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Original article

Characteristics of chitosan from *Penaeus monodon* on chitosan-gelatin suspension viscosity

Widyasri Prananingrum[1], Grace Caroline Setiawan[1], Vindya Meinda Putri[1], Baiq Nadia Risna Wahyuning[1], Fitria Rahmitasari[1], Sularsih[1], Nurina Rizka Ramadhania[2], Sri Fatmawati[3]
[1]Department of Dental Materials, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia
[3]Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia

ABSTRACT

Background: *Chitosan synthesized from Penaeus monodon shells was developed into a chitosan-gelatin suspension as an injectable bone substitute for socket preservation.* ***Purpose:*** *To investigate the characteristics of chitosan from P. monodon shells and their influence on the viscosity of a chitosan-gelatin suspension.* ***Methods:*** *P. monodon shells from Tarakan Waters were prepared using three methods: Group 1) deproteinization-depigmentation-deacetylation, Group 2) demineralization-depigmentation-deacetylation, and Group 3) deproteinization-demineralization-depigmentation-deacetylation. The chitosan was characterized by morphology, moisture and ash content, molecular weight (MW), deacetylation degree (DD), and viscosity. This chitosan was made into a chitosan-gelatin suspension with a ratio of 45:55 (w/w%) (95 ml:110 ml). The differences in viscosity of the chitosan-gelatin suspension were determined using Kruskal–Wallis and Mann–Whitney tests. The effects of the chitosan's MW and DD on the viscosity of the chitosan-gelatin suspension were analyzed using Spearman's correlation.* ***Results:*** *Group 2 had the highest moisture content (10.63%), MW (159.68 kDa), viscosity of the chitosan powder (5.53 dPa.s), and viscosity of the chitosan-gelatin suspension (40.20 cps). Group 1 had the highest ash content (27.83%) and DD (93.72%). Group 3 showed the lowest ash content (1.06%), MW (37.12 kDa), and DD (86.22%), but it had good viscosity of the chitosan-gelatin suspension (37.25 cps). A significant difference in viscosity was found between the chitosan-gelatin suspension groups. Spearman's correlation coefficients between the viscosity of the chitosan-gelatin suspension and MW and between the viscosity of the chitosan-gelatin suspension and DD were 0.389 and −0.195, respectively.* ***Conclusion:*** *The viscosity of a chitosan-gelatin suspension is influenced by the MW and DD of the chitosan powder. Chitosan from P. monodon can potentially be an injectable bone substitute in socket preservation.*

Keywords: *characteristics; chitosan; chitosan-gelatin suspension; Penaeus monodon shell; medicine*
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Correspondence: Widyasri Prananingrum, Department of Dental Materials, Faculty of Dentistry, Universitas Hang Tuah. Jl. Arif Rahman Hakim 150 Surabaya, 60111 Indonesia. E-mail: widyasri.prananingrum@hangtuah.ac.id

INTRODUCTION

The incidence of tooth extraction generally will have a repercussion on alveolar bone resorption. Alveolar bone resorption may occur horizontally or vertically. Nonetheless, a large shrinkage will occur in the horizontal dimension, especially on the buccal side of the alveolar ridge. The changes in the alveolar bone's dimension in post-extraction human tooth sockets show a decrease in the height of the alveolar crest by 1.53 mm, a decrease in the width of the alveolar bone by 3.87 mm, and a decrease in the alveolar height on the buccal side by 1.67 mm.[1] The process of socket wounds healing after tooth extraction results in a decrease in volume and changes in the shape of the alveolar bone. Thus, socket prevention treatment must be carried out immediately to be able to reconstruct the alveolar bone in preparation for implant treatment so that results are obtained that meet the criteria for successful implant treatment, both aesthetically and functionally.[2]

The use of bone substitute material in the post-extraction socket can prevent alveolar ridge shrinkage and help the bone healing process. A scaffold is one of the substitute materials that is commonly used, but it has several disadvantages, as it is brittle and porous, has poor

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mechanical strength caused by weak bonds between the particles, is easy to biodegrade, and has a limited and rigid shape. A study using a biphasic calcium phosphate scaffold combined with gelatin with high porosity and a porous size of ±200–300 μm showed low fracture toughness and strength.[3] Some studies used a chitosan scaffold, and the combination proved that porosity with various porous sizes would impact biological properties, such as VEGF and FGF expression.[4,5] The disadvantages of bone substitute materials in a scaffold have prompted researchers to seek a form that is effective, can cover these disadvantages, and gain more leverage advantages. One of the forms of bone substitute materials considered to gain improvement in covering the lack of scaffold form is injectable gel suspension. The advantages of administering bone substitute material in the form of a suspension are evident, as it can set into the pores of the bone, resulting from its high mechanical properties, execution to fulfill all parts of the bone, adaptability to the anatomical shape of the bone, sterility, and attainability. A study using hydroxyapatite as an injectable bone substitute (IBS) showed setting ability according to the desired shape after injection with sufficient pore size to ensure bio-transportation into the bone.[6] IBSs can be found as bone grafts in dentistry, such as injectable resin-based for load-bearing sites and injectable ceramic-based for bone defect sites.[7]

IBS is often used to treat osteoporosis, a disorder of bone fragility that can increase the risk of fractures. The two types of IBS are IBS that contains ionic hydraulic cement, which can harden in vivo after being injected, and IBS that contains a suspension, which can harden when injected. The role of IBSs can also be alleviated by drug agents that facilitate the healing process of bone defects. A synthesizing IBS material based on HA-gelatin with the addition of 10% alendronate with a suspension ratio of 45:55, which has the best viscosity value, is not toxic and can harden through the setting time test.[8] The manufacture of IBS suspensions based on hydroxyapatite composites with gelatin has an applicable viscosity value, especially at 25°C, of 30.4–39.4 dPa.s, and it has an injectability percentage value of 98.22–98.64%.[6] A suspension of IBS material can be applicable if the viscosity value is ≤40 dPa.s because the suspension will be able to flow over a wider area and penetrate the pores of the damaged bone.[9] Viscosity is a material property that determines the thickness of a liquid and is related to the ability of a material to flow.[8] Viscosity is influenced by pressure, temperature, additional ingredients in suspension, size, and molecular weight. The presence of additional materials, such as suspension materials, will increase viscosity because binding and bonding reactions will occur between the substances. This also explains that the greater the size and molecular weight of a substance, the greater the increase in viscosity.[10,11]

Materials that can be used as bone substitute materials include natural polymers, such as chitosan.[4,12] Chitosan is produced through the process of deacetylation of chitin compounds. Chitin is the main component of the shells of hard crustacean animals with exoskeletons, such as crabs, shrimps, and clams. Chitosan has a quality standard, namely the degree of deacetylation. The deacetylation degree (DD) is the number of acetyl groups from chitin that is converted into the active group NH_2 in chitosan so that it can affect the work of chitosan in its application. For use in the biomedical field, chitosan must have a DD of ≥80%.[13,14] The ideal requirement for chitosan to be used in the biomedical field is a DD of ≥70%. The optimum deacetylation rate will be obtained if the concentration of NaOH used is 50%. A concentration of 50% produces better deacetylation if it is carried out at a low temperature.[15] The higher the degree of deacetylation, the more chitosan will have cationic properties, which can stimulate cell adhesion as a cell modulator, differentiation, cell movement, synthesis, and cell function.[16,17] The DD of the chitosan will depend on the source, preparation procedure, and condition.[18]

The chitosan preparation procedure commonly involves deproteinization, demineralization, and deacetylation stages. In the deproteinization stage, which aims to remove protein, a low concentration of a strong base is used. Conversely, in the deacetylation stage, a high concentration of a strong base is used. The use of two stages in the preparation procedure results in more wastage of strong base solutions and increases the preparation time. Therefore, investigating the elimination of the deproteinization stage is necessary to obtain a more efficient preparation process that will also affect the chitosan's quality. Furthermore, the demineralization stage involves the removal of minerals, and the mineral content of different types of crustacean shells varies. Therefore, it is important to investigate whether including the demineralization stage will impact the chitosan's quality.

This research aimed to develop chitosan synthesized from the shells of tiger shrimps (*Panaeus monodon*) from Tarakan Waters, East Kalimantan, Indonesia. These shrimps are traditionally cultivated; hence, they have good quality and are much thicker and bigger than other types of shrimp, resulting in higher chitosan content.[19] Based on the above, the researcher analyzed the characteristics of chitosan synthesized from *P. monodon* with various methods and evaluated its effect on viscosity and cytotoxicity of a chitosan-gelatin suspension as an ideal requirement for a candidate to be an IBS material in socket preservation.

MATERIALS AND METHODS

This research was carried out by making chitosan powder from *P. monodon* shells from Tarakan Waters, East Kalimantan, Indonesia. The *P. monodon* shells were cleaned with water and then dried under the sun. Afterward, the shells were blended carefully and homogenized through 200 mesh to obtain a fine powder (<74 μm).

This research was divided into three synthesized method groups that were treated at a 75°C deacetylation temperature: Group 1) deproteinization, depigmentation,

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and deacetylation stages; Group 2) demineralization, depigmentation, and deacetylation stages; and Group 3) deproteinization, demineralization, depigmentation, and deacetylation stages. Figure 1 shows the synthesized stages of the chitosan powder from *P. monodon* shells. Deproteinization stage: The stage was initiated by placing 200 g of *P. monodon* shell powder into 2,000 ml of NaOH 3.5% (Merck, USA) 1:10 (b/v). This solution was stirred for 2 h at 75°C on a magnetic stirrer and filtered with filter paper. Afterward, the solution was cleaned with 1,000 ml of aqua dest until it became a neutral-pH powder. Lastly, it was roasted inside a 75°C oven for 24 h until it became a powder, which was chitin. Demineralization stage: The stage was undertaken by adding 3,000 ml of HCI 1 M (Merck, USA) 1:15 (b/v) to 200 g of chitin powder. This solution was stirred for 1 h at room temperature on a magnetic stirrer and filtered with filter paper. Afterward, the solution was filtered and cleansed with 1,000 ml of aqua dest until it became a neutral-pH powder. Finally, the powder was roasted inside a 75°C oven for 1 h until it dried, which produced chitin powder. Depigmentation stage: The stage involved adding 200 g of chitin powder obtained from the deproteinization or demineralization stage to 2,000 ml of acetone (Merck, USA) 1:10 (b/v) for 20 h. Afterward, the solution was filtered and cleaned with aqua dest until a neutral-pH powder was obtained. The powder was then roasted in a 75°C oven for 1 h until it dried. Deacetylation stage: The stage entailed placing 100 g of powder obtained from the depigmentation stage into 1,000 ml of NaOH 50% (Merck, USA) 1:10 (b/v). Afterward, the solution was soaked at a temperature of 75°C. The solution was filtered and cleansed with 1,000 ml aqua dest until it became a neutral-pH powder. Finally, the solution was roasted inside a 75°C oven for 1 h until it dried and became chitosan.

The physicochemical properties of chitosan synthesized from *P. monodon* shells using various synthesized methods were characterized to analyze the chitosan's morphology, moisture content, ash content, molecular weight, deacetylation degree, and viscosity. The chitosan's morphological characteristics were analyzed by using a scanning electron microscope (SEM) (Hitachi Type TM-3000, Japan) with a 15-kV voltage to evaluate the morphology illustration by 200 resolution.

To measure the moisture content, gravimetric analysis was carried out. A 0.5-g sample in the porcelain cup was heated inside a 100–105°C oven for 1–2 h. Then, it was refrigerated inside a desiccator for 30 min. Afterward, it was weighed and heated inside the oven and refrigerated inside a desiccator; the process was repeated according to the constant weight. The procedure was replicated three times for each sample group, and then the calculations

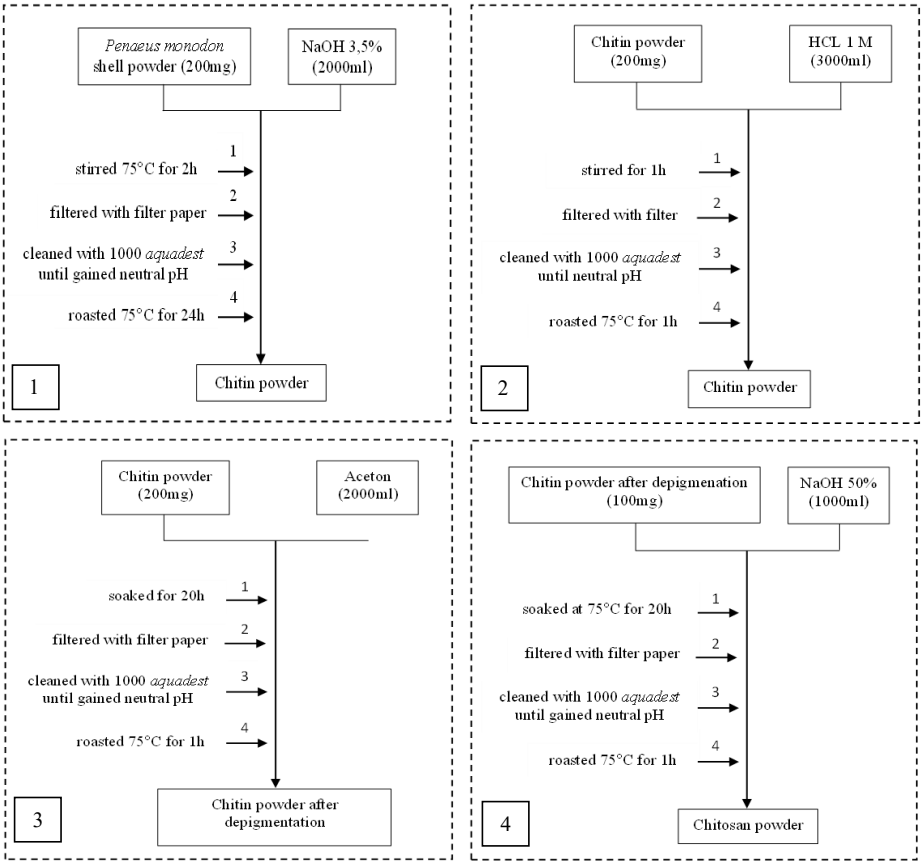

Figure 1. Synthesized stages of the chitosan powder from *P. monodon* shells: deproteinization stage (1), demineralization stage (2), depigmentation stage (3), and deacetylation stage (4).

DOI: 10.20473/j.djmkg.v58.i2.p135–143

were averaged. This moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{[\text{Initial weight (g)} - \text{Dry weight (g)}]}{\text{Initial weight (g)}} \text{ x } 100$$

The ash content was tested with gravimetric analysis. The porcelain cup was weighed, and then 1 g of chitosan was placed inside that cup. Afterward, the chitosan was soaked by adding 1 mL of concentrated H_2SO_4 and slowly heated until it became very burnt. When it finally cooled, 1 mL of H_2SO_4 was added to the cup. It was heated slowly until it reached 600 ± 50°C and then retained at a similar temperature until the residual became burnt. Finally, the cup was refrigerated inside the desiccator and weighed. The heating and cooling process was repeated until a constant weight resulted. The ash content was calculated as follows:

$$\text{Ash content(\%)} = \frac{(W_2 - W_1)}{W} \text{ X } 100$$

W_1 is the initial weight tested (g), W_2 is the ignited crucible weight (g), and W_3 is the residue ignited with the crucible (g). The process was replicated three times for each sample, and then the calculations were averaged at the end.

The Ostwald viscosity method was used to measure the molecular weight. The initial chitosan powder was weighed, placed in a centrifuge tube, and dissolved in 50 ml of 1% acetate acid. The sample was then placed in an Ostwald viscometer. Afterward, it was smoked until the upper line flowed into the lower line. The flow time was calculated. Finally, the result was connected to the weight molecule by Mark and Houwink equality as follows:

$$[\eta]SC = \frac{[2(\eta sp - \ln \eta r)]^{0.5}}{C}$$

[Η]SC is the intrinsic viscosity score, Ηsp is the specific viscosity score (Ηsp = Ηr – 1), Ηr is the relative viscosity, ln is the natural log, and C is the solvent.

$$[\eta] = k\,[M_v]^{\propto}$$

M_v is the chitosan molecule's average weight viscosity, ∝ and k are constants (∝ = 0.83 and k = 1.4 x 10^{-4} acetate acid solution, and [Η] is the intrinsic viscosity.

Infrared spectrophotometry was used to determine the chitosan's functional groups and DD. The method used in this study was the Fourier transform infrared (FTIR) spectroscopy instrument (Perkin Elmer, UK) method. The DD was calculated by using Baxter's formula equation as follows:

$$DD\ (\%) = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times \left(\frac{100}{1.33}\right)\right]$$

A_{1655} is the absorption degree at 1,655 cm^{-1} of the amide I band, which measures group N-acetyl content. A_{3450} is the absorption degree at 3,450 cm^{-1}, which measures the hydroxyl band. Factor 1.33 is ratio A_{1655} dan A_{3450} for fully N-acetylated chitosan.

The chitosan-gelatin suspensions were prepared by mixing the two solutions with a gelatin with an Na-CMC ratio of 3:1. The gelatin solution that had been made with a concentration of 5% was placed in a 225-ml beaker and mixed with 75 ml of 2% Na-CMC solution. The solution was stirred until it was homogeneous. A chitosan-gelatin suspension for each group was prepared by mixing chitosan and gelatin with a ratio of 45:55. The preparation of gelatin and chitosan suspension (ratio 45:55) was performed by mixing 5% (w/v) gelatin solution that had been mixed previously with 300 ml of 2% (w/v) Na-CMC solution with 1% (w/v) chitosan solution. As much as 270 ml was added to the beaker and stirred continuously for 6 h at 40°C.

The viscosity of the chitosan powder and chitosan-gelatin suspension was tested using a Brookfield viscometer. The measurements for each group were carried out six times. Each sample was immediately placed in a 300-ml glass beaker and heated to room temperature (25°C). Then, the sample was measured for viscosity with rotor number 1. The results displayed as numbers indicated the viscosity level of the chitosan suspension in cps units as a function of temperature.[6]

Spearman's correlation coefficients between the molecular weight and the viscosity of the chitosan-gelatin suspension and between the DD and the viscosity of the chitosan-gelatin suspension were analyzed using the Statistical Package for Social Science (SPSS) software (Version 26.0; SPSS Japan Inc., Tokyo, Japan).

RESULTS

The morphology of the *P. monodon* shells and the chitosan isolation method's effect on the sample morphology were observed using the SEM, as shown in Figure 2. The *P. monodon* shell powder exhibited an irregular and rough morphology. The morphology of the *P. monodon* shell powder was almost similar to that of the isolated chitosan powder in Group 1. Group 2 showed a more delicate material surface presented by a well-defined round shape. Group 3 showed a more delicate and fibrous surface morphology.

The percentage yield of chitosan was determined by using the ratio of the obtained chitosan (dry/wet) after the various synthesis methods and the starting material of *P. monodon*. Table 1 shows that the percentage yield of chitosan in Group 1 was 10.5%. Group 3 showed the lowest percentage yield of chitosan at 8.2%, while Group 2 showed the highest percentage yield of chitosan at 20%.

The moisture content, ash content, and viscosity of the chitosan synthesized from *P. monodon* using various methods are shown in Table 1. In this study, the synthesized chitosan from *P. monodon* of Group 1 showed the lowest moisture content of 6.53%, but this group also had the highest ash content of 27.83%. Group 2 showed the highest moisture content of 10.63%, as well as a 1.2% ash content. The moisture content of Group 3 was 8.73%. Furthermore, Group 3 showed the lowest ash content at 1.06%. The viscosity of Group 3 was 5.07 dPa.s. Meanwhile, Group 2 showed the highest viscosity at 5.53%, and Group 1 showed the lowest viscosity at 4.6%.

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Table 1 also illustrates that the chitosan synthesized from *P. monodon* using various methods produced different molecular weights and DDs. Group 2 showed the highest molecular weight of 159.68 kDa. The DD of Group 2 was 87.87%. The lowest molecular weight was from Group 3, which was 37.12 kDa; this group's DD was 86.22%. The highest DD was observed in Group 1, which is 93.72%. The molecular weight of Group 1 was 65.98 kDa.

FTIR analysis was employed to identify the functional groups of the chitosan derived from *P. monodon* shells through various synthesis methods. In Figure 3, the infrared spectrum of the chitosan reveals distinctive features. The determination of the DD of the chitosan involved a robust band appearing at 3,444.7 cm^{-1} (Group 1); 3,365 cm^{-1} (Group 2); and 3,437.7 cm^{-1} (Group 3), affirming the presence of NH and OH stretching, along with intramolecular hydrogen bonds. Additionally, the existence of acetylated residues of amide I (NHCOCH3) was confirmed in the bands around 1,655.8 cm^{-1} for all groups. Notably, in proximity to this band, several peaks emerged, attributable to the N-H bending of amide II bands and indicating C–H bending vibrations of −CH2. These findings align with the results reported in previous studies.[18,20,21]

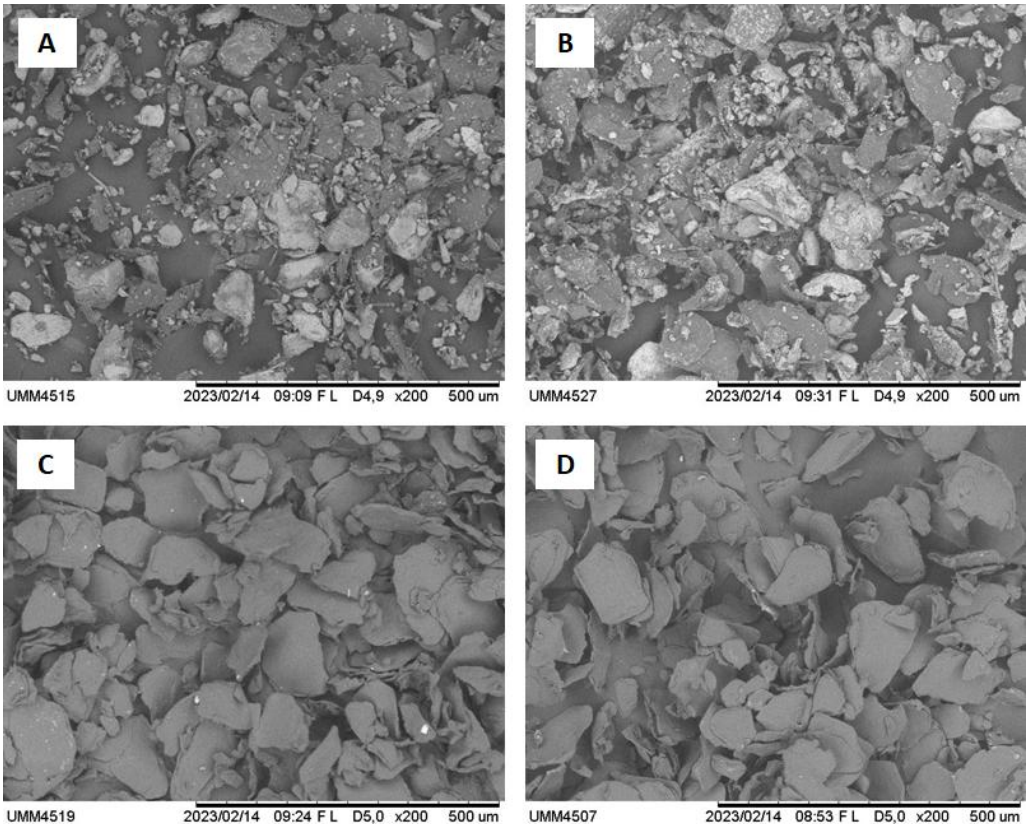

Figure 2. Morphology of the *P. monodon* shell powder (A) and the chitosan powders of Group 1 (B), Group 2 (C), and Group 3 (D), as seen through a scanning electron microscope with 200 magnification.

Table 1. Yield and physicochemical characteristics of chitosan from *P. monodon* shells

	Group 1	Group 2	Group 3
Yield (%)			
Chitosan powder from *P. monodon* shells (g)	100	100	100
Yield of chitosan (%)	10.50	8.20	20.00
Physicochemical properties (%)			
Moisture content (%)	6.53	10.63	8.73
Ash content (%)	27.83	1.20	1.06
Viscosity (dPa.s)	5.07	5.53	4.60
Molecular weight (kDa)	65.98	159.68	37.12
Deacetylation degree (%)	93.72	87.87	86.22

Table 2. Viscosity of chitosan-gelatin suspensions

	Group 1	Group 2	Group 3
Viscosity of chitosan-gelatin suspension (cps)	27.30	40.20	37.25

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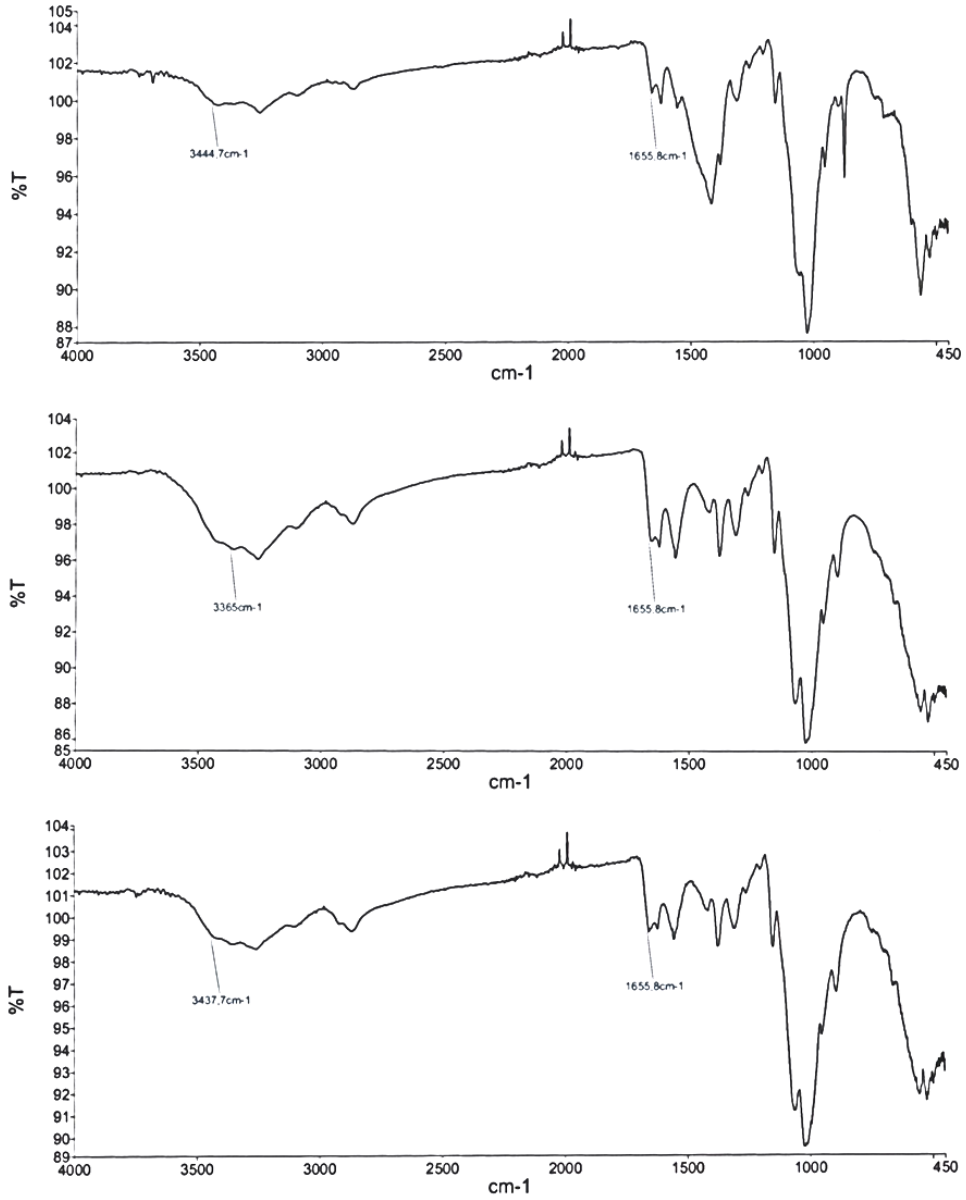

Figure 3. Fourier transform infrared spectra of the chitosan powders of Group 1, Group 2, and Group 3.

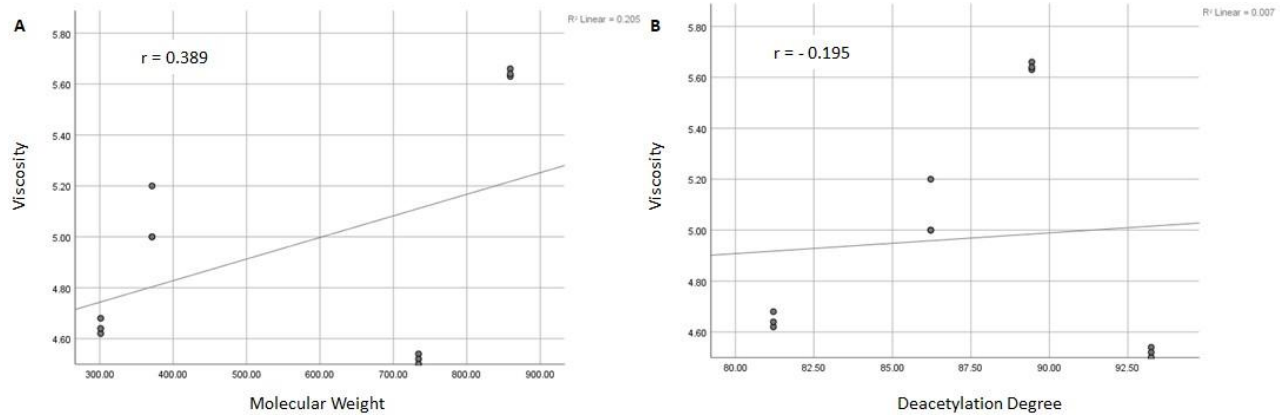

Figure 4. Results from Spearman's correlation tests between viscosity and molecular weight (A) and between viscosity and deacetylation degree (B).

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Table 2 shows the viscosity of the chitosan-gelatin suspension. The lowest viscosity of 27.30 cps was from the chitosan (Group 1)-gelatin suspension. The highest viscosity of 40.20 cps was seen in the chitosan (Group 2)-gelatin suspension. The viscosity of the chitosan (Group 3)-gelatin suspension was 37.25 cps. Statistical analysis showed significant differences between the groups ($p = 0.00$). Spearman's correlation test was carried out to determine the effect of the DD and molecular weight of the chitosan on the viscosity of the chitosan-gelatin suspension. Figure 4 shows the results from Spearman's correlation test. The correlation coefficient between the molecular weight and the viscosity of chitosan-gelatin suspension was 0.389, which has a moderate correlation level and is positive. Meanwhile, the correlation coefficient between the DD and the viscosity of the chitosan-gelatin suspension was −0.195, which has a weak correlation level and is negative.

DISCUSSION

The morphology of the material and the effect of various methods on synthesized chitosan powder from *P. monodon* was observed using an SEM. The difference in the surface materials can be seen in Figure 1. This study proved that the demineralization stage of the method affected the form and the chitosan material's surface. The *P. monodon* shell powder as the starting material and the Group 1 powder that was synthesized without the demineralization stage showed similar morphology, such as the irregular form and rough surface. By contrast, Groups 2 and 3, which were synthesized with the demineralization stage, exhibited more delicate surface morphology. As one of the stages of synthesizing chitosan powder, demineralization aims to remove mineral content, especially $CaCO_3$ and $Ca_3(PO)_4$ contained in the shrimp's shell, using an acid treatment.[21] Deproteinization disrupted the chemical bonds between the chitin and the proteins, demineralization removed all the minerals, and deacetylation removed the acetyl groups.[22] The demineralization process had a significant effect on the surface morphology and size of the chitosan powder. Group 3, which used the four-step method, presented a smooth surface with a fibrous structure and was smaller than the other groups. These results indicated that the synthesis method of the chitosan caused changes in the shape and surface morphology.

During storage, commercial chitosan may be affected by moisture absorption due to its characteristic as a hygroscopic material. This study showed that chitosan's moisture content is affected by the synthesis process, specifically the deproteinization step. This was proven by Group 2, in which a three-step method without deproteinization was used; it showed the highest moisture content compared with Groups 1 and 3, which included the deproteinization step. A similar study also proved that the moisture content of chitin from shrimp shells decreased after deproteinization compared to before deproteinization.[23] Commercial chitosan should contain less than 10% moisture.[24]

The demineralization step affects the ash content of chitosan powder. The ash content percentage of chitosan describes the effectiveness of the demineralization process to eliminate minerals. The ash content should not exceed 1% to be declared as good-quality chitosan.[24] The ash content of chitosan is an important indicator in determining chitosan purity. The more ash content is removed, the higher the degree of purity of the chitosan. This is because chitosan ash content is calculated from the residue inorganic weight to chitosan weight ratio. Group 1, which used the three-step method without the demineralization process, had the highest ash content because this method had no demineralization stage. Groups 2 and 3, which used the demineralization process, had lower ash content than Group 1 at 1.2% and 1.06%, respectively.

The molecular weight of chitosan is also an important parameter because it affects the physiochemical characteristics and chitosan biology, such as hydrophilicity, viscosity, moisture absorption, biodegradability, antimicrobial activity, and adhesion. These results showed that the deproteinization and/or demineralization steps influence chitosan's molecular weight. The highest molecular weight of chitosan was presented by Group 2, which used the three-step method without deproteinization, which disrupts the chemical bonds between chitin and proteins. Furthermore, chitosan's molecular weight depends on the number of monomeric units in the biopolymer. When the biopolymer only contains monomeric forms of 2-amino-2-deoxy-D-glucopyranose, the DD is 100%. The DD is the relation between units of 2-acetamido-2-deoxy-Dglucopyranose.[25] This study proved that Group 1, which used the three-step method, and Group 3, which used the four-step method, had lower molecular weight yet higher DD. The properties and uses of chitosan are influenced by the DD and molecular weight. The repeated use of NaOH in the deproteination and deacetylation processes is more effective in breaking off more acetyl groups so that the DD will be lower, as it produces longer polymer chains and greater molecular weight. The isolation process of the deproteinization, depigmentation, and deacetylation methods only uses NaOH at the deacetylation stage; thus, it tends to cut the chitin's main chain and produces chitosan with a short polymer chain. The use of NaOH alone in the deacetylation stage will result in the release of a few acetyl groups and a low molecular weight so that the DD will be higher.[26]

Chitosan is a product of chitin deacetylation, which is a long-chain polymer of glucosamine (β-1,4-2 amino-2-deoxy-D-Glucose). FTIR was used to identify the functional group present in the chitosan. The DD was determined by two absorption bands, a characteristic band that represents the acetylated residues of amide I that were seen for the three groups. It was also confirmed that carboxymethylation took place only at both amino groups and hydroxyl groups with a DD from chitosan of about >86% for the three

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groups. However, the intensities differed due to the varied reactions of the acid treatment (demineralization stage), except in Group 1. Some bands were weakened after deproteination and demineralization.[20]

The viscosity value of chitosan is directly proportional to its molecular weight. The greater the viscosity value, the greater the chitosan's molecular weight. In this research, chitosan powder produced using the deproteinization, demineralization, depigmentation, and deacetylation process (Group 3) had the lowest molecular weight and lowest viscosity value of 4.6 cps. Meanwhile, chitosan powder produced using the demineralization, depigmentation, and deacetylation processes only (Group 2) had the highest molecular weight and highest viscosity value of 5.53 cps. This means that Group 2's chitosan polymer molecular chain was longer than that of Group 3. When chitosan is added to a solvent, the solvent gradually diffuses into polymer aggregates, resulting in the swelling of the polymer, and all chain segments of the polymer molecule in solution are unfolded and fully solvated.[27]

This study also showed the correlation between the molecular weight and the viscosity of a chitosan-gelatin suspension using Spearman's correlation test. The positive correlation value indicated a positive relationship, which means a relationship in the same direction in which the greater the molecular weight, the greater the viscosity. A higher molecular weight of chitosan may increase the viscosity of a chitosan-gelatin suspension. However, Spearman's correlation test in this study showed a moderate level of correlation between the molecular weight of the chitosan powder and the viscosity of the chitosan-gelatin suspension. This is possible due to the intermolecular interactions between chitosan and gelatin. Gelatin molecules bind to chitosan macromolecules through electrostatic interactions and hydrogen bonds; it is thought that a single chitosan macromolecule binds to 3–4 gelatin macromolecules. Chitosan is a lyophilized component, and gelatin is a blocking polyelectrolyte. The electrostatic interactions between the charged amino groups in chitosan and carboxyl groups of gelatins contribute to the stabilization of chitosan-gelatin complexes.[28,29] A similar study reported that the viscosity of a chitosan solution is influenced by its molecular weight, but electrolytes also affect the viscosity. The viscosity is sharply reduced in the presence of sodium acetate with a concentration of about 2 g/dl.[27]

The result from Spearman's correlation test between the DD and the viscosity of the chitosan-gelatin suspension was −0.195. As this level of correlation is weak and negative, the relationship can be said to be opposite. That is, as the DD increases, the viscosity decreases, and vice versa. This is possible because the three chitosan synthesis methods in this research can produce chitosan powder with a high DD in the range of 86.22–93.72%. Therefore, even though there appears to be a tendency for a correlation between DD and the viscosity of a chitosan-gelatin suspension, the level of correlation is weak. A higher DD of chitosan may lower the viscosity of the chitosan-gelatin suspension. Increasing the value of the DD results in the stiffness of the chitosan chain in solution decreasing and the chitosan's solubility increasing. A high DD causes the cationic charge of the chitosan to also increase. Increasing the cationic charge in the solution will produce a repulsive force, which will make the chitosan polymer, which was previously coiled, open into straight chains, and the viscosity of the solution will decrease.[30]

With regard to the results of this study, the chitosan synthesized from *P. monodon* with four stages—deproteinization, demineralization, depigmentation, and deacetylation—was the most suitable to be used as an IBS material for socket preservation based on the yield and physicochemical characteristics of the chitosan powder and the viscosity test of the chitosan-gelatin suspension. The viscosity of the chitosan-gelatin suspension is influenced by the chitosan powder's molecular weight and DD. While it can be concluded that a chitosan-gelatin suspension can be applied to socket preservation treatment, further study regarding the appropriate setting time of a chitosan-gelatin suspension is suggested.

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