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Preparation and Characterization of Chitosan Membranes from Crab Shells (*Scylla olivacea*) for Beverage Preservative

Reni Mulyani, Dikdik Mulyadi, Nopriyana Yusuf

Chemistry Study Program, Faculty of Science and Technology, Muhammadiyah University Sukabumi 43113, Sukabumi Indonesia

**Corresponding author: jauja_20@yahoo.co.id*

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Abstract

Chitosan can extend the shelf life of food and can be used in food preservation. Chitosan is derived from the shell of the animal crustacean, and is a derivative of the chitin polymer. This study aimed to determine the effectiveness of the use of chitosan membrane as an antibacterial compound and its application in pineapple juice products. Chitosan isolation is carried out through three stages, namely deproteination, demineralization, and deacetylation. Determination of the degree of deacetylation using the infrared spectroscopy method and the preservative effectiveness test was carried out based on SNI 01-2332.3-2006 concerning the testing of the Total Plate Count (ALT). Crab chitosan obtained from white-brown isolation results, soluble in 1% acetic acid and the value of the degree of deacetylation (DD) of chitosan crab is 81%. The addition of 1.5% crab chitosan membrane to pineapple juice can reduce bacterial growth until the 20th day with a total plate value (ALT) of 9.1x103 CFU / ml.

Keywords: Chitosan, pineapple juice, total plate count (TPC).

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1. INTRODUCTION

Fruit juice is one drink that is widely consumed and liked by the public. Generally, fruit juice has storage limitations such as nutrient loss by high temperatures, long shelf life and bacterial contamination. Most processed fruit products on the market are preserved using heating, the heating treatment of the juice causes the color to darken. Currently, research on the use of chitosan polymers as membranes is developing. Chitosan membranes are widely used in the separation, purification, and concentration of solutions. Chitosan membrane is easier to obtain compared to chitin membrane because of its relatively high solubility in acetate, making it easy to obtain membrane products after the solvent has been evaporated (Meriatna, 2008). Department of Marine and Fisheries in 2000 report, Indonesia produces waste crab shells, skins or heads of shrimp, and other marine animals of approximately 56.200 metric tons or 56.200 kg. Shrimp shell waste and crab shells contain chitin and have the potential to become more valuable products such as chitosan. Chitosan has a structure [β-(1-4)-2-amine 2-deoxy-D-glucose] is the result of deacetylation of chitin (Honarkar *et al.,* 2009).

According to Hafdani (2011) chitosan can inhibit the growth of pathogenic bacteria and spoilage microorganisms such as fungi, bacteria, gram-negative and gram-positive bacteria. Therefore chitosan has the potential as an antibacterial. Chitosan compounds that have the potential as an antibacterial can be used as food additives because they are not harmful to humans. Chitosan cannot be digested in the human body and will be released directly by the body through feces (Helander *et al.,* 2003). Chitosan has a functional group of amines ($-NH_3^+$) which are positively charged and highly reactive, so that they can bind to the cell walls of negatively charged bacteria.

Chitosan structure resembles peptidoglycan, where the peptidoglycan is the main structure of cell wall compilers in gram-positive bacteria (Hafdani *et al.,* 2011). Chitosan isolation begins with deproteination

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stage, namely removal of protein in the sample using a NaOH solution with a concentration of 3.5%. The next process is the demineralization step to purify chitin from the mineral using 1.5 M HCl solution. Furthermore, chitosan is obtained from the deacetylation stage by heating in a solution of NaOH with high concentrations (> 40%) and high temperatures ranging from 90-120 °C (Hong *et al.*, 1989) and characterization using FTIR.

The way to find out the presence of microbial contamination in food products is microbiological examination. This examination is an indicator of microbial contamination that exceeds the maximum limit standard (Suriawiria, 1996). Methods in microbiology, especially for fruit drinks one of total plate count (TPC) (BSNI, 2014).

2. MATERIALS AND METHODS

Materials and Instruments

The instrument used in this research was a set of glassware available in the laboratory, magnetic stirrer, glass mold, filter paper, universal pH and Bruker FT-IR spectroscopy. The ingredients used were pineapple juice processed by themselves, crab shells, HCl, NaOH, distilled water, CH_3COOH.

Sample Preparation

Crab shell was cleaned with running water until clean, dried in the sun, so that the pulverized into powder, and sieved to 60 mesh size.

Isolation of Crab Shell Chitosan

Deproteination: 60 grams of crab shell added with 3.5% NaOH solution (1:10) was heated for 2 hours at 65 °C. Filtered and neutralized using distilled water. Solids obtained in the oven at 40 °C.

Demineralization: the obtained solid was added with a 1.5 M HCl solution (1:10) heated for 2 hours at room temperature. Filtered and neutralized using distilled water. Chitin obtained was dried in an oven at 40 °C.

Deacetylation: The resulting chitin was added with 70% NaOH solution (1:10), heated for 8 hours at 120 °C. Filtered and neutralized using distilled water. Chitosan was obtained in an oven at 40 °C.

Preparation of Chitosan Membranes

One gram of shrimp and crab chitosan was dissolved in 100 mL CH_3COOH 1% then spread over glass molds and allowed to dry through the evaporation process at room temperature.

Chitosan Membrane Application

Pineapple juice was heated for 20 minutes, then chitosan membrane (1%, 1.5%, 2%) was added per 100 mL of juice. 100 mL juice without chitosan as a control. All treatments were stored in glass bottles at room temperature.

Chitosan Characterization

Chitosan characterization was performed using FT-IR (Fourier Transform Infrared) spectroscopy to see functional groups.

Analysis of Total Bacteria (Total Plate Count)

One mL of each dilution was put into a sterile petri dish and added 12-15 ml PCA (plate count agar) which was cooled to 45 ºC ± 1ºC into each cup containing the sample and then incubated in reverse position for 48 hours at 37 °C.

3. RESULTS AND DISCUSSION

Isolation of Chitosan from Crab Shells

Table 1. The yield of chitin isolation becomes chitosan from crab shells

Stages	Yield (% w/w)
Deproteination	88.85
Demineralization	65.87
Deacetylation	37.72

The initial stage of chitin isolation into chitosan is a deproteination process aimed at eliminating the protein content using a 3.5% base solution (NaOH). At this stage, the solution becomes thick, that indicates the protein content of chitin which is released and binds to Na+ ions in the solution and forms Na-proteinate. The equation of the reaction can be seen in Figure 1 The end of the negatively charged protein (polyamide) chain will react with a base (NaOH) to form an amino salt.

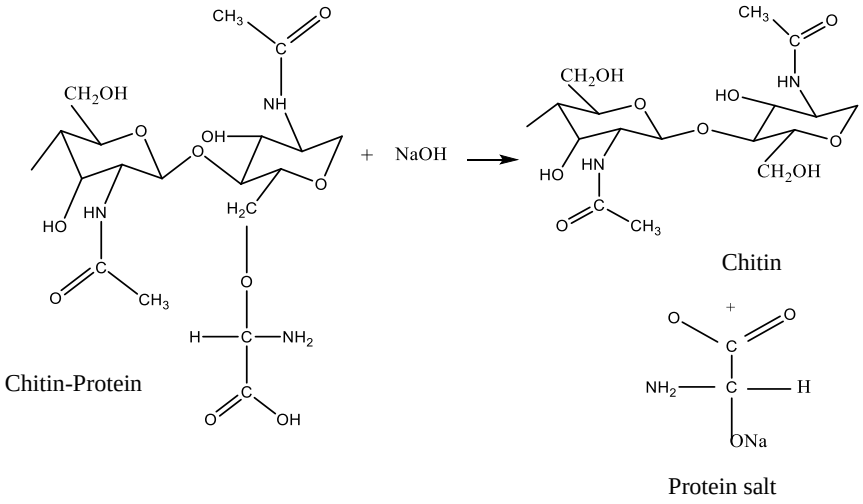

Figure 1. Deproteination reaction (Riswan, 2014).

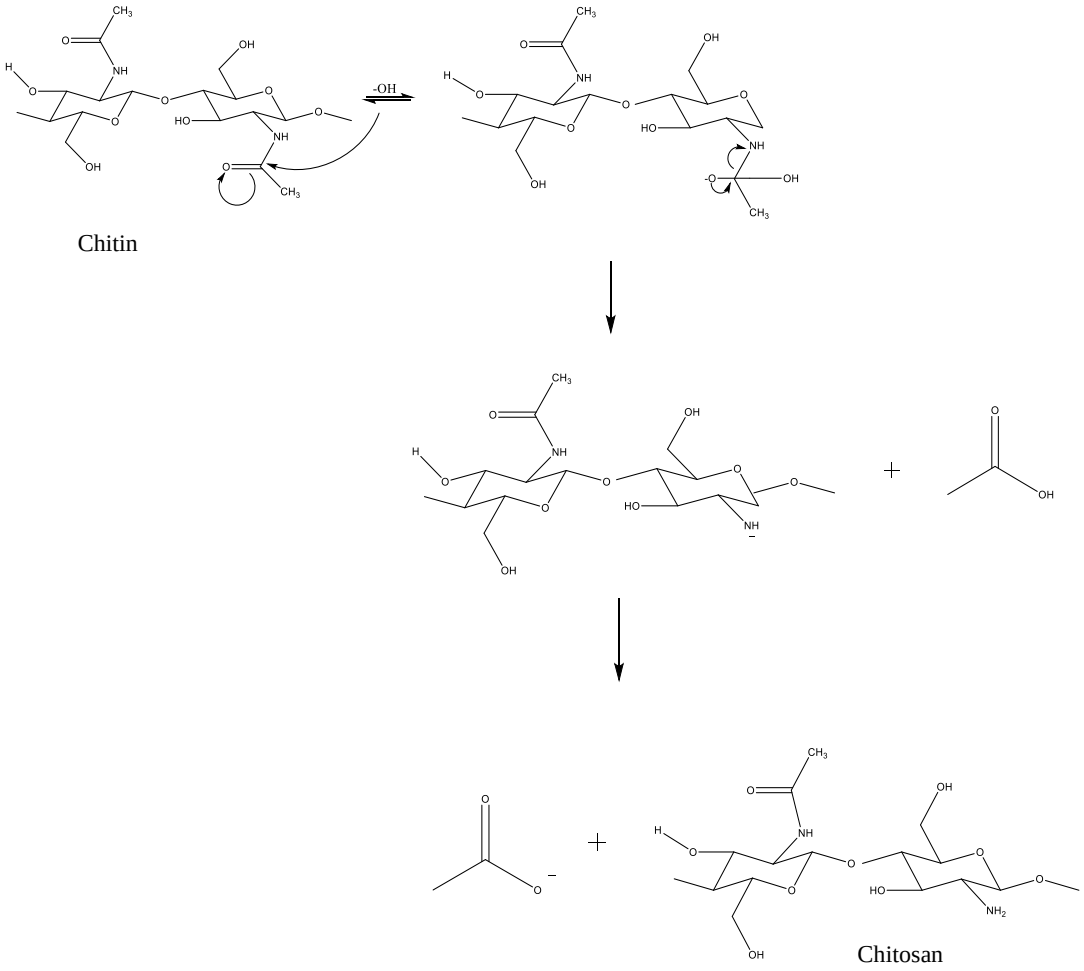

Figure 2. Deacetylation reaction of chitin (Champagne, 2002)

The second stage is demineralization which aims to separate the organic minerals that are bound to the basic ingredients, namely $CaCO_3$ as the main mineral and $(Ca_3(PO_4)_2)$ in small amounts. The process that occurs in the demineralization stage is that the minerals contained in the crab shell will react with HCl so that the mineral separation occurs marked by the formation of foam and air bubbles. This indicates the formation of CO_2 and H_2O gas on the surface of the solution (Hendry, 2008). The demineralization process uses 1.5 M HCl

because the use of acid with low concentrations and constant stirring will lead to complete mineral separation. The reaction equation is as follows:

$$Ca_3(PO_4)_{2(s)} + 6HCl_{(aq)} \rightarrow 3CaCl_{2(aq)} + 2H_3PO_{4(aq)}$$

$$CaCO_{3(s)} + 2HCl_{(aq)} \rightarrow CaCl_{2(aq)} + H_2CO_{3(g)}$$

$$H_2CO_{3(g)} \rightarrow CO_{2(g)} + H_2O_{(l)}$$

The third step, deacetylation of chitin using 70% NaOH aims to break the bond between the nitrogen atom and the acetyl group so as to produce an amine group ($-NH_2$). The higher the concentration of NaOH used causes the acetyl group to be separated from the chitin polymer structure to be greater so that the degree of deacetylation is higher (Kim *et al.*, 2004). The equation of the reaction can be seen in Figure 2.

The isolation results show that the color of chitosan obtained from the crab shell has a brownish white color as shown in Figure 4. This is due to the presence of carotenoid pigment content in the crab shell, among others, β-carotene, zeaxanthin, astaxanthin, astaxanthin dieter, and astaxanthin monoester (Ross, 2001).

Figure 3. Chitosan crab

Figure 5 shows the presence of OH stretching (3448.72 cm^{-1}), NH bending (R-NH_2) (1564.27 cm^{-1}), CH stretching (2887.44 cm^{-1}), C=O stretching on the bond (-$NHCOCH_3$) (1660.71 cm^{-1}), CO stretching (1026.13 cm^{-1}) (Silverstein *et al.*, 1989). Based on the analysis of the spectrum characteristics above between chitosan isolated from standard chitosan there was no significant difference in stretching in the range 896.90 cm-1 indicating the existence of β-1,4-glycosidic bonds.

Chitosan Characterization

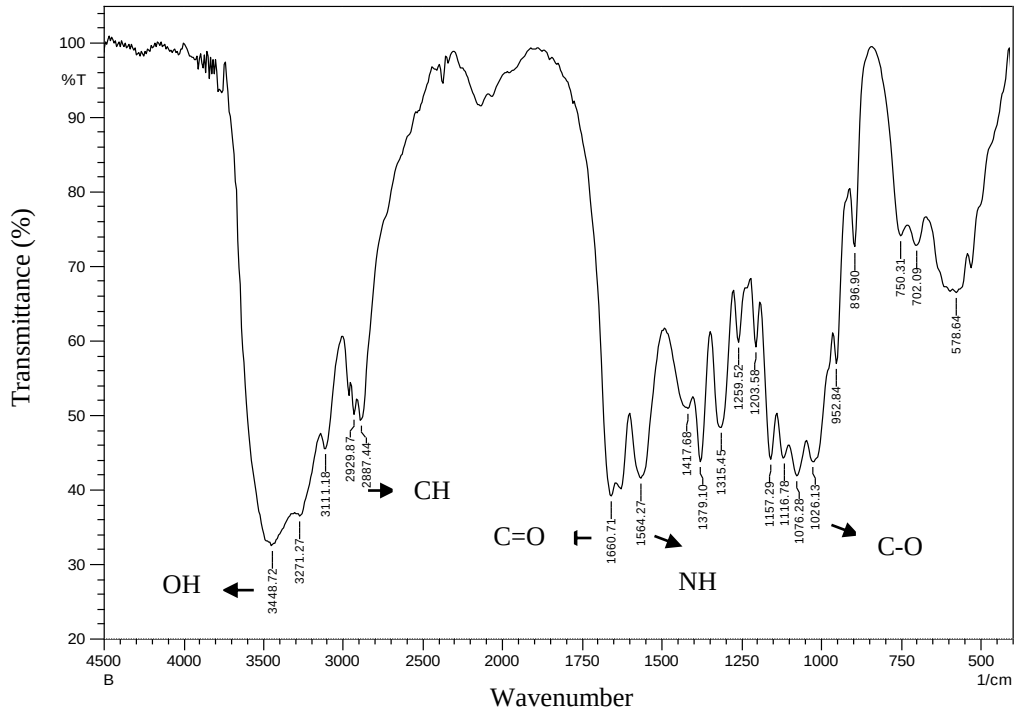

Figure 4. FT-IR Spectrum of crab chitosan

Determination of The Degree of Deacetylation

The degree of deacetylation is obtained by calculating the absorption at wavelengths of 1655 cm^{-1} and 3450 cm^{-1}. The degree of deacetylation obtained from chitosan crab isolation was 81%. This shows that chitin has not been completely deacetylated into chitosan because there is still an acetyl content. Perfect deacetylation is achieved if the value of the degree of deacetylation (DD)> 90% (Srijanto, 2003).

Test Total Plate Count

Addition of chitosan membrane can reduce the growth rate of the number of bacterial colonies in pineapple juice, but different chitosan membrane concentrations would affect the growth of bacteria in pineapple juice. Chitosan membrane with a concentration of 1%, 1.5%, and 2% has its own ability to suppress bacterial growth. Test the total plate numbers for chitosan from crab shells can be seen in Table 2.

Table 2. Test total plate count

Chitosan Concentration (w/v)	Results CFU/mL
1%	16 x 105
1.5%	9.1 x 103
2%	0
Control	55 x 106

Another mechanism that may occur positively charged chitosan will interact with the negatively charged phosphate group in theatric acid, causing intracellular leakage of cells which ultimately leads to cell death. Teichoic acid itself is a constituent of bacterial cell walls (Tyagi *et al.*, 2014). Illustration of inhibitory mechanism by chitosan can be seen in Figure 5.

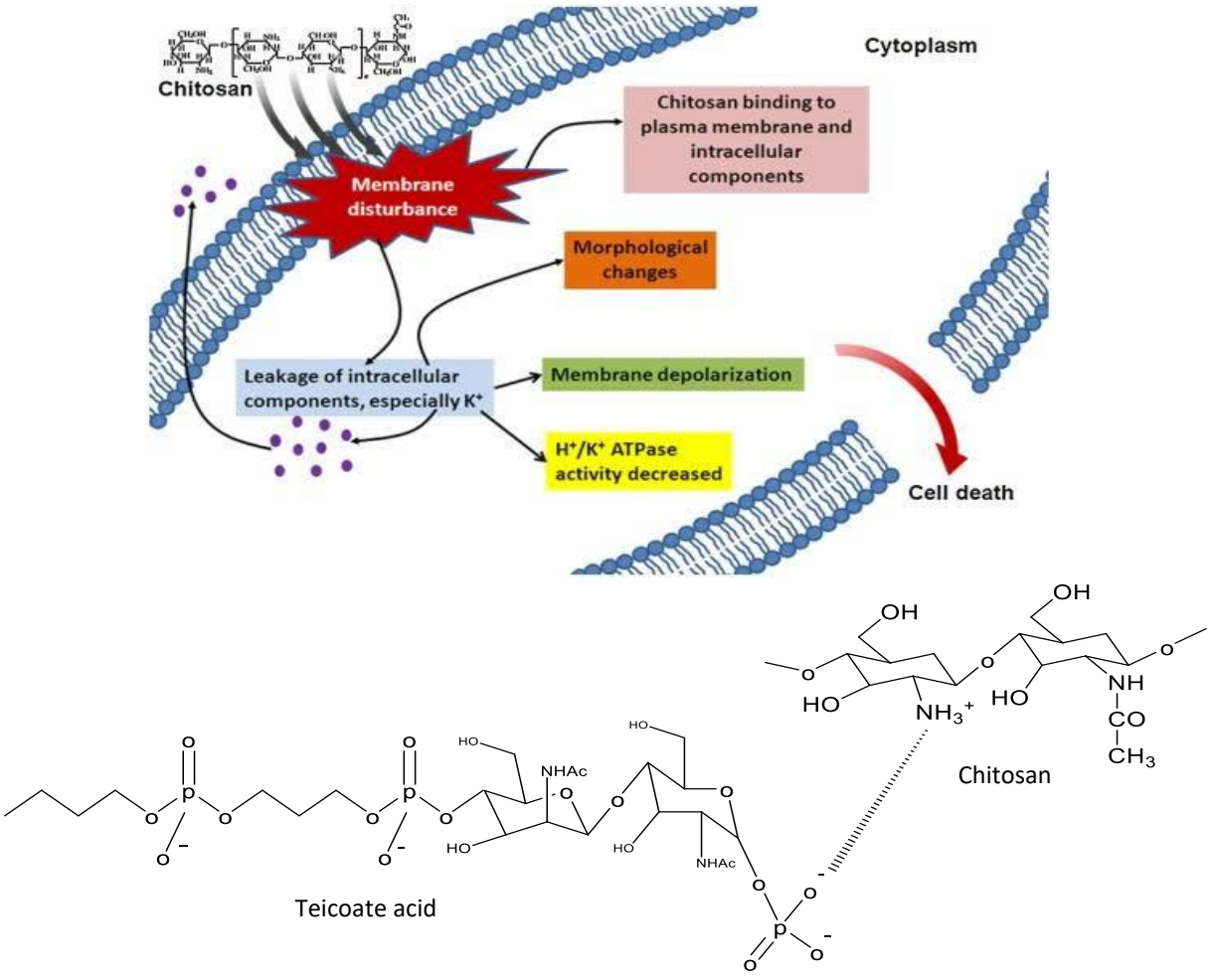

Figure 5. Mechanism of bacterial inhibition by chitosan (Tyagi *et al.*, 2014)

From the research results obtained using the membrane chitosan crab able to suppress bacterial growth, i.e. at the use of a concentration of 1.5% the results of testing the total plate number (ALT) obtained by 9.1 x 10^3 CFU / mL the value is still below the SNI set threshold of 1x104 CFU / mL (7338-2009).

4. CONCLUSION

Chitosan is obtained through three stages, namely deproteination, demineralization, and deacetylation. Chitosan crab obtained in the form of light brown powder and soluble in 1% acetic acid. The degree of deacetylation produced by 81%. Chitosan membrane made from the shells of crabs (*Scylla olivacea*) is able to suppress the growth of bacteria that is at a concentration of 1.5% can be used as an antibacterial against pineapple juice until the 20th day to the value of Total Plate Count (TPC) of 9.1x10^3 CFU/mL

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