Heliyon 8 (2022) e11168

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Preparation, physicochemical properties and antimicrobial activity of chitosan from fly pupae

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HIGHLIGHTS

- Chitosan was prepared from fly pupae through an improved traditional method.
- The physicochemical properties corresponded with the characteristics of chitosan.
- Oligochitosan exhibited stronger antimicrobial activity than that of chitosan.

ARTICLE INFO

Keywords:
Chitosan
Fly pupae
Preparation process
Physicochemical properties
Antimicrobial activity

ABSTRACT

As an alternative chitosan source, edible insects have been proposed as an unconventional but viable option. Taking fly pupae as an example, this work performed chitosan extraction through a traditional chemical method with some modifications, and investigated its physicochemical properties and antimicrobial activity. The results showed that adding 0.5% sodium sulfite (Na_2SO_3, w/w, Na_2SO_3/fly pupae) synergized with sodium hydroxide (NaOH) for deproteinization was more effective than lye alone. Acid leaching was applied for desalination, and the optimal concentration of hydrochloric acid (HCl) was determined as 2 mol/L by ash content. For decoloration, the optimal decolorization oxidant was sodium hypochlorite (NaClO) with a concentration of 1.0%. For the deacetylation of chitin to chitosan, both the yield and degree of deacetylation (DD) using segmented treatment with alkali-NaOH were higher than those of traditional one-time deacetylation. The established physicochemical properties corresponded with the typical characteristics of chitosan. The determination of antimicrobial activity of chitosan by the turbidimetric method showed that chitosan exhibited notable activity in the order of *Staphylococcus aureus* > *Escherichia coli* > *Saccharomyces cerevisiae*, and this effect decreased with the increase in viscosity-average molecular weight (Mη). These results proved the viability of our improved method for the preparation of chitosan, a valuable antimicrobial agent, using an alternative natural source.

1. Introduction

Chitin, a linear polysaccharide made from polymerized β-(1–4)-N-acetyl-D-glycosamine, is considered the most abundant polysaccharide in nature after cellulose. It is commonly found in crustacean exoskeletons, fungal cell walls, and insect cuticles. Chitosan, described as a functional derivative of chitin, is a straight-chain polysaccharide composed of N-acetyl-D-glucosamine and D-glucosamine units, usually obtained by the partial deacetylation of chitin with NaOH [1]. In recent years, as a kind of highly valuable biopolymer material, chitin-chitosan has attracted more and more attention with potential applications in the food, cosmetics, composite materials, wastewater treatment, and biomedical industries due to its preferable properties including biocompatibility, biodegradability, non-toxic and bioactive nature, etc. [2].

At present, the raw materials for preparing chitosan are derived mainly from certain marine crustaceans such as shrimp, pen, lobster, and crab [3, 4, 5], and the relevant preparation process essentially includes deproteinization, desalination, decolorization, and deacetylation [4, 6]. According to the existing reports, shrimp and crab shells have a chitin content of 20%–30% and protein content of 30%–40% [7, 8]. In addition, some scholars found that the chitin content in some insects is also high at around 20%–50% of their dry weight [9], such as *Apis mellifera*, dung beetles, and cicadas at 30–40%, 28.7% and 36%, respectively [10, 11]. Moreover, insects are unconventional but feasible resources because of

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https://doi.org/10.1016/j.heliyon.2022.e11168
Received 13 January 2022; Received in revised form 14 March 2022; Accepted 5 August 2022

their high reproductive rate and ease of cultivation. Therefore, the extraction of chitosan from these resources has recently gained popularity; however, fly pupae have been hardly utilized. Fly pupae, the next development stage of housefly after larvae do not undergo shedding nor shrink after maturation in the process of metamorphosis. They are mostly barrel-shaped and colored brown and black, with high protein levels at about 52%–64% in dry weight, while the chitin content of puparia has been reported as 21%–33% [12, 13]. In this sense, this study aimed to use fly pupae to prepare chitosan by the above-mentioned established facile chemical treatment procedures with some modifications.

According to the traditional approach, chitosan is obtained by chemical methods that involve the use of strong alkalis, acids and oxidants to dissolve proteins, calcium salts, and pigments for deproteinization, deacetylation, desalination and decolorization [4, 14]. In this paper, the traditional preparation technology was employed with certain alterations, as described in Figure 1. Reductants were employed to synergize with alkali-NaOH instead of traditional lye alone for deproteinization of fly pupae. Meanwhile, the effect of different oxidants were compared to find the optimal decolorant in the decolorization process. In the deacetylation of chitin to chitosan, traditional one-time deacetylation using alkali-NaOH was also performed instead by segmented treatment with alkali-NaOH. As for the properties of chitosan, antimicrobial activity is the most representative one, which is greatly dependent on its physical characteristics, most notably Mη and DD [15, 16]. Hence, the degradation of prepared chitosan to oligochitosan was carried out with an emphasis on the effect of Mη on antimicrobial activity. The long-term purpose of this work was to exploit the chitin content of fly pupae as a novel resource and thus expand the source range of chitosan using this type of raw material.

2. Materials and methods

2.1. Materials and reagents

Fly pupae were purchased from Puyang Agricultural and Sideline Products Purchasing Station (Henan, China). Commerical chitosan was obtained from Zhejiang Golden-Shell Pharmaceutical Co., Ltd (Taizhou, China). Dithiothreitol (DTT), Na_2SO_3, sodium dithionite ($Na_2S_2O_4$) and thioglycolic acid ($C_2H_4O_2S$) were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and all reagents used were analytical grade.

2.2. Deproteinization

1.0 g of fly pupae powder was immersed in a 10% (w/v, g/mL) NaOH solution with a solid-liquid ratio of 1:20 (w/v, g/mL), and different reductants (DTT, Na_2SO_3, $Na_2S_2O_4$, $C_2H_4O_2S$) were added simultaneously in 1.0% proportion (w/w, reductant/fly pupae), synergized to react with lye for 2 h at 60 °C. To evaluate the extent of deproteinization, the protein content in the extract solution was determined according to the Kjeldahl method by calculating the removal rate using Eq. (1) for the optimal synergistic reductant. Meanwhile, the effects of different added amounts (0%, 0.3%, 0.5%, 0.7%, 1.0%, 1.5%) of optimal reductant were also investigated.

$$\text{Protein removal rate}\ (\%) = \frac{\text{Kjeldahl nitrogen mass}\ (g) \times 6.25}{\text{Raw material mass}\ (g)} \times 10 \quad (1)$$

2.3. Desalination

The salt-containing chitin prepared after deproteinization was washed with distilled water to neutrality, immersed in different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mol/L) of HCl solution and stirred at low speed with a magnetic stirrer to react for 1 h. After the reaction was completed, the chitin solid was collected by filtration and washed to neutrality, and its ash content was calculated by the fire weighing method using Eq. (2) to evaluate the desalination effect.

$$\text{Ash}(\%) = \frac{m_2}{m_1} \times 100 \quad (2)$$

where m_2 denotes the residual weight and m_1 denotes the initial weight of samples.

2.4. Decolorization

The chitin prepared after desalination was melted into 2% or 1% potassium permanganate ($KMnO_4$), NaClO, and hydrogen peroxide (H_2O_2) at a solid-liquid ratio of 1:20 for decolorization at 30 °C for 2 h, respectively. After decolorization, the chitin was filtered, washed with distilled water to remove the residual oxidant, and dried. Then, precise color quality control (PCQC) software (Konica Minolta China) was used to measure the whiteness of chitin for evaluating the decolorization effect of different oxidants, and commercial chitosan (DD >90%) was used as control.

2.5. Deacetylation

The chitin prepared after decolorization was immersed in a 20% NaOH solution at a solid-liquid ratio of 1:15 and left to react at 100 °C for 1 h. After the reaction was completed, the solid was obtained by filtration and immersed in a 50% NaOH solution for a secondary reaction. The solid was filtered again, washed to neutrality with distilled water, and freeze-dried to obtain crude chitosan. This was dissolved in 10% acetic acid solution with a solid-liquid ratio of 1:10 at 70 °C. After dissolution, the obtained solution was centrifuged at 8000 r/min for 15 min, and the supernatant was neutralized with 30% NaOH solution. Next, the chitosan was precipitated, the precipitate was centrifuged, washed with distilled water, freeze-dried, and weighed to calculate the chitosan yield by Eq. (3).

$$\text{Yield}\ (\%) = \frac{\text{Chitosan extraction}}{\text{Raw material}} \times 100\% \quad (3)$$

2.6. Degradation of chitosan to oligochitosan

The degradation process of chitosan to oligochitosan was conducted according to method of Li et al. [17] with some modifications. First, 1 g of chitosan powder was dissolved into 20 mL 3% acetic acid, then 10 mL 30% H_2O_2 was added to the chitosan solution. The degradation was assisted with microwave radiation at 800 W power for 10 min. Subsequently, the reaction mixture was cooled to room temperature and adjusted to pH 7.0 with 10 mol/L NaOH solution, and then precipitated by adding ethanol at 4 °C overnight to remove salts and the rest of H_2O_2. The precipitate was collected by centrifugation at 8000 rpm for 10 min and lyophilized to yield powdered oligochitosan.

2.7. Analysis of physicochemical properties

2.7.1. Determination of moisture and ash content

The moisture content was measured by drying the samples in an oven at 105 °C for 24 h, and the ash content was determined by burning the samples at 600 °C in a muffle furnace.

2.7.2. Determination of viscosity

In this step, 2 g of the sample was accurately weighed and immersed into 50 mL of 2% acetic acid, then stirred until completely dissolved for determination with a NDJ-79 rotary viscometer (China).

2.7.3. Determination of intrinsic viscosity and Mη

The average molecular weight of chitosan and oligochitosan were determined according to a previous study [6]. 1 g of powder sample was

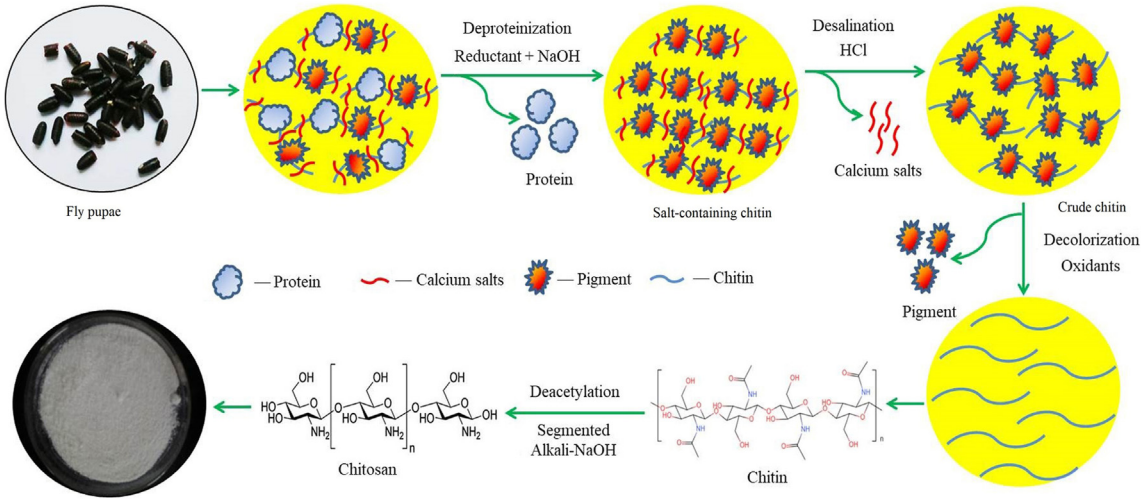

Figure 1. Chitosan prepared from fly pupae based on the traditional chemical extraction method with some modifications.

dissolved into 50 mL acetic acid (0.1 mol/L) - sodium chloride (0.2 mol/L) solution. Subsequently, the intrinsic viscosity was determined by a Ubbelohde viscometer. The Mη value was calculated according to the Mark–Houwink empirical formula $[\eta] = KM\eta^{\alpha}$, where $[\eta]$ means the intrinsic viscosity, and $K = 1.81 \times 10^{-3}$ cm^3/g and $\alpha = 0.93$ are constants that depend on the solvent-polymer system [18, 19].

2.7.4. Determination of DD

Alkalimetry was performed to determine the DD in chitosan samples. 0.2 g of lyophillized chitosan was dissolved into 25 mL of standardized HCl (0.1 mol/L), and the resulting solution was stirred continuously for 1 h. Subsequently, 2–3 drops of methyl orange was added as an indicator, then a standardized solution of 0.1 mol/L NaOH was used to neutralize excess acid by acid-base titration until the solution changed from red to orange-yellow. The calculation of the degree of deacetylation was obtained using Eq. (4).

$$DD\% = \frac{(C_1V_1 - C_2V_2) \times 0.016}{G(100 - W) \times 0.0994} \times 100\% \quad (4)$$

where C_1, C_2 respectively represent the concentration of standardized HCl and NaOH (mol/L); V_1, V_2 respectively represent the volume of standardized HCl and NaOH (mL); G represents the weight of the sample (g); W represents the moisture content of the sample (%); 0.016 is the amino equivalent to 1 mL hydrochloric acid of 1 mol/L (g); 0.094 is the theoretical amino content in chitosan (16/161).

2.7.5. Infrared spectroscopy

The Fourier-transform infrared (FTIR) spectra of prepared chitosan and oligochitosan were detected on a Bruker-Vector 22 spectrometer (German). The samples were mixed with KBr powder, ground, and pressed into 1-mm pellets in the frequency range of 4000–500 cm^{-1}.

2.8. Determination of antimicrobial activity

2.8.1. Microbial strains and their culture method

The microbial strains of *Escherichia coli, Staphylococcus aureus,* and *Saccharomyces cerevisiae* used in this study were obtained from Jiangsu Provincial Key Laboratory of Veterinary Bio-pharmaceutical High-tech Research (Taizhou, China). The *Escherichia coli* and *Staphylococcus aureus* strains were cultured at 37 °C, 200 rpm for 12 h in Luria-Bertani medium, and the *Saccharomyces cerevisiae* strain was grown in yeast peptone dextrose medium at 30 °C as seed broth.

2.8.2. Determination of antimicrobial activity

A total of 200 mg of the prepared chitosan and oligochitosan were separately weighed, dissolved in 0.4 mL of acetic acid, and then diluted with the corresponding above-mentioned media to 100 mL to prepare 0.2% chitosan solution. Then, the double broth dilution method was used to dilute the chitosan solution to the concentrations of 0.1%, 0.05%, 0.025%, 0.0125%, 0.00625%, separately. Next, 100 μL of seed broth was inoculated into 5 mL of the corresponding medium, followed by the addition of 5 mL of chitosan at different concentrations mentioned above

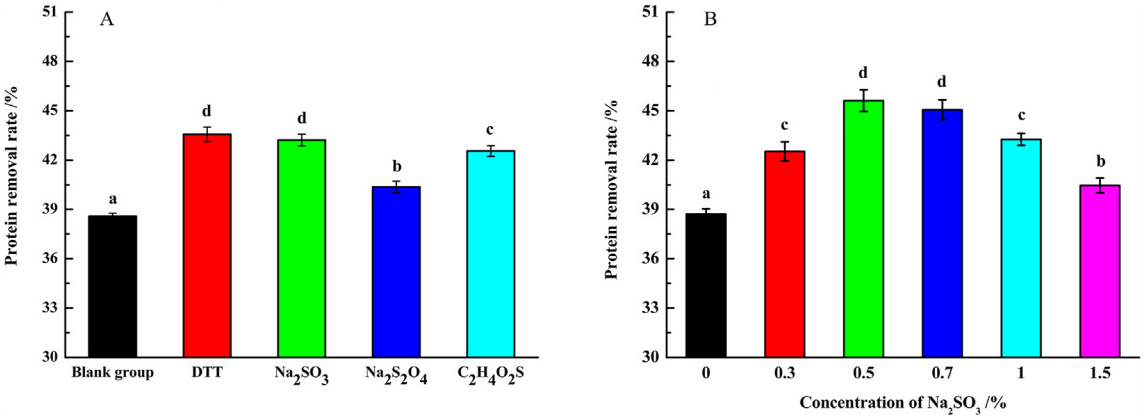

Figure 2. Effect of different reductants (A) and Na_2SO_3 concentrations (B) on the removal rate of deproteinization. Note: The bars without the same superscript (a–c) show significant differences ($P < 0.05$).

to culture at 37 °C (*Saccharomyces cerevisiae* at 28 °C) for 12 h. After culture, absorbance at 600 nm was measured by a spectrophotometer (UV2700, Shimadzu, Japan), and potassium sorbate and commercial chitosan (1.70×10^5 Da) were used as reference controls. Acetic acid medium was used as blank control.

2.9. Statistical analysis

All experiments were conducted in three replicates, and the results were repeated three times and expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was carried out to compare the mean values, followed by Duncan's multiple-range tests, and the significant differences in the mean values were defined as $P < 0.05$ (SPSS 22.0).

3. Results and discussion

3.1. Deproteinization

The solubility of native proteins is generally poor due to their heterogeneity and the influence of non-protein impurities. Therefore, it is usually not sufficient to remove proteins using alkaline solution alone, as the purity of chitosan will be affected. At present, the main principle to increase protein solubility is to destroy the interaction forces in protein-polymer conjugates by relevant reagents, including reductants, chaotropes, detergents, etc., such that the protein-polymer conjugates can be lysed into single polypeptides with high solubility. Among such reagents, reductants are the most common [20, 21]. The main reduction mechanism lies in the destruction of disulfide bond in the protein through a reaction to form sulfydryl, which will reduce the crosslinking degree, enhance the dispersivity of protein network structure, and improve the protein solubility [22, 23]. As shown in Figure 2, the protein removal rate increased significantly with the reductants synergized compared with the blank group of alkali-NaOH alone ($P < 0.05$). The comparison of different reductants revealed that the protein removal rates of DTT and Na_2SO_3 were significantly higher than that of other groups ($P < 0.05$), but no significant difference was found between the two groups ($P > 0.05$). Considering cost-effectiveness, Na_2SO_3 was selected as the optimal reductant synergized with alkali-NaOH for the deproteinization process.

Considering the effect of Na_2SO_3 in different doses on the deproteinization process, the result showed that the protein removal rate increased firstly and then decreased with the elevated dose of Na_2SO_3 synergized with alkali-NaOH. When the dose was 0.5%, the protein removal rate reached the peak value (45.61%). Compared with the blank group without Na_2SO_3 (38.71%), the efficiency increased by 17.82%, indicating that Na_2SO_3 could effectively promote protein dissolution and improve protein solubility. When the dose exceeded 0.5%, the protein removal rate decreased, which was caused by the breakage of excessive disulfide bond within peptide chains, such that the spatial structure of proteins expanded from a folded state to linear polypeptides, and the loose structure exposed many hydrophobic groups embedded in molecules, leading to protein interaction and aggregation and resulting in decreased solubility. Therefore, the optimal concentration of Na_2SO_3 synergized with alkali-NaOH was suitably controlled at 0.5% for deproteinization. Our experiment confirmed that the protein removal rate of fly pupae could be effectively improved by adding an appropriate amount of Na_2SO_3 to synergize alkali-NaOH.

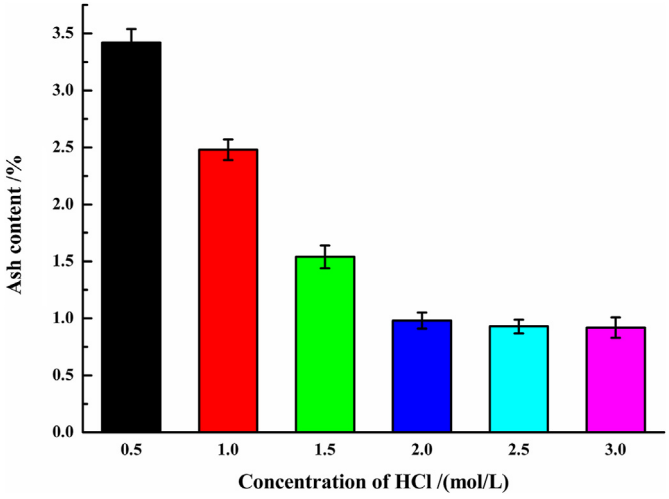

Figure 3. Effect of different concentrations of HCl on desalination.

3.2. Desalination

In nature, chitin is always closely associated with proteins, calcium carbonate and calcium phosphate, etc., resulting in its low solubility [24]. Therefore, these inorganic salts should be removed during the extraction process. Currently, the widely used method to achieve this is

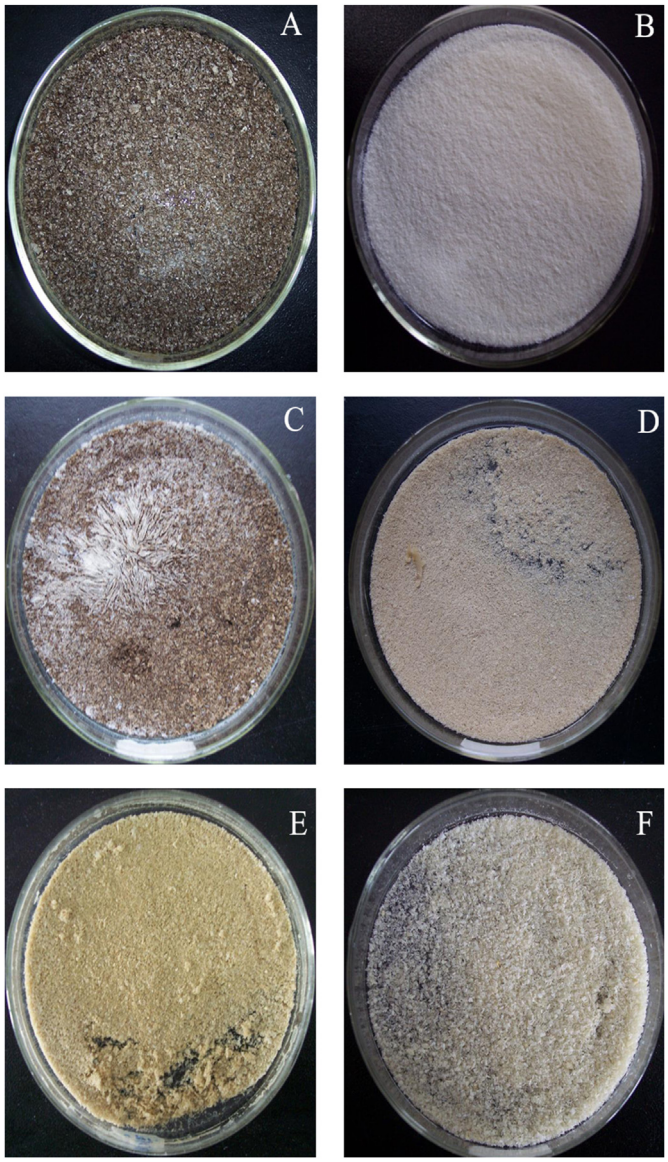

Figure 4. Effects of different oxidants on decolorization (A: without decolorization; B: commercial chitosan; C: 2.0% $KMnO_4$; D: 2.0% NaClO; E: 2.0% H_2O_2; F: 1.0% NaClO).

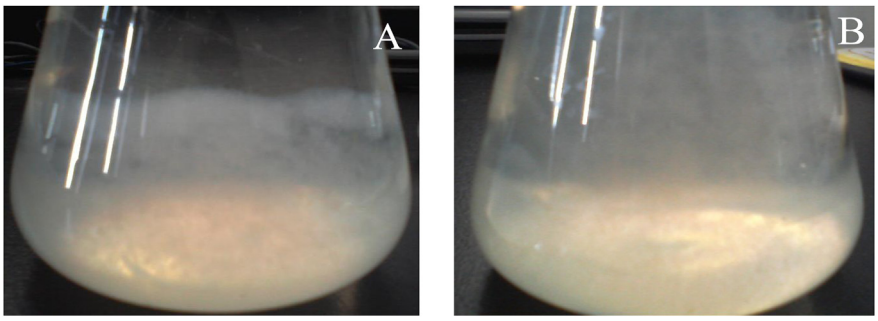

Figure 5. Comparison of deacetylation effect between traditional one-time (A) and segmented treatment (B) with alkali-NaOH.

Table 1. Physicochemical characterization of chitosan and oligochitosan prepared from fly pupae.

Samples	Moisture content (%)	Ash content (%)	Viscosity (mPa·s)	Intrinsic viscosity	Mη (Da)	DD (%)
Chitosan	2.46	0.93	505.32	36.12	4.20×10^4	88.29
Oligochitosan	2.17	1.08	96.52	4.81	4.80×10^3	91.35

acid leaching [4, 6, 10, 14]. As shown in Figure 3, when the concentration of HCl was controlled within 0.5–3 mol/L, the ash content decreased continuously with the increase in HCl concentration, which indicated that salt components were continuously dissolved. When the concentration of HCl exceed 2 mol/L, the ash content basically stabilized, suggesting that salt dissolution reached the limit. Moreover, too high a dose of HCl was not conducive to cost reduction and environmental protection. Therefore, the optimal concentration for the desalination process was selected at 2 mol/L, and the final ash content was determined to be 0.96%.

3.3. Decolorization

In this part, the decolorization effects of three common oxidants $KMnO_4$ [10], NaClO [4] and H_2O_2 [12] are compared. As shown in Figure 4, at a concentration of 2% $KMnO_4$, the decolorization whiteness was 14.28%, which was lower than that of NaClO (23.09%) and H_2O_2 (21.21%). $KMnO_4$ solution itself is purplish red and dark, and fly pupae were easily stained by the $KMnO_4$ solution during decolorization, so its decolorization effect was weaker than that of NaClO and H_2O_2. Morever, if $KMnO_4$ was not removed completely in the later stage, the color of prepared chitin would be deepened. Among the three oxidants, the decolorization effect of NaClO was the best. However, it should be noted that when the concentration of NaClO was too high, the oxidation was too intensive, and the raw materials were easily denatured. On the other hand, when the concentration was too low, the oxidative decoloration would not be completed. Generally, the concentration of 0.5%–1.0% was suitable [25]. The results showed that the decolorization effect of 1.0% NaClO was the most optimal one, with a detection whiteness of 36.90%, which was 1.60 times of that for the concentration of 2%. Therefore, it was more suitable to select NaClO as the oxidant for the decolorization process, with its concentration controlled at 1.0%.

3.4. Deacetylation

The traditional one-time deacetylation method usually uses strong alkali-NaOH at a high concentration to remove the acetyl group of chitin; however, is difficult to prepare a chitosan product with high DD [26]. In this experiment, deacetylation was carried out using segmented treatment with alkali-NaOH, so that the residual chitin or chitin with low DD

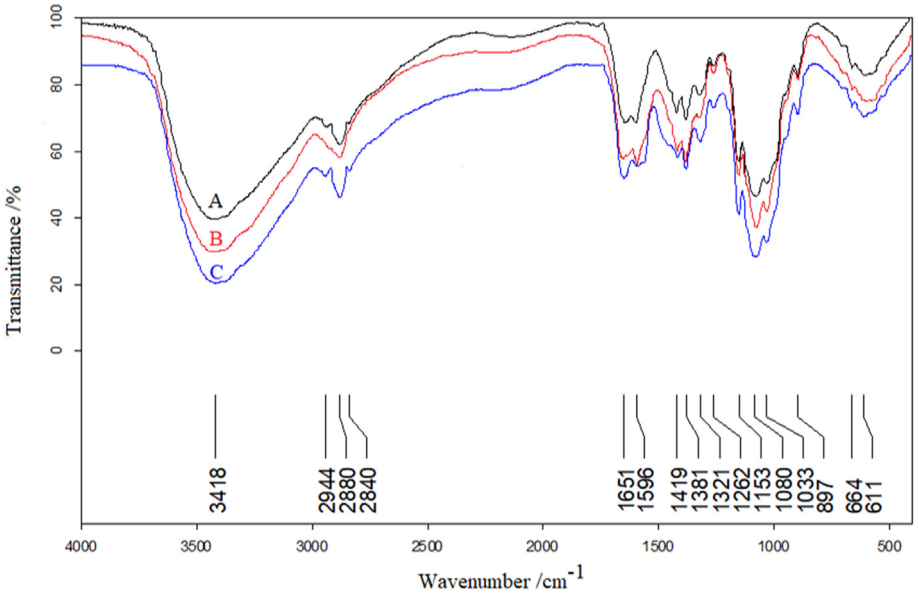

Figure 6. FTIR spectra of chitosan (A), oligochitosan (B) prepared from fly pupae, and commercial chitosan (C).

could be deacetylated more thoroughly, and high-quality chitosan could be obtained. The solid obtained by deacetylation with two segmented treatments of strong alkali–NaOH was crude chitosan, which also contained a lot of impurities. According to the property of chitosan of being soluble in dilute acid but insoluble in alkali, partial impurities could be removed by firstly dissolving the prepared chitosan in acetic acid, and then neutralizing and precipitating it with alkali-NaOH solution. As shown in Figure 5, the yield of chitosan by deacetylation using segmented treatment with strong alkali-NaOH was obviously higher than that of traditional one-time treatment, determined as 3.22%, which was higher than that of the traditional deacetylation method (2.42% when only treated by 50% NaOH), and the DD between the two methods was also determined as 88.29% and 68.55%, respectively, indicating that segmented alkali-NaOH treatment for deacetylation was more effective than the traditional method.

3.5. Analysis of physicochemical properties

Table 1 shows the physicochemical properties of prepared chitosan and oligochitosan prepared from fly pupae. It was found that the moisture content of prepared chitosan and oligochitosan was 2.46% and 2.17%, respectively. In general, lower moisture content causes higher thermal stability due to the fact that water molecular is adsorbed on the polymeric chains, exerting a plasticizing effect on the thermal stability and structure [27]. Ash content, an important index for evaluating the purity of chitosan, was lower in the chitosan from fly pupae (<1%) when compared to commercial chitosan (2.2 ± 0.6%) (considered pure) [9], up to the quality requirement of chitosan; low ash content is also a key factor for the better solubility of chitosan [28]. As previously reported, the viscosity of chitosan is closely related to Mη; the higher the Mη, the higher the viscosity [29, 30]. The viscosity obtained for chitosan before degradation was 505.32 mPa s with 4.20 × 10^4 Da, whereas that of oligochitosan obtained after degradation was 96.52 mPa s with 4.80 × 10^3 Da. The DD is a significant parameter of chitosan, influencing its biological, physicochemical, and mechanical properties dependent on the method of extraction [31]. In our study, the DD of inital and degraded chitosan was 88.29% and 91.35%, respectively, which was much higher than that of chitosan from traditional material shell waste reported in the relevant literature [4, 32, 33].

According to the analysis of FTIR spectra, as shown in Figure 6, both chitosan and oligochitosan had similar characteristic absorption peaks as commercial chitosan. The broad and intense band at 3418 cm^{-1} was due to the stretch vibration of N–H. The signal at 2944–2840 cm^{-1} was assigned to the stretching of C–H bond, which is similar to the typical characteristic absorption peaks of sugar. The signals at 1651 cm^{-1}, 1596 cm^{-1}, 1321cm^{-1} were attributed to the amide I, II and III band, respectively, especially at 1596 cm^{-1}, which indicated that the DD of chitosan was higher than 70%. The signals at 1419 cm^{-1} and 1381 cm^{-1} were attributed to the bending of $–CH_2$ and the deformation of $–CH_3$, respectively. The signal at 1153 cm^{-1} was the asymmetrical stretching of C–O–C, the signals at 1080 cm^{-1} and 1035 cm^{-1} were the stretching of secondary and primary alcohol hydroxyl (C–O), respectively, and the signal at 897 cm^{-1} was indicative of the stretching of ring [34].

3.6. Antimicrobial activity

Several factors have been reported to influence the antibacterial activity of chitosan, including chitosan Mη, DD and positive charge content, the temperature and pH of the reaction conditions, individual microbial structural characteristics, and cell age [35]. The most notable of these are DD and Mη. In this study, as shown in Figure 7, the antimicrobial effect of chitosan with low Mη was significantly higher than that of chitosan with high Mη. The reason might be that chitosan with high Mη is mainly adsorbed on the cell surface to form a polymer film to prevent the transportation of nutrients into the cell, thereby playing a bacteriostatic and bactericidal effect [36]. Meanwhile, chitosan with low Mη not only prevents the transportation of substances, but also can enter the cell through osmosis, and adsorb anionic substances (DNA and RNA) in the cell body due to the positive charge of protonated amino group. This leads to flocculation that interferes with the normal physiological metabolism of the cell and in turn inhibits the reproduction of microorganisms [37, 38]. Furthermore, it is known that chitosan degradation occurs during the deacetylation reaction. This commonly involves molecular weight decrease and the gradual increase of deacetylation degree

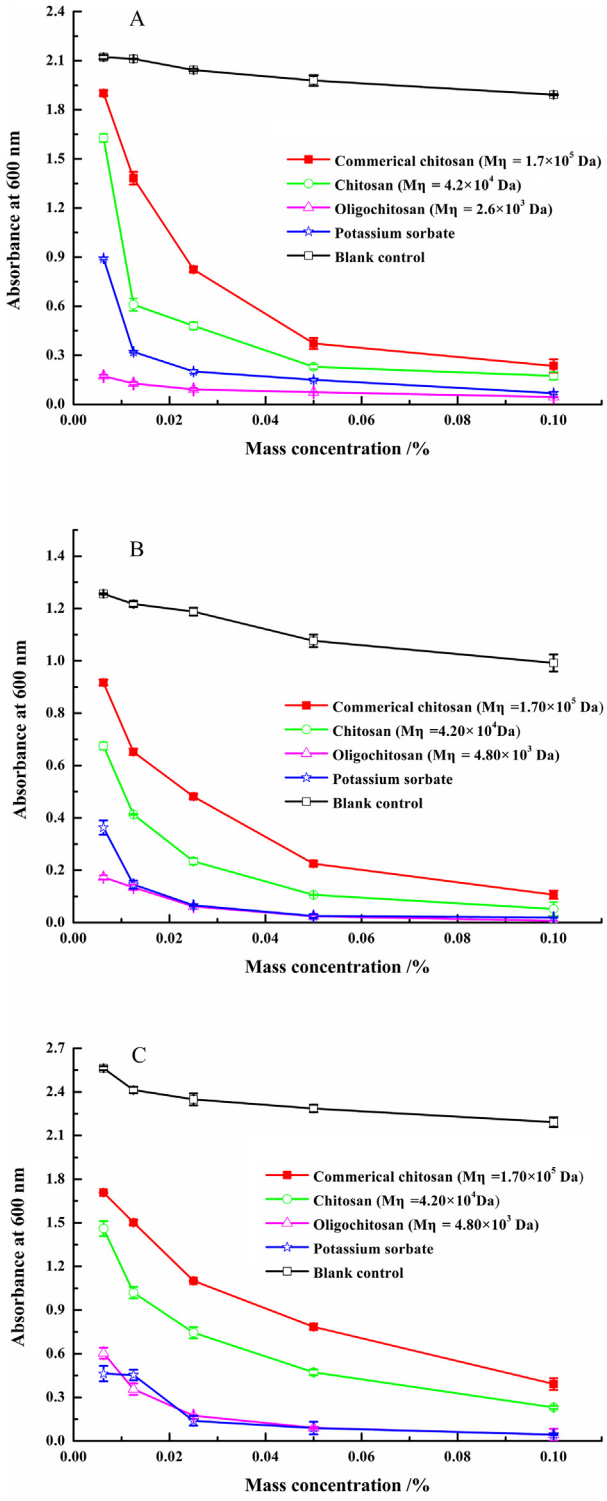

Figure 7. Antimicrobial activity of chitosan and oligochitosan prepared from fly pupae (A: *Escherichia coli;* B: *Staphylococcus aureus;* C: *Saccharomyces cerevisiae*).

[39]. The DD of chitosan has an important effect on the antimicrobial properties of chitosan; the higher the DD, the higher the positive charge after the amino protonation of chitosan, and the stronger its antimicrobial activity [40]. Therefore, the inhibitory effect was stronger than that of chitosan with high Mη. When the chitosan concentration reached 0.025%, chitosans with different Mη values all showed an obvious antimicrobial effect, especially low-Mη oligochitosan with 4.80×10^3 Da, which almost completely inhibited the growth of microorganisms. The antimicrobial effect on *Escherichia coli* was even stronger than that of potassium sorbate, a commonly used food preservative, which might indicate that low-Mη oligochitosan could be used as a natural preservative. Meanwhile, other chitosan samples required larger concentrations to inhibit bacterial growth completely. The inhibitory effect of chitosan with different Mη levels against the three selected microorganisms was in the order of *Staphylococcus aureus* > *Escherichia coli* > *Saccharomyces cerevisiae*, indicating that chitosan's ability to inhibit Gram-positive bacteria was better than that for Gram-negative bacteria and fungi, which was consistent with the reports of No et al. [41] and Jeon et al. [42].

4. Conclusions

In this work, the preparation process, physicochemical properties and antimicrobial activity of chitosan from fly pupae were studied under modified extraction conditions. The results indicated that the optimal process for chitosan extraction was as follows: adding 0.5% Na_2SO_3 synergized with alkali-NaOH for deproteinization, 2 mol/L HCl for desalination, NaClO of 1.0% for decolorization, and segmented treatment with alkali-NaOH for deacetylation. The physicochemical properties including moisture, ash content, viscosity, Mη, DD, and infrared spectrum were determined. The quality and chemical properties of both chitosan and oligochitosan were highly performant and representing commercial value. The analysis of FTIR spectra showed the typical characteristic absorption peaks of chitosan. The antimicrobial activity of chitosan was obvious against Gram-positive and Gram-negative bacteria, as well as fungi, and closely related to its Mη value; the lower the Mη, the higher the activity. Accordingly, this study proposes an improved strategy based on the traditional chemical extraction method and broadens the source of raw materials for preparing chitosan.

Declarations

Author contribution statement

Zhicun Sheng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ainan Guo: Conceived and designed the experiments; Contributed analysis tools or data.

Jing Wang: Performed the experiments; Analyzed and interpreted the data.

Xiaolan Chen: Conceived and designed the experiments; Contributed reagents, materials; Wrote the paper.

Funding statement

This work was supported by the National Natural Science Foundation of China (31902317), Project of Qinglan Engineering of Jiangsu Province (2021), Agricultural Project of Taizhou City (TN202006), and Project of Jiangsu Agri-animal Husbandry Vocational College (NSF2022ZR05).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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