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Article

Study of the optimum conditions to prepare chitosan from Iraqi shrimp wastes and some of it is physicochemical and functional properties

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ABSTRACT

The study included the preparation of chitin from the wastes of *Penaeus semisulcatus* shrimp, which represented the headshells and the shells of the meaty part of shrimp, and the study of its chemical composition, such as moisture, ash and nitrogen content, which reached to 3.2,2.5,4.66% respectively, while the yield was 34.39% based on the dry weight of the shells powder. Two chitosan symbols, A and B, were prepared from chitin by chemical method and under different conditions. Chitosan A was prepared by alkali treatment by removing acetyl groups at the temperature of 100°C for 3h, while chitosan B for 9h. The percentage of the yield of Chitosan A was 25.56% and Chitosan B 20.15% based on the dry weight of the shell powder. Then, the chemical composition of chitosan A, such as moisture, ash and nitrogen content, reached 4.3,0.66,2.1%, respectively, while the moisture, ash and nitrogen content of chitosan B were 4,0.33,2.8%, respectively. The physicochemical and functional properties of chitosan A and B were studied, and the degree of deacetylation using the FTIR technique for chitosan A, B, and commercial chitosan was 49.9,81.8,70.2%, respectively. Chitosan A gave high viscosity 480cP while chitosan B had low viscosity 380cP. The molecular weights of chitosan A and B were 678.715kDa and 527.732kDa, respectively. Chitosan B showed the highest solubility in 1% glacial acetic acid solution, which was 100%, and it was lower for Chitosan A, which was 88.6%. Chitosan B showed a high water binding capacity of 446.5%, while it was lower for Chitosan A, reaching 388.8%. Chitosan A gave a high-fat binding capacity of 397.6%, while it was lower for Chitosan B, which reached 385.2%. SEM scanning electron microscopy was used to determine the morphology of chitosan A, B and commercial chitosan.

Keywords: Chitin, Chitosan, Degree of deacetylation, Shrimp shells, Shrimp wastes, Penaeus semisulcatus

INTRODUCTION

Brackets, bands, archwires and auxiliaries (elastic ligatures, elastic chains, etc.) are the main components of fixed orthodontic appliances that make them under the effect of biodegradation and ions release when placed inside the mouth of the patients, the most changeable parts in the fixed orthodontic appliance are archwires, as the orthodontists choose the required gauge and type in accordance to the stage of orthodontic treatment. Therefore, Nickel-Titanium and heat-activated archwires are utilized during the leveling and alignment stage while stainless steel is utilized during working stages [1–2].

The metallic and silvery appearances of most orthodontic appliances are unaesthetic; therefore, with the manufacturing of aesthetic brackets (composite and ceramic), the aesthetic problem is partially solved. But the conventional archwires also have an unaesthetic metallic appearance, and in order to overcome this aesthetic problem, several companies manufactured coated metallic and fiber-reinforced archwires [3–4].

Several previous studies found that the coating material is unendurable and could be damaged by the forces of mastication and enzyme activities inside the mouth. Moreover, this coating material might cleave and crack, showing the underlying metallic wire, and its color might change during orthodontic treatment [5–6].

A technique usually used to close spaces called orthodontic sliding mechanic is formed by the motion of orthodontic brackets along the archwire or by archwire sliding through brackets and molar tubes. The frictional resistance that results from the contact between orthodontic brackets and archwires is considered the primary problem that affects sliding mechanics [7]. The amount of frictional resistance is higher when using plastic and ceramic brackets than when using metallic brackets [8]. The frequency of tooth brushing and the type of toothpaste used during brushing may affect the surface characteristics of the materials used in dentistry. These types of toothpaste usually have bleaching agents, fluoride, abrasive systems, certain pigments and other materials that aim to enhance the tooth quality. Different types of toothpaste (specially manufactured for orthodontic treatment) have been introduced to the markets that may have an effect on orthodontic brackets and archwires [9–10]. For these reasons, this study was prepared in order to evaluate and compare the effect of brushing with different kinds of toothpaste on the frictional resistance between aesthetic (ceramic) brackets and aesthetic archwires (Epoxy and Teflon-coated archwires) to find which toothpaste generates the least amount of friction during sliding mechanics in a patient undergoing aesthetic orthodontic treatment.

The null hypothesis in this study is that there are no significant differences in frictional resistance between aesthetic brackets and aesthetic archwires after brushing with different types of toothpaste.

Materials and Methods

Prepare shrimp wastes representing headshells and the meaty part shells

The shrimp *Penaeus semisulcatus* wastes, representing the headshells and the meaty part shells, were collected from Kadhimiya city in Baghdad Governorate and transported in polyethylene bags. The dirt was removed by washing them with tap water repeatedly. Then, the shells were transported in trays and dried in an air-heating oven at a temperature of 60°C until constant weight. Then, the dry shells were ground to a fine powder using a grinder at a speed of 25000 rpm and a power of 650 watts.

Extraction of chitin from shrimp wastes

Deproteinization

The protein was removed from shrimp shells according to the method [of 14] with some modification by changing the concentration of the solution. A solution of sodium hydroxide with a concentration of 3% was added to the shrimp shell powders at a ratio of 1:10 (w:v). The mixture was placed in a beaker at a temperature 65 °C for two hours using a heater with a magnetic stirrer the precipitate washed with deionized and distilled water until the filtrate reached to pH = 7 by vacuum pump and using filter paper then dried the precipitate in air heating oven at a temperature 60 °C for 24 h.

Demineralization

The minerals were removed from the shrimp shells according to method [15] with some modifications by changing the volume of the solution and the reaction time. A solution of hydrochloric acid at a concentration of 1M was added to the deproteinized shells, and a ratio of 1:10 (w:v) and the mixture was placed in a beaker using a magnetic stirrer at room temperature for 20 minutes. Then, the precipitate was washed with deionized and distilled water until the filtrate reached pH = 7 by vacuum pump and using filter paper. Then, the precipitate was dried in an air-heating oven at a temperature of 60 °C for 24 h, and the result was chitin.

Converting chitin to chitosan

Deacetylation

The chitin extracted from shrimp shells was modified into chitosan by two methods, the first by 16, with some modification by changing the volume of the solution. The acetyl groups were removed from chitin using an alkali solution of sodium hydroxide NaOH 50% and a ratio of 1:10 (w:v) at a temperature of 100 °C for 2 h. Then, the precipitate was washed by a vacuum pump several times with deionized and distilled water until the filtrate reached pH = 7 using filter paper. Then, the precipitate was placed in an air-heating oven at a temperature of 60 °C for 24 h, and the result was chitosan A. As for the second method, which was mentioned by [17] with some modification by changing the volume of the solution and the temperature, it was done by removing the acetyl groups from the chitin using alkali sodium hydroxide solution NAOH 50% and ratio 1:20 (w:v). at 100°C for 3 h. Then the precipitate was washed by a vacuum pump

several times with deionized and distilled water until the filtrate reached pH = 7 using filter paper. Then, the precipitate was dried in an air-heating oven at 60 °C for 24 hours, and the process was repeated three times. The result was chitosan B.

Determination of the physicochemical and functional properties of chitosan

Determination of moisture

The moisture of chitosan was determined according to the method mentioned by [18] weighing 1gm of the sample and put it in a crucible, and it was dried in an air-thermal oven at a temperature of 60 °C for 24 h. Then, the crucible was placed in a Desiccator to cool at room temperature and was weighed, and the percentage of moisture was calculated from the following equation:

$$\%\ \text{Moisture} = \frac{\text{weight of sample before drying} - \text{weight of sample after drying}}{\text{sample weight}} \times 100$$

Determination of yield

The percentage of the yield was Determined according to [11] from the following equation:

$$\%\ \text{Yield} = \frac{\text{chitosan dry weight}}{\text{shells powder dry weight}} \times 100$$

Determination of ash

The ash was Determined for chitosan according to the method mentioned by 19, weighed 1gm of the sample, and placed in a crucible heated in an oven to 550 °C until the appearance of the white or light gray color of the sample. Then, the crucible was placed in the desiccator. Cool until it reaches room temperature and weighs, and the percentage of ash was calculated from the following equation:

$$\%\ \text{Ash} = \frac{\text{Weight of ash}}{\text{sample weight}} \times 100$$

Protein determination

The protein was estimated by Determining the total nitrogen in chitosan, and the micro Kjeldahl method was followed[19].

Determination of viscosity

The viscosity of chitosan was determined using a Brookfield viscometer model RVT (Brookfield Engineering Laboratories, Inc., Stoughton, Mass., USA). Chitosan solution was prepared at a concentration of 1% using glacial acetic acid at a concentration of 1% and using spindle No. 5 at a speed of 100 rpm. On chitosan solution at 25 °C[20].

Determination of molecular weight

The molecular weight of chitosan was determined by using the Mark–Houwink equation, as mentioned [21].

$$[\eta] = KMw^{a}$$

Where the value of k and a are constants $K=1.81\times10^{-3}$ cm3/g and a=0.93, [η] = intrinsic viscosity centi-poise (cP), Mw = molecular weight (daltons).

Determination of the binding ability of water and fat

The water binding capacity (WBC) and the fat binding capacity (FBC) of chitosan were determined as mentioned [22]. The centrifuge tube contains 0.5 g of the sample, and 10 ml of water or olive oil is added. Then, the tubes were mixed well with a vortex electric mixer for one minute to disperse the sample. Then, the tubes were left at room temperature for 30 min, with the tubes mixed every 10 min for 5 seconds. Then, the tubes were centrifuged at 3500 rpm for 25 min. After centrifuge, the filtrate was neglected, then the tubes were weighed again, and the WBC and FBC were calculated from the following equations:

$$\%\ \text{water binding capacity} = \frac{\text{water bound (g)}}{\text{sample weight (g)}} \times 100$$

$$\%\ \text{fat binding capacity} = \frac{\text{fat bound (g)}}{\text{sample weight (g)}} \times 100$$

Determination of solubility

The solubility of chitosan was determined, as mentioned, by placing 0.1 gm of the sample in a centrifuge tube of known weight and adding 10 ml of 1% glacial acetic acid solution to it. Then, the tubes were placed in a shaker incubator at a speed of 240 rpm for 30 min and at a temperature of 25°C. The tubes were placed in a water bath at boiling temperature for 10 min then cooled to 25°C. Then, centrifuged at 10,000 rpm, the supernatants were discarded, and the undissolved particles were washed in distilled water 25ml and centrifuged again at the same speed. After that, the supernatants were discarded, and the insoluble pellets were dried at 60°C until the weight constant and the solubility percentage were calculated from the following equation:

$$\%\ \text{solubility} = \frac{(\text{initial weight of tube + chitosan}) - (\text{Final weight of tube + chitosan})\,g}{(\text{initial weight of tube + chitosan}) - (\text{weight of empty tube})\,g} \times 100$$

Fourier Transform Infrared Spectroscopy FTIR

Chitosan was diagnosed using the FTIR technique as mentioned[12] by diagnosing functional groups. The made disk of the sample was mixed well with the chitosan powder with potassium bromide KBR in the ratio of 5:1(w:w). It was pressed using a hydraulic press. Then, 50mg of the mixture was taken and placed in a special disk of the FTIR device. The

disk was placed in the FTIR device for analysis with a frequency ranging between 400 - 4000 cm^{-1}.

Determination of the degree of deacetylation

Absorbance values of the FTIR spectroscopy determined the value of the DD. The absorbance value for wavelength 1655 (A1655) was dependent, and it represents the absorbance value of the amide band as a measure of the content N-acetyl groups. The absorbance value of the wavelength 3450 (A3450) represents the absorbance value of the hydroxyl band as an internal measure for these group hydroxyl groups that are not affected by the chemical reactions on chitin upon extraction and modification to chitosan. Then, the DD was calculated according to the following equation mentioned by[23].

$$\%DD = 100 - [\ A_{1655}/A_{3450}\ ] \times 100/1.33$$

Scanning Electron Microscopy

The morphology of chitosan prepared from chitin extracted from shrimp waste was examined by scanning electron microscopy SEM. Images photographed at 100000x magnification and using a voltage of 5kV, as mentioned [24].

RESULTS

Chemical composition of chitin

Table 1 shows the yield of chitin extracted from shrimp wastes for this study was 34.39%, while the moisture percentage was 3.2%, and the result was identical with 27. The moisture ranged from 1.2-8.4% with different treatments. As for the Nitrogen content, it was found the nitrogen content was 4.66%. In the same role, the percentage of ash was 2.5%, and the result was identical with 30. The result was 2.25% with different treatments, while the percentage of ash in the fresh shells was 24.5%.

Chemical composition%			
Yield	**Moisture**	**Nitrogen**	**Ash**
34.39	**3.2**	**4.66**	**2.5**

Table 1. Chemical composition of chitin

Chemical composition of chitosan

Table 2 shows two types of chitosan, A and B. The yield percentage was 25.56% and 20.15%, respectively, based on the dry weight of the shell powder. The same table shows the moisture percentage for two types of chitosan, A and B, reached 4.3% and 4%, respectively. Similarly, the nitrogen content for two types of chitosan for this study, A and B, were 2.1% and 2.8%, respectively, and the results agreed with the results of [38] the nitrogen content for two types of chitosan A and B for their study 1.9% and 2.9%. Table 2 shows the ash for chitosan A and B were 0.66% and 0.33% respectively. [39] Explained the ash is an indicator of an effective demineralization process, which affects the solubility of chitosan and contributes to a decrease in its viscosity. Also, it may affect the features of the produced chitosan.

chitosan	**chemical composition%**			
	Yield	Moisture	Nitrogen	**Ash**
A	25.56	4.3	2.1	**0.66**
B	20.15	4	2.8	**0.33**

Table 2. Chemical composition of chitosan

Physicochemical and functional properties of chitosan Fourier transform infrared spectroscopy

Table 3 shows the wave numbers of the functional groups of commercial chitosan, chitosan A and chitosan B prepared from shrimp waste using infrared technology, which is one of the most easy and fast techniques used in detecting the functional groups of chemical polymers. It does not need many additional materials or special solvents except for the integration with potassium bromide to make the diagnosis and wide range of wave numbers for chitosan was recorded with a range between 400-4000cm^{-1} and hydroxyl groups (OH) for chitosan A, B and commercial appeared on wave numbers (3444,3444,3560 cm- 1) respectively, and as shown in (figure 1,2 and 3) and it is one of the important functional group that is not affected by the conditions of the reactions when extracting chitin from shrimp shells and transforming it into chitosan. And considered as an internal measure to determine the degree of deacetylation. While the free amine (NH_2) groups of A, B and commercial chitosan located on C-2 of the polymer molecule are considered one of the most important functional groups for detecting the properties of chitosan and indicate the success or achievement of the deacetylation process and its absorbance appeared in the wave numbers (3385,3377,3423cm^{-1}) respectively. The chemical (CH_2) groups of A, B and commercial chitosan belonged to the primary alcohol group (CH_2OH) appeared on wave numbers (2879,2877,2883cm^{-1}) respectively. The wave numbers (1645,1656cm^{-1}) were for A and commercial chitosan, respectively, and belong to the amide carbonyl group (C=O) whose absorbance value for

the wave number range (1640-1700cm^{-1}) depends on the conditions of deacetylation process, were the more harsh treatment conditions, the more it was not visible on this wave number due to the decrease in its concentration in the polymer and vice versa. The degree of deacetylation for chitosan B reached 81.8%. It was superior to chitosan A, while chitosan B was not recorded by the infrared spectrum absorbance of the carbonyl group, as shown in (figure 2). The reason is due to the harsh conditions of the deacetylation process, which was repeated three times on chitin to remove many acetyl groups than chitin and obtaining chitosan with unique properties distinct on chitosan A. chemical amides (NH) groups appeared that carrying acetyl group (C=O-CH_3) on the wave numbers (1591,1597,1560cm^{-1}) for chitosan A, B and commercial respectively. The absorbance of the methyl chemical group (CH_3) was represented in acetyl group on the wave numbers (1377, 1379, 1379cm^{-1}) for chitosan A, B and commercial, respectively.

Functional group	**Wave namber (cm^{-1})**		
	chitosan A	chitosan B	**commercial chitosan**
OH	3444	3444	**3560**
NH_2	3385	3377	**3423**
CH_2	2879	2877	**2883**
C=O in $NHCOCH_3$	1645	-	**1656**
NH amide band	1591	1597	**1560**
CH3 in $NHCOCH_3$	1377	1379	**1379**

Table 3. Functional groups of chitosan.

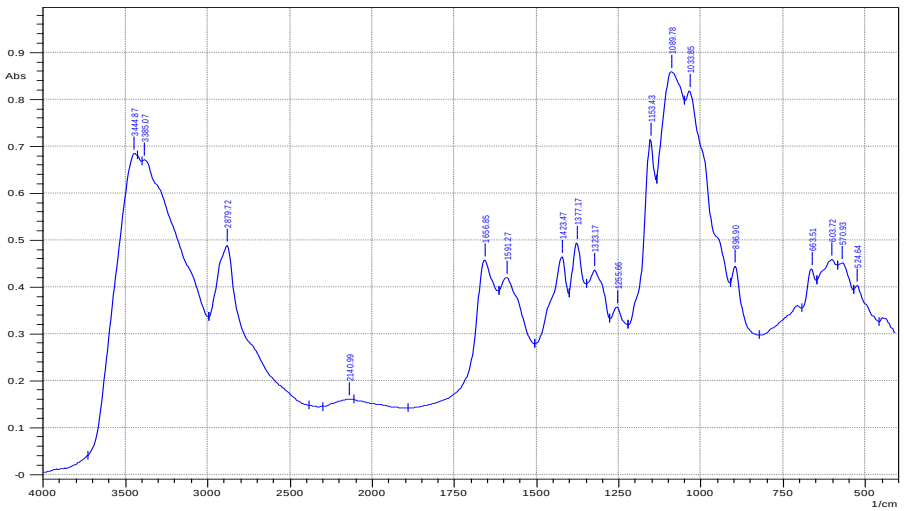

Figure 1. Fourier Transform Infrared spectroscopy (FTIIR) of extracted chitosan A absorbance

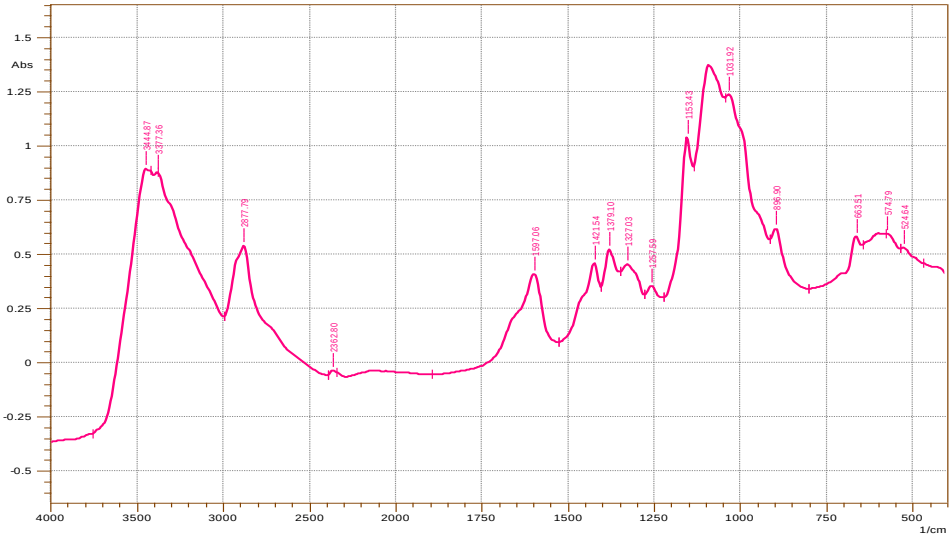

Figure 2. Fourier transform infrared spectroscopy (FTIIR) of extracted chitosan B absorbance

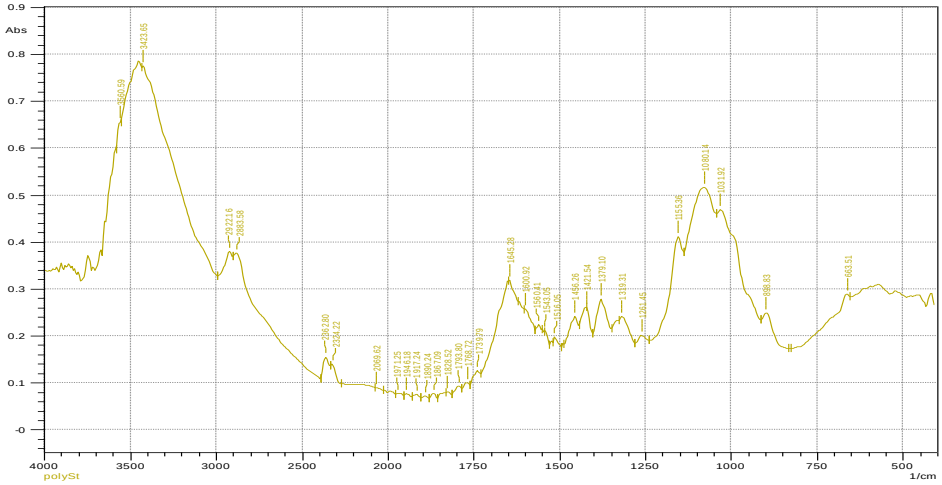

Figure 3. Fourier transform infrared spectroscopy (FTIIR) of commercial chitosan absorbance

Scanning Electron Microscopy

The microscopic images in (figure 4) of chitosan A, (figure 5) of chitosan B and (figure 6) of commercial chitosan show the morphology of chitosan molecules using a scanning electron microscope.

The microscopic image of chitosan A shows the non-homogeneous and nonsmooth surface of chitosan molecules, and its surfaces appear in granules form with different sizes and irregular shapes. The microscopic image of chitosan B showed the external appearance of the molecule surface was smooth compared to chitosan A and commercial chitosan, and it contains pores with different sizes spread throughout most parts of chitosan.

The microscopic image of commercial chitosan showed the surface of chitosan molecules was rough and contained several layers of flakes in some parts of chitosan irregular in shape, and did not contain pores.

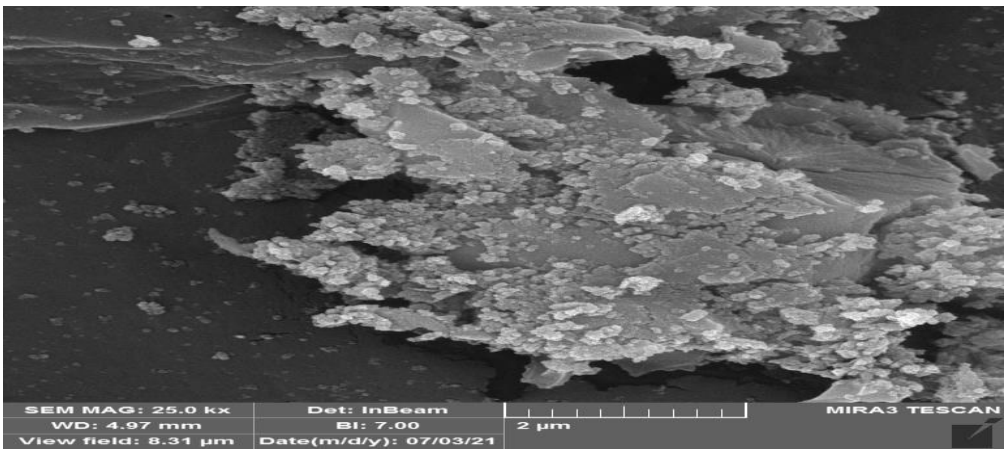

Figure 4. Morphology of Chitosan A prepared from shrimp shells under scanning electron microscope.

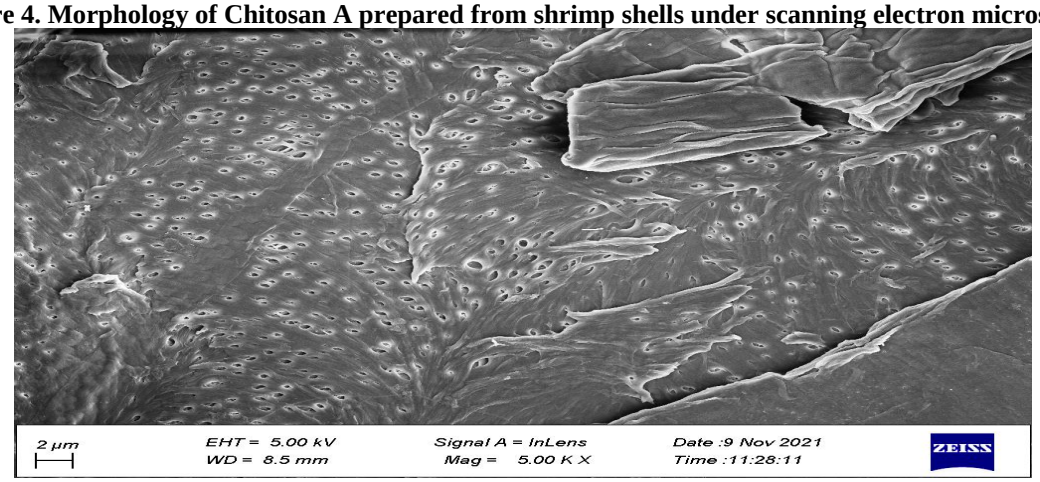

Figure 5. Morphology of Chitosan B prepared from shrimp shells under scanning electron microscope.

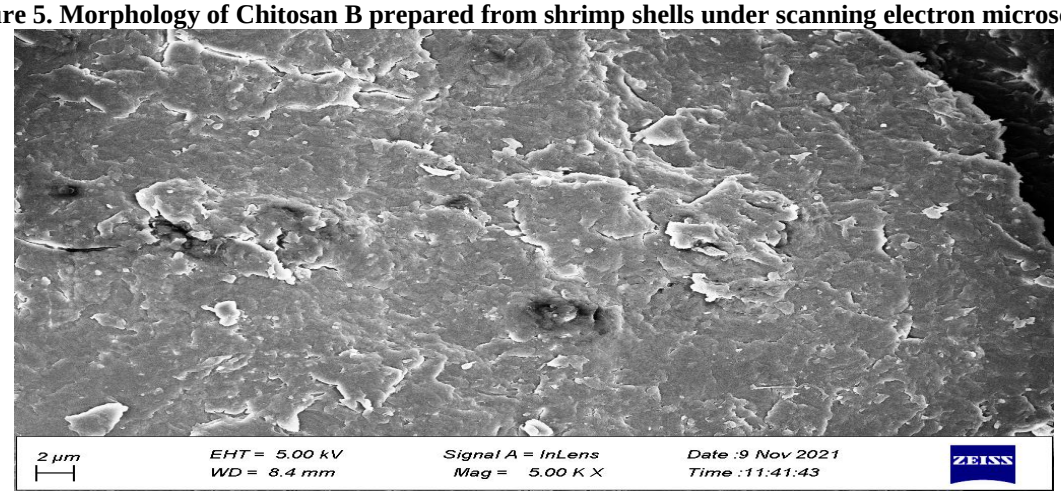

Figure 6. Morphology of commercial Chitosan under scanning electron microscope.

Degrees of deacetylation

(Table 3) show DD for chitosan A and B for this study, and commercial chitosan reached 49.9%, 81.8% and 70.2%, respectively. The result of chitosan A was identical to that of [45] the DD, which was 50.6%. The result of the chitosan B agreed with the result of the percentage of 81.7% with different treatments.

Viscosity

(Table 3) show two types of chitosan for this study, A and B. The viscosity ratios were 480cps and 380cps, respectively, and the results were identical to the results of 42. The viscosity ratio ranged from 106-6370cps with different chemical treatments. The results also agree with 50. The ratio ranged 72-1928cps.

Molecular weight

(Table 3) show two types of chitosan for this study. A and B molecular weights were 678.715 kDa and 527.732 kDa, respectively. The results were identical to what was mentioned.[51] *chitosan samples give different DD with a range between 75-95% and have a molecular weight between 50-2000 kDa.*

Solubility

(Table 3) show two types of chitosan for this study: A and B, the solubility was 88.6% and 100%, respectively. [42,34] Clarify the amount of insoluble pellets of chitosan in acetic acid solution is due to the insufficient removal of acetyl groups from it as well as the incomplete removal of protein from chitin. The result of chitosan A is identical to the result of 54. The solubility ratio was 85.4%, and with different treatments, 60% NaOH was used in deacetylation.

chitosan	**physicochemical properties**			
	Degree of deacetylation	viscosity	Molecular weight	solubility
A	49.9%	480cps	678.715kda	88.6%
B	81.8%	380cps	527.732kda	100%
commercial	**70.2%**	-	-	-

Table 4. Physicochemical properties of chitosan

Water binding ability

(Table 4) show two types of chitosan for this study. A and B water binding abilities were 388.8% and 446.5% respectively, and the results were identical to the results of 20. The water binding ability ranged from 355-611%, and the researchers explained in their study the possible explanations for the variation in the water binding ability are due to the degree of crystallization of chitosan, the difference in the number of residual minerals and protein content.

Fat binding ability

(Table 4) show two types of chitosan for this study A and B fat binding ability were 397.6% and 385.2%, respectively,

chitosan	functional properties	
	water binding capacity%	Fat binding capacity (olive oil)
A	388.8	397.6
B	446.5	385.2

Table 5. Functional properties of chitosan

DISCUSSION

The chemical composition of chitin for the yield result was agreed with [25], where the percentage was 34.4% based on the dry weight of the shells. [26]Clarify causes the difference in yield due to the reaction time, which is directly proportional to the value of the yield and the difference in the type of shrimp used, as well as the variation in the method of preparation. Furthermore, the result was identical with 2, as the moisture ranged from 1.2-8.4% with different treatments. the nitrogen rate was agree with28, where the percentage reached to 4.16%. [29]Clarify protein is naturally linked with chitin in shrimp shells and forms with it chitin protein. Simple methods can remove some of this protein, but the other part cannot be removed because it binds with chitin through strong covalent bonds.

The above results show an evident decrease in the proportions of ash and protein. The reason is due to the efficiency of processes of deproteinization and demineralization on the shrimp shells for this study. The long demineralization process using HCL for more than 24 h leads to the breakdown of the polymer chain. It affects the purity of the resulting chitosan because inorganic materials and calcium carbonate from the chitin in the form of calcium chloride and large foam were formed due to CO_2 release[31].

$$2HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + Co_2\uparrow$$

The process of demineralization has a significant impact on the functional and physicochemical properties of chitosan. The chitosan prepared from chitin without the demineralization process is low in solubility, ability to bind water and fat, and DD. It is also high in molecular weight, viscosity and ash[32].

The variation in the value of the yield is attributed to the difference in the method of preparation. The alkali treatment using a high concentration of sodium hydroxide solution in which chitin is exposed during the preparation of chitosan as well as the temperature and the long time of the treatment which have a significant impact on the amount of yield and lead to the dissolution of the polymer chains when the acetyl groups of chitin are removed and this leads to decrease in mass or weight the resulting chitosan also causes decrease in molecular weight[33]. The result for chitosan B agreed with the yield ranged from 16.4-19.6% with different treatments for several types of chitosan. The result for chitosan A was identical to the result of the percentage of 22% with different treatments.

[36]Explained that chitosan has a naturally high ability to absorb moisture from the atmosphere. It's hygroscopic. It also explained the difference in moisture content is

due to the percentage of DD. The lower the DD, the higher the moisture content of the chitosan, which agrees with A and B chitosan for this study. The results were also identical. With results of 37, the moisture ranged from 4-4.6%.

Researchers showed the nitrogen content in the produced chitosan depends on the alkali treatment and the duration of the deacetylation process. The nitrogen content increased with the length time of treatment, and this was identical to the results for this study and the proportion of type B increased, while the percentage decreased for type A.

[40]Clarify that the high percentage of ash in chitosan is due to the presence of calcium carbonate in shrimp shells with many quantities. The result of chitosan B agreed with the result of 41. The ash percentage of chitosan prepared from shrimp shells was 0.3% with different treatments. The researchers explain high quality chitosan should have less than 1% ash content. In addition, abandoning the necessary demineralization process will lead to the production of chitosan, which has a high ash content ranging from 31-36%. The result of chitosan A was identical to the result of 42. The ash of chitosan prepared from shrimp shells of black tiger (*Penaeus* monodon) was 0.68% with different treatments.

All the above results for the Functional groups were identical [to those of 43] when studying two types of chitosan prepared from shrimp shells and crab shells. Also, the results were identical with [44] when they studied the preparation of chitosan and loaded it with drugs. The results also agreed with the results of[11] when they studied the efficiency of the heavy metal removal process using chitosan prepared from the wastes of shrimp shells.

The researcher proved the method used to prepare chitosan affects the DD for the produced chitosan. Generally, the high DD control through alkali treatment by using NAOH and increasing treatment time and temperature. [47]Proved repeated deacetylation process with increased reaction time and temperature leads to a decrease in the molecular weight of the resulting chitosan and also leads to a decrease in the protein and ash content, and this is identical to chitosan B for this study. And[48] showed the high DD represents the purity of chitosan. The result of commercial chitosan was agreed with 49, indicating the ratio ranged from 70-90%. And[11] explained that DD is one of the important factors that affect the properties of the resulting chitosan, such as solubility, chemical reaction, and biodegradability. The value of the DD depends on several factors, such as the source of chitosan, the procedures used to prepare chitosan, and the type of methods used.

[21]Announced viscosity of chitosan decreases with the increase of the hydrolysis time for chitin extraction treatments (deproteinization and demineralization) and treatment of deacetylation, as well as result to high temperature of deacetylation process. The viscosity ratio of chitosan varies with its source, preparation method, and residual aggregates in the chitosan solution. [33]Explained that the intrinsic viscosity of chitosan is closely related to the DD and temperature as well. As the researcher explained in his study, the increase in viscosity is due to the high content of acetyl groups in chitosan. [34] Explained that low-viscosity chitosan has several advantages over high-viscosity chitosan for use in the foods and drugs industry.

The result of chitosan B agreed with the result of 26 different treatments, reaching 526 kDa. The result of chitosan A was convergent to the result of 38, and the molecular weight reached 720 kDa. [52]Announced the harsh conditions of preparation processes for chitosan, such as high temperature, concentration of the alkali substance, reaction time, and acid concentration, lead to breaking the polymer chain and consequently decrease its molecular weight and this identical to the result of chitosan B for this study. [53]Also reported DD plays a vital role in influencing the molecular weight of chitosan. The researchers explained DD and molecular weight have an inverse relationship to each other, and this agrees with the chitosan B for this study.

The solubility of chitosan B agrees with the result of 38. The solubility was 100% using 50% NaOH for 20 h in the deacetylation process. [39]Explained that chitosan, which has a high solubility in acetic acid, should have DD at least 85%. The high solubility of chitosan is an important feature in medicine and food applications. [51]Attributed solubility of chitosan, which depends on the pH, is due to the presence of free amine groups (-NH2) for chitosan, which becomes positively charged (-NH3+) (protonated) as a result of loss proton at pH less than 6, leading to an electrical repulsion between the molecules to result about that soluble polysaccharide.

The results agreed with the results of [55] water binding ability ranged 274.2-941.5% with different sequences of chitosan preparation steps. The researcher mentioned reversing the sequence of chitosan preparation steps led to a clear effect on the water-binding ability of the produced chitosan. [21]Explained the water binding ability of chitosan is generally related to the molecular weight and the DD. [56]Explained that the difference in the binding ability of chitosan to water is due to the free amine groups (-NH2) for chitosan, which form hydrogen bonds with water, leading to an increase in the binding ability of chitosan to water and is identical with the chitosan B for this study which is more water bound from chitosan A.

Fat binding ability results agreed with results of 20. The fat binding ability ranged from 217-403%. [55] Mentioned changing the sequence of steps when chitin is extracted from its sources, such as demineralization followed by deproteinization. Finally, deacetylation leads to an increase in the fat-binding ability of the resulting chitosan. In contrast, the fat-binding ability of chitosan decreases if deproteinization is performed first and then demineralization is performed.

CONCLUSION

The percentage of the yield of Chitosan A was 25.56% and Chitosan B 20.15% based on the dry weight of the shell powder. Then, the chemical composition of chitosan A, such as moisture, ash and nitrogen content, reached 4.3,0.66,2.1%, respectively, while the moisture, ash and nitrogen content of chitosan B were 4,0.33,2.8%, respectively. The physicochemical and functional properties of chitosan A and B were studied, and the degree of deacetylation using the FTIR technique for chitosan A, B, and commercial chitosan was 49.9,81.8,70.2%, respectively. Chitosan A gave high viscosity 480cP while chitosan B had low viscosity 380cP. The molecular weights of chitosan A and B were 678.715kDa and 527.732kDa, respectively. Chitosan B showed the highest solubility in 1% glacial acetic acid solution, which was 100%, and it was lower for Chitosan A, which was

88.6%. Chitosan B showed a high water binding capacity of 446.5%, while it was lower for Chitosan A reached 388.8%. Chitosan A gave a high-fat binding capacity of 397.6%, while it was lower for Chitosan B, which reached 385.2%. SEM scanning electron microscopy was used to determine the morphology of chitosan A, B and commercial chitosan.

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