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Fabrication of Chitosan Biopolymer from Pearl Oyster Shells (*Pinctada maxima*) for Medical Applications

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Abstract

Chitosan is one of the biopolymers that has recently been developed in the medical field. Chitosan is biocompatible, biodegradable, and non-toxic, so it is safe for the human body. This study aimed to identify the characteristics of chitosan isolated from pearl oyster shells. Chitosan is obtained through three stages: deproteination, demineralization, and deacetylation. Characterization of chitosan is done physically and chemically including organoleptic test, yield calculation in each step of isolation, FTIR, and XRD. Isolated chitosan identified deacetylation degree and functional group with FTIR. While the crystal structure was determined using XRD. The results showed that chitosan powder has a beige color with a final yield of 7,06%. The characterization of FTIR shows that synthesized pearl oyster shells have successfully formed chitosan compounds with a deacetylation degree of 81,50%. Another characteristic is the crystal structure obtained; chitosan has orthorhombic unit cells with a degree of chitosan crystallinity of 36,94%. Based on the result, chitosan has met several standards in medical applications as a biomaterial.

Introduction

The scientific development of the medical field can not be separated from the development of materials science. Medical materials science have become popular, especially as a substitute for bone and teeth. Several materials have been developed as medical materials, including bioceramics, biometals, biopolymers, and others. Polymers are materials formed by structural

units in the form of repeating monomers. Polymers are derived from synthesis, semisynthesis, and polymers available in nature or naturally occurring [1]. Synthetic polymers are polymers that are widely and commonly used. However, problems arise related to consumable products (waste) and the source of raw materials for the synthesis. Synthetic polymer waste creates environmental pollutants that are difficult to decompose. In contrast, synthetic polymer raw materials come from petroleum, a non-renewable source that can be leveraged at any time [2]. Therefore, many applications and research on polymers switch to natural polymers. Utilization of polymers based on natural materials for various applications is believed to be more economical because the availability of abundant raw materials is also more environmentally friendly. In recent years, biopolymers based on natural materials have been widely developed to overcome these problems [3]. One of the natural polymers that are currently widely developed is chitosan biopolymer. Chitosan is a polysaccharide biopolymer resulting from the chitin deacetylation process and is composed of monomers with a 2-amino-2deoxy-D chain of glucose with β-(1-4) as a glycoside bond [4].

Sources of chitosan insulation raw materials are abundant in nature; chitin, as a source of chitosan, can be obtained from crustaceans, mollusks, and insects. Crustacean animals such as clam shells, shrimp shells, and crab shells are more widely used as isolated sources of chitosan because they are believed to have high chitin levels [4]. This study used pearl oyster shells (*Pinctada maxima*) from the waters of West Nusa Tenggara because it contains chitin, protein, glycoprotein, peptide, lignin, and pigment [5]. The chitin content in pearl oyster shells is relatively high, amounting to 14%-35%, so that it can be used as a source of chitosan isolation [6]. The Chitosan isolation process includes several stages: deproteination, demineralization, and deacetylation. The deproteination stage removes protein levels using low-concentration robust base solutions such as NaOH and KOH. The demineralization stage is the stage of removal of mineral levels by using acid solutions such as HCl. The deacetylation stage changes the acetyl group in chitin to an amine group using a high-concentration robust base solution [7]. Research conducted by Kurniawidi et al. (2022) performing chitosan isolation with the same stages produces chitosan with a deacetylation degree of 81,20% [8].

Chitosan can be obtained in various morphological forms, including the irregular structure and crystalline or semicrystalline properties. It can also be a white amorphous solid with a fixed crystalline structure of the initial state of pure chitin [9]. The formation of chitosan is characterized by the appearance of hydroxyl and amine functional groups. Based on the Indonesian national standard, chitin's derivative can be called chitosan if it has a value of deacetylation degree ≥ 75% [10]. In research conducted by Nurlaili et al. (2022), utilizing chitosan as an adsorbent dye, methylene blue obtained chitosan with a deacetylation degree of 57,50% [10]. The degree of deacetylation dramatically affects the quality of chitosan; the higher the value of deacetylation, the better the quality of chitosan, so the wider its use.

Chitosan has many benefits in various fields of modern industry, such as pharmaceuticals, biochemistry, cosmetics, food, and textile [11]. Chitosan has wide use in everyday life; for example, chitosan is used as a coating (film) on various foodstuffs due to the nature of chitosan, which is not harmful to health [12]. The antibacterial activity of chitosan can inhibit putrefactive bacteria in local foods containing pathogenic bacteria [13]. Another great potential

of chitosan is as an ingredient in pharmaceutical preparations such as antimicrobial, antiviral, anticholesterol, and antitumor [14][15]. Research on chitosan in the field of health is most widely used for its application as a bone graft. Srividya, dkk., (2014) succeeded in making bone grafts from a mixture of calcium phosphate with chitosn, based on the tets that have been done chitosan used as a mixture of bone graft can increase osteochonductivity [16]. Chitosan from oyster shells was isolated and used as an absorbent in a liquid substance. Research conducted by Handayani et al. (2022) successfully separated chitosan utilized as a Fe metal adsorbent, showing that chitosan from oyster shells has good adsorption ability [17].

The widespread use of chitosan in various fields, especially in the medical field, encourages researchers to improve the quality of chitosan as a biopolymer. Several studies have been carried out with with the raw material of shells as a source of chitosan biopolymer, which is utilized in several applications. Therefore, this study conducted isolation and further characterization of chitosan from oyster shells (Pinctada maxima) for medical purposes such as a mixture of bone graft and drug delivery. Further characterization was carried out to identify the characteristics of isolated chitosan so it can be known for its potential use in the medical field

Experimental Method

Tools and materials

The research used several equipment ranging from the preparation process to characterization. The tools used in this study included grinder (SY-150 Pulp Grinder Yamamoto, Indonesia), and magnetic stirrer with hot plate (IKA C-MAG HS, Indonesia). The characterization tool used is Fourier Transform Infrared (FTIR) (Perkin-Elmer, USA) and X-Ray Diffractometer (XRD) (Shimadzu, Japan). The main material used in this research is nacre (*Pinctada maxima*) from West Nusa Tenggara. Chemicals used include NaO 60% pro analyst (Merck, Germany), HCl 1M pro analyst (Mallinckrodt, USA), aquades, and whatman 42 filter paper (PT. Bensara Sukses, Indonesia).

Research Procedures

The research procedures included the preparation of pearl oyster shells, isolation of chitosan through deproteination, demineralization, deacetylation, and characterization of chitosan powder. The initial practice of pearl oyster shells involves cleaning them using a brush and washing them clean. Then the pearl oyster shells were dried in an oven at 70 °C for 2 hours. Next, the clam shells were crushed using a hammer to become smaller. To produce fine shell powder, grinding was carried out using a grinder, and the obtained shell powder was sieved using a 100-mesh sieve [18].

The chitosan isolation process refers to research conducted by Bahri et al., (2015) went through three stages: deproteination, demineralization, and deacetylation. The deproteination process was carried out by mixing 80 grams of pearl oyster shell powder with 4% NaOH in a ratio of 1:10 (w/v). The demineralization process was carried out by combining the results of deproteination with a 1 M HCl solution with a balance of 1:15 (w/v). Then the deacetylation process was carried out by mixing chitin with a 60% NaOH solution in a ratio of 1:15 (w/v) at 120 °C for 3 hours with stirring [7].

Characterization of Chitosan

The resulting chitosan was subjected to organoleptic and yield analysis. Organoleptic testing on chitosan to determine chitosan quality following Indonesian National Standard, including shape, color, and odor. Yield analysis to determine the amount of lost content in each isolation process uses equation (1), with m_2 as the final mass and m_1 as the initial mass [19].

$$Yield\ (\%) \quad = \quad \frac{m_2}{m_1} \times 100\% \tag{1}$$

Analyses of functional groups and the degree of deacetylation were carried out by FTIR testing. The results of the FTIR test are in the form of absorption bands expressed by wave number (cm^{-1}) and percent transmittance (%T). The results of the wave spectrum formed can be determined by functional groups. The determination of functional groups was done by matching the IR spectrum obtained with the IR spectrum of chitosan references from the study of Handayani et al., (2022). Chitosan can be categorized as successful if it forms OH (bending) and NH (stretching) functional groups, indicating the presence of primary amine (NH_2). An analysis of the degree of deacetylation can be carried out by comparing the absorbance at wave numbers (1650-1500) cm^{-1} for the hydroxyl groups with the absorbance at wave numbers (3500-3200) cm^{-1} for primary amine groups. Calculation of the degree of deacetylation using equation (3).

$$A \quad = \quad log\frac{r_0}{r} \tag{2}$$

$$DD\ (\%) \quad = \quad \left[100 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{100}{1{,}33}\right)\right] \tag{3}$$

Declaration:

r_0	=	Distance between baseline and tangent
r	=	Distance between the baseline and the lowest trough
A_{1655}	=	Absorbance value at 1655 cm^{-1}
A_{3450}	=	Absorbance value at 3450 cm^{-1}
1,33	=	Ratio of A_{1655}/A_{3450} for fully acetylated chitosan

Through XRD characterization, crystal structure, phase changes, and degree of crystallinity were acquired. The phase changes that occur can be observed through the formation of the peaks that are formed. The sample peaks were compared with the JCPDS Card No. 39-1894 database to determine the phase for pure chitosan. The degree of crystallization of the compound can be obtained through equation (4) [20] :

$$Crystallinity = \frac{Area\ Fraction\ of\ Crystal}{L\ Fraction\ of\ Crystal\ +\ L\ Fraction\ of\ amorphous} \tag{4}$$

The area fraction of a crystal is multiplied by the intensity of β. β is the apex's FWHM (full width at half maximum). The value of β can be obtained through the equation:

$$\beta = \frac{1}{2}(2\theta_2 - 2\theta_1) \tag{5}$$

Result and Discussion

Isolation of chitosan from pearl oyster shells was successfully carried out through three stages, namely deproteination, demineralization, and deacetylation. The results of chitosan isolation can be seen in Figure 1.

Figure 1. Chitosan isolated powder

Based on Figure 1, the resulting chitosan is in the form of a white powder. The results of organoleptic testing for each isolation process are more clearly shown in Table 1.

Table 1. Organoleptic data for each isolation stage

Isolation Stage	Test Parameters		
	Color	Smell	Shape
Deproteination	Beige	Slightly smelly	Powder
Dermineralization	Pale brown	Ordoless	Powder
Deacetylation	Creamy white	Ordoless	Powder

Organoleptic observations show that each stage of isolation produces different characteristics. The difference in color at each stage occurs due to the use of varying solution concentrations. Table 1 shows that the shape and color of chitosan particles have met the quality standards for chitosan based on the 2013 National Standardization Agency Number 7949, which is powder in the form of light brown to white [21].

The yield of the isolated chitosan powder was calculated using equation (1). Yield is the percentage resulting from the weight of the initial raw material. The yield data for each isolation stage is contained in Table 2.

Table 2. Yield for each isolation stage

Isolation stage	Initial mass (gram)	Final mass (gram)	Yield (%)
Deproteination	80	77.44	96.80
Dermineralization	77.44	27.39	35.37
Deacetylation	27.39	5.65	20.63

Based on Table 2, there was a decrease in powder yield along with the isolation process. The reduced product of powder was due to the protein and mineral content lost in pearl oyster

shells during the deproteination and demineralization processes. The most reduction in yield occurs in the demineralization process because the mineral content in the surfaces is the most abundant content. In addition, the high concentration of NaOH in the deacetylation process causes the yield to be smaller. This is because more and more NaOH molecules are added to chitin molecules, thereby reducing the output of chitosan powder. So that the total result of chitosan obtained from the initial raw materials is 7.06%. The low chitosan yield increases chitosan's purity because more acetyl groups are released from the chitin [22].

Using FTIR, the outcome of chitosan powder was then analyzed for functional groups and the degree of deacetylation (DD). The following graph shows the results of the FTIR test data for chitosan, as shown in Figure 2.

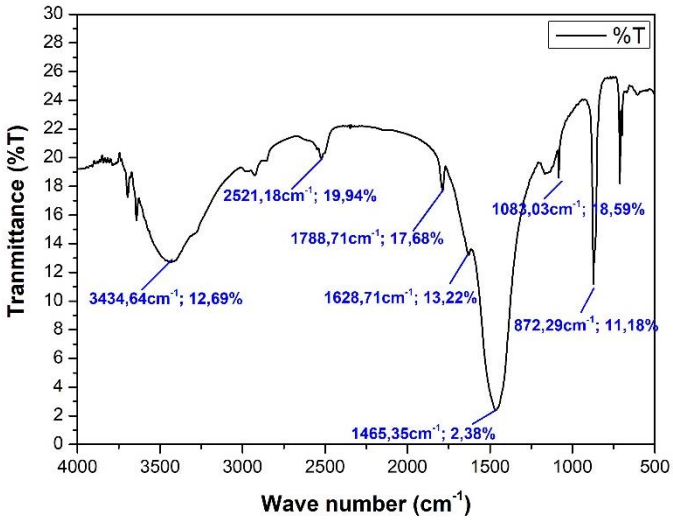

Figure 2. Chitosan FTIR spectrum

In Figure 2, there is an absorption band indicating the functional groups present in chitosan. The identification results of the formation of chitosan functional groups from Figure 2, which have been analyzed more clearly, are shown in Table 3.

Table 3. Functional group of chitosan based on literature and research

Functional Group	Wave Number (cm^{-1})	
	Dompeipen et.al (2016)	Research result
O-H *(stretch)*	3377.95	3434,64
C-H *(stretch)*	2922,80	2521,18
$N\text{-}H_2$ *(bend, primary amine)*	1660.55	1628,71
$C\text{-}H_3$ *(bend)*	1422,73	1465,35
C-N (*stretch)*	1154,64	1166,55
C-O-C *(glucosamine ring)*	1103,85	1083,03
β-1,4- glycosidic	879,41	872,29

The FTIR results in Figure 2 and Table 3 display the absorption pattern at wave numbers 3434,64 cm^{-1} and 1628,71 cm^{-1}, which indicates the presence of the OH functional group with the vibration mode of stretching and the NH_2 active group with the vibration mode of bending (bend). The formation of hydroxyl and amine groups is essential because these two functional groups show the loss of acetyl group content or indicate the formation of chitosan. Difference in wavenumbers obtained in the results of research with literature wavenumbers due to difference in chitin sources used [23].

In addition to determining the functional groups from the FTIR test results, the purity level of chitosan can be determined by assessing the degree of deacetylation using equation (3). The value of the degree of deacetylation of chitosan is 81.50%, which meets the quality standard of chitosan with a degree of deacetylation of ≥75% [21]. The large degree of deacetylation obtained was due to the high concentration of NaOH used in the deacetylation process, increasing the number of hydroxyl groups, which could cause an addition reaction to occur in the chitin carbonyl group, resulting in the formation of more amines and an increase in the degree of deacetylation [7].

The main parameter that determines the characteristics of chitosan, besides the degree of deacetylation, is crystallinity. XRD analysis was used to support the FTIR analysis that was carried out. From the chitosan XRD readings, a graph of the relationship between the peak intensity on the y-axis and the measured diffraction angle on the x-axis can be seen in Figure 3.

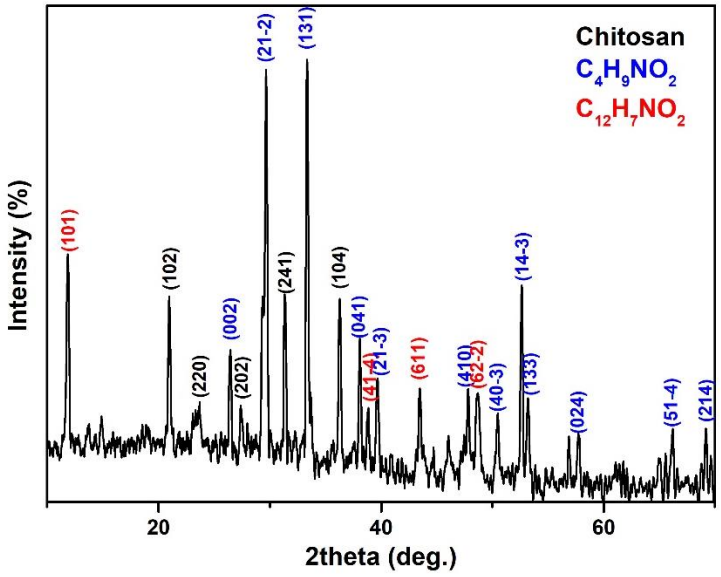

Figure 3. Chitosan XRD pattern

Figure 3 shows chitosan formed at peaks 2θ 20,98°; 23,74°; 27,42°; 31,34°, and 36,52°. The figure clearly shows that the identified chitosan peaks have a wide shape. So, this indicates that the chitosan compound phase formed is a semicrystalline polymer. The resulting chitosan crystal structure shows an orthorhombic unit cell with lattice parameters $a = 8{,}24; b = 16{,}48; c = 10{,}39$. It can be seen from the graph that the peaks of chitosan based on the JCPDS

Card Database No. 39-1894 have miller indexes (102), (220), (202), (241), dan (104). Miller index aims to indicate the crystallinity of a material. The results of the crystallinity calculation using equation (4) showed that the degree of crystallinity of chitosan was 36.94%. The crystallinity of chitosan is strongly influenced by the strength of intramolecular and intermolecular hydrogen bonds in chitosan polymers [24]. So, the XRD pattern of chitosan is characteristic of an amorphous polymer [25]. Chitosan with an amorphous structure is preferred because it is easily absorbed and has better bioavailability, so it is widely used in the medical field [26].

Conclusion

Chitosan biopolymer was successfully isolated from pearl oyster shells (*Pinctada maxima*). The resulting chitosan is in the form of white powder with a yield value of 7.06%. The characteristics of chitosan include forming functional groups OH and NH_2 at wave numbers 3434,64 cm^{-1} and 1628,71 cm^{-1}, with a deacetylation degree percentage of 81,50%. The crystal structure of chitosan shows an orthorhombic unit cell with a degree of crystallinity of 36,94%. Based on these characteristics, chitosan biopolymer can be used as a candidate material in medical applications.

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