Dental Journal
Majalah Kedokteran Gigi
Vol. 45. No. 1 March 2012

Research Report

Degrees of chitosan deacetylation from white shrimp shell waste as dental biomaterials

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ABSTRACT

Background: *Chitosan is biomaterial improved for various dentistry applications because it is biocompatible, degradable, non-toxic, and not carcinogenic. The main parameter affecting the characteristics of chitosan is deacetylation degree.* ***Purpose:*** *This study is aimed to determine the degree of deacetylated of chitosan derived from white shrimp shell waste used as dental biomaterial.* ***Methods:*** *White shrimp shells were crushed into powder. Next, deproteination process was conducted with 3.5% NaOH solution, demineralized with 1N HCl solution, and then depigmented with 90% acetone solution into chitin powder. Deacetylation process was then conducted by soaking the chitin powder in 50% NaOH solution for 6 h at 65° C to produce white powder of chitosan. Afterwards, deacetylation degree test was conducted by using Fourier Transform Infrared Spectrophotometer (FTIR) to calculate the ratio of the absorption bands between the absorbance peak of amide group about 1655 cm^{-1} and the absorbance peak of hydroxyl group about 3450 cm^{-1}.* ***Results:*** *The result of the deacetylation degree test on the chitosan powder derived from white shrimp shell waste was high, about 85.165%, and had the eligible form, solubility, and pH.* ***Conclusion:*** *It can be concluded that the deacetylation degree of chitosan from white shrimp shells could reach 85.165%.*

Key words: *Chitosan, shrimp shell waste, deacetylation degree*

ABSTRAK

Latar belakang: *Kitosan merupakan biomaterial yang dikembangkan untuk berbagai aplikasi kedokteran gigi karena biokompatibel, dapat didegradasi, tidak toksik dan tidak karsinogenik. Parameter utama yang mempengaruhi karakteristik kitosan adalah derajat deasetilasi.* ***Tujuan:*** *Tujuan dari penelitian ini adalah mengetahui derajat deasetilasi kitosan dari limbah kulit udang putih sebagai biomaterial kedokteran gigi.* ***Metode:*** *Kulit udang putih dihaluskan menjadi serbuk. Setelah itu dilakukan proses deproteinasi dengan larutan NaOH 3,5%, demineralisasi dengan larutan HCl 1N, depigmentasi dengan larutan aseton 90% sehingga menjadi serbuk kitin. Proses deasetilasi dilakukan dengan merendam serbuk kitin dalam larutan NaOH 50% selama 6 jam pada suhu 65° C sehingga dihasilkan serbuk putih kitosan. Uji derajat deasetilisasi menggunakan metode spektrofotometer Fourier Transform Inframerah (FTIR) dengan menghitung nilai perbandingan pita serapan antara puncak absorbansi gugus amida sekitar 1655 cm^{-1}, dan puncak absorbansi gugus hidroksil sekitar 3450 cm^{-1}.* ***Hasil:*** *Hasil uji derajat deasetilasi serbuk kitosan dari limbah kulit udang putih adalah tinggi yaitu sebesar 85.165% dan memiliki bentuk, kelarutan dan pH yang memenuhi syarat.* ***Kesimpulan:*** *Dapat disimpulkan derajat deasitilasi kitosan dari kulit udang putih adalah 85,165%.*

Kata kunci: *Kitosan, limbah kulit udang, derajat deasetilasi*

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INTRODUCTION

Indonesian marine are actually considered as the shell source of marine invertebrate animals (crustaceans) containing a lot of chitin. Chitin contained in crustaceans can be in high levels ranging from 20–60% depend on the species. Actually, Indonesia currently produces chitin derived from approximately 56,200 tons of wastes per year.[1]

Shrimp is one of Indonesia's main commodities for non-oil exports. The average of world consumers for shrimp per year has even been increasing.[2] One of nine shrimp species which has high commercial value and is very popular is white shrimp (*Peneaus merguiensis*). The results of shrimp processing are shell and head wastes. However, these wastes have still not been utilized properly and efficiently, and most of them even participate in polluting environment. Thus, chitin processing as an alternative effort is needed to utilize the shrimp shell wastes in order to make them have high value.[3]

Chitin is the major polysaccharide found in shrimp and crab shell wastes, but it can actually be derived from fungi and insect exoskeleton. Chitosan with the structure of β-(1-4)-2-amino-2-deoxy-D-glucose is a natural product derived from chitin polysaccharide. Chitosan is produced from deacetylation process of chitin. The deacetylation process of chitin is a process in which most of acetyl groups in chitin is substituted by hydrogen into amide group. The percentage of acetyl groups (COO-) replaced by amide (-NH2) then shows the magnitude of the deacetylation degree of chitosan.[3,4] The deacetylation degree of chitosan is a quality parameter of chitosan. The deacetylation degree is related to the ability of chitosan to form isoelectric interaction with other molecules. High degree of chitosan deacetylation will make more amide group formed, so chitosan will become increasingly active.[3,5,6]

Chitosan with the structure of β-(1-4)-2-amino-2-deoxy-D-glucose, is one of the abundant natural polymers dispersed in nature.[5] Chitosan is a cationic polymer with 2000-3000 monomers, which are biocompatible, degradable, and less toxic to the LD 50 = 16 g/kg of body weight. Chitosan can interact with charged materials, such as proteins, anionic polysaccharides, fatty acids, bile acids, and phospholipid.[6] Chitin and chitosan are actually beneficial for health and industry, such as textiles, photography, medicine, fungicides, cosmetics, food processing, and waste handling.[4]

The use of chitosan as biomaterial for medical applications has rapidly been growing as shown by many studies. Chitosan does not only have a role in closing wound, prompting bone regeneration, and accelerating healing process of burns, but is also considered as antibacterial, antitumor, anti-cholesterol, antioxidant, antidiabetic, anti-HIV, anti-inflammatory, and matrix metalloproteinase (MMP) inhibition.[7] The study results even conclude that chitosan is able not only to stimulate macrophage cells, but also to increase transforming growth factor increased bheta 1 (TGF β1), Platelets release transforming growth factor (PDGF), fibroblasts growth factor 2 (FGF-2),[8] bone morphogenetic protein expression of mRNA on the seventh day,[9] and deacetylation degrees of chitosan concentration 1% (w/v), over 80%, that can stimulate collagen synthesis in incision wound healing.[10]

In dentistry, the use of chitosan as biomaterial has also been growing rapidly. The results of antimicrobial chitosan test with high deacetylation degree even show that the growth of *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*) is little.[11] Chitosan at the concentration of 2% is able to remove plaques that stick to maxillary and mandibulary complete denture, about 9,9169 µg and 9,3021 µg, after immersion for 60 minutes.[12] Chitosan with molecular weight of 5-6kDa and deacetylation degree of 50-50% could inhibit the initial adhesion of *S. mutans* on tooth surface.[13]

Applications of chitosan in the medical field are determined by the specification of the deacetylation degree. Thus, the higher the deacetylation degree of chitosan is, the more active chitosan is. The purpose of this study, therefore was to investigate the characteristics of the deacetylation degree of chitosan derived from white shrimp shell wastes in order to be used as biomaterials in dentistry field.

MATERIALS AND METHODS

To make chitosan, 1 kg of shrimps were washed and boiled in boiling water for 15 minutes. They were dried for 3 hours, and then crushed and mashed. Then they were sieved by using sieve with size of 60 mesh, so powder obtained was about 200 mg. After that the extraction of chitin involving deproteination, demineralization, and depigmentation processes was conducted. At the stage of deproteination, white shrimp shell powders were sieved, put into beaker glasses, added with 3.5% NaOH solution with a ratio of 1:10 (w/v), and then stirred for 2 hours at 65° C. Tthe solution was filtered with filter paper, and then precipitation was obtained in the form of pellets. Pellets were washed with distilled water until pH became neutral, and then dried in oven at 65° C for 24 hours. The results obtained were chitin located on shells of the head and chest.

At the stage of demineralization, the following steps were conducted: chitin located on shells of the head and chest was put into beaker glasses, added with 1 N HCl solution with a ratio of 1:15 (w/v), and then stirred for 1 hour at room temperature. The results of the solution were filtered with filter paper. The precipitation obtained in the form of pellets was washed with distilled water until pH became neutral, and then dried in oven at 65° C for 24 hours until it was dry and produced chitin powders.

At the stage of depigmentation, those chitin powders were soaked in 200 ml of 90% acetone for less 20 hours. The results of the immersion were washed with distilled water until they were clean and then filtered by using filter paper. The precipitation obtained then was 36 grams of white chitin powders.

To extract chitosan, 36 grams of chitin powders were soaked in solution of 50% NaOH with a ratio of 1:10 (w/v) for 6 hours at 65° C, and then washed with distilled water and filtered with filter paper. The precipitation obtained then was dried in oven at 80° C. Chitosan obtained later was about 23 grams. Next, chitosan obtained was stored in a clean and dry room at room temperature until it was ready to be tested by deacetylation degree test.

For deacetylation degree test, 10 mg of chitosan powders were grounded with 50 mg of KBr powders until they were smooth, and then pressed with a hydraulic pressure of 1 atm. The mixture of chitosan and KBr powders were analyzed by using a spectrophotometer method, fourier transform infrared (FTIR). How to determine the degree of deacetylation was then calculated with the ratio of the absorption band between the absorbance peak of amide group, about 1655cm^{-1}, and the absorbance peak of the hydroxyl group, about 3450cm^{-1}. The comparison of those two groups was determined by making a straight line from 1800 cm^{-1} to 1600 cm^{-1} as a baseline for the band of amide group, and another straight line from 4000 cm^{-1} to 2500 cm^{-1} as a baseline for the band of hydroxyl group. The degree of deacetylation was then calculated by the following equation.[14]

The degree of deacetylation (DD)
= 100 - [(A amide/A hydroxyl) × 115]
A3450 = Log (T 0 hydroxyl/T hydroxyl)
A1655 = Log (T 0 amide/T amide)

The next stage, chitosan powders already known for their deacetylation degrees were finally analyzed for their characteristics concerning with their shape, solubility, and pH.

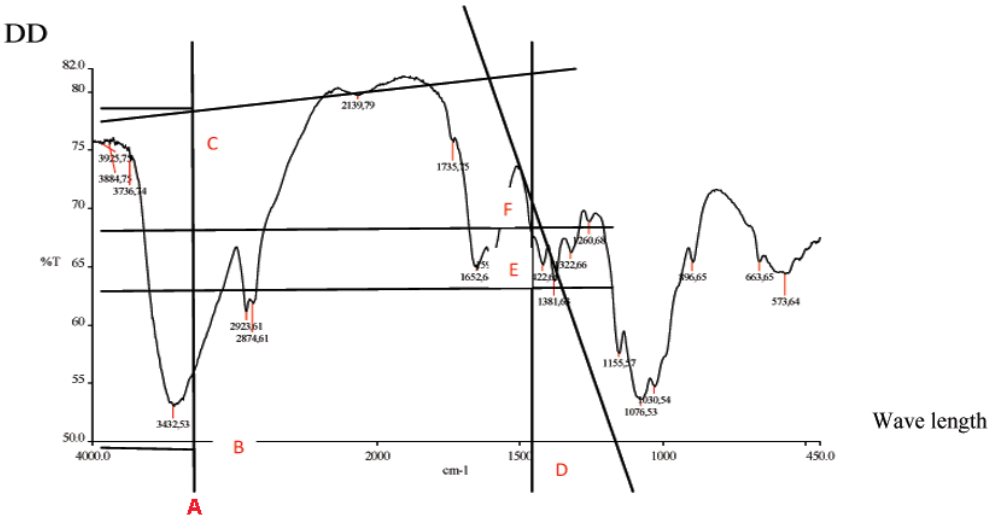
RESULTS

The deacetylation degrees of chitosan derived from white shrimp shells were tested by using FTIR spectrophotometry method, and the results were shown in figure 1.

Chitosan has hydroxyl group and amide group. The degree of deacetylation was then calculated with the ratio of the absorbance band between the peak absorption of amide group and the peak absorbance of hydroxyl group. It is known that the peak of hydroxyl group was at 3432.53 cm^{-1} (point B). Meanwhile, the peak of amide group was at 1652.64 cm^{-1} (point E). Based on the results of quantitative analysis by using FTIR spectrophotometry, it is finally known that the deacetylation degree of chitosan derived from white shrimp shells was 85.165%. The results of the characteristics of chitosan with that deacetylation degree, 85.165%, were in the following form, solubility, pH as shown in table 1.

Table 1. The characteristics of chitosan derived from white shrimp shells with 85.165% of deacetylation degree

No	Characteristics	Requirements	Observation results
1	Form	slightly yellow-white powders, odorless, and tasteless	Qualified
2	Solubility	Not soluble in water, but soluble in acetic acid	Qualified
3	pH	7.0 – 9.0	7.4
4	Deacetylation Degree	More than 70 %	85.165 %



chitosan.sp 3551 4000 450 53 81 4 %T 3 0 REF 4000 75 2000 80
3925 75 3884 75 3736 74 3432 53 2923 61
2874 61 2139 79 1735 75 1652 64 1599 65
1422 65 1381 63 1322 66 1260 68 1155 57
1076 53 1030 54 896 65 663 65 573 64

Figure 1. Graph of deacetylation degree test results.

DISCUSSION

Chitin is mostly found in insects, microorganisms, and vertebrates' heads and shells, such as shrimps, crabs, oysters, and squids. Chitosan is made of shrimp shells classified into crustacean group containing a lot of chitin. Chitosan is a compound with chemical structure of poly (2-amino-2-dioksi-β-D-glucose) that can be derived from hydrolysis process of chitin by using strong alkaline. Nowadays, there are more than 200 applications of chitin and chitosan as well as their derivatives in food industry, food processing, biotechnology, agriculture, pharmaceuticals, healthcare, and environment.[3,5]

During the deproteination process, the making process of chitosan derived from chitin, used a strong alkaline (NaOH) to remove protein content. Meanwhile, during the demineralization process, a strong acid (HCl), was used to remove mineral contents. Calcium compounds then would react with chloride acid soluble in water. Protein, fat, phosphorus, magnesium, and iron were actually also wasted in this process. In the depigmentation process, 90% acetone solution was finally used to deacetylate with a strong alkaline, NaOH solution, in order to produce chitosan.

In the process of deacetylation, furthermore, most of acetyl group (COO-) on chitin was then substituted by hydrogen to amide group (-NH_2). The percentage of the acetyl group replaced by the amide one indicates the magnitude of the deacetylation degree (DD) of chitosan since the main parameter affecting the characteristics of chitosan is deacetylation degree.[14] Therefore, the deacetylation degree of chitosan can be considered as a quality parameter indicating the percentage of acetyl group that can be removed from the yield of chitin and chitosan. In other words, the higher the deacetylation degree of chitosan is, the lower the chitosan acetyl groups is. As a result, the interaction between the ions and their hydrogen bonds will be stronger. It means that the release of the acetyl group of chitosan causes positive chitosan that is able to bind negative compounds, such as proteins, polysaccharide anion, to form neutral ions.[4]

The degree of deacetylation that is more than 75% is actually considered as the high degree of deacetylation.[3,5] And, the characteristics of the deacetylation degree of chitosan obtained in this study were high, about 85.165%. This is because the researchers observed several factors affecting the deacetylation degree of chitosan, such as temperature, the concentration of NaOH solution used, and the duration of chitin powder immersion into NaOH solution.[3] In this study, the deacetylation process of chitin powder used 50% NaOH solution with a ratio of 1:10 (w/v) for 6 hours at 65° C to produce chitosan powder with the degree of deacetylation more than 75% because the higher the concentration of NaOH is, the more increasing the degree of deacetylation (DD) is.

Temperature and the duration of NaOH immersion, furthermore, can affect the molecular chain of chitin. The use of high temperature, above 150° C, for example, can cause the breaking of polymer bond (depolymerization) of chitosan molecular chains resulting in lowering molecular weight chitosan. The high concentration of NaOH solution, more than 40%, can break the bonds of carboxyl group and nitrogen atoms of chitin that have thick and long crystal structure. The high concentration of NaOH then can lead to the functional group of amine (NH_3^+) substituting the acetyl groups of chitin, so the solution in the system is more active, and the deacetylation is better.[3,15,16] Therefore, the use of higher concentrations of NaOH can make the degree of deacetylation greater, but this does not always make the degree of deacetylation significantly increasing. In the largest concentration of NaOH, 60%, the degree of deacetylation even becomes decreasing. This is because in 60% NaOH the solution becomes more viscous resulting in imperfect mixing process which means that some parts of chitin can not perfectly react with NaOH solution, so the amino group formed is little or has decreased DD value.[17]

Chitosan is actually a polysaccharide that is very hard to be immersed at neutral pH, like in water, since chitosan contains high density of polymer chains that are bonded to each other with very strong hydrogen bonds. Chitosan solution is considered as strong alkaline solution, so it will be more soluble in the aqueous solution, acetic acid. Acetic acid is actually classified as weak carboxylic acid containing carboxyl group (-COOH). Carboxyl group contains carbonyl group and hydroxyl group. It even reaches its boiling point at 118° C and its smell is very sharp. The increasing of the solution is even linear to the increasing of deacetylation degree. This is because the acetyl group in chitin cut by the deacetylation process will leave the amine group. H ions on the amine group of chitosan, as a result, will lead to easily interact with water through hydrogen bonding.

In this study 2% acetic acid used to dissolve chitosan powder so that it becomes gel. It is because the higher the degree of deacetylation is, the higher the solubility of chitosan in acetic acid solution is. It means that there is hydrogen interaction between the carboxyl group in acetic acid and the amide group in chitosan.[3,15] Therefore, the solubility of chitosan is also influenced by the type of crustacean that is used. This type of crustaceans, such as white shrimp, used in this study, as a result, could produce high deacetylation degree of chitosan. Another type of crustaceans, such as crab shell, can also make amide group formed little, less reactive, and unpredictable for the duration of deacetylation process.[3,15,18]

Some studies on chitosan derived from shrimp shells have been conducted concerning with the benefit of dentistry. Based on the results, it is known that there is no toxicity effect on the cell culture test,[19] and for the biocompatibility test, skin patch test is needed to be used. Chitosan with concentration of 1–4%, as a result, does not cause allergic reactions in individuals with allergic or not allergic to seafood history. The results of another study even concludes that the number of osteoblast like cell and

collagen synthesis type I in the formation of reparative dentin is increased in direct pulp calping on *Rattus norvegicus* teeth by using chitosan.[20] It indicates that 1% chitosan gel with deacetylation degree, about 85.165%, can increase the proliferation of fibroblasts, osteoblasts, and collagen type 1 in healing wounds of tooth extraction of *Rattus norvegicus* at 7 and 14 days of the observation.[21]

In other words, chitosan with higher deacetylation degree will make more amide group of chitosan formed, so it will become more active, have high chemical reactivity, and be able to interact with proteins and other organic matrices, such as anionic glycosaminoglycans and proteoglycans, and extracellular matrix macromolecule. In addition, the positive chitosan is able to react with the surface of negative anionic polymer, so it can facilitate the migration of inflammatory cells.[22,23] Based on several studies on chitosan as described above, it can be said that chitosan still needs to be developed since it has some potential functional characters for broad use in dentistry. Finally, it can be concluded that the deacetylation degree of chitosan derived from white shrimp shells could reach 85.165%.

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