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Effect of NaOH-based deproteinisation on chemical and biological properties of *Moina* sp. chitosan

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Abstract

Chitosan is a biopolymer which is isolated from the crustacean exoskeleton of *Moina* sp. with particular antibacterial properties. Alkali-sodium hydroxide deproteinisation has a huge role in ensuring the chitosan quality by removing protein to enhance its dispersivity. Modification of sodium hydroxide (NaOH) concentration by 3N and 6N was performed in this study to observe the effect on chemical and biological characteristics of chitosan. The highest concentration treatment of 6N NaOH exhibited the best composition of protein, fat, and ash by 6.09%, 3.77% and 0.9%, respectively. The deacetylation degree of chitosan treated with 3N NaOH depicted the highest solubility of 73.25% compared to the solubility of 6N NaOH by 61.06%. The antibacterial activity of *Moina* sp. chitosan was considered as having a weak and fluctuated activity by different concentrations of NaOH. The 6N NaOH treatment chitosan showed the best antibacterial activity against the gram-positive bacterial strain of *Staphylococcus aureus* with the widest inhibition zone diameter of 4.34±0.55 mm, while the 3N NaOH treatment chitosan showed the best antibacterial activity against the gram-negative bacterial strain of *Escherichia coli* with widest inhibition zone diameter of 4.47±0.78 mm.

1. Introduction

Chitosan, a natural polymer composed of a repetitive combination between D-glucosamine (deacetylation units) and N-Acetyl-D-glucosamine (acetate units), is described as a non-toxic, biodegradable and biocompatible polymer with alluring biological properties, such as permeation enhancement, mucoadhesive ability, anticoagulant and antimicrobial activity. Those biological properties illustrate chitosan compatibility for medical applications (Park and Kim, 2010; Aranaz *et al*., 2010; Yang, 2011; Morin Crini *et al*., 2019). Various types of chitosan substantially exhibit stronger bactericidal effects against gram-positive bacteria than gram-negative bacteria, even at a low concentration of 0.1% (No *et al*., 2002).

Chitosan has wide applications in various industrial sectors. In the food industry, chitosan is used as a preservative, packaging additive, food supplement and encapsulation base (Gutiérrez, 2017). In the cosmetic industry, chitosan is utilised as an antioxidant and antibacterial agent in skin protection products, toothpaste, and mouthwash, and as a film-forming agent in shampoos and lotions (Aranaz *et al*., 2018). In agriculture, chitosan is not only primarily used for plant protection against bacterial, fungal, and viral pathogens, but also as plant growth regulator and fertiliser additive (El Hadrami *et al*., 2010; Gumilar *et al*., 2017; Malerba and Cerana, 2018; Sharif *et al*., 2018). In addition, chitosan can bind fat which is suitable as a chelating agent for the wastewater treatment industry (Nechita, 2017; Morin Crini *et al*., 2019).

Chitosan is generally derived from chitin, a linear polysaccharide found in the cell walls of fungi and crustacean exoskeletons (Paul *et al*., 2014). Chitin is a major component of cell walls in fungi and arthropods exoskeleton of crustaceans, insects, molluscs, cephalopods, and fish scales (Morin-Crini *et al*., 2019). Chitosan synthesis from chitin is carried out by changing

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the acetamide group (–$NHCOCH_3$) in chitin into an amine group (–NH_2), with initially isolating chitin through the deproteinisation and demineralisation stages (Terbojevich and Muzzarelli, 2000). The deproteinisation process was reported to affect the final product quality, as the usage of alkali-NaOH might optimally remove protein, fat, and pigment, while also increasing chitosan solubility (Suptijah, 2004). The increase of NaOH concentration at 60% exhibited the highest deacetylation degree up to 90% and the lowest protein residue of 0.05%, but the high NaOH concentration utilisation is likely to be environmentally unsustainable (Abdel-Rahman *et al*., 2015). Hence, the NaOH concentration adjustment for the deproteinisation process is required to determine the optimal concentration for obtaining chitosan with the best properties.

Several studies showed that chitosan could be obtained from various fisheries by-products, such as shrimp carapace (Suptijah, 2004), shrimp shells (Kurniasih and Kartika, 2009; Hendrawati *et al*., 2015; Dompeipen *et al*., 2016; Setha *et al*., 2019), squid internal shell (Rochmawati *et al*., 2018), *Artemia* cysts (Tajik *et al*., 2008) and fish scales (Rumengan *et al*., 2017). Aquatic zooplankton organisms were reported as potential alternative sources of chitin, as reported by Kaya *et al*. (2013) that zooplankton *Daphnia magna* cysts contained approximately 18-21% chitin. Tseng *et al*. (2021) added that the utilisation of *Daphnia similis* from the *Cladocera* group as a source of chitin showed a positive effect on improving the *Vannamei* shrimp growth performance. As an aquatic organism that also belongs to *Cladocera* crustaceans, *Moina* sp. has prominent potential for alternative sources (Okunsebor, 2014), as it has an average protein content of 50% per dry weight with respective total fat of 20-27% and 4-6% per dry weight for breed stock and juveniles (Islam *et al.,* 2017). Besides that, *Moina* sp. cultivation is accessible with cheaper media, fertiliser, and organic feed, even Mubarak *et al.* (2017) used bran suspension as feed to produce *Moina* sp. biomass. Therefore, this study aimed to evaluate the characteristics of chitosan isolated from *Moina* sp. which is deproteinised with different NaOH concentrations of 3N and 6N to achieve higher quality on chemical and biological properties.

2. Materials and methods

2.1 Materials and equipment

The equipment used was centrifuge (Merck Rotanta 460), analytical balance (Merck PIONEER PX224/E), Erlenmeyer flask (Iwaki, Germany), measuring cylinder (Merck Herma), beaker glass (Merck Iwaki), measuring flask (Merck Iwaki), hot plate stirrer (Merck Stirrer Thermo Scientific Cimarec), moisture analyzer (BEL i-Thermo 163L, India), soxhlet extractor apparatus 250 mL (Merck Duran), three-neck flask (Merck Iwaki), oven (Merck Thermo Scientific), micropipette (Merck Thermo Scientific), spectrophotometer (Merck Perkin Elmer Spectrum One, USA), amino acids (Acquity UPLC), Petri dish (Iwaki), laminar airflow, and autoclave (Hirayama HVE-50). The materials used in this study were Moina sp., NaOH (Merck, Germany), HCl (Merck PA, Germany), NaOCl (Merck, Germany), acetone (SmartLab, Indonesia), Kjeldahl tablets (Merck 1.10958.0250 Kjeld, Germany), distilled water, mannitol salt agar (MSA) (Oxoid, Ireland), eosin methylene blue agar (EMBA) (Oxoid, Ireland), and bacterial isolates of *S. aureus* and *E. coli.*

2.2 Moina sp. preparation

Moina sp. was initially cleaned by aerating samples on a container filled with distilled water for 6 hrs. The cleaned raw materials were dried using a storage box that had been coated with aluminium foil on the inside so that heat could be distributed evenly. The heat source came from a 5-Watt yellow light bulb (brand Procyon). The drying process was carried out for 2-3 days under the steady temperature of 62-68°C, until obtaining a constant weight. The dried *Moina* sp. was pulverised for around 1 min using a dry food grinder (Fomac, DE100G). The grinding process was conducted to obtain fine powder form for the convenience of further process.

2.3 Deproteinisation

Moina sp. powder was immersed in an alkaline-NaOH solution at different concentrations of 3N and 6N, with the sample to solution ratio of 1:10. The samples were regularly stirred for 2 hrs using a magnetic stirrer on a hotplate at 70-75°C. The deproteinised samples were washed using distilled water until it reached neutral pH and dried using an oven until obtaining constant weight.

2.4 Demineralisation

Demineralisation was carried out using 1N HCl for 2 hrs on a hotplate at 90°C. The demineralised samples were filtered using a calico-cloth and washed using distilled water until reached neutral pH. The samples required a drying process until getting constant weight.

2.5 Decolourisation

The demineralised samples were submerged in acetone for 10 mins and dried for 2 hrs, followed by bleaching out using 0.32% NaOCl solution for one hr at room temperature. The decolourised samples were filtered using a calico-cloth and washed using distilled

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water until reached neutral pH. Decolourised samples are supposed to have whiter colour compared to before treatment.

2.6 Chitosan deacetylation

Deacetylation was carried out using 50% NaOH solution for one hr while being heated on a hotplate at 140°C. Samples that had been heated were filtered and washed with distilled water until the pH was neutral, then dried in an oven at 55°C for 8 hrs.

2.7 Amino acids composition

Amino acid compositions were analysed by using the UPLC (Ultra Performance Liquid Chromatography) method. The collagen samples were put in a test tube for 0.1 grams to be hydrolysed with 5-10 mL of 6N HCl and dried in the oven at 110°C for 22 hrs. The samples were added with 70 µL of AccQ Fluor Borate reagent and incubated at 55°C for 10 mins. The incubated sample was then injected at UPLC for 1 µL, using ACCQ-Tag Ultra C18 column chromatography, at a temperature of 49°C. Amino acid concentrations were calculated using the ratio of analyte area to the internal standard, with the following formula:

$$\text{Sample ratio} = \frac{\text{Amino acid analyte area}}{\text{Internal standard area}}$$

$$\text{Amino acid level (mg/kg)} = \frac{\frac{\text{Sample ratio}}{\text{Standard ratio}} \times \frac{\text{C std}}{1000000} \times \text{MW} \times \text{Va} \times \text{Df}}{\text{Weight or Volume}}$$

The mg/kg data was converted into a percentage (%) with the following formula:

$$1 \text{ mg/kg} = 1 \text{ ppm}, 1 \text{ ppm (\%)} = \frac{1}{10000} = 0.0001\%$$

2.8 Yield calculation

Moina sp. chitosan yield was calculated based on the weight percentage of the weight of resulting chitosan divided by the weight of raw material before treatment and multiplied by one hundred per cent. The yield calculation was determined by the formula below:

$$\text{Yield} = \frac{\text{Final Weight (g)}}{\text{Initial Weight (g)}} \times 100\%$$

2.9 Proximate compositions

Moina sp. chitosan samples were tested for protein content, ash content, and fat content following the AOAC proximate testing guideline (2005). The moisture content was analysed using a moisture analyser.

2.10 Determination of deacetylation degree

The deacetylation degree test was carried out referring to Czechowska-Biskup *et al*. (2012), with diluting 6.1 mg chitosan powder in 0.1M HCl and injected to a spectrophotometer with a wavelength range of 201 nm. The deacetylation degree equation was calculated with the equation below:

$$\text{DA} = \frac{(161{,}1.A.V) - (0{,}0218.m)}{(3{,}3615.m) - (42{,}1.A.V)}$$

$$\text{DD} = 1 - (\text{DA}) \times 100\%$$

Where DA: degree of acetylation, DD: degree of deactylation, A: absorbent, V: chitosan volume (L) and M: chitosan weight.

2.11 Antimicrobial activity

Bacterial isolates of *Staphylococcus aureus* and *Escherichia coli* were isolated from selective media inoculation on mannitol salt agar (MSA) and eosin methylene blue agar (EMBA). The isolated bacteria were re-cultured in tryptic soy broth (TSB) and incubated at 37±1°C for 24 hrs. The re-cultured isolates were centrifuged, and then the supernatant was washed three times using physiological NaCl solution. The bacterial suspension was diluted until it reached a concentration of 10^8 CFU/mL following the 0.5 McFarland standard.

In this study, 0.01 g of *Moina* sp. chitosan and shrimp shell chitosan were dissolved in 1 mL of 1% acetic acid to obtain a sample concentration of 10 mg/mL (100 µg/disk). The sample solutions were serially diluted with 1% acetic acid to obtain concentrations of 5, 2.5, 1.25, 0.6, 0.3 and 0.15 mg/mL. 10 µL of each diluted solution was taken and dripped onto a paper disk. The paper disks were placed on Mueller Hinton Agar (MHA) media which were already inoculated by test bacteria, and incubated at 37±1°C for 24 hrs. The inhibition zone diameter was measured with a vernier calliper.

3. Results and discussion

3.1 Yield and chemical characterisation of Moina sp. chitosan

Chitosan from *Moina* sp. was resulting in low yield in all treatments (Table 1) because of the utilisation of strong hydrochloric acid and strong sodium hydroxide, which causes the perpetual dissolution of mineral components, proteins, and other inorganic materials during deproteinisation and demineralisation processes (Patria, 2013). High temperatures during the deacetylation process between 115-120°C might also affect the produced yield. According to Tanasale (2010), the heating temperature in the deacetylation process which was high temperature to degrade the polymer into a lower molecular weight polymer. Apriani *et al*. (2012) added that temperature accelerates the deacetylation reaction. The high temperature induces the excessive release of the acetylation chain in chitin and stimulates its dissolvability in an alkaline solution, causing a

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Table 1. Chemical properties of *Moina* sp. chitosan.

Parameter	Percentage (%)		
	Shrimp Shell Chitosan	*Moina* sp. Chitosan (3N NaOH)	*Moina* sp. Chitosan (6N NaOH)
Yield	-	4.58	4.21
Protein	0.5	7.62	6.07
Ash	0.5	1.2	0.9
Fat	-	4.99	3.77
Moisture	8.5	7.63	1.64
Deacetylation degree	88.5	73.25	61.06

decrease in resulting mass. The yield of *Moina* sp. chitosan was relatively low, around 4.58%, when compared to chitosan from shrimp which ranges from 15.21–18% (Islam *et al*., 2011). Chitosan from *Moina* sp. has an indistinguishable yield amount from *Artemia* chitosan, which is 4.5% (Tajik *et al*., 2008), while the yield of chitosan extracted from *Daphnia similis* is around 9% (Tseng *et al*., 2021).

The protein residual content of *Moina* sp. chitosan was still relatively high on both treatments of 3N and 6N by 7.62% and 6.02% NaOH, when compared to shrimp shell chitosan protein residue of 0.5%. This is likely caused by the nonoptimal deproteinisation process, as the large quantity of protein residues is prone to be left behind. The remaining protein residue has a distinctive characteristic of chitosan from α-chitin which was predominantly found in endocuticles of crustaceans and difficult to remove even through the deproteinisation process (Lucas *et al*., 2021). The increase in NaOH concentration has been shown to reduce protein levels in *Moina* sp. chitosan by 1.6%, aligned with the research outcome by Zhao *et al*. (2004), which reported that higher concentrations and temperatures of NaOH would be more effective in the degrading rigid structure of the protein. In this study, chitosan treated with 3N NaOH was composed of 7.62% protein content and further decreased to 6.02% while treated with a higher concentration of 6N NaOH. Protein content optimally decreased with temperature assistance at 90°C during the deproteinisation process to accelerate the binding of the protein end chains by NaOH, so the protein degradation and deposition transpire perfectly (Pillai *et al*., 2009).

Ash content is associated with the demineralisation process's effectiveness in eliminating organic mineral content, such as calcium, phosphorus, and toxic minerals which are commonly found in crustacean chitosan (Al-Shaqsi *et al*., 2020). The demineralisation process requires acid treatment to reduce mineral content such as HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH solutions. Longer submerging time is apt to enhance its effectiveness in reducing mineral content, although it comes with a risk of losing more final yield. Two molecules of hydrochloric acid are stoichiometrically required to convert calcium carbonate into calcium chloride to complete the demineralisation process. The amount of acid solution is supposed to be comparable to or greater than the number of samples to be demineralised (Al-Shaqsi *et al*., 2020; Kou *et al*. 2021). Chitosan treated with 6N NaOH was opted as the best treatment having the lowest ash content of 0.9% in comparison with a lower concentration of 3N of 1.2% NaOH. This result depicted the optimal demineralisation process in the chitosan manufacturing with a little amount of mineral content remaining, although it was still higher when compared to the shrimp shell chitosan ash content of 0.5%. The escalating concentration of HCl solution during demineralisation may affect the optimising reactions of minerals to form soluble salts and CO_2 gas which can be separated from the mixture by forming air bubbles (Sinardi *et al*., 2013).

The fat content on the chitosan *Moina* sp. which was treated with 3N NaOH in the deproteinisation process was 4.99%, while 6N NaOH treatment portrayed lower fat content of 3.77%. The use of 6N NaOH concentration was proven to be able to reduce fat content more optimally when compared to 3N NaOH concentration. Fat degradation is likely affected by the acid concentration in the demineralisation process and the alkaline concentration during the deproteinisation process. The solution concentration has a proportional ability to denature proteins, fats, pigments, organic materials and discharge minerals (Cahyono, 2018).

Chitosan extracted from *Moina* sp. had a moisture content of 7.63% and 1.64% at respective concentrations of 3N and 6N NaOH, which was lower than the moisture content of shrimp shell chitosan by 8.5%. According to data from BSN (2013), the maximum amount of moisture content in chitosan is 12%, so the moisture content of *Moina* sp. chitosan was still within a reasonable range. The amount of moisture in chitosan needs to be kept at a minimum to optimally resist the microorganism growth (Rochima *et al*., 2004). The moisture content in chitosan was not affected by the concentration of NaOH and HCl solution, but affected by the drying process duration.

The deacetylation degree of chitosan deproteinated

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using 3N and 6N NaOH were 73.25% and 61.06%. The deacetylation degree of *Moina* sp. chitosan was lower than shrimp shell chitosan at 88.5%, but higher when compared to *Artemia urmiana* chitosan at 55% (Asadpour *et al*., 2007). According to Lv (2016), the deacetylation degree is conceivably utilized as a solubility indicator of chitosan. A deacetylation degree of 55-70% is defined as a low deacetylated degree of chitosan, which is almost completely insoluble in water. A deacetylation degree of 70-85% is considered a middle deacetylation degree of chitosan, which may partly dissolve in water. A high deacetylation degree of chitosan of 85-95% is identified as having good solubility in water, and a 95-100% deacetylated degree is called the ultrahigh deacetylation degree of chitosan, which is difficult to achieve. The deacetylation degree is allegedly affected by the amount of alkaline solution, extracting duration and reaction temperature. Higher concentration of NaOH and temperature increases during the deacetylation process will result in a higher deacetylation degree due to the lower molecular weight properties of the produced chitosan (Abuzaytun *et al*., 2005; Lv, 2016; Sheng *et al*., 2022). The outcome of this study portrayed a low deacetylation degree, caused by the incomplete degradation of the acetyl chitin group due to a high concentration of alkaline solution which restrains the amine groups formation that indicates chitosan solubility (Hargono *et al*., 2008).

3.2 Amino acids composition of Moina sp.

The amino acid proportion of *Moina* sp. was dominated by the non-essential amino acid of glutamic acid by 13.10% (Table 2), not too different from the research outcome from Khudyi *et al*. (2018) who found the major proportion of glutamic acid in *Moina macrocopa* was 16.69%. Another study on *Artemia* sp. amino acid reported an extremely higher glutamic acid content of 78,200 mg/kg compared to *Moina* sp. which was only 19,900 mg/kg (Anh *et al*. 2023). The essential amino acids composition, such as leucine, valine, and alanine were also found in *Moina* sp. by 11.12%, 9.72% and 9.42%, respectively (Table 2). These three amino acids belong to the branched-chain amino acids (BCAA) group, because of their distinctive aliphatic side chains with branched methyl groups. This type of amino acid is predominantly found in animals with skeletal and muscular characteristics which are apt to be catabolised and utilised for biosynthetic processes (Engelking, 2015). Each organism is composed of distinctive amino acid proportions which are heavily influenced by specific dietary feed, activity intensity and metabolic rate (Shimomura *et al*., 2015).

Table 2. Amino acids composition of *Moina* sp.

No	Amino Acid Profile	Content (mg/kg)	Percentage (%)
1	L-Glutamic Acid	19934.72	13.10
2	L-Leucine	16912.18	11.12
3	L-Valine	14788.57	9.72
4	L-Alanine	14383.18	9.45
5	L-Aspartic Acid	13943.36	9.17
6	L-Isoleucine	10902.83	7.17
7	Glycine	10236.67	6.73
8	L-Lysine	9280.21	6.10
9	L-Phenylalanine	8262.70	5.43
10	L-Threonine	7853.56	5.16
11	L-Arginine	6240.08	4.10
12	L-Proline	5873.59	3.86
13	L-Serine	5474.94	3.60
14	L-Histidine	4094.92	2.69
15	L-Tyrosine	3948.24	2.60

3.3 Antimicrobial activity of Moina sp. chitosan

The inhibition zone of *Moina* sp. chitosan against *S. aureus* bacteria exhibited greater diameter when treated with 6N NaOH than with 3N NaOH (Table 3). On the other hand, the chitosan inhibition zone against *E. coli* bacteria portrayed a larger inhibition zone diameter in the treatment with 3N NaOH compared to 6N NaOH (Table 4). According to Zheng *et al*. (2022), the antibacterial activity of chitosan is strongly influenced by the deacetylation degree value. A higher deacetylation degree is associated with lower molecular weight, which can prevent the osmotic transport of substances into cells while also absorbing anionic substances (DNA and RNA) in the cell body due to the positive charge of protonated amino groups. This causes flocculation which disrupts the normal physiological metabolism of cells and inhibits the microorganism's replication.

The results of the antibacterial activity in this study exhibited the enlarging diameter of the inhibition zone along with the increase of sample diluting concentration. Chitosan with highest diluting concentration of 100 µg/ disk generated the largest diameter of inhibition zone amongst all treatments compared to smaller sample diluting concentrations. In the assessment against *S. aureus,* the largest inhibition zone diameter on chitosan treated with 3N and 6N NaOH were 2.64±0.57 mm and 4.34±0.55 mm, considerably larger than shrimp shell chitosan inhibition diameter of 1.57±0.46 mm (Table 3). On the other side, the antibacterial activity against *E. coli* showed the largest inhibition diameter of each chitosan treated with 3N and 6N NaOH was 4.47±0.78 mm and 3.77±0.10 mm, which was slightly larger than shrimp shell chitosan inhibition diameter of 3.87±0.36 mm (Table 4). According to Mellegård *et al*. (2011), the effectiveness of antimicrobial performance on chitosan is

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Table 3. Diameter of inhibition zone by *Moina* sp. chitosan against *Staphylococcus aureus.*

Concentration	Diameter (mm)		
	Shrimp Shell Chitosan	*Moina* sp. Chitosan (3N NaOH)	*Moina* sp. Chitosan (6N NaOH)
100 µg/disk	1.57±0.46	2.64±0.57	4.34±0.55
50 µg/disk	0.84±0.21	1.80±0.25	2.97±0.00
25 µg/disk	0.60±0.21	1.60±0.30	2.37±0.20
12,5 µg/disk	0.54±0.06	1.20±0.20	1.87±0.62
6 µg/disk	0.44±0.12	1.04±0.12	1.47±0.10
3 µg/disk	0.44±0.06	0.84±0.26	1.14±0.15
1.5 µg/disk	0.37±0.1	0.47±0.79	0.84±0.21

Values are presented as mean±SD.

Table 4. Diameter of inhibition zone by *Moina* sp. chitosan against *Escherichia coli.*

Concentration	Percentage (%)		
	Shrimp Shell Chitosan	*Moina* sp. Chitosan (3N NaOH)	*Moina* sp. Chitosan (6N NaOH)
100 µg/disk	3.87±0.36	4.47±0.78	3.77±0.10
50 µg/disk	3.44±0.42	3.60±0.38	2.80±0.12
25 µg/disk	3.00±0.25	3.27±0.00	2.74±0.12
12,5 µg/disk	2.67±0.44	2.87±0.10	2.17±0.36
6 µg/disk	2.64±0.25	2.67±0.53	2.04±0.06
3 µg/disk	2.0±0.38	2.60±0.15	1.70±0.06
1.5 µg/disk	1.47±0.10	2.47±0.26	1.34±0.15

Values are presented as mean±SD.

highly dependent on its concentration, molecular weight, and biopolymer acetylated fraction. The concentration of chitosan imposes an important role in the capacity of antimicrobial activity, where the greatest antimicrobial effect on arthropod chitosan was started at a concentration of 250 µg/disk against *B. cereus* and *E. coli.*

Chitosan extracted from *Moina* sp. with the concentration of 3N and 6N NaOH profoundly exhibited a weak antibacterial activity, because no inhibition zone exceeded 5 mm in diameter. The largest diameter of the inhibition zone was opted from chitosan treated with 3N NaOH with diameter of 4.47±0.78 mm against the gram-negative bacterial strain of *E. coli*. According to Sumilat (2019), the ability of antibacterial activity is generally determined based on the inhibition zone diameter. The antimicrobial activity are classified into four groups, weak activity with inhibition diameter less than 5 mm, moderate activity which generates inhibition zone diameter of 5-10 mm, strong activity with diameter of inhibition around 10-20 mm, and antibiotic-alike activity with widest diameter of inhibition zone more than 20 mm.

The effectiveness of antibacterial ability may differ in accordance with the distinct bacterial isolate strains. In this study, the inhibition zones of shrimp shell and *Moina* sp. chitosan treated with 3N NaOH generated the largest diameter against the *E. coli* gram-negative strain. This is comparable with the research outcome by Zheng *et al*. (2022), who found a stronger antimicrobial effect on chitosan toward gram-negative bacteria like *E. coli* compared to gram-positive bacteria such as *B. cereus* and *S. aureus*. This happened due to the chitosan's ability to interfere with cell wall synthesis (such as β-lactams and glycopeptide agents) and inhibit bacterial metabolic pathways (Tenover, 2006). Kumar *et al*. (2006) added that the diameter of the inhibition zone is usually larger against gram-negative *E. coli* bacteria due to the main chitosan properties by having a positive charge of pH 6.3 to form polycationic structures, which can pierce through the thick outer membrane of gram-negative bacteria cells and inhibit anionic components syntheses such as lipopolysaccharides and proteins. However, chitosan-treated 6N NaOH had a larger diameter toward gram-positive bacterial strain of *S. aureus* which may happen due to miscellaneous factors such as the electronegativity of the cell surface which is affected by cell age, cell surface structure along with the chemical reaction rate which is affected by temperature, and protonation which is affected by pH value (Nurainy, 2008). Chitosan nanoparticle was also reported to have strong antibacterial activity while showing a synergistic effect with gentamicin, ampicillin, tetracycline, and streptomycin against *E. coli, S. aureus,* and *P. aeruginosa* (Bonde *et al*., 2012). In another study, chitosan was reported to be utilised as an alternative to antibiotics with larger concentration doses, due to differences in molecular weights that likely affect the effectiveness of bacterial growth inhibition. Therefore,

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chitosan solubility is considered an essential factor in determining its antimicrobial activity, as higher solubility guarantee permission to enter the bacterial cell without any difficulty and interact with the cell membrane of both gram-positive and gram-negative bacteria, causing conditions of inhibition (Mohanbaba *et al*. 2016).

4. Conclusion

Different concentrations of NaOH during deproteinisation have been shown to affect the chemical characteristics of *Moina* sp. chitosan. The antibacterial activity of *Moina* sp. chitosan showed fluctuating weak activity along with different concentrations of NaOH.

Conflict of interest

The authors declare no conflict of interest.

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