Synthesis method of chitin become chitosan polymer from shrimp shells for enhanced oil recovery

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Abstract. The purpose of this study was to examine the benefits and procedures for making chitosan polymer from chitin found in shrimp shells. This initial research aims to synthesize chitosan membrane from chitosan powder and its characteristics. Chitin is the main organic material found in groups of crustaceans, insects, fungi, molluscs and arthropods animals. Crab, shrimp and lobster shells have long been known as sources of chitin production as their chitin content is quite high. Chitin is the second most abundant natural biopolymer after cellulose. This non-toxic natural biopolymer is produced commercially from shrimp and crab shell waste. Chitosan powder is prepared from shrimp shells through deproteination, demineralization and deacetylation processes. Chitosan is characterized by conducting tests of water content, ash content, nitrogen content, viscosity, degree of deacetylation, analysis of functional groups by IR spectroscopy and crystallinity by X-ray diffraction. Chitosan characterization is seen from physical appearance, functional group analysis and crystallinity. The potential application of chitosan, as a deacetylated chitin derivative is multidimensional in nature, such as in food and nutrition, biotechnology, materials science, medicine and pharmacy, agriculture and environmental protection. Chitin isolation from shrimp shells and its synthesis into chitosan, makes shrimp shells more beneficial for other fields. One of the applications of chitin is as chitosan polymer, which is used as an injection fluid in the field of Enhanced Oil Recovery.

1. Introduction

Shrimp is one of the most favored foods for the community because it contains high nutrition, has a distinctive aroma and delicious taste. The part of shrimp utilized as food is mainly the meat part. Several shrimp parts which are not commonly consumed and often become shrimp waste come from the skin, head and tail of shrimp [1].

Shrimp head waste reaches 35%-50% of the total weight of shrimp. In Indonesia, some shrimp waste has been used for making shrimp crackers, shrimp paste, and animal feed ingredients. In developed countries such as America and Japan, shrimp waste has been used in various industrial fields namely, pharmaceutical, biochemical, biomedical, food, agriculture, and health industries [2]. It is simply because shrimp waste can be used as a chitosan maker. The main constituent of shrimp shells is chitin, a natural polysaccharide that has many uses, such as chelating, emulsifying and adsorbent materials [3].
The non-toxic and easily degraded nature of chitin encourages chitin modification with the aim of optimizing the use and expanding the field of chitin application. One of the compounds derived from chitin which is widely developed because of its vast benefit is chitosan. Chitosan is a polysaccharide amine produced from chitin deacetylation process. This compound is an important natural biopolymer and is polycationic so that it can be applied in various fields. The biocompatible, biodegradable and non-toxic properties of chitosan are highly recommended to be utilized as basic compound in environmentally friendly industries. Shrimp waste has great potential to be processed into chitosan because its availability is quite large and easily obtained [4]. Shrimp skin waste consists of three main components, namely protein (25%-44%), calcium carbonate (45%-50%), and chitin (15%-20%) [5].

Chitin content in shrimp shell waste is around 20% -50% of its dry weights. Chitin is a group of carbohydrates classified as structural homoglycans. Chitin is the second world’s most available natural polymer in the world after cellulose [6]. There are three sources of chitin namely crustaceans, insects and microorganisms. Commercial sources of chitin are crustacean shells such as shrimp, crab, lobster and krill (a type of small shrimp) [7]. Chitin is the second world’s most available organic compound in the nature after cellulose and is widely distributed in various marine invertebrates, insects, fungi, and yeast. Chitin has a high economic value because of its availability to be utilized in biological, industrial and biomedical applications [8].

The difference between chitin and chitosan is defined by their nitrogen content, if the nitrogen is less than 7%, the polymer is called chitin and if the total nitrogen content is more than 7%, it is called chitosan [9]. Commercially produced chitin and chitosan have both the acetamide group and the amide group on their polymer chains, with various compositions in the group. Chitin and chitosan are the names of two groups of compounds that are not restricted by definite stoichiometry. Chitin is a slightly acetylated poly-N-acetyl glucosamine, while chitosan is deacetylated chitin at the highest level. Chitosan is called polyglucosamine if chitin is completely deacylation (100%). Transformation of chitin into chitosan occurs through deacetylation process. The deacetylation process is the process of acetyl groups (-COCH3) removal from chitin by using an alkaline solution which turning it into amine group (-NH2). Chitosan is a product formed from chitin deacetylation through hydrolysis process in an alkaline solution [10]. Chitosan is a derivative of chitin with \[\beta-(1-4)-2\text{-Deoxy-D-glucose}\] structure and included into polycationic polymers class. Chitosan is a compound that is insoluble in water, a strong base solution, slightly soluble in HCl and HNO3 and insoluble in H2SO4. Chitosan comes from animals with hard outer skin such as crabs, shrimp, oyster shells, insects and other marine animals [11]. Chitosan is a derivative of chitin which is a major component in shrimp shells, and crabs, squid cartilage, and insect outer skin, as well as those that form the structural components of the anthropods exoskeleton or fungal and yeast cell walls.

Chitosan nature is connected with the existence of amine function groups, primary hydroxyl group, and secondary hydroxyl group. The existence of these groups causes chitosan to possess a high chemical reactivity than chitin. The function groups cause chitosan to be able to interact with organic compounds such as protein, so it is relatively more popular to be used in various applied industry and healthcare fields [12]. Chitosan can be used in food processing, medical and biotechnological industries, and also can be an interesting material in biomedical and pharmaceutical applications. It is simply because of its non-toxic, biological activity, biocompatibility, biodegradability, and can be chemically and physically modified [13]. A number of chitin sources have been tested by isolating chitin and chitosan are snail shell with deacetylation degree of 74.78-77.99%, shrimp shell with deacetylation degree of 79.57%, and sea crab shell with deacetylation degree of 40.90% [14]. In shrimp shell, chitin acts as mucopolysaccharides that bind together with inorganic salts, especially calcium carbonate (CaCO3), protein and lipid, including pigments. Because of that, to obtain chitin from shrimp shell would involve deproteination and demineralization processes. Meanwhile, to obtain chitosan we can continue the process with deacetylation process [15]. Chitosan formation reaction from chitin is a hydrolysis reaction of an amide by alkali. Chitin acts as amide and NaOH as alkali. First, addition reaction occurs where OH- group enters NHCOCH3 group where elimination of CH3COO- group happens that produce an...
amide, which is chitosan. Figure 1 and Figure 2 show chitin compound structure and chitosan formation reaction from chitin respectively.

![Figure 1. Chitin compound structure [4,16].](image1)

![Figure 2. Chitosan formation reaction from chitin [17].](image2)

2. Materials and method
The important chitosan characteristic is the deacetylation degree (DD). DD value can be determined with FTIR (Fourier-Transform Infrared). FTIR is a function group or compound characterization method based on infra-red radiation uptake by vibrating atom. This frequency occurs in infra-red electromagnetic spectrum on wave number of 4000 to 400 cm\(^{-1}\).

Chitin deacetylation degree can be determined by using baseline method. This method is based on absorption value on absorption tape of infra-red spectrum on wave number of 1,655 cm\(^{-1}\) and 3,450 cm\(^{-1}\). Absorbance (A) is stated as formula (1), meanwhile DD value is stated as formula (2).

\[
A = \log \frac{P_0}{P}
\]

where:

\[P_0 = \% \text{ transmitter on base-line (maximum absorption)}\]
\[P = \% \text{ transmitter on maximum absorption}\]

\[
\% \text{DD} = 100 - [(A_{1665}/A_{3450}) \times 115]
\]

A1655 and A3450 values are A values suitable for absorption bands 1,655 cm\(^{-1}\) and 3,450 cm\(^{-1}\). The absorption band 1,655 cm\(^{-1}\) is the absorption band of the carbonyl Nausetil group while the absorption band 3450 cm\(^{-1}\) is the absorption band of the NH2 group [18,19]. The process of deacetylation of chitin can be done by heating chitin in a high concentration of strong base solution [16,20].

The tools utilized in this research are glassware, blender, oven, pH paper, filter paper, FTIR spectrophotometer, 40-60 mesh sieves, analytical balance, measuring flask (1 L, 100 mL, 10 mL), micro pipette, chemical beaker (1 L, 250 mL, 50 mL), watch glass, funnels and test tubes. The ingredients used are shrimp shells, NaOH, KOH, aquades, acetone and Na2S2O4.
The main steps of this research are: (a) preparation of shrimp shell samples; (b) chitin isolation from shrimp shells, and (c) deacetylation, with the following description:

- **Sample preparation:** Shrimp shell waste is cleaned from the remaining dirt and shrimp meat left on the skin. Shrimp shells washed thoroughly with water for several times. Shrimp shells are dried in an oven at 40 °C for 3 hours and coarsely ground.
- **Chitin isolation:** Chitin isolation and chitin deacetylation process were carried out according to the Hang method [21]. Chitin isolation from shrimp shells includes deproteination and demineralization and decolorization stages.
- **Deacetylation:** One gram of chitin is added with 100 mL of KOH or NaOH with varying concentrations. The reaction is carried out at 100 °C for 5 hours. The solution is separated from the residue and the residue is neutralized by washing with distilled water for several times. The residue is dried in an oven at 60 °C for 4 hours. After drying, the residue is finely ground and sieved with a size of 20 - 40 mesh. The residue was characterized by FTIR.
- **Measurement by FTIR spectrophotometer:** Chitin or chitosan are stored in a desiccator for one day before made into KBr pellets. KBr pellets production is done by mixing 1 mg of sample with and KBr from 10 - 100 mg. The powder mixture is crushed until it reaches homogeneous level and pressed with a hydraulic pump. The pellets were analyzed by FTIR spectrophotometer.

3. Results and discussion

Based on the discussion above, we acknowledge that there are several literatures and a number of researches that have already conducted on chitosan polymer synthesis processes. Chitin isolation process from a number of materials such as shrimp shell, small crab shell, crab shell, or squids has been conducted. Minda et al [1] researched on NaOH and KOH concentrations on deacetylation degree of chitin made of shrimp waste by using sequential method consists of sample preparation, chitin isolation and chitin deacetylation process by deproteination and demineralization, and also component measurement by using FTIR spectrometer. Deacetylation process was conducted by using 40% and 50% of NaOH and KOH solutions proved to be able to produce chitosan. The research result shows that 40% and 50% of KOH and NaOH concentrations is not significantly influence chitin DD. Chitin DD produced from isolation process is at 3.3165%.

This result shows that chitin isolation still produces chitin. If DD value is at 40 to 100%, it can be categorized as chitosan [22]. The highest chitin DD value was resulted at 50% NaOH. Deacetylated DD chitin is proven higher when using KOH than NaOH. With that, it can be said that KOH is a stronger alkali than NaOH. Alkali strength is connected with the number of OH- that can be added in water. The higher number of OH- will be released on water. Chitin DD is affected by several factors which are alkali strength, alkali concentration, reaction time, and temperature. NaOH and KOH concentration variations show a number of OH- will react during deacetylation process and also show a concentration variation of Na+ and K+ ions in the medium. The higher the OH- number in the solution, the higher the possibility of carbonyl attack probability on acetamide will be; which would lead to a more probability of acetyl release [1].

The result of a research conducted by Edward et al. [23] by utilizing 4 previously mentioned methods such as deproteination, demineralization, decolorization, and deacetylation, from physically and chemically characterized compound, identified that the produced compounds are chitin and chitosan [23]. This result shows that the chitosan polymer synthesis procedure has gone through the right process, which is through 4 stages of deproteination, demineralization, decolorization, and deacetylation. Another research conducted by Rani et al [3], on comparison of chitosan production from tiger shrimp waste and pure chitosan by using 4 stages of chitosan synthesis which are deproteinization, demineralization, depigmentation, and deacetylation.

This research has been able to isolate chitin and chitosan with chitin quality that possesses water content of 6.89%, and ash content of 7.8%, and chitosan quality that possesses water content of 9.28%, ash content of 1.49%, protein content of ≤ 0.5%, perfect solubility on acetic acid, yield of 63% and deacetylation degree of 83.25% [23]. Other research conducted by Sry et al [24] aimed to isolate chitin,
synthesizing, and characterize chitosan from shrimp shell. Chitin isolation stages include
demineralization process by 1.5M HCL and deproteination with 3.5% NaOH. Chitin transformation into
chitosan was conducted through a deacetylation stage with 60% NaOH [24,25].

The result of the research shows the following chitosan characteristic: chitin transformation yield
into chitosan is at 67.08%, has a texture in the form of white powder, odorless, contain 1.55% water
content, soluble on 2% acetic acid with deacetylation degree of 84.85%. The result of research by Rani
et al., stated that tiger shrimp waste chitosan purity is lower than pure chitosan purity. Protein and
mineral contents and the existence of acetyl group bind by nitrogen cause an indecent chitosan [3].
Based on that, to obtain a decent purity, a more detailed chitosan synthesis to overcome acetyl group
chain binding is required. The result of a research conducted by Fikriatun et al. [17] reveals that 100g
of shrimp shell powder was able to be utilized to produce 15.33 g of chitin with chitin yield of 15.33%.
The obtain chitosan of this research is at 9.94g with chitosan yield of 9.94% on a deacetylation degree
of 69.87% [17].

Besides utilizing shrimp shell, there are similar research which utilizes mussel shell, a type of clam,
which is processed as material to produce chitin and chitosan [26]. This research was conducted on two
stages, which are mussel shell preparation and main research that includes chitin purification which
continued by chitosan production. The resulted product is acknowledged through FTIR test. The wide
and strong absorption peak on wave range of 3200-3600 cm-1 indicated the existence of O-H and NH2
groups as markers of chitin and chitosan products. From chitosan spectrum residue, red mussel shell
chitosan deacetylation degree measurement result is at 76.30%. This means that 76.30% of chitin residue
was deacetylated into chitosan. The commercial chitosan acetylation degree was at 20-25% [26], or
deaacetylation degree is at 75-80%. Based on that, deacetylation condition in this research shows a decent
result.

4. Conclusion
Deacetylated chitin DD is higher when using KOH solution than NaOH solution. Shrimp shell or other
shells processing process always going through 4 stages of chitosan synthesis which are; (a)
deproteination; (b) demineralization; (c) depigmentation; and (d) deacetylation. To achieve decent
chitosan purity, a more detailed chitosan synthesis method is required to overcome acetyl group chain
binding. Because of that, chitosan production from shrimp shell needs to be reexamined to obtain higher
deaacetylation degree by increasing the temperature, deacetylation time, and higher reagent
concentration.

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