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Physicochemical Properties of Golden Apple Snail (*Pomacea canaliculata*) Shell Chitosan

Sekar Ayu Larasati[1,2], Dedin Finatsiyatull Rosida[1,2]*, Jariyah[1,2], Teeradate Kongpichitchoke[3], Anugerah Dany Priyanto[1,2], Andre Yusuf Trisna Putra[1,2]

[1]Department of Food Technology, Faculty of Engineering,
University of Pembangunan Nasional Veteran East Java, Surabaya, 60294, Indonesia
[2]Center Innovation of Appropriate Technology for Lowland and Coastal Food, Surabaya, East Java, Indonesia
[3]Faculty of Biotechnology,Assumption University, Bangkok, Thailand
Corres Author Emai: dedin.tp@upnjatim.ac.id

ABSTRACT

Pomacea canaliculata, an animal that lives rice fields, is one of the agricultural pests that frequently affects and may retard the growth of rice plants due to its rapid development. The golden snail shell contains a chitin polysaccharide which can be reduced to chitosan (β1-4 N-acetyl D-glucosamine) through the deacetylation stage, namely the process of taking the acetamide group in chitin (CH_3CONH) so that it becomes an amine group (NH2) in chitosan. The manufacture of chitosan is carried out through 3 stages, namely the process of deproteination, demineralization, and deacetylation. The purpose of this study was to determine the characteristics and quality of chitosan produced from golden snail shells. Based on the analysis conducted, chitosan has a yield of 53.91%; water content 1.68%; ash content 12.31%; molecular weight 640.83 kDa; solubility 95.53; and deacetylation degree 82.33%.

Keywords: *Chitin; Chitosan; Deacetylation; Golden Snail*

INTRODUCTION

The golden snail (*Pomacea canaliculata*) is an animal that can reproduce quickly, which lives in agricultural areas and eventually becomes the most common pest of rice plants in several regions in Indonesia. The golden snail pest usually attacks young rice plants that are less than 1 month old (Sulistiono, 2020). The golden snail lays 1,000-1,200 eggs per month can reduce the productivity of rice plants by up to 16-40% (Purnamaningsih, 2010). The use of snails is limited to the consumption of snail meat by some people, which causes the shells to be very abundant and easy to find. Until now, rice field snail shell waste is mostly processed as animal (poultry) feed (Nurhaeni et al., 2019).

Chitin is one of the polysaccharides which are one of the main constituents of the shells of the crustacean group of animals (crabs, shrimp, crabs); molluscs (clams, snails), insects (cockroaches, scorpions, spiders, beetles), as well as several types of functions, are naturally non-toxic and biodegradable (Islam et al., 2017). Chitin ($C_8H_{13}O_5N$) or ((1-4)-N-acetyl-D-glucosamine) is the second most abundant natural biopolymer besides cellulose in nature. Applications of chitin are limited compared to chitosan because chitin is chemically inert and insoluble in both water and acid, while chitosan is relatively reactive and can be produced in various forms (Lodhi et al., 2014). Naturally the level of acetylation of chitin is still low, so that in its utilization in the food or medical sector, it needs to be converted into chitosan first. Chitin and chitosan contain 5–8 % nitrogen, in which chitin is in the form of acetylated amine groups and in chitosan in the form of primary aliphatic amine groups, which makes chitin and chitosan suitable for typical reactions of amines (Islam et al., 2017).

The chitin obtained can be converted into chitosan by changing the acetamide group (-NHCOCH3) in chitin into an amino group (-NH2). Chitosan is a poly-(2-amino-2-deoxy-β-(1-4)-D-glucopyranose) compound which is bonded to each other by (1-4) β-glycosidic bonds,

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with the molecular formula $(C_6H_{11}NO_4)n$, which is an abundant sources biopolymers (Bahri, 2015). Structurally, chitosan contains primary and secondary hydroxyl groups for each repeating unit, and an amine group for each deacetylated unit, which makes chitosan more reactive (Islam et al., 2017). Chitosan can be obtained in various forms including irregular structures, crystalline or semi-crystalline forms. Besides that, it can also be a white solid with a fixed crystal structure from the initial form of pure chitin. The solubility of chitosan in acidic solutions and the viscosity of the solution depend on the degree of deacetylation. Currently, chitosan has received considerable attention for its commercial applications in the biomedical, food, and chemical industries. Due to its unique biological characteristics, including biodegradability and nontoxicity, many applications have been found either alone or blended with other natural polymers (starch, gelatin, and alginates) in the food, pharmaceutical, textile, agriculture, water treatment, and cosmetics industries (Lodhi et al., 2014). The purpose of this study was to determine the physicochemical characteristics of the golden snail shell chitosan, including the functional groups contained.

METHODS

Material

The materials used is golden snail shell obtained from East Jakarta (purchased through online market), Indonesia, hydrocloric acid 37% (Smart-Lab A 1050), NaOH (Merck 106498), acetic acid 100% (Smart-Lab A 1001), and aquadest.

Tool

The tools used in this research are crusher (wooden pestle), blender (Philips), 100 mesh sieve, magnetic stirrer, beaker glass, funnel, filter paper (Whatman), measuring flask, erlenmeyer glass, stirring rod, spoon, scales, pH paper (Merck), ostwalt viscometer (Iwaki), and drying oven (Memmert).

Production of Chitosan

Chitosan Preparation (Making of Golden Snail Shells Powder)

The stage of making chitosan was done by making gold snail shell powder with a size of 100 mesh. The golden snail shell first sorted and thoroughly cleaned under running water to remove any dirt or dust. In order to make the snail shell easier to destroy, then dried at 60°C for a further 2-3 hours. Then, using of 100 mesh sieve, the dry, clean snail shells were crushed. The powder has been sifted and now prepared for processing to create chitosan.

Deproteination Process

The process of making chitosan was based on Nurhaeni et al., (2019). The deproteination process was carried out by weighing 100 mesh gold snail shell powder and reacted with 4% NaOH solution, with a ratio (w/v) of 1:10. Then, the shell powder and NaOH solution were heated for two hours at 65°C on a hotplate. After the stirring stage was finished, the residue was filtered through filter paper and rinsed with distilled water until it reaches a neutral (±pH 7). The neutral residue is then put in a cabinet dryer with a temperature of 60^{o}C for 2-3 hours or until dry.

Demineralization Process

This step was carried out by mixing the deproteination residue and 1 M HCl in a ratio of 1:15 (w:v). The solution was then stirred using a magnetic stirrer for 3 hours at room temperature (25-30^{o}C), and then filtered using filter paper. The demineralized residue

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obtained usually still with a pH of 5-6, and must be washed with distilled water until neutral (±pH 7). The neutral residue is then put in a cabinet dryer with a temperature of 60°C for 2-3 hours or until dry. The residue obtained after the deproteination and demineralization process is chitin.

Deacetylation Process

The deacetylation process was carried out by mixing the obtained chitin with a NaOH solution with a concentration of 60%, and stirring on a hotplate stirrer at a temperature of 120°C for 3 hours. After the process completea, it cooled at room temperature for ±1 hour. Chitosan obtained was then washed using distilled water until a neutral pH was obtained (±pH 7). The neutral residue then put in a cabinet dryer with a temperature of 60°C for 2-3 hours or until dry. The chitosan obtained was ready to be used for other analyzes.

Test Parameters

Yield

The yield was calculated based on the weight of the chitosan (product weight after deacetylation) and compared with the weight of chitin (product weight before deacetylation). Final yield could be calculated with given formula (Agustina et al., 2015):

$$\text{Yield (\%)} = \frac{\text{Chitosan Weight (gr)}}{\text{Chitin Weight(gr)}} \text{ x } 100\%$$

Water content

Testing the water content was carried out by placing 1 gram of sample into a weighing bottle whose constant weight is known. The samples were then heated in an oven at 100-105°C for 8 hours, then removed, put in a desiccator, and weighed. The process was then repeated every 2 hours to obtain a constant weight. Water content can be calculated using given formula (AOAC, 2005) :

$$\text{Water Content (\%)} = \frac{(\text{Initial Sample Weight} - \text{Final Sample Weight})(\text{g})}{\text{Initial Sample Weight (g)}} \text{ x } 100\%$$

Ash content

The ash content test was carried out by weighing 0.5 gram of the sample and putting it in a weighing bottle whose constant weight was previously known. The sample was then burned in a furnish for 6 hours at a temperature of 600°C degrees before being taken out, after 6 hours the sample was removed and heated at 100 degrees to lower the temperature, then placed in a desiccator for 30 minutes, and weighed. The ash content can be calculated with given formula (AOAC, 2005):

$$\text{Ash Content (\%)} = \frac{(\text{Initial Sample Weight} - \text{Final Sample Weight})(\text{g})}{\text{Initial Sample Weight (g)}} \text{ x } 100\%$$

Solubility

Chitosan dissolution was carried out by dissolving 1 gram of chitosan in 100 ml of 2% acetic acid. The solution is stirred until homogeneous or vortex for 10 seconds. The solution was then centrifuged for 15 minutes, then filtered to get residue on filter paper. The filter paper was then in the oven at 100-105°C for 2 hours, the process was repeated until a constant weight was obtained. Solubility is obtained by insert into the formula (Shon et al, 2011) :

$$\text{Insolubility (\%)} = \frac{\text{Final Weight (g)}}{\text{Initial Weight (g)}} \text{ x } 100\%$$

$$\text{Solubility (\%)} = 100\% - \text{Insolubility}$$

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Molecular weight

Molecular weight testing was carried out using an Ostwald viscometer, a chitosan solution (which was dissolved in 1% acetic acid) was prepared with a concentration of 0.2%; 0.4%; 0.6%; 0.8%; up to 1%. After that, the solution is pumped through the viscometer's pipe as the flow rate is calculated. The same thing was done with 1% acetic acid (without chitosan). Determination of the molecular mass of chitosan was carried out by entering the intrinsic viscosity data obtained from the curve between reduced viscosity and concentration into the Mark-Houwink equation (Mujianto, 2012), namely :

$$[\eta] = k.M.v^a$$

Descriptions :
η = intrinsic viscosity, obtained from the curve (intercept value)
k = 1.46 x 10^{-4}
M = Molecular Weight
a = 0.83

FTIR functional groups

First, 0.02 g sample mixed with 200 mg KBr until homogeneous. The mixture was put into a pellet to be compacted and vacuumed. The pellet then inserted into the cell placement chamber, then shouted with an IR beam. The FTIR histogram recordings was produced on the monitor during detection using the detector button. The histogram displays the peak data of a sample's functional groups. To gather both qualitative and quantitative data, the histogram was first obtained and then examined (Muyonga et al., 2004)

Degree of deacetylation

The degree of deacetylation can be determined using “Base Line” method. The absorbance value at wave number 1655 cm^{-1} (amide absorption band) and wave number 3450 cm^{-1} was compared to determine the degree of deacetylation (DD) (hydroxyl band absorption). Value of deacetylation degree could be measured with given formula (Czechowska et al., 2012) :

$$\text{Deacetylation Degree (\%)} = \left(\left(100 - \frac{A\,1655}{A3450}\ x\ \frac{100}{1{,}33}\right)\right)$$

Description:
A1655 = Amide group (CH_3CONH) absorption band at wave number 1655 cm^{-1}
A3450 = Hydroxyl/amine group (CH_3CONH) absorption band at wave number 3450 cm^{-1}
1,33 = Constant for perfect degree of deacetylation

Data Analysis

Data analysis using Microsoft Excel software in calculating the average in each parameter

RESULT AND DISCUSSIONS

Physcochemical Characteristics of Chitosan

The golden snail shell chitosan as a result of analysis has a light brown powder form, comparable to research by Dewi et al., (2016) who used escargot snail shells and produced chitosan's final product in the form of a brownish white powder. This complies with the

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standards set by SNI in 2013, that the color of chitosan powder is light brown to white. Further list of chitosan characteristics can be seen in Table 1.

Table 1. Golden apple snail shells chitosan characteristics

Parameter	Chitosan	
	Analysis Result	**Reference**
Form	Powder	Powder[a]
Colour	Light brown	White-ish brown[a]
Yield (%)	53.91 ± 0.79	34.66[a]
Water Content (%)	1.68 ± 0.10	3.26 ± 0.45[b]
Ash Content (%)	12.31 ± 0.17	10.11 ± 0.38[b]
Solubility (%)	95.53 ± 0.38	97,65[c]
Molecular Weight (kDa)	640.83 ± 0.64	640.48[d]
Deacetylation Degree (%)	82.33	83.23[d]

Reference : [a)] Dewi et al., (2016), [b)] Kusumaningsih et al., (2004), [c)] Hossain and Iqbal (2014) [d)] Nurhaeni et al., (2019)

The yield obtained was 53.91%. The results of this chitosan yield are greater than the comparative studies, namely research by Dewi et al. (2016). The difference in yield is most likely caused by the difference in raw materials used as well as the difference in the process of making chitosan, namely by deacetylating chitosan at 110^{o}C for 1 hour. Based on Mursal et al. (2022) which deacetylated chitin with various temperature variations, namely 110^{o}C, 120^{o}C, and 130^{o}C, with 60% NaOH (1:20) for 4 hours stated that temperature causes a rise in the number of reactions, and the more reactions are occurred in the process, the more they affect the yield of the finished product.

Thus according Table 1, the water content of the golden snail shell chitosan obtained was 1.68%, this figure was relatively lower when compared to the figure in Kusumaningsih et al., (2004), which was 3.26%, the resulting water content was meet the standards set by SNI, namely a maximum of 12%. The existence of differences in numbers can be influenced by raw materials and differences in the deacetylation process carried out, research belonging to Kusumaningsih et al., (2004) used snail (*bekicot*) shell raw materials that just had been dried for only 3 hours. Although the numbers obtained in this study were low, they still met the standards of Protan Laboratories (1989), namely ≤10%. According to Mursal et al., (2022) the drying process or method, drying duration, quantity of dried chitosan, and surface area of dried chitosan are all factors that influence the moisture content of chitosan. Drying time and the reduction in water content are closely linked. The water content will decrease more quickly as the material dries since the amount of water in it reduces.

The ash content of chitosan is a parameter that indicates the total mineral content and measures the efficiency of demineralization. The ash content found in the golden snail shell chitosan was 12.78%. The figure obtained is still higher when compared to the literature belonging to Kusumaningsih et al., (2004), which is 10.11%, but when compared to other studies that also use snail shells, namely Dewi et al., (2016) it is 87.40% and Puspitasari (2007) at 95%. The percentage of ash content obtained was very high, so it did not meet the standard of Protan Laboratories (1989), namely ≤2%. The ineffectiveness of the washing process after the deacetylation process and the high levels of minerals, particularly calcium carbonate that incorporate with chitin in the snail shell, are the two factors that affect the ash content of chitosan. The first one as per Mursal et al., (2022), the chitosan washing process can affect the ash content. The process of neutralization will be affected by the higher NaOH content. Na atom will stay in the chitosan, giving it a relatively high ash content, if the washing procedure is improperly done and the pH level does not reach neutrality. According

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to Islami and Anita (2014), the golden snail shell's high mineral content is the second contributing component. Their ash content is 65.96 %, it is likely that the high ash content in the final chitosan is caused by the high total minerals in the golden snail shell because the chitosan neutralization process has produced a neutral pH of 7-8. As a result, a higher concentration of HCl is required so that the demineralization process is more efficient.

Solubility is one of the standards used to determine the quality of chitosan, the higher the solubility of chitosan, the better the quality. The resulting chitosan has a solubility of 95.53%, this figure is still smaller than the literature belonging to Hossain and Iqbal (2014) which is equal to 97.65% with chitosan from shrimp shells. The nature of chitosan limits its ability to dissolve in water and dissolve in dilute acids such as acetic, formic and citric. The example of a weak acid in the carboxylic acid group with a carboxyl group is acetic acid (COOH). A carbonyl group and a hydroxyl group are both present in the carboxyl group. Chitosan will dissolve more easily due to hydrogen bonding interaction between the amine groups in chitosan and the carboxyl groups in acetic acid (Rochima, 2007).

The molecular weight of chitosan was calculated based on the calculation of the intrinsic viscosity value, using the Mark-Khun Houwing equation. Based on Table 1 the molecular weight of chitosan from the research results is 640.83 kDa or 640.836 Da. The figures obtained are not significantly different from the research of Nurhaeni et al., (2019) with chitosan of field snail shells, which is 640.48 kDa. The molecular weight of chitosan obtained complies with commercial chitosan standards, with a moderate molecular weight category, based on Santoso et al, (2020). Chitosan is grouped into three based on its molecular weight, low molecular weight (<100 kDa), medium molecular weight (100–1000 kDa), and high molecular weight (>1000 kDa).

The degree of deacetylation is the percentage ratio of chitosan formed when compared to chitin. Based on the results of the analysis, the degree of deacetylation of golden snail shell chitosan was 82.33%. The figures obtained are not much different from the research of Nurhaeni et al., (2019), which found out that chitosan of rice field snail is 83.23%. The resulting degree of deacetylation of chitosan complied with the SNI chitosan standard (2013), namely ≤70%. The high NaOH concentration and longer deacetylation time of chitin to chitosan were responsible for the high number that was obtained. According to Bahri et al., (2015) the increase quantity of NaOH used, the more hydroxyl groups available for the deacetylation process, thereby increasing the possibility of elimination of the carbonyl group due to addition by hydroxyl, resulting in the formation of more and more amine groups.

Functional Group of Chitosan Identification

The deacetylated golden snail shell chitosan was then characterized using infrared spectroscopy (FTIR) (Figure 1) and then compared with the infrared spectrum of chitosan based on the research of Darman et al., (2016) who synthesized chitosan from mangrove snail shells, the comparation results can be seen in Table 2.

Based on Table 2, it can be seen that there is absorption at the wavelength number 3200-3500 cm^{-1} which indicate the presence of OH and NH functional groups, based on Pavia et al., (2001) in Hayati (2020) the amine group (NH) appears at a wavelength of 3500 -3300 cm^{-1}, while the hydroxyl group appears at a wavelength of 3400-3300, there is a peak at wave number 3136.25; 3234.62 and 3275.13 cm^{-1}, the peak widens and shifts to shorter wave numbers as a result of hydrogen bonding, which results in overlap with the amine groups or NH groups, this is in accordance with Fessenden (1989) in Darman et al., (2016) OH and NH groups are found between wave numbers 3000-3700 cm^{-1}. There is also an absorption at a

FOODSCITECH, Vol. 6 No. 1 July 2023
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wavelength of 2515.18 which is an absorption of the carboxylic acid OH group which is visible at a wavelength of 2500-3600 cm^{-1}, based on Darman et al., (2016) this group has a weak absorption band because to the NH stretching vibration of the amine

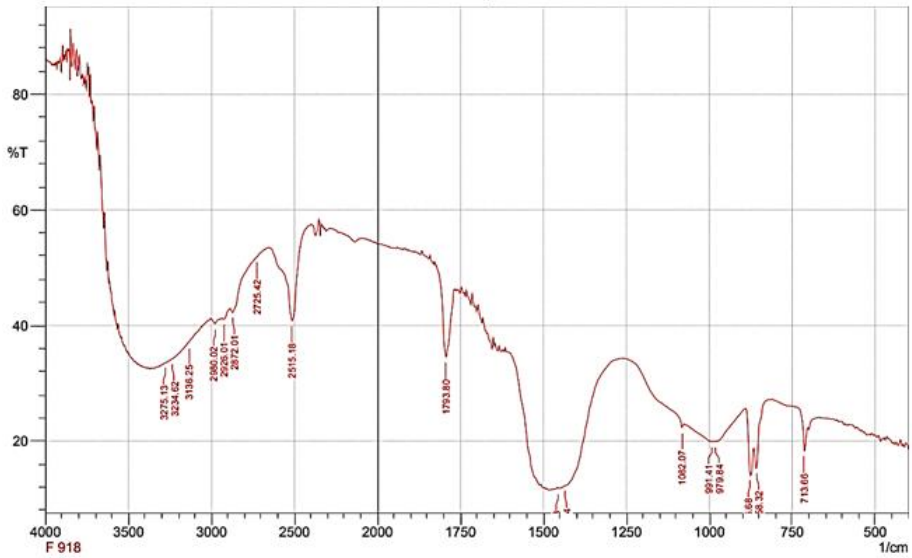

Figure 1. Infrared Spectrum of Golden Apple Snail Shells Chitosan

Table 2. Characterization of the FTIR spectrum of golden snail shell chitosan

Functional Groups	Wavelength (cm^{-1})	
	Chitosan Obtained	Reference Chitosan (Darman et al., 2016)
OH (Hydroxyl) dan NH	3136.25 3234.62 3275.13	3448.72
CH (Alkanes)	2725.42 2872.01 2926.01 2980.02	2922.16 2856.58
OH (Carboxylic acid)	2515.18	2515.18 2362.8 2320.37
C=O	1793.8	1741.72
CH (Bending)	1435.04 1454.33	1462.04
C-O-C	1082.07	-
NH (Swish)	991.41 979.84 875.68 858.32	999.13 871.82

Absorption at a wavelength of 2725.42; 2872.01; 2926.01 and 2980.02 cm^{-1} indicate the presence of CH groups from alkanes, which according to Darman et al., (2016) associated with CH_2 stretching vibrations. Meanwhile, the absorption at wavelengths of 1435.04 and 1454.33 cm-1 shows an asymmetrical C-H vibration of CH3. There is absorption at a wavelength of 1793.8 cm-1 which is the absorption stretch of the C=O group from the secondary amide, according to Silverstein (1989) in Dompeipen (2017) there is a weak stretch at 1650 cm^{-1} which indicates a C=O group on the (-NHCOC3) bond. Absorption at a

wavelength of 1082.07 cm^{-1} is a stretching vibration of the C-O-C group, based on Hayati (2020) which denotes the presence of a glycosidic bond.

CONCLUSION

Chitosan is a chitin-derived compound that can be found in various sources. In this study, chitosan was extracted from golden snail shells, which were obtained through 3 stages, namely demineralization, deproteination, and deacetylation. Based on the analysis conducted, chitosan has a yield of 53.91%; water content 1.68%; ash content 12.31%; molecular weight 640.83 kDa; solubility 95.53; and deacetylation degree 82.33%. Results revealed that the water content and level of deacetylation met SNI criteria, but not the ash content, which was significantly higher than the standard, indicating the ineffectiveness of the mineral removal procedure in chitin. The FTIR analysis results additionally indicated that the extracted compound was chitosan because it contained the typical chitosan groups of hydroxyl (OH) and amine (NH), but there remains chitin compounds that exist in chitosan as demonstrated by the presence of the C=O group in the acetamide group (-NHCOC3).

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