite strong. The effectiveness of chitosan on the quality of catfish meatballs with 7% chitosan immersion and storage at room temperature showed that the TPC value of catfish meatballs with chitosan could prolong the shelf life of meatballs until day-2.

Keywords: Mantis Shrimp, Chitosan, Bacterial Inhibition Zone, TPC

1. INTRODUCTION

Tanjung Jabung Barat Regency is one of the regencies in Jambi Province which is located on the easternmost coast of Jambi Province, the capital city is Kuala Tungkal. One of the fishery resources of Tanjung Jabung Barat Regency, Jambi Province is mantis shrimp (*Harpiosquilla raphidea*). The amount of mantis shrimp production in the waters of Tanjung Jabung Barat Regency is 537,200 tons[1].

The high production of mantis shrimp results in an increased amount of mantis shrimp waste. Mantis shrimp waste is obtained from the by-products of the fishing sector or the results of dead mantis shrimp that are not used in the export process. According to Rianta[2], the shrimp processing process produces waste and by-products in the form of skin and head by 60-70%.

Waste products in the form of heads, skins, and shells of mantis shrimp have the potential to be utilized, one of which is the manufacture of chitosan. According to Taufan & Zulfahmi[3], the chitin content in crab shells is around 71.4%. Chitin is a derivative compound of glucose and has non-toxic and easily degradable properties. One of the compounds derived from chitin and widely developed due to its wide application is chitosan.

Meatballs have a pH neutral and high water content so it is a good growth

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medium for microorganisms. This causes the shelf life of meatballs at room temperature to be very low, namely 12 hours to 24 hours, so meatball traders often add dangerous preservatives such as formalin or borax to extend the shelf life[4]. The utilization of chitosan as a natural preservative can be an alternative to increase the durability of meatball products without any health concerns.

Chitosan has benefits in everyday life such as heavy metal waste absorbents, dyes, preservatives, antifungals, antibacterials, cosmetics, pharmaceuticals, flocculants, and anticancer, chitosan can be active and interact with cells, enzymes, or negatively charged polymer matrices[5]. Meanwhile, according to Rianta[2], chitosan can be applied in the food field to function as a natural preservative, dye absorber, and antioxidant. This study aims to determine the quality characteristics of mantis shrimp shell chitosan, get the best heating time treatment, see the potential of chitosan as an antibacterial, and see the potential of chitosan in increasing the durability of catfish meatballs.

2. RESEARCH METHOD

Method

The mantis shrimp carapace flour obtained was then demineralized and deprotonated to obtain chitin. Chitin from mantis shrimp carapace flour was deacetylated with heating time K1 (90 minutes), K2 (120 minutes), and K3 (150 minutes) to obtain chitosan. The chitosan obtained was analyzed for a zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* bacteria and the activity of mantis shrimp carapace chitosan on the shelf life of catfish meatballs.

Procedure

The research consisted of 3 stages, namely: 1) preparation and proximate analysis of mantis shrimp shell flour, 2) chitin and chitosan extraction, 3) chitosan inhibition testing against *E.coli* and *S.aureus* bacteria, and chitosan application of mantis shrimp carapace chitosan on the shelf life of catfish meatballs.

Raw Materials Preparation

Mantis shrimp shells were washed under running water and brushed to remove any remaining dirt and debris. The washed shrimp shells were drained and dried in the sun until they shrimp shells were dry. The dried shrimp shells were reduced in size with a blender and sieved with a 60 mesh size to obtain fine mantis shrimp shell flour.

Chitin Extraction

The chitin isolation process consists of 3 stages, namely the deproteination stage, demineralization stage, and decolorization stage. The deproteination stage of mantis shrimp shell powder was added with 3.5% NaOH in a ratio of 1:10 (b/v). The mixture was heated at 60-700C for 2 hours while stirring. After the mixture cooled, it was filtered and washed with distilled water until neutral and then mined. The demineralization stage continued with the results in the deproteination process. At this stage, 1N HCl was added to the tube in a ratio of (1:10). Then the tube was heated at 60-70^{o}C while stirring for 2 hours. After completion, the precipitate was filtered and washed with distilled water until neutral and dried at 60^{o}C. The decolorization stage is pigment removal, the residue of the demineralization stage is added with 0.315% NaOCl in a ratio of 1:10 (b/v) and heated at 60-70^{o}C while stirring for 2 hours. After completion, the precipitate was filtered and washed with distilled water until neutral and dried at 60^{o}C

Chitosan Extraction

The obtained chitin was put into an *Erlenmeyer* tube, then added 50% NaOH, with a ratio of 1:10 (b/v), and heated at 100°C with varying lengths of heating time (90 minutes, 120 minutes, and 150 minutes). The solid obtained was washed repeatedly using distilled water until the PH was neutral, then dried in an oven.

Well Diffusion Method

The media that had been inoculated with the test bacterial culture was poured into a cup and left to freeze. Then five holes (wells) were made aseptically with a diameter of 7 mm and 60 µL of chitosan solution (K1, K2, K3) (9% b/v) was inserted. Incubate the media that has been given chitosan for 24 hours. The calculation of the inhibition zone in the well diffusion method can use the following formula:

$$\text{IP (cm)} = \frac{(Clear\ zone\ diameter - Well\ diameter}{Well\ diameter}$$

Data Analysis

In addition, observations were also made on the yield of carbon dioxide, water content, ash, and protein referring to the AOAC method[6], the degree of Deacetylation referring to the Winarti method[7], and the TPC test.

The research method used was experimental. The experimental design used for chitosan isolation was a completely randomized design (CRD) consisting of various heating reaction times (90 minutes, 120 minutes, and 150 minutes) as treatments during the chitosan deacetylation process. Parameters measured on mantis shrimp shell flour, chitin, and chitosan activity on the quality of the shelf life of catfish meatballs were analyzed descriptively

3. RESULT AND DISCUSSION

Chemical Composition Analysis of Mantis Shrimps Carapace Flout

The mantis shrimp carapace flour obtained is slightly reddish ash in color, fine grain texture, and has a distinctive shrimp aroma. The obtained mantis shrimp carapace flour was subjected to chemical analysis to determine the composition of moisture, ash, protein, and fat content. The chemical analysis of mantis shrimp carapace flour is presented in Table 1.

Table 1. Chemical analysis of mantis shrimp carapace meal

Content	Percentage (%)
Water	3.11±0.37
Ash	35.67±0.38
Protein	28.88±0.28
fat	0.61±0.02

The highest chemical composition of mantis shrimp carapace is ash content of 35.67% followed by protein content of 28.88%, the water content of 3.11%, and fat content of 0.61%. High ash content indicates high mineral (calcium carbonate) content. The moisture content is influenced by the dryness of the sample during the drying process and the drying method used. The method of drying materials using ovens and microwave ovens can produce controllable product moisture content than drying with sun drying methods.

Characteristics of Mantis Shrimps Carapace Chitin

The chitin extraction process goes through several stages, namely mineral removal (demineralization) and protein removal (deproteination). The mantis shrimp carapace chitin obtained has characteristics in the form of brownish-white color, the form of flakes, and crystals. The characteristics of mantis shrimp carapace chitin are presented in Table 2.

Table 2. Characteristics of mantis shrimp carapace chitin

Characteristics	Chitin (%)	Chitin Quality Standard (SNI[8])
Yield	31,38	
Water (bb)	3.02 ± 0,13	< 12 %
Ash (wt)	3,37 ± 0,25	< 5 %
Degree of Deacetylation	30,06	< 70 %

The results of the analysis of chitin characteristics produced have met the chitin quality standards set[8]. Water content is one of the most important parameters in

determining the quality of chitin. The lower the moisture content of chitin, the better the quality of chitin produced. The moisture content of chitin is influenced by the drying method, drying time, and the amount of chitin to be dried. Low water content also affects the growth of microorganisms in the media. The deacetylation degree of mantis shrimp carapace chitin produced is lower than the deacetylation degree of rama-rama shrimp which has a deacetylation degree of 43.30%[9]. The high degree of deacetylation indicates that chitin contains few acetyl groups. The fewer acetyl groups present in chitin, the stronger the interaction between ions and hydrogen bonds in chitin[7].

Characteristics of Mantis Shrimps Carapace Chitosan

The resulting chitosan has a yellowish-white color, has a crystalline shape, and is odorless and tasteless. The characteristics of the chitosan produced are presented in Table 3.

Table 3. Characteristics of mantis shrimp chitosan

Treatment	Characteristics			
	Yield (%)	Water Content (%)	Ash Content (%)	Deacetylation Degree (%)
K1	91,25	3,72	2,38	54,31
K2	85,93	4,65	2,53	78,04
K3	83,63	5,24	2,93	79,51

In the deacetylation process of chitin into chitosan, the highest yield was produced at a heating time of 90 minutes (K1) with a percentage of 91.25% and followed by a heating time of 120 minutes (K2) with a percentage of 85.93% and a heating time of 150 minutes (K3) with a percentage yield of 83.63%. The length of heating time can reduce the yield of chitosan, this is due to the long contact time of the NaOH solution so that the acetyl group changes to an amine group.

Moisture content is one of the most important parameters in determining the quality of chitosan. The lower the moisture content of chitosan, the better the quality of chitosan produced. Heating at different times gives different chitosan moisture content values, this is because the longer the heating time is carried out, the more water content is produced. The difference in chitosan moisture content is thought to be in the process of washing the chitosan with distilled water so that the PH is neutral which also causes an effect on the amount of chitosan moisture content. The water content of mantis shrimp carapace chitosan produced from the treatment showed results that met the standard of <10%[2].

Ash content is a parameter to determine the minerals contained in a material. The ash content of mantis shrimp carapace chitosan is higher than the chitosan quality standard of <2% [2]. Low ash content indicates low mineral content and the success of the deacetylation process. The lower the ash content, the higher the quality and purity of chitin or chitosan.

The degree of deacetylation is the percentage or number of acetyl groups released after deproteination, demineralization, and deacetylation processes. The degree of deacetylation states the number of free amino groups in polysaccharides. Prasetyaningrum et al.[10] stated, the higher the degree of deacetylation, the better the ability of chitosan as a food preservative. The highest degree of deacetylation in the 150-minute heating time treatment (K3) was 79.51% and the lowest was in the 90-minute heating time treatment (K1). The K1 treatment has a low degree of deacetylation because the NaOH compound does not have enough time to eliminate the acetyl group so the formation of amines is not much.

Zone of Inhibition of Mantis Shrimps Carapace Chitosan Extract against *E.coli* and *S.aureus* Bacteria

The bacteria used in this study were *E.coli* and *S.aureus* reason that it is a comparison because bacteria are grouped based on the arrangement of their cell walls into two, namely gram-positive and gram-negative bacteria. The inhibition zone activity of chitosan extract against *E.coli* and *S.aureus bacteria* can be seen in Table 4.

Table 4. Zone of inhibition activity of chitosan extract against *E.coli* and *S. aureus* bacteria by the well diffusion method

Treatment	Well Diffusion (mm)	
	E.coli	*S. aureus*
C^-	9.25	7.35
C^+	14.64	14.02
K1	10.11	10.18
K2	11.52	11.48
K3	12.15	11.70

The results showed that each treatment had bacterial inhibition. From each treatment, K3 showed great bacterial inhibition, this was due to the deacetylation degree of K3 treatment of 79.51%. The higher the degree of deacetylation of chitosan, the better the inhibition of chitosan against bacteria. The strength of an antibacterial substance based on its inhibition zone is classified into 4, namely very strong with an inhibition zone diameter >20mm, strong with an inhibition zone diameter of more than 10-20 mm, moderate activity if it produces an inhibition zone diameter of 7-10 mm, and weak activity if it has an inhibition zone diameter of less than 7 mm[11]. The results of inhibition zone measurements; each chitosan treatment is classified as strong.

The antibacterial activity of chitosan depends on the degree of deacetylation, molecular weight, the concentration used, the solvent used, and the species of target microorganisms. The positive charge of the NH3+ group in chitosan can interact with the negative charge on the surface of bacterial cells[12]. Damage to the cell wall results in a weakening of cell wall strength, abnormal cell wall shape, and enlarged cell wall pores. This causes the cell wall to be unable to regulate the exchange of substances from and into the cell, and then the cell membrane becomes damaged and lysed so that metabolic activity will be inhibited and will eventually die.

The difference in the cell wall structure in gram-negative and gram-positive bacteria causes differences in the bacterial response to chitosan. According to Yuliana[13], the greater inhibition of gram-negative bacteria is due to the thinner cell wall of gram-negative bacteria consisting of 10% peptidoglycan and high lipid content (11-22%). While Gram-positive bacteria have thick cell walls consisting of peptidoglycan of more than 50% and low lipid content (1-4%).

The Activity of Mantis Shrimps Carapace Chitosan on the Shelf Life of Catfish Meatballs

The content of bacteria in a product is one of the microbiological parameters in determining whether the product is suitable for consumption. Analysis of the number of bacteria aims to determine the total number of bacteria in a product and to determine the growth rate during storage. The observation results of chitosan activity on the TPC value of catfish meatballs during storage are presented in Table 5.

The provision of chitosan (K1, K2, K3) can extend the quality of catfish meatballs at room temperature storage until storage on day 2. Mohanasrinivasan et al[14]. explained that polycation compounds

owned by chitosan have bacteriostatic properties (inhibit microbial growth and development). Microbial/bacterial cell membranes (negative charge) are pulled by polycation compounds (positive) so that microbial growth is inhibited. In addition, other antimicrobial compounds in the form of acetic acid can inhibit microbial growth.

Table 5. TPC value of catfish meatballs during storage

Day	Treatment				
	C-	C+	K1	K2	K3
0	3.22	2.48	2.60	2.76	2.67
1	6.27	5.10	3.72	3.08	3.17
2	6.38	6.38	5.17	5.21	5.20
3	7.36	7.23	6.31	6.23	6.24
4	7.33	7.25	7.38	7.34	7.30

The positive control treatment (C+) using 1% acetic acid in the storage of catfish meatballs can maintain the quality of catfish meatballs on day 1 storage. According to Kurniasih & Kartika[15], acetic acid can inhibit bacteria.

4. CONCLUSION

The content of mantis shrimp carapace flour produced was 9.91% moisture content, 63.21% ash, 27.81% protein, 0.57% fat, and 31.85% yield. Chitin produced from mantis shrimp carapace flour has characteristics in the form of brownish-white color, the form of flakes, and crystals. The yield, water content, ash, and deacetylation degree of chitin produced have met the chitin standard. Chitosan from mantis shrimp carapace has yellowish-white color characteristics, has a crystalline shape, odorless and tasteless. The ash content of chitosan produced by each treatment ranged from 2.38-3.69%, where the ash content of chitosan exceeded the quality standard of <2%. Treatment K1 has a low degree of deacetylation and does not meet the quality standard of chitosan > 70%, this is due to the short heating time, so the NaOH solution does not have time to eliminate the acetyl group so the formation of amines is not much.

The chitosan sensitivity test on the inhibition zone of *Escherichia coli* and *Staphylococcus aureus* bacteria showed that the chitosan produced was relatively strong. Soaking catfish meatballs with a soaking time of 60 minutes with 7% chitosan concentration can maintain the quality of catfish meatballs for 2 days at room temperature storage.

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