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The Effects of Demineralization Process on Diameter, Tensile Properties and Biodegradation of Chitosan Fiber

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Abstract

Chitosan fiber is one of the potential fibers that can be used as absorbable monofilament suture in biomedical application. In chitosan synthesis, aside from deproteination and deacetylation, demineralization is a crucial step where the major minerals within crustacean shells are removed. This demineralization process is carried out with two parameters, i.e. time and temperature. This research studies the influence of demineralization time on the diameter, tensile properties and biodegradability of chitosan fibers. Chitosan was synthesized from shrimp shells using 1 × 2 h and 3 × 2 h demineralization process. Chitosan fibers were produced by means of wet spinning. The chemical properties of chitosan fibers were characterized by means of Fourier Transform Infrared (FTIR) spectroscopy and X-Ray Diffractometry (XRD) technique. Physical properties characterization was carried out to measure the fibers' diameter, density and viscosity. Tensile properties were evaluated by means of tensile test. The results were compared to standard of absorbable suture from the United States Pharmacopoeia (USP). Furthermore, *in vitro* degradation testing was also performed for analyzing biodegradation properties. Chitosan fibers were successfully made with diameter and maximum tensile force of chitosan fibers in a range of 364 - 460 μm and 5.6 - 8.3 N, respectively. The results of this research pointed that adding demineralization time would increase the diameter of chitosan fiber. However, the degradation occurred in prolonged demineralization process broke the bonds within the fiber which lead to a decrease in fiber's density. It is due to the degradation of chitosan occurred during extended demineralization process, which leads to degree of crystallinity reduction. Extensive demineralization process has been found to lower fibers' tensile strength from 80.4 MPa to 38.4 MPa (52.2%), but increase their biodegradability by 17% and maximum elongation from 6.9% to 16.2% (136%). Despite that extensive demineralization process lowered chitosan fiber's tensile strength, both fibers made could still fit the standard for synthetic absorbable suture from USP number 0 and 1.

Keywords: Chitosan fiber; demineralization; tensile strength; biomaterials; biodegradation; *in vitro*.

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1. Introduction

Biomedical application needs biomaterials. One of the examples of biomedical applications of natural polymers is absorbable suture, made of collagen, gut,chitosan, polyglactin, polyglycolic acid and polydioxanone [1-4]. Aborbable sutures have been widely utilized in various medical procedures, due to the benefits they offer. For instance, Karounis *et al.* [5] and Luck *et al.* [6] have proven that absorbable suture promotes better cosmesis and higher patient compliance, respectively. Chitosan as the second most abundant natural biopolymer, is derived from the exoskeletons of shellfish and exhibits many bioactive properties that make it an ideal material for use as biomaterial such as antibacterial, biodegradable, non-toxic, and the ability to attract and promote cell growth and formation [1,3]. Compared to other raw materials for absorbable sutures, chitosan is also desirable due to its low immune response, as proven by VandeVord *et al*. [7]. The common standard used for classifying sutures according to their strength and diameter is from the *United States Pharmacopoeia (USP)* [8]. This standard is applicable to both non-absorbable and absorbable sutures, either natural (e.g. collagen) or synthetic (e.g. chitosan) polymers.

Research on chitosan monofilament fibers have been carried out in correlation to drying process by Knaul *et al.* [9], biodegradation control by Yang *et al.* [10]. In the process of chitosan isolation, demineralization is one of the crucial steps (aside from deproteination and deacetylation), as written by Khor [11], in which major minerals within the crustacean shells are removed. This demineralization is carried out by using time and temperature as its parameters. Research on the effects of demineralization process on chitosan fiber production has not been achieved.

In this research, the influence of demineralization time on physical properties, tensile properties, and biodegradability of chitosan fiber will be analyzed. The diameter and tensile strength of fibers produced will also be compared to standard of absorbable suture from the (USP) [8].

2. Experiment

The process of isolating chitosan involves three steps, i.e. deproteination, demineralization and deacetylation. Deproteination was carried out by immersing shrimp shells in a 1% w/w aqueous solution of sodium hydroxide for 2 h at 85 °C. The shrimp shells then were washed in distilled water until reaching neutral pH and dried for 2 h at 105 °C. After that, the shrimp shells were immersed in a 3% v/v aqueous solution of hydrochloric acid for at 50 °C for 2 h and 3 × 2 h. The shrimp shells were washed and dried again as above. The last step, deacetylation, was achieved by immersing the samples in a 50% w/v solution of sodium hydroxide for 2 × 4 h. Washing and drying were also performed after the completion of deacetylation process. Shrimps shells were obtained from Padalarang Beach (West Java, Indonesia). All the other chemicals used in this study were analytical grade and used as received.

Chitosan fibers were prepared by wet-spinning procedure. The dope was prepared by 20 g chitosan in 400 ml of 2% v/v aqueous acetic acid-methanol solution. Viscosity measurement was carried out before performing wet-spinning process by means of falling ball viscometer. A laboratory scale wet-spinning machine, comprised of a reservoir, two rolling wheels (5 rpm and 9 rpm, respectively) and a spinneret (1 hole, 2000 μm), was used. The polymer was passed through a wheel-powered (9 rpm) metering pump by gravitational force, and then pumped to a stainless steel spinneret, which was immersed in a 1-meter coagulation bath containing 5 L of NaOH 14% w/w at 50 °C. Secondary coagulation bath containing 5 L of NaOH 2% w/w at 50 °C was also used. Fibers were collected by take-up rollers, washed and dried at room temperature for 3 days.

The chitosan made were divided into two types, i.e. 1 × 2 h demineralization (1DD) and 3 × 2 h demineralization (3DD). The degree of deacetylation (DD) of chitosan samples was determined using Fourier Transform Infrared (FTIR) spectroscopy Shimadzu Prestige 21 according to procedure and

formula from Khor [11] and Domszva [12]. FTIR was also performed to confirm whether the fiber produce was chitosan. Density calculation was done by performing fiber weighing and diameter measurement beforehand. The structure of chitosan fibers was analyzed by Scanning Electron Microscopy Philips XL-20 after coating with gold, before and after *in vitro* degradation test.

In order to evaluate the crystallinity level of chitosan fiber, X-ray diffraction testing was performed by means of Philips XL-20 X-ray diffractometer. The samples were irradiated with monochromatized Cu K_α radiation (1.54 Å) and analyzed between 5 and 60° (2θ) to evaluate the XRD pattern and crystallinity level of chitosan fibers. The current and voltage used were 30 mA and 40 kV, respectively. Crystallinity level of chitosan fiber was calculated dividing the area under peak region and the total area [13].

Tensile tests on two types of chitosan fibers were performed on Textechno Statimat ME Test with a 10 N load cell, using a cross-head speed of 300 mm/min and a gauge length of 100 mm. Average values of tensile strength and maximum elongation were determined after repeating the test 15 times for each type of chitosan fiber. The tensile test results were compared to standard of absorbable suture from the United States Pharmacopoeia (USP) [8].

The *in vitro* degradation of two types chitosan fibers was followed in 2 mL phosphate-buffered solution (pH 7.4) at 37 °C containing 2 mg/mL lysozyme (hen egg-white, Sigma-Aldrich, Singapore). The concentration of lysozyme was chosen to correspond to the concentration in human serum [14]. Briefly, 10 mg of chitosan fibers sterilized and then incubated in the lysozyme solution for 24 hours each day. The lysozyme solution was refreshed daily to ensure continuous enzyme activity according to the procedure carried out by Yang *et al.* [10]. After 7 days, samples were removed from the medium, washed carefully with distilled water, dried under vacuum and weighed. *In vitro* degradation rate was expressed as percentage of weight loss of the fibers after lysozyme treatment.

3. Results and Discussion

3.1. Chitosan and chitosan fiber production

According to the FTIR spectra shown in Figure 1, a characteristic band at 3300-3500 cm^{-1} is attributed to –OH and –NH2 stretching vibration groups and a characteristic band at 1637 cm^{-1} account for the existence of amide I in chitosan structure [15]. At 1570 cm^{-1}, a peak is shown to be an attributed to –NH amide II bending groups. Another peak which was also important was the peak at 1118 cm^{-1}, since it is attributed to the C-O-C link in glucopyranose structure of chitosan [15].

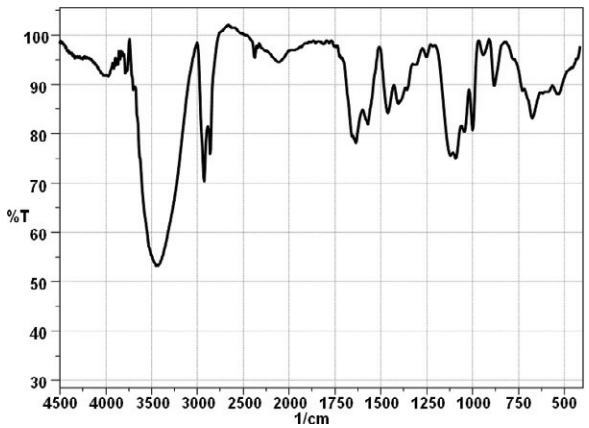


Fig. 1. IR spectra of chitosan fiber

The degree of deacetylation (DD) of chitosan fiber 1DD (1 × 2 h demineralization) and 3DD (3 × 2 h demineralization) were 70.6% and 67.4%, respectively. The viscosities of chitosan fibers were 4.5 Pa.s and 0.7 Pa.s, respectively. From the results obtained it can be clearly seen that demineralization process of chitosan greatly affects the viscosity of chitosan solution by decreasing the viscosity by 84.4%, whereas it affects the change of %DD to a much lesser extent by decreasing the %DD only by 4.26%. This occurred because excess demineralization can break glycosidic bond along chitosan polymer chains. These broken chains will then lead to a decrease of viscosity.

3.2. Physical properties characterization

Density and diameter of two type chitosan fibers are shown in Figure 2. Figure 2a showed that there is a decrease in the density of chitosan fiber due to the increase of demineralization duration. By prolonging the demineralization process to 3 × 2 h (3DD), fiber density will decrease by 5% from 1.29 g/mL to 1.23 g/mL. This decrease occurred because of the breakage by hydrolysis reaction [11] happening along chitosan polymer chains during extended demineralization process. The breakage causes polymer chain to be shorter, which leads to the decrease in the number of polymer chains per volume unit along chitosan fiber.

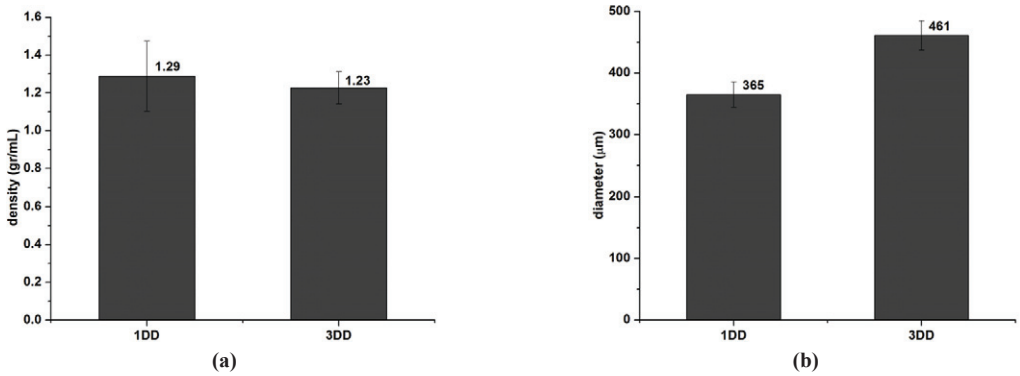
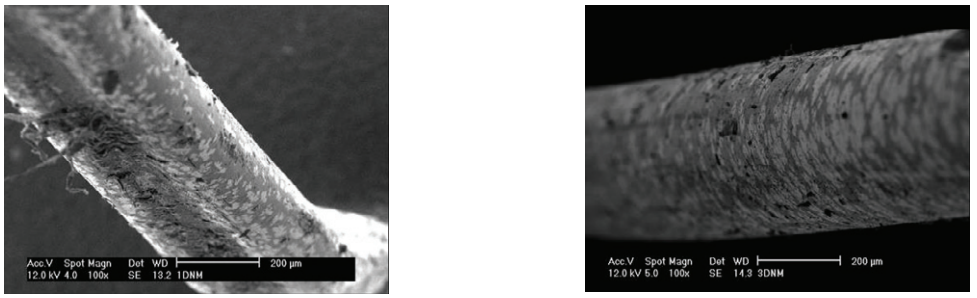


Fig. 2. (a) Chitosan fiber density results (b) Diameter measurement results of chitosan fiber

From Figure 2b it can be seen that the diameter of chitosan fiber 3DD is larger (461 μm diameter) than that of fiber 1DD (365 μm diameter). This means that prolongation of demineralization extended the diameter of chitosan fiber. This is due to the degradation occurred in extensive demineralization process. Degraded chitosan polymer from this process has more amorphous regions. This makes the polymer chains more mobile when it is spun. This mobility gives polymer chains the ability to move farther when it is spun, which then makes it harden to a bigger fiber. The larger diameter of the chitosan fibers 3DD compared to 1DD is also shown from SEM data on Figure 3.

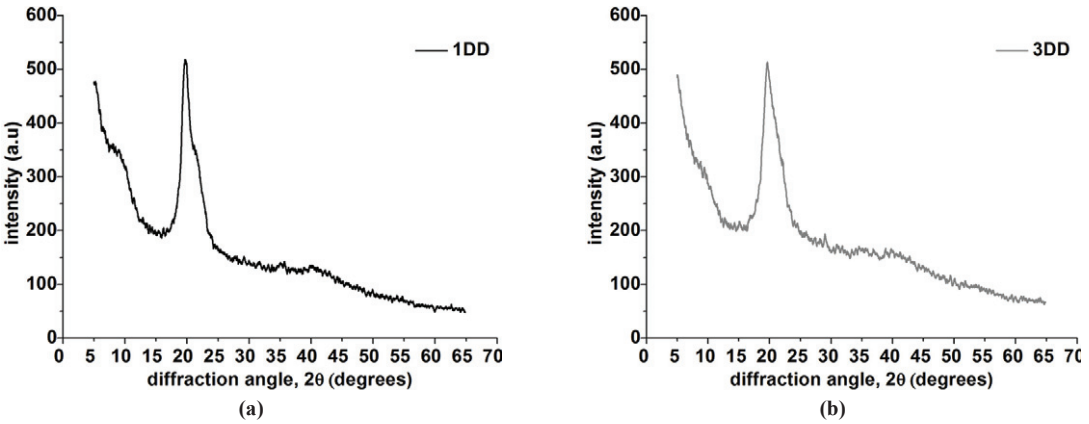
3.3. X-ray diffraction (XRD) analysis

XRD spectra of two types chitosan fibers, Figure 4 showed two crystalline peaks at $2\theta = 10°$ and 19.5-19.7° for chitosan fiber 1DD. For 3DD specimen, the crystalline peak at $2\theta = 10°$ does not appear. This is due to the breakage of hydrogen bond between amino group and hydroxyl group in chitosan polymer chain [16].



(a) **(b)**

Fig. 3. SEM results of chitosan fibers (a) 1 × 2 h demineralization - 1DD and (b) 3 × 2 h demineralization - 3DD



(a) **(b)**

Fig. 4. XRD patterns of two types chitosan fiber (a) 1 × 2 h demineralization - 1DD and (b) 3 × 2 h demineralization - 3DD

The calculation of chitosan fiber crystallinity level, Table 1 were performed by comparing the crystalline area in XRD spectra of chitosan fiber at 2θ = 7-12° and 15-25° with total area of the XRD spectra. The results showed that 3DD fiber had lower crystallinity level than 1DD fiber. There was a decrease in the crystallinity degree of chitosan fiber by 21.5%, from 12.0% to 9.5%. This decrease occurred because of the degradation of chitosan polymer chains happening in excess demineralization process. When this degradation is occurring, both amorphous and crystalline regions within chitosan will be deteriorated. It will then lead to the decrease of crystallinity level or the increase of amorphous level of chitosan fiber. The higher crystallinity level of 1DD chitosan fiber confirms the higher density of 1DD chitosan fiber compared to 3DD. The higher amorphous level of 3DD chitosan fiber corroborates with the diameter increased of 3DD chitosan fiber.

Table 1. Crystallinity level of chitosan fiber

Chitosan Fiber	Crystalline Area	Total Area	% Crystallinity
3DD	1033	10929	9.5
1DD	1182	9817	12.0

3.4. Mechanical properties analysis

Figure 5 shows tensile strength and maximum elongation of two type chitosan fibers. From Figure 5a, it can be seen that there is a decrease in chitosan's tensile strength. Prolonging the demineralization process from 1 × 2 h (1DD) to 3 × 2 h (3DD) can decrease the tensile strength, from 80.4 MPa to 38.4 MPa (52.2%). This phenomenon occurred due to the polymer degradation in excess demineralization process. Polymer degradation due to hydrolysis reaction cuts the polymer chains inside chitosan fiber to shorter ones [11]. Hence, these shorter polymer chains have lower entanglement level in which polymer chains are more likely to slip one another. This then leads to the decrease of fiber tensile strength.

Figure 5b showed that there was an increase in chitosan fiber's maximum elongation. By adding the number of hours for demineralization process to 3 × 2 h, the maximum elongation of chitosan fiber increased 136% from 6.9% to 16.2%. This increase happened due to polymer degradation resulting from hydrolysis reaction [11]. When chitosan is degraded, the polymer chains are cut to shorter chains. This creates more amorphous regions within chitosan fiber, in which there will be more empty spaces within the fiber to be filled by water molecules during fiber spinning. Water molecules between chitosan's shorter polymer chain makes these chains move easier, which then leads to the increase of the fiber's maximum elongation.

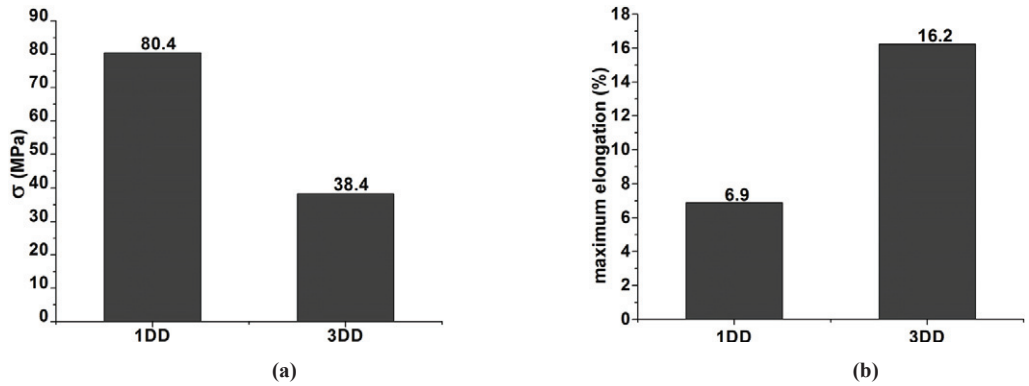


Fig. 5. (a) Tensile strength of chitosan fibers (b) Maximum elongation of chitosan fibers

On Figure 6, data showed that diameter and average tensile force of chitosan fiber produced in this research could fit in the absorbable suture standard from USP 32 – NF 27. The fiber produced in this research can be classified as absorbable suture USP number 0 for 1DD fiber and USP number 1 for 3DD fiber. From the data, it is shown that the average tensile force of fiber 1DD far exceeded the standard of USP 1. However, since the diameter of this fiber is smaller than that of USP standard fiber number 1, it then falls to USP number 0.

3.5. In vitro degradation results

Figure 7 shows the decrease of fiber weight for 7 days due to *in vitro* degradation on two types of chitosan fibers. The weight of chitosan fiber decreased from 7.9 mg to 6.55 mg (17%) by prolonging the demineralization process from 2 h (1DD) to 6 h (2DD), which means that the biodegradability of chitosan fiber increased. This is due to the degradation of chitosan in the prolonged demineralization process. Extensive demineralizaiton process will result degradated chitosan, which has lower crystallinity level. This makes chitosan has more spaces to entrap water molecules. Hence, having more water molecules

within its structure will make chitosan fiber more prone to lysozyme degradation. Besides crystallinity level, deacetylation degree is also the main cause of the decrease. The lower the degree of deacetylation of chitosan, the higher the degradation is [10,14]. Since the deacetylation degree of fiber 3DD is lower than that of fiber 1DD, it has lower weight after *in vitro* degradation

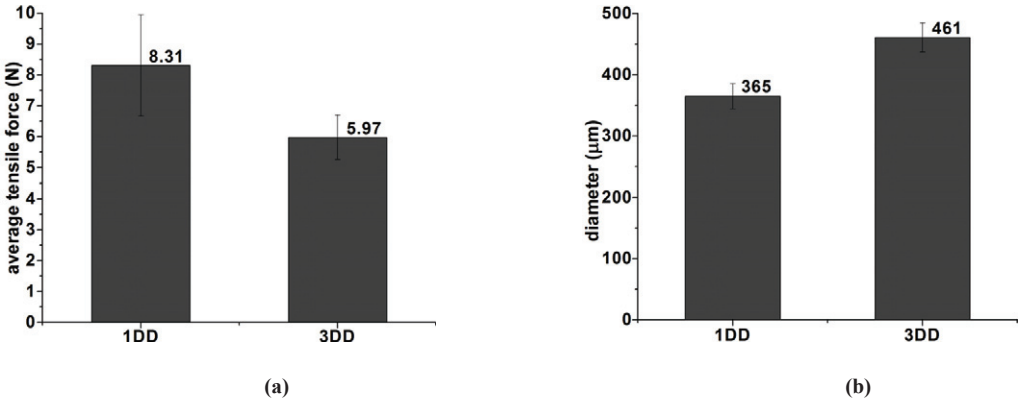


Fig. 6. Tensile data comparison of chitosan fiber with USP standard (a) fiber tensile force (b) diameter of fiber

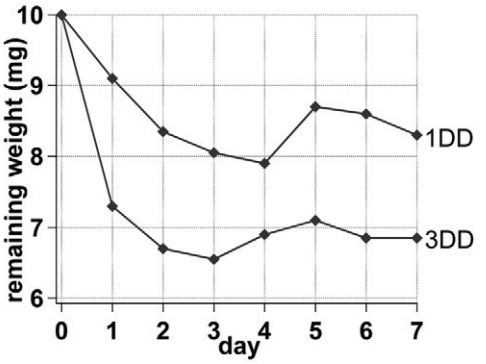


Fig. 7. *In vitro* degradation result of two types chitosan fibers

4. Conclusion

Chitosan fibers were successfully made with diameter and maximum tensile force of chitosan fibers in a range of 364 - 460 μm and 5.6 - 8.3 N, respectively. The results of this research pointed that adding demineralization time would increase the diameter of chitosan fiber. However, the degradation occurred in prolonged demineralization process broke the bonds within the fiber which lead to a decrease in fiber's density. It is due to the degradation of chitosan occurred during extended demineralization process, which leads to degree of crystallinity reduction. As for other properties, extensive demineralization process has been found to lower fibers' tensile strength, but increase their biodegradability and maximum elongation. The fibers' tensile strength decreased by 52.2% from 80.4 MPa, the fiber's maximum elongation increased by 136% from 6.9% to 16.2% and the fiber's biodegradability increased by 17%. Despite that extensive demineralization process lowered chitosan fiber's tensile strength, both the fibers made could still fit the standard for absorbable suture from USP number 0 and 1.

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