Chitosan Membrane from Shrimp Shells (*Panaeus Modonon*) as an Antibacterial Food

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Abstract: Chitosan can extend the shelf life of food, so that it 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 aims to determine the effectiveness of the use of chitosan membrane as an antibacterial compound and its application in pineapple juice products. Chitosan enters and damages the cell walls of gram-negative bacteria that cause death in bacteria, gram-negative bacteria have the ability to interact and absorption of chitosan is greater than gram-positive bacteria. 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 testing of the Total Plate Count (ALT). Shrimp chitosan obtained from the insulation results is white, soluble in 1% acetic acid, and the value of the degree of deacetylation (DD) 72%. Test the total plate count with bacterial control 55 x 106 CFU / mL at the addition of 1% chitosan worth <2 x103 CFU / mL, Addition of 1.5% chitosan worth 30x 105, CFU / mL.

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 contamination by microbes. Most processed fruit products on the market are preserved using heating. The heating treatment of pineapple juice causes the color of the juice to change [6,12].

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 membranes are more easily obtained than chitin membranes because of their relatively high solubility in acetate making it easy to obtain membrane products after the solvent has been evaporated [10]. The Ministry of Maritime Affairs and Fisheries reported in 2000 that Indonesia produced approximately 56,200 metric tons or 56,200 kg of crab shells, shrimp shells or shrimp heads and other marine animals. Shrimp skin waste is proven to be rich in chitin and has the potential to become a more valuable product such as chitosan. Chitosan has the structure of [β- (1-4) -2-amine-2-deoxy D-glucose] which is the result of deacetylation of [5].

Chitosan can inhibit the growth of pathogenic bacteria and spoilage microorganisms such as fungi, gram-negative bacteria 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 [3]. Chitosan cannot be digested in the human body and will be released directly by the body through feces [4]. Chitosan has a functional group of amines ($-NH_3^+$) that 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 [3]. Chitosan isolation begins with deproteination stage, namely removal of protein in the sample using a NaOH solution with a concentration of 3.5%. The next stage, demineralization process to purify chitin from minerals using a 1.5 M HCl solution. Furthermore, chitosan was obtained from the deacetylation stage by heating in a NaOH solution with high concentrations (> 40%) and high temperatures ranging from 90-120^{o}C [5] 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). The method in microbiological examination especially for fruit drinks is one of them is the total plate number (TPN)[1].

2. Research Method

2.1. Tools and materials

The instrument used in this study is a set of glassware available in the laboratory, magnetic stirrer, glass mold, filter paper, universal pH and bruker FT-IR spectroscopy. The ingredients used are pineapple juice, shrimp shells; crab shells, HCl, NaOH, distilled water, CH_3COOH.

2.2. Sample preparation

Shrimp skin is cleaned with running water until clean, dried in the sun, mashed so that it becomes powder, and sieved with a size of 60 Mesh.

2.3. Chitosan isolation of shrimp skin

The deproteination of 60 grams of shrimp shells added with 3.5% NaOH solution (1:10) was heated for 2 hours at 65^{o}C. Filtered and neutralized using distilled water. Solids obtained in the oven at 40^{o}C. Demineralization of 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^{o}C. Deacetylation of chitin produced was added with 70% NaOH solution (1:10), heated for 8 hours at 120^{o}C. Filtered and neutralized using distilled water. Chitosan is obtained in an oven at 40^{o}C.

2.4. Making chitosan membranes

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

2.5. Chitosan membrane applications

Pineapple juice was heated for 20 minutes then chitosan membrane (1%, 2%, and 3%) was added per 100 mL of fruit juice and 100 mL of fruit juice without chitosan membrane as a control. All treatments were stored in glass bottles at room temperature for 20 days.

2.6. Chitosan Characterization

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

2.7. Analysis of Microbial Numbers (Total Plate Figures)
One mL of each dilution is put into a sterile petri dish and added 12-15 ml PCA (plate count agar) which has cooled to 45ºC ± 1ºC into each cup containing the sample and then incubated in an upside down position for 48 hours at 37ºC .

3. Results and discussion

Chitosan yield after deacetylation for shrimp shells was 36.69%. This is close to the results of Muzarelli's (1985) study that the percentage of chitin in shrimp shells is 20-30%.

Table 1. Yield of chitin isolation into chitosan from shrimp shells

Stages	Chitosan Yield (%b/b)
	Shrimp
Deproteination	86,78
Demineralization	60,34
Deacetylation	36,69

The initial process of chitin isolation into chitosan is a deproteination process that aims to eliminate the protein content using a 3,5% base solution (NaOH). At this stage the solution becomes thick, this 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 sTPN. 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.

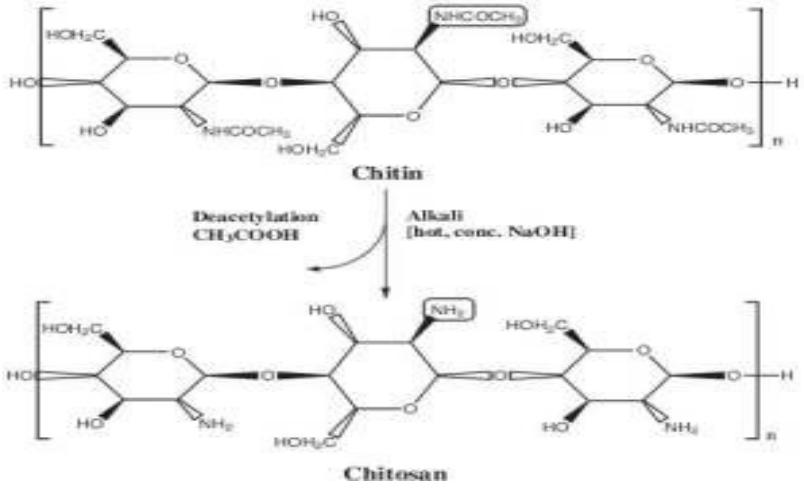

Figure 1. Chemical structure of chitosan and its production from chitin [14]

The process that occurs in the demineralization stage is that the minerals contained in the shrimp shells will react with HCl so that the mineral separation is 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 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 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 [7]. The isolation results show that the color of chitosan obtained from white shrimp shells is brighter (Figure 2). This proves that shrimp shells have different pigment contents. Carotenoid pigments contained in shrimp shells include echinonen, lutein, cantaxanthin, β-carotene, astaxanthin, phenoxanthin, and zeaxanthin [9].

Figur 2. Chitosan shrimp

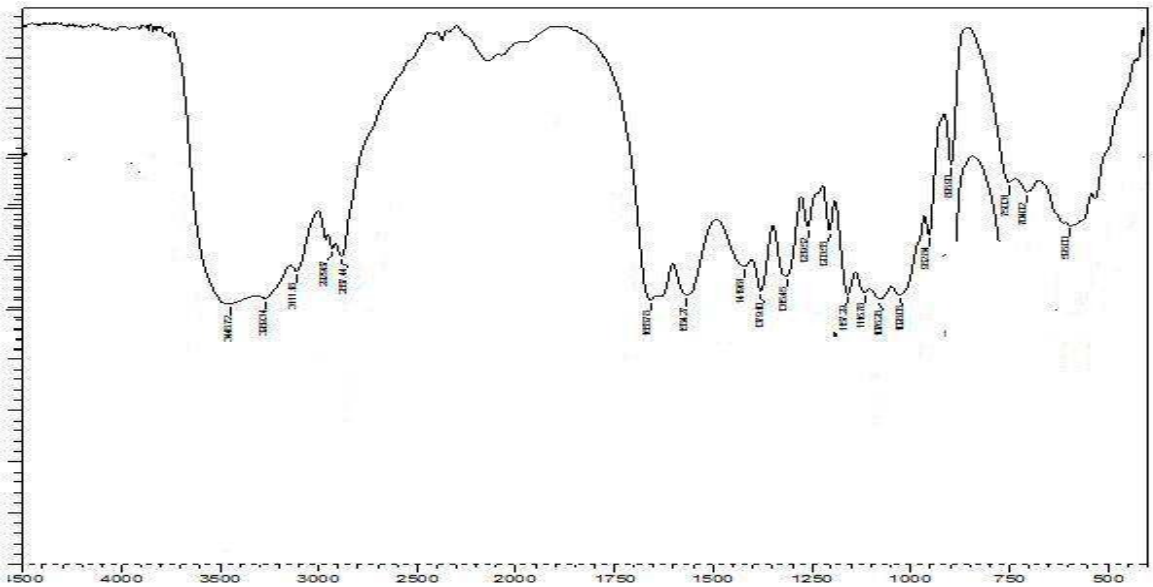

Figure 3. FT-IR spectrum of shrimp chitosan

Figure 3, shows that the chitosan absorption band in the range 3448.72 cm-1 is OH stretching. The presence of -NH bending ($R-NH_2$) at 1564.27 cm-1 which is a typical absorption for chitosan. C-H stretching ($-CH_2$) for shrimp chitosan. Weak stretching in the range of 1658.71 cm-1 for shrimp chitosan, Stretching in the range of 896.90 cm-1 indicates the existence of β-1,4-glycosidic bonds.

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 the isolation of shrimp chitosan 72%. This shows that chitin has not been completely deacetylated into chitosan because there is still an acetyl content. Perfectly, deacetylation if the value of the degree of deacetylation (DD)> 90% [13]

Table 2. Test the total plate figures

Chitosan concentration	CFU / ml results
	Chitosan shrimp
1 %	$< 2 \times 10^3$
1,5 %	30×10^5
Control	55×10^6

The addition of chitosan membrane can reduce the growth rate of the number of bacterial colonies in pineapple juice. However, pineapple juice with the addition of chitosan membrane has a thicker texture than pineapple juice without chitosan membrane, viscosity occurs due to chitosan which is a polysaccharide that can become a natural fiber (mucilage) when dissolved. The mechanism of bacterial inhibition by chitosan is not known with certainty until now. However, the most widely accepted and used mechanism is due to the interaction of chitosan positive charge with negative charge on the bacterial surface that will cause changes in cell surface permeability. This will cause the loss of some of the cell constituents such as protein, amino acids and glucose which results in inhibited metabolism and ultimately causes death of bacterial cells [8].

The mechanism of inhibition of bacteria by chitosan is different between gram-negative bacteria and gram-positive bacteria. The cell wall of gram negative bacteria is thinner consisting of 10% peptidoglycan and high lipid content (11-12%). While gram-positive bacteria have thick cell walls consisting of more than 50% peptidoglycan and low lipids (1-4%) so thicker cell walls are difficult to damage [11]. Therefore the inhibitory effect of chitosan is better in gram negative bacteria.

Chitosan is easier to enter and damage the cell walls of gram-negative bacteria that cause the death of bacteria, this is because gram-negative bacteria have the ability to interact and absorption of chitosan is greater than gram-positive bacteria [2].

4. Conclusion

Chitosan is obtained through three stages, namely deproteination, demineralization, and deacetylation. Chitosan produced shrimp is in the form of white powder. Percent degrees of deacetylation produced for chitosan shrimp and crabs respectively 72% and 81%. Chitosan membrane made from crab shells (*Scylla olivacea*) is more effective in suppressing bacterial growth ie at a concentration of 1,5% can be used as an antibacterial against pineapple juice because it is able to suppress bacterial growth until the 20th day with the value of the Total Plate Number (TPN) 9.1x103 CFU / mL.

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References

[1] Indonesian National Standard Agency (BSNI). (2014). *Fruit Drinks, SNI 3719-2014*. National Standardization Agency, Jakarta: BSN

[2] Alessandro F. Martins, Suelen P. Facchi, Heveline D. M. Follmann, Antonio G. B. Pereira, Adley F. Rubira Edvani C. Muniz. [2014]. *Antimicrobial Activity of Chitosan*

Derivatives Containing N-Quaternized Moieties in Its Backbone: A Review, *Int. J. Mol. Sci. 15*(11), 20800-20832

[3] Hafdani, FN. and Sadeghinia. N. (2011). *A Review on Application of Chitosan as a Natural Antimicrobial.* World Academy of Science. Engineering and Technology 50.

[4] Helander IM, Nurmiaho EL, Ahvenainen R, Rhoades J, Roller S. (2001). *Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria.* International Journal of Food Microbiology 71: 235-244.

[5] Hakima El Knidri, Raja Belaabed, Abdellah Addaou, Ali Laajeb, Ahmed Lahsini.(2018). *Extraction, chemical modification and characterization of chitin and chitosan,* International Journal of Biological Macromolecules, Volume 120, Part A, December 2018, Pages 1181-1189.

[6] Kamal Rai Aneja, Romika Dhiman, Neeraj Kumar Aggarwal, and Ashish Aneja.(2014). *Emerging Preservation Techniques for Controlling Spoilageand Pathogenic Microorganisms in Fruit Juices*, International Journal of Microbiology, Volume 2014, Article ID 758942, 14 pages

[7] Kim, SOF. (2004). *Physicochemical and Functional Properties of Crowfish Chitosan As Affected by Defferent Processing of Paper.* Seoul : Seoul University. [Thesis]

[8] Kurniasih, Mardiyah, Dwi Kartika. (2009). *Antibacterial Activity of Chitosan on S.aureus Bacteri*a. Molecular Journal. Vol 4. No. 1: 1-5 pp.

[9] Kouchi, H.H, Marzieh, M.N, and Bahareh, S. (2012). *Extraction of Carotenoid From Crustacean,* Science and Natural Resource.

[10] Meriatna. (2008). *The Use of Chitosan Membranes to Reduce the Levels of Chrome (Cr) and Nickel (Ni) Metals in Metal Coating Liquid Waste*. USU: Medan. [Thesis].

[11] Rabea, E.E, Badawy, M.E.T, Stevens, C.V, Smagghe, G and Steurbout, W. (2003). *Chitosan as Antimicrobial Agent* : Aplications and Mode of Action. Biomacromolecul. 4 : 1457-1465.

[12] Rattanathanalerk, M.N. Chlawchan, and W. Srichumpoung. (2005). *Effect of thermal processing on the quality loss of pineapple juice*. J. Food Engin : 269-275.

[13] Srijanto, B.(2003). *Chemical Technology Development Process Study of Chitin and Chitosan*. Proceedings of the Indonesian Chemical Engineering National Seminar. [On line].

[14] Stephen O.Majekodunmi.[2016].*Current Development of Extraction, Characterization and Evaluation of Properties of Chitosan and its Use in Medicine and Pharmaceutical Industry*, American Journal of Polymer Science, 6(3):86-91.