Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(SI-1): 286-294. **OPEN ACCESS**

Effect of different deacetylation parameters on the physicochemical properties of chitosan from shrimp shell waste

Yusof Nurhayati[1]*, Engku Nurul Nor Imas Engku Ibrahim[1], Asmaliza Abd Ghani[1] and John Yew Huat Tang[1]

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, **Malaysia**

***Correspondence:** nurhayatiyusof@unisza.edu.my **Received 25-07-2022, Revised: 31-08-2022, Accepted: 01-09-2022 e-Published: 20-10-2022**

Chitin is primarily used for the production of chitosan by a deacetylation process using chemical hydrolysis. Deacetylation is the non-enzymatic process of extracting the residue of chitin which is acetamide groups at high temperatures and treating it with a strong alkali to produce different degrees of deacetylation (DDA) of chitosan. The production of shrimp shell waste from shrimp processing industries has undergone a dramatic increase in recent years that causing disposal and contamination problems. The purpose of this study was to investigate the effect of different concentrations of NaOH (10, 30 and 50%) and time of heating (15 and 30 minutes) during the deacetylation process on the yield and physicochemical properties of chitosan. The obtained chitosan was evaluated by their DDA, moisture content, viscosity, solubility, water binding capacity, and fat binding capacity. The best yield of chitosan produced was observed by using 10% of NaOH and 30 minutes of deacetylation was 93.43%. The viscosity of extracted chitosan obtained from shrimp shell waste in this study ranged from 240 to 764 cPa.s. The highest degree of deacetylation i.e., 62.23% was obtained by 50% of NaOH at 15 minutes of heating. Moisture content produced in this study was from 5.58 to 9.57%. The solubility of chitosan extracted from shrimp ranged from 0.9 to 11.27%. Water binding capacity and fat binding capacity of chitosan were obtained in the range of 113.49- 1137.4% and 460.18-783.84%, respectively. Chitosan produced in this study has the potential to be used as a colour stabilizer, dye-binding properties, thickener, and stabilizer for sauces and flavour extenders in the food industry.

Keywords: Chitosan, Shrimp shell waste, degree of deacetylation, physicochemical properties

INTRODUCTION

Chitin is a polysaccharide found in abundance in the shell of crustaceans such as crayfish, lobster, crabs, prawns and shrimp (Manni et al. 2010). The shrimp shells have 15–40% of chitin that can be used for chitosan production (Pal et al. 2014). In addition, shrimp head and shell are a good source of proteins and also contain several dietary minerals such as Calcium (Ca), Ferum (Fe), Magnesium (Mg), and Sodium (Na) which are beneficial to human and animal diets (Singh et al. 2018).

Chitosan is the deacetylated derivative of chitin and it is a polysaccharide composed of β-1, 4-linked 2-acetamido-D-glucose and β-1, 4-linked 2-amino-D-glucose units, where the chitin acetyl groups are substituted by amino groups on the C-2 position in the carbon chain (Domard 2011).

Chitosan deacetylated by a chemical method employs concentrated alkaline solutions for long periods to produce chitosan with a modified structure.

The biological, functional, and physicochemical properties of chitosan are closely related to the degree of deacetylation (DDA) or degree of acetylation (DA), and molecular weight (MW), parameters that directly influence its pKa, viscosity, gelling capacity, and solubility (Domard 2011, Harris et al. 2011). Chitosan is insoluble in most familiar organic solvents (acetone, ethanol, and glycerol) and water. It can be dissolved easily in acidic aqueous solutions below pH 6.3. Even in very small concentrations, the aqueous solution of chitosan commonly has great viscosity. The chitosan with a DDA in the range of 55-70% is indicated to have low chitosan deacetylated degree that is almost completely insoluble in water. The chitosan with a DDA in the range of 70-85% is the middle chitosan deacetylation degree that is partly dissolved in water. Chitosan with 85-95% of DDA is known as high chitosan deacetylation degree and it has good solubility in water. Finally, 95%-100% is an ultrahigh DDA of chitosan and it is difficult to achieve (Lv 2016).

Chitosan can be applied in medicine as antibacterial and wound healing biomaterials (Muxika et al. 2017), antibacterial activity against Gram-positive and Gram-negative bacteria (Ali et al. 2021), and chelating agent because of its ability to bind to fats, proteins, cholesterol and metal ions (Ibrahim and El-Zairy 2015). The application of chitosan tends to increase the shelf life of food such as chitosan coating on fruits and vegetables (Xing et al. 2016) and prebiotics (Yusof et al. 2012, Nurhayati et al. 2016). The antioxidant activity of chitosan is due to the scavenging effect on free radicals. This ability depends on the DDA and increases with the increase in unsubstituted amino groups (Kabanov and Novinyuk 2020).

Research on chitosan has been conducted in many fields. Nurhayati and Ali (2020) reported on the use of response surface methodology in producing chitosan oligosaccharides. The use of modelling and simulation during the process can also be applied (Wan Mokhtar et al. 2013). Therefore, this study aimed to investigate the effect of the deacetylation of chitosan using different concentrations of NaOH (10, 30, and 50%) and time (15 and 30 minutes) from shrimp waste. Apart from that, the yield and physicochemical characteristics of chitosan from shrimp waste were also determined.

MATERIALS AND METHODS

Materials

Shrimp shell waste was obtained from Aji-Aji Seafood Restaurant in Besut, Terengganu. Acetic acid solution (99.8%) (Darmstadt, Germany), sodium hydroxide (Merk, Germany) and 37% hydrochloric acid (Merk, Germany were used in deacetylation.

Sample collection and preparation

The sample was cleaned and washed using tap water. Then, the sample was put in an oven drying at 60 °C overnight. The next day, the chitosan was extracted from the dried shrimp shell waste. Chitosan extraction was done in three major steps; demineralization, deproteination, and deacetylation.

For demineralization, the shrimp's waste sample (10 g) was treated with 2N hydrochloric acid at a ratio of solid to solvent which is 1:15 with a constant stirring at room temperature at 150 rpm in the incubator shaker for 2 hours. To avoid frothing, the acid was added slowly to prevent the formation of gases due to the calcium carbonate content from the shell reacting with the acid and the carbon dioxide. The sample was washed with clean water after demineralization until the sample achieved a neutral pH (pH 7.0). The final wash was conducted with hot distilled water and the sample was kept drying overnight at 80°C (Varun et al. 2017).

For deproteination, shrimp shell waste that has been demineralized was treated with 2N NaOH at a solid to solvent ratio of 1:10 with constant stirring at 150 rpm at 50°C in an incubator shaker for 2 hours followed by systematic drying and washing steps., The end product produced was known as chitin.

For deacetylation, 1g of chitin was treated with alkali at various concentrations and times. The treatment was done in an autoclave at 121 °C at 15 psi as shown in Table 1. The samples were washed with clean water until the sample achieved a neutral pH (pH 7.0). Then, the samples were dried in an oven drying at 80 °C overnight. The final product was called chitosan.

Table 1. The concentration of NaOH and time for deacetylation

Concentration of NaOH (%)	Time (min)	Temperature (°C)

10	15	121°C
10	30	121°C
30	15	121°C
30	30	121°C
50	15	121°C
50	30	121°C

Determination of Total Extraction Yield

Total extraction yield is a measurement of extract mass compared with the original mass of raw material. It was calculated as the weight (g) of sample extract gained from raw material and expressed as a percentage. The per cent yield was calculated by the following equation;

$$Yields\ (\%) = \frac{W_1}{W_2} \times 100 \quad \textbf{Eqn.1}$$

where;
W_1= Weight of sample after extraction
W_2 = Weight of raw material

Moisture Content

The moisture content of each sample was determined by using the gravimetric method (AOAC, 1990).

Determination of degree of deacetylation using a titration method

Chitosan of 0.3 g was dissolved in 30 mL of 0.1 M hydrochloric acid and stirred for 30 minutes until it dissolved. Two drops of methyl orange indicator were added to the solution. The solution was titrated with 0.1 M of NaOH until the colour of the solution turns from pink to orange yellowish and the volume of NaOH titrated was recorded. The degree of deacetylation was calculated by the following equation;

$$NH_2[\%] = \frac{[(C_1V_1 - C_2V_2) \times 0.016]}{[G(100-W)]} \times 100 \quad \textbf{Eqn.2}$$

where:
C_1: HCl concentration, mol·dm^{-3}
C_2: NaOH concentration, mol·dm^{-3}
V_1: volume of HCl solution, cm^3
V_2: volume of NaOH solution, cm^3
0.016: molecular weight of NH2 in 1 cm^3 0.1 mol·dm^{-3} HCl [g].
G: the sample weight [g]
W: the water percentage of the sample [%]

The DDA value was calculated by the following equation;

$$DDA[\%] = \frac{NH_2\,\%}{9.94\%} \times 100 \quad \textbf{Eqn.3}$$

where:
9.94 % is the theoretical NH_2 percentage
Chitosan theoretic NH_2 content % = (16/161) × 100% = 9.94%

Solubility

The solubility of the sample was tested by dissolving 1 g of the sample in 20 ml of 0.2 M acetate buffer at pH 3.8, 4.5 and 5.5. The sample solution was stirred for 1 hour at room temperature. Then, the sample was filtered by using a filter paper with a pore size of 110 mm and the sample was dried in an oven at 80℃ for 8 h and weighed (Divya et al. 2014). The following equation was used to determine the solubility;

$$\%Solubility = 1 - \frac{Weight\ of\ insoluble\ part}{Total\ Weight\ of\ sample} \times 100 \quad \textbf{Eqn.4}$$

Viscosity

The viscosity of the chitosan was determined by using a Brookfield Digital Rotary Viscometer (WVS-2M, Made in). Chitosan solution was prepared in 1% acetic acid at 1% concentration on a dry basis. Measurement was made in triplicates using spindle number 4 at 60 rpm on solutions at room temperature (27℃) in centipoises (cPs) units (Ocloo et al. 2011).

Fat Binding Capacity (FBC)

FBC analysis was carried out by weighing a centrifuge tube containing 0.5 g of sample according to Ocloo et al. (2011). 10 ml of vegetable oil was added and was mixed on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s at every 10 min and centrifuged at 3200 rpm for 25 min. When the supernatant was decanted, the tubes were weighed again. FBC was calculated using the following equation:

$$FBC\ (\%) = \frac{fat\ bound\ (g)}{initial\ sample\ weight\ (g)} \times 100 \quad \textbf{Eqn.5}$$

Water Binding Capacity (WBC)

Water absorption was carried out by weighing a centrifuge tube containing 0.5 g of the sample according to Ocloo et al. (2011). 10 ml of water was added and mixed on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s at every 10 min and centrifuged at 3200 rpm for 25 min. When the

supernatant was decanted, the tubes were weighed again. WBC was calculated as follows:

$$WBC\ (\%) = \frac{water\ bound\ (g)}{initial\ sample\ weight\ (g)} \times 100 \quad \textbf{Eqn.6}$$

Statistical Analysis

To verify the statistical significance of all parameters, the values of mean ± standard deviation (SD) are calculated by using SPSS software. One way of variance (ANOVA) was used to perform the physicochemical properties in the sample of chitosan at different concentrations of NaOH and time of deacetylation. A p-value of less than 0.05 ($p<0.05$) is considered statistically significant.

RESULTS AND DISCUSSION

Yield of chitosan

Table 2 shows the result of the yield percentage of chitosan deacetylated with different NaOH concentrations (10, 30 and 50%) for 15 minutes and 30 minutes. The yield of chitosan extracted varied depending on the concentration of NaOH and the time used during the deacetylation step. The highest extraction yield was 10% NaOH at 30 minutes of deacetylation time which was 93.43%. The yield of chitosan extracted for 15 and 30 minutes was decreased as the concentration increased from 10% to 50% of NaOH and the reductions were not significant. The decrease in yield with NaOH concentration is due to the excessive loss of sample mass/weight from the excessive removal of an acetyl group from the polymers during deacetylation and the loss of chitosan particles during washing. Martín-López et al. (2020) reported that the range of yield for chitosan extracted from white shrimp by the ultrasonic method (amplitude 75-90%, 65% NaOH) was in good agreement with the finding in this study which was between 80.3%-87.31%. Hence, a different method of treatment during deacetylation results in a different percentage yield of chitosan.

Table 2. Percentage of yield in chitosan

NaOH Concentration (%)	Deacetylation Time	
	15 min	30min
10	92.12 ± 0.62[a]	93.43 ± 1.85[a]
30	91.05 ± 1.89[a]	92.21 ± 1.33[a]
50	85.82 ± 7.46[a]	88.66 ± 4.77[a]

Mean ± standard deviation of triplicates determination. Values of the same letters in the columns are not significantly different at P>0.05.

Moisture content

Table 3 shows the range of moisture content obtained for each sample extracted at different concentrations of NaOH and time during the deacetylation process. For 15 minutes of deacetylation, there was a significant ($p<0.05$) decrease in the moisture content with NaOH concentration from 9.57% (10% NaOH) to 5.89% (50% NaOH)). For 30 minutes of deacetylation, the moisture content also decreased significantly with NaOH concentrations at 10%, 30% and 50% which were recorded at 9.20 %, 7.73 % and 5.58%, respectively. This result is in good agreement with Ocloo et al. (2011). They reported that the moisture content for chitosan extracted from shrimp at 50% of NaOH with 5-6 hours at 100°C of deacetylation time was 8.69%. Chitosan properties are hygroscopic so they can be affected by moisture absorption during storage (Sarbon et al. 2015). In addition, the lower value of moisture content may also be affected by storage conditions such as relative humidity and the intensity of sunlight (Divya et al. 2014). A study done by Szymańska and Winnicka (2015) proposed that the moisture content of chitosan must be low which ranges from 6-10% so that the capability to form hydrogen bonding is greater. Furthermore, the damage in the polymer of the chitosan structure was faster when it contains higher water content due to hydrolysis reactions (Viljoen et al. 2014).

Table 3. Percentage of moisture content in chitosan.

NaOH Concentration (%)	Deacetylation Time	
	15 min	30 min
10	9.57 ± 1.77[a]	9.20 ± 0.06[a]
30	8.88 ± 0.96[ab]	7.73 ± 0.49[b]
50	5.89 ± 0.37[b]	5.58 ± 0.67[c]

Mean ± standard deviation of triplicates determination. Values of different letters (a)–(c) in the same columns are significantly different at P<0.05.

Degree of Deacetylation (DDA)

Table 4 showed the result of DDA for this study. There were significant differences ($p<0.05$) among all samples in terms of DDA. In this study,

increasing the concentration of NaOH from 10% to 50% NaOH in the deacetylation process of chitosan for 15 and 30 min, resulted in increasing in DDA from 55.03 to 67.90%, and from 11.19 to 51.39%, respectively. Meanwhile, increasing deacetylation time (15 minutes to 30 minutes) resulted in a decrement of DDA for all NaOH concentrations. This result showed that the DDA obtained was affected by the different concentrations of NaOH and also a different time of heating in the autoclave during the deacetylation process (Kumari et al. 2017). Previous literature suggests using 60% of NaOH to obtain a higher deacetylation grade of chitosan which was 81.24% (Kumari et al. 2017).

The degree of deacetylation is an important parameter that affects the solubility of chitosan. DDA may range from 65.9% to 82% from squid pen samples, depending on the source and preparation procedure (Ocloo et al. 2011). Hossain and Iqbal (2014) reported the DDA of chitosan deacetylated using different concentrations of NaOH (30, 40, 50 and 60%) in shrimp waste was from 45.5% to 81.24%. Many factors affect the DDA of chitosan such as crustacean species, preparation methods and NaOH concentration. Acetyl groups bound in chitin are difficult to remove, thus it needs a high concentration of alkalines such as sodium hydroxide or potassium hydroxide (NaOH/KOH) and a high temperature (>100 °C) to remove the acetyl group in the crustacean sample (Hossain and Iqbal 2014).

Table 4. Degree of Deacetylation of chitosan.

NaOH Concentration (%)	Deacetylation Time	
	15 min	30 min
10	55.03 ± 1.75^{c}	11.19 ± 0.89^{c}
30	60.56 ± 0.56^{b}	34.84 ± 0.76^{b}
50	67.90 ± 0.45^{a}	51.39 ± 1.46^{a}

Mean ± standard deviation of triplicates determination. Values of different letters (a)–(c) in the same columns are significantly different at P<0.05.

Viscosity

Table 5 shows the viscosity of chitosan extracted with different concentrations of NaOH and at different times of deacetylation. The viscosity of the chitosan for all samples obtained in this study ranged from 240 cPa.s to 764 cPas. There was a significant difference ($p<0.05$) in viscosity when the concentration of NaOH was increased from 10 to 50% for 15 and 30 minutes. The viscosity of the chitosan increased from 461 to 764 and 240 to 609 cPas when the concentration of the NaOH increased from 10% to 50% at 15 and 30 min of deacetylation time, respectively. In contrast, the viscosity of the chitosan decreased when time increased from 15 to 30 min. The viscosity of chitosan increases with the increase of DDA because in concentrated alkaline reactions, free amino groups tend to form cations with water and that will increase the viscosity (He et al. 2016).

Ocloo et al. (2011) reported the viscosity of radiated and non-radiated process shrimp chitosan from 26.2 to 711.9 cPs depending on the species and the preparation method used. The different value obtained for the viscosity is due to different condition during the extraction method and also different type of crustacean used as the sample for chitosan extraction. Chitosan viscosity decreases with an increased time of demineralization and deacetylation time. The viscosity of chitosan increases when the chitosan particle size is smaller (Tokatlı and Demirdöven 2018).

Table 5. The viscosity of chitosan at different concentrations of NaOH and deacetylation time

NaOH Concentration (%)	Deacetylation Time	
	15 min	30 min
10	461 ± 0.23^{b}	240 ± 0.04^{c}
30	544 ± 0.63^{b}	450 ± 0.25^{b}
50	764 ± 0.09^{a}	609 ± 0.17^{a}

Mean ± standard deviation of triplicates determination. Values of different letters (a)–(c) in the same columns are significantly different at P<0.05.

Solubility

The solubility of chitosan is one of the important parameters for the quality of the chitosan produced. Good quality chitosan has good solubility. There are several critical factors affecting chitosan solubility including temperature, alkali concentration, time of deacetylation, the particle size of chitin and the ratio of chitin to alkali solution. The solubility is controlled by the DDA and must be at least 85% complete to achieve the desired solubility (Hossain and Iqbal 2014). The solubility of the chitosan prepared with 10-50% of NaOH for 15 and 30 minutes of deacetylation time at different pH is shown in Table 6. The highest solubility is in pH 3.8 at 50% of NaOH at 15 minutes of deacetylation time which is 11.27%. In contrast, the lowest solubility is in pH 5.5 at 10% of NaOH for 30 minutes of deacetylation time which is 0.63%. The solubility of chitosan increased as the concentration of NaOH was increased due to the

increasing DDA value that affects the solubility of chitosan (refer to Table 4. Condition for chitosan to be solute in an acidic solution below pH 6.0 due to it being a strong base because it has primary amino groups with a pKa value of 6.3. The lower value of the pKa solution would be able to protonate the chitosan and increase the solubility of chitosan. From the result, the solubility of chitosan decreased as the pH value increased. This is due to a higher pKa value when the pH value is increased. The amine groups are unable to get protonated causing the chitosan to become less soluble (Zargar et al. 2015).

Table 6. Solubility of chitosan at different pH for 15 and 30 minutes of deacetylation

NaOH Concentration (%)	pH		
	pH 3.8	pH 4.5	pH 5.5
15 minutes			
10	9.36 ± 1.59[a]	5.33± 0.35[a]	1.36± 0.01[a]
30	10.17± 0.69[a]	6.13± 0.86[a]	1.63± 0.87[a]
50	11.27± 0.56[a]	7.35± 1.34[a]	2.83± 0.83[a]
30 minutes			
10	6.44 ± 0.86[b]	3.80 ± 0.64[b]	0.63 ± 0.38[b]
30	8.37 ± 0.86[ab]	5.83 ± 0.34[ab]	1.53 ± 0.15[ab]
50	9.83 ± 0.52[a]	6.55 ± 0.81[a]	2.42 ± 0.82[a]

Mean ± standard deviation of triplicates determination. Values of the same letters in the columns are not significantly different at P>0.05.

Water Binding Capacity (WBC)

The significance of water binding capacity (WBC) analysis was conducted to measure the ability of the chitosan to hold the water even after treatment with external forces. The higher WBC showed that the chitosan can retain water when external forces were applied. Also, the WBC of chitosan is related to the integrated hydrophilic ability that presents well deal of interest for hydrogel structure. Chitosan-based hydrogels are the potential for engineering scaffolds to provide tissue repair achievements and can be used for drug delivery to the oral cavity, intestine, stomach and colon (Ahmadi et al. 2015).

Table 7 shows the WBC of chitosan at 15 and 30 minutes of deacetylation time. The results for the WBC of the chitosan samples ranged from 942.65% to 1374.91% in the chitosan sample extracted from shrimp waste. The highest WBC was 1374.91% for 10% NaOH at 15 min of deacetylation time. The WBC obtained by this study was higher compared to the study by Ocloo et al. (2011), which used different concentrations of acid at demineralization (1N HCl) and different concentrations of alkaline at deproteinization steps (3.5% *w/w* of NaOH) and WBC range from 582.40% to 656.75%. In this study, the concentration used is 2N of HCl for demineralization and 2N of NaOH for deproteinization steps. The WBC mainly depends on the demineralization and deproteinization steps thus, varying the concentration of acid (HCl) and alkaline (NaOH) during the steps will affect the WBC (Kumari et al. 2017). Other factors that explained the differences in water uptake between chitinous polymers include differences in the crystallinity of the products, different protein content in the chitosan and particle size of the chitosan (Elieh-Ali-Komi and Hamblin 2016).

Table 7. Water binding capacity of chitosan

NaOH Concentration (%)	Deacetylation Time	
	15 min	30 min
10	1374.91± 120.76[a]	1090.12 ± 258.86[a]
30	1118.36± 117.76[a]	1032.03± 120.34[a]
50	942.65± 96.12[a]	1113.49± 55.84[a]

Mean ± standard deviation of triplicates determination. Values of the same letters in the columns are not significantly different at P>0.05.

Fat Binding Capacity (FBC)

Fat binding capacity (FBC) analysis was conducted to measure the amount of oil absorbed by the chitosan whereas the FBC properties are useful in fat, flavour retention and texture such as application in batter, flavour and emulsions. Therefore, FBC is affected by the protein content, size of the particle, number of polar amino acids, processing method, and protein-lipid interactions (Yada 2017).

Table 8 showed the fat binding capacity (FBC) for chitosan prepared with different concentrations of NaOH at two different times. The FBC of the chitosan sample derived from shrimp waste ranged from 460.18% to 783.84%. Previously, Kumari et al. (2017) reported that the FBC value in shrimp chitosan is 246%, lower than the FBC obtained in this study when the extraction

is done by deproteinization, demineralization and deacetylation. The FBC obtained in this study is higher because it depends on the demineralization and deproteinization steps which when the demineralization process was carried out first, resulted in increasing fat binding capacity. Hence, changing the sequence of the method during the extraction will also affect the result of FBC (Kumari et al. 2017).

Table 8. The fat binding capacity of chitosan

NaOH Concentration (%)	Deacetylation Time	
	15 min	30 min
10	537.56 ± 16.00[a]	656.46 ± 38.77[a]
30	573.26 ± 100.23[a]	783.84 ± 162.34[a]
50	460.18 ± 36.39[a]	657.60 ± 5.08[a]

Mean ± standard deviation of triplicates determination. Values of the same letters in the columns are not significantly different at P>0.05.

CONCLUSION

In conclusion, the extraction of chitosan using different concentrations of NaOH (10, 30 and 50%) and different times (15 and 30 minutes) was feasible. Yields of chitosan decreased with increasing alkaline concentration (10 to 50% of NaOH) during the deacetylation step. Meanwhile, the yields of chitosan increased with the increase in deacetylation time (15 to 30 minutes). The moisture content for all samples of chitosan was in the range of 5.58% to 9.57%. The highest DDA was obtained from chitosan samples at 15 minutes of deacetylation that were treated with 50% of NaOH with a value of 67.90%. The viscosity of chitosan increased with the increasing concentration of NaOH during deacetylation. All chitosan samples demonstrated a low solubility range at three different pHs (3.8 pH, 4.5 pH and 5.5 pH). All chitosan samples showed excellent functional properties for water and fat binding capacity.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors would like to acknowledge Universiti Sultan Zainal Abidin for funding this study (Dana Penyelidikan Universiti UniSZA/2021/DPU1.0/03).

AUTHOR CONTRIBUTIONS

NY and EIENNI designed, performed the experiments, and collected and analysed data. AGA and TJYH improved the experiments and reviewed the manuscripts. All authors have read and approved the final version of the manuscripts.

REFERENCES

Ahmadi F, Oveisi Z, Samani SM, Amoozgar Z, 2015. Chitosan Based Hydrogels: Characteristics and Pharmaceutical Applications. Research in Pharmaceutical Sciences 10(1): 1.

Ali NA, Mamat WAW, Johari SATT, Aziz MYA, Othman AS, 2021. The Antibacterial Activity of *Catunaregam tomentosa* against Selected Gram-Positive and Gram-Negative Bacteria. Asian Journal of Medicine and Biomedicine 5(S1): 34-38.

Divya K, Rebello S, Jisha M, 2014. A Simple and Effective Method for Extraction of High Purity Chitosan from Shrimp Shell Waste. Proceedings of the International Conference on Advances in Applied Science and Environmental Engineering-ASEE.

Domard A, 2011. A Perspective on 30 Years Research on Chitin and Chitosan. Carbohydrate Polymers 84(2): 696-703.

Elieh-Ali-Komi D, Hamblin MR, 2016. Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials. International Journal of Advanced Research 4(3): 411.

Harris R, Lecumberri E, Mateos-Aparicio I, Mengíbar M, Heras A, 2011. Chitosan Nanoparticles and Microspheres for the Encapsulation of Natural Antioxidants Extracted from *Ilex paraguariensis*. Carbohydrate Polymers 84(2): 803-806.

He X, Li K, Xing R, Liu S, Hu L, Li P, 2016. The

Production of Fully Deacetylated Chitosan by Compression Method. The Egyptian Journal of Aquatic Research 42(1): 75-81.

Hossain M, Iqbal A, 2014. Production and Characterization of Chitosan from Shrimp Waste. Journal of the Bangladesh Agricultural University 12(1): 153-160.

Ibrahim H, El-Zairy E, 2015. Chitosan as a Biomaterial—Structure, Properties, and Electrospun Nanofibers. Concepts, Compounds and the Alternatives of Antibacterials 1(1): 81-101.

Kabanov VL, Novinyuk LV, 2020. Chitosan Application in Food Technology: A Review of Recent Advances. Food Systems 3(1): 10-15.

Kumari S, Annamareddy SHK, Abanti S, Rath PK, 2017. Physicochemical Properties and Characterization of Chitosan Synthesized from Fish Scales, Crab and Shrimp Shells. International Journal of Biological Macromolecules 104: 1697-1705.

Lv S, 2016. High-Performance Superplasticizer Based on Chitosan. Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials, Elsevier**:** 131-150.

Manni L, Ghorbel-Bellaaj O, Jellouli K, Younes I, Nasri M, 2010. Extraction and Characterization of Chitin, Chitosan, and Protein Hydrolysates Prepared from Shrimp Waste by Treatment with Crude Protease from *Bacillus cereus* SV1. Applied Biochemistry and Biotechnology 162(2): 345-357.

Martín-López H, Pech-Cohuo SC, Herrera-Pool E, Medina-Torres N, Cuevas-Bernardino JC, Ayora-Talavera T, Espinosa-Andrews H, Ramos-Díaz A, Trombotto S, Pacheco N, 2020. Structural and Physicochemical Characterization of Chitosan Obtained by UAE and its Effect on the Growth Inhibition of *Pythium ultimum*. Agriculture 10(10): 464.

Muxika A, Etxabide A, Uranga J, Guerrero P, De La Caba K, 2017. Chitosan as a Bioactive Polymer: Processing, Properties and Applications. International Journal of Biological Macromolecules 105: 1358-1368.

Nurhayati Y, Ali AM, 2020. Production of Chitosan Oligosaccharides using B-Glycosidic Degrading Enzyme: Optimization using Response Surface Methodology. Malaysian Journal of Applied Sciences 5(2): 30-44.

Nurhayati Y, Manaf AA, Osman H, Abdullah ABC, Tang JYH, 2016. Effect of Chitosan Oligosaccharides on the Growth of *Bifidobacterium* Species. Malaysian Journal of Applied Sciences 1(1): 13-23.

Ocloo F, Quayson E, Adu-Gyamfi A, Quarcoo E, Asare D, Serfor-Armah Y, Woode B, 2011. Physicochemical and Functional Characteristics of Radiation-processed Shrimp Chitosan. Radiation Physics and Chemistry 80(7): 837-841.

Pal J, Verma HO, Munka VK, Maurya SK, Roy D, Kumar J, 2014. Biological Method of Chitin Extraction from Shrimp Waste an Eco-friendly Low Cost Technology and its Advanced Application. International Journal of Fisheries and Aquatic Studies 1(6): 104-107.

Sarbon N, Sandanamsamy S, Kamaruzaman S, Ahmad F, 2015. Chitosan Extracted from Mud Crab (*Scylla Olivicea*) Shells: Physicochemical and Antioxidant Properties. Journal of food science and technology 52(7): 4266-4275.

Singh S, Siddhnath BR, Aziz A, Verma N, Chriwatkar B, 2018. Shrimp Waste Powder–Potential as Protein Supplement. International Journal of Pure and Applied Bioscience 6: 401-406.

Szymańska E, Winnicka K, 2015. Stability of Chitosan—A Challenge for Pharmaceutical and Biomedical Applications. Marine Drugs 13(4): 1819-1846.

Tokatlı K, Demirdöven A, 2018. Optimization of Chitin and Chitosan Production from Shrimp Wastes and Characterization. Journal of Food Processing and Preservation 42(2): e13494.

Varun TK, Senani S, Jayapal N, Chikkerur J, Roy S, Tekulapally VB, Gautam M, Kumar N, 2017. Extraction of Chitosan and its Oligomers from Shrimp Shell Waste, their Characterization and Antimicrobial Effect. Veterinary World 10(2): 170.

Viljoen JM, Steenekamp JH, Marais AF, Kotzé AF, 2014. Effect of Moisture Content, Temperature and Exposure Time on the Physical Stability of Chitosan Powder and Tablets. Drug Development and Industrial Pharmacy 40(6): 730-742.

Wan Mokhtar WMF, Taip F, Abdul Aziz N, Mohd Noor S, 2013. Modeling, Simulation and Control of Pink Guava Puree Pasteurization Process with Fouling as Disturbance. Journal of Food Process Engineering 36(6): 834-845.

Xing Y, Xu Q, Li X, Chen C, Ma L, Li S, Che Z, Lin H, 2016. Chitosan-based Coating with Antimicrobial Agents: Preparation, Property, Mechanism, and Application Effectiveness on Fruits and Vegetables. International Journal of Polymer Science 2016.

Yada RY, 2017. Proteins in Food Processing, Woodhead Publishing.

Yusof N, Muhammad K, Mohd Adzahan N, Matanjun P, 2012. Functional Properties of Spray-dried White Dragon Fruit (*Hylocereus undatus*) Juice. VII International Postharvest Symposium 1012.

Zargar V, Asghari M, Dashti A, 2015. A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives, and Applications. ChemBioEng Reviews 2(3): 204-226.