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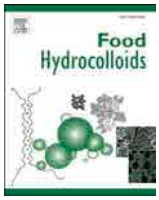

Study on emulsification property of chitosan with strong antibacterial activity

Junqing Qian *, Junlan Yin, Peilong Ma, Chenghong Mo, Yan Chen, Hui Guo

College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, 310000, China

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ABSTRACT

Chitosan of different molecular weight had a large difference in antibacterial activity. Study on emulsification property of chitosan with stronger antibacterial activity, which beneficial to expand the application of chitosan in the field of food. Chitosan with series molecular weights were prepared by acid degradation, the molecular structure of degraded chitosan was characterized by infrared spectroscopy, X-ray diffraction and nuclear magnetic detection, indicating that the pyran ring of chitosan was not destroyed after acid degradation. Through antibacterial activity and minimal inhibitory concentration of chitosan with different molecular weights were measured, chitosan with molecular weights of 52.5-167 kDa had strong antibacterial activity. The emulsification performance evaluation showed that the emulsifying effect of chitosan with strong antibacterial activity was similar to chitosan with higher molecular weight, the emulsion droplet size of oil was smaller, and the emulsifying stability was similar under different pH, temperature and ionic strength. The solution viscosity of chitosan with strong antibacterial activity was suitable for application in food emulsification, and the viscosity of emulsion system had higher stability. The correlation between emulsification and antibacterial activity of chitosan with strong antibacterial activity was investigated through simulated emulsifying, the chitosan concentration 0.5 g/L, pH 6.0, and emulsification time 20 min, significant antibacterial activity could be shown. The morphological distribution of chitosan with strong antibacterial activity in simulated emulsification was detected, and it was found beneficial to antibacterial. The results of the study provided new strategies for the application of chitosan as an emulsifier in the food field.

1. Introduction

Chitosan is a safe and non-toxic natural cationic polysaccharide with good thickening properties (Soares LS et al., 2019), which has been widely used as a food thickener. Chitosan has antibacterial activity (Martau GA et al., 2019), and biological affinity (Hu ZY et al., 2019), which can be used for food preservation (Sugiyanti D et al., 2018). Besides, chitosan can be employed as coating material to prolong the storage time of food, due to have antibacterial effect (Li JH et al., 2020). Chitosan is added to food can improve stability, and act as emulsifier (Li JH et al., 2019). Chitosan composited with other materials such as starch can improve emulsifying stability in food system (Basit HM et al., 2020), that has a variety of applications in the food industry.

The natural chitosan has higher molecular weight, reaching more than 700 k Da, has higher viscosity compared to other natural polysaccharides (Eduardo MF et al., 2014). Chitosan with high molecular weight can increase solution viscosity (Klinkesorn U., 2013). The emulsification property of chitosan correlate with its solution viscosity (Rodriguez MS et al., 2002), both excessively high or low viscosity impair emulsion stability (Bhattarai N et al., 2005). The antibacterial activity of chitosan not only correlates with molecular weight but also shows association with solution viscosity (Motiei M et al., 2017).

Extensive studies have been conducted on the correlation between chitosan molecular weight and antibacterial activity. Prasertsung reported chitosan with molecular weights of 55-155 kDa had strong antibacterial activity (Prasertsung I et al., 2012), while literature reported that chitosan with molecular weights of 79.5-128.5 kDa had strong antibacterial activity against *C. albicans* (Silva GLG et al., 2018). Liu (Liu XL et al., 2020) founded that chitosan with molecular weights of 42.5~135 kDa had strong antibacterial activity. Liu N (Liu N et al., 2006) observed that chitosan with 130 kDa molecular weight can complete inhibited *B. cereus* and *V. parahaemolyticus* at 125 ppm. Bernabe (Bernabé et al., 2020) reported that low-viscosity chitosan can prevented the formation of *S. epidermidis* biofilm, and exhibited strong

* Corresponding author.
E-mail address: qjq_2023@163.com (J. Qian).

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antibacterial activity. Younes (Younes I et al., 2014) reported chitosan with molecular weights of 104-201 kDa were found to have strong antibacterial activity against Listeria、 *Staphylococcus aureus*, and *Pseudomonas fluorescens*. Li (Li JH et al., 2020) founded that the film made of low molecular weight chitosan had strong antibacterial activity and can be used as food preservative material. Minh and Dragland reported that chitosan with low molecular weight had lower viscosity, enhancing the adhesion to the cell membrane (Minh NC et al., 2018), and the penetration to the cell membrane (Dragland IS et al., 2016), which improved the antibacterial activity. The viscosity of the low molecular weight chitosan solution was reduced, and chitosan was easy to penetrate into the bacterial biofilm, gave play to strong antibacterial activity (Wang WQ et al., 2020). But, Jeon (Jeon Y., 2001) reported that chitosan with molecular weight below 10 kDa did not exhibit antibacterial activity. Summarizing the above series of literature reports, the molecular weight range of chitosan with strong antibacterial activity was basically 42.5~55 kDa.

Chitosan had significant application value as an emulsifier. The emulsification property of chitosan was related to the molecular weight (Asfour MH et al., 2017). Chitosan with molecular weights of 410~600 kDa had good emulsification property, and compared with chitosan with strong antibacterial activity, had higher molecular weight (Li & Xia, 2011). The emulsification property of chitosan composite with casein were investigated, the emulsion property was better with low molecular weight chitosan, which was founded the viscosity was the main influence factor for the emulsion system (Zhang F et al., 2021). Tabatabaei (Tabatabaei M et al., 2022) reported that fatty acids attached to chitosan with molecular weights of 50-190 kDa can enhanced emulsification. The emulsification property of modified chitosan was reported, which improved the water solubility of chitosan, reduced the viscosity of solution, can conducive emulsifying stability(Chung C C et al., 2022). The study of the above literatures shown that chitosan with lower molecular weight can reduce viscosity of solution, and enhance emulsification (William, W M et al., 2016).reported that chitosan particles with small particle size had higher emulsion stability, as well as, chitosan with lower molecular weight can be used to prepare nanoparticles (Seo S et al., 2008). Based on the above literatures reported, chitosan was applied to emulsification, the molecular weight and the viscosity of solution were important influence elements, chitosan with lower molecular weight was beneficial to emulsification stability.

The literature reported that the molecular weight range of chitosan with strong antibacterial activity was about 42.5~155 kDa, the viscosity of solution was significantly lower than natural chitosan (Benchamas G et al., 2021). Based on the literature of research on emulsification property of chitosan, there was no reported on the study of the properties in oil-in-water (o/w) classic emulsions and multilayer emulsions of chitosan with 42.5~155 kDa molecular weights(Yang Y et al., 2023). The classic emulsions were widely used in the food industry, to explore chitosan with strong antibacterial activity as emulsifier of classic emulsions, which can play a role in the antibacterial effect of chitosan, it was great significance for food processing and improving safety of food. The preparation of microparticles with chitosan to improve the stabilization of oil-in-water (O/W) Pickering emulsions had reported in literature study (Tian H et al., 2019; William, W M et al., 2016). Literature (Pan CL et al., 2020) reported that chitosan with molecular weights of 50~100 kDa was prepared nanoparticles by ion cross-linked with particle size of nearly 100 nm and uniform distribution. So chitosan with molecular weight range of strong antibacterial activity was prepared microparticles were promising to obtain smaller and uniformly distributed particles, which were beneficial improving the stability of Pickering emulsions. Therefore, it was well worth to study the property of chitosan with strong antibacterial activity as food emulsifier.

In this study, a series of chitosan with different molecular weights were prepared by degradation with dilute hydrochloric acid. The molecular structure of the degraded chitosan was characterized using fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and nuclear magnetic resonance (NMR). Chitosan with stronger antibacterial activity was obtained by detection antibacterial activity, and the performance of oil-in-water (o/w) classic emulsions was explored for chitosan with strong antibacterial activity. According to the reported on the correlation between the emulsification performance and the viscosity of solution of macromolecular emulsifier(Liu J et al., 2021), the viscosity change of chitosan solution within the range of emulsification concentration was evaluated. The correlation between the chitosan with strong antibacterial activity application for emulsifying and antibacterial effects was explored. The results of the study provided new strategy for the application of chitosan as a food emulsifier.

2. Materials and methods

2.1. Materials and reagents

Chitosan (728.5 k Da, deacetylation degree: 94.0 %) was purchased from Zhejiang Golden Shell Biochemistry Co., Ltd. *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S.aureus*) and *Candida albicans* (*C.albicans*), provided by the Institute of Biotechnology, Zhejiang University of Technology. LB agar and Sabouraud agar medium were purchased from Hangzhou Microbial Reagent Co., Ltd., all reagents are domestic analytical pure or biochemical reagents.

X-ray diffractometer, PNAlytical, Netherlands. Fourier transform infrared spectrometer (Tensor 27),BRUKER, Germany. nuclear magnetic resonance spectrometer (AVANCE III 500 MHz), BRUKER, Germany. Particle size analyzer (Zetasizer Nano S90), Malvern. Homogenizer (AH-BASIC), ATS Engineering Limited. Rotational viscometer (NDJ-5S), Shanghai China. LABNCO Freeze Dryer, Beijing Zhaoshenghang Instrument Equipment Co. Ltd. China。

2.2. Preparation of chitosan with a series molecular weight and detection of antibacterial activity

2.2.1. Preparation of a series of different molecular weight chitosan

1.0 g chitosan (728.5 kDa) was accurately weighed and then added with 100 mL 0.096 M HCl solution, fully stirred in oil bath at 105 °C, chitosan with different molecular weights could be obtained through the control of degradation time. On the basis of the method mentioned in literature (Chen RH and Hua HD., 1996), the intrinsic viscosity[η] of chitosan was measured with a 0.5 mm Ubbelohde viscometer, and the viscosity-average molecular weight Mv of chitosan was calculated from the intrinsic viscosity. The correlation between molecular weight and viscosity of chitosan was investigated. The Mv is calculated by the following formulas (Chen RH and Hua HD., 1996; Yang TC et al., 2005):

$$[\eta] = K \times Mv^{\partial} \quad \text{Eq (1)}$$

$$K = 1.64 \times 10^{-30} \times DD^{14} \quad \text{Eq (2)}$$

$$\partial = -1.02 \times 10^{-2} \times DD + 1.82 \quad \text{Eq (3)}$$

where K and ∂ are Mark-Houwink constants, which are related to polymer type, solvent type and temperature. [η] is the intrinsic viscosity; Mv is the viscosity average molecular weight of chitosan; DD is the degree of deacetylation of chitosan.

2.2.2. Structural characterization of low molecular weight chitosan

The chitosan raw material (Mv: 728.5 kDa) and two low molecular weight chitosan (Mv: 102 kDa, 48.5 kDa) obtained by dilute hydrochloric acid degradation were selected for comparative characterization. The structure was identified by infrared spectroscopy (resolution of 4 cm^{-1}, scanning range of 4000-400 cm^{-1}, scanning 13 times). The structure of low molecular weight chitosan obtained by degradation was analyzed by XRD and NMR. The ^{1}H NMR spectra was detected with $CDCl_3$ as solvent, and the spectra were analyzed by MestReNova

software.

2.2.3. Antibacterial experiment of chitosan with a series of different molecular weight

2.2.3.1. Determination of antibacterial activity of chitosan with different molecular weight. The determination method of antibacterial activity depended on the literature (Pan CL et al., 2020). The culture medium to be sterilized at 121 °C for 20 min was poured into the culture dish, after solidification, the bacterial solution was inoculated on the culture medium, then sealed and cultured at 37 °C for 24 h. *E. coli*、*S. aureus* and *C. albicans* were selected as the test bacteria, and colonies of bacteria were picked and put into the culture, and cultured at 30 °C and 200 r/min for about 18 h, controlling the bacterial concentration at $8.96 \times 10^6 \sim 1.24 \times 10^7$ CFU/mL, for subsequent use.

A series of molecular weight chitosan was prepared into 0.75 g/L solution with 0.1 M CH_3COOH, added to a sterile tube, adjusted to pH 6.0, and 0.1 mL bacterial solution($8.96 \times 10^6 \sim 1.24 \times 10^7$ CFU/mL) was added to a sterile tube and mixed for 30 min. 0.2 mL of the above mixture was diluted to the optimal concentration, coated on the agar plate, cultured at 37 °C for 24 h, the 0.1 M CH_3COOH aqueous solution was used as a control, the antibacterial rate (CFU %) of the sample was calculated according to the following formula.

$$CFU\% = (B\text{-}A)/B \times 100\% \quad \text{Eq (4)}$$

In the formula, A is the number of bacterial colonies (CFU/mL) on the test plate, and B is the number of colonies on the control plate (0.1M CH3COOH aqueous solution adjusted to pH 6.0 with ammonia). The same concentration was repeated three times, and the number of bacterial colonies was the average number of bacterial colonies on three plates.

2.2.3.2. Determination of minimum inhibitory concentration. The minimum inhibitory concentration (MIC) was determined by turbidity method and broth dilution method with sterilized water as blank control (Schön T et al., 2020; Zhang YH et al., 2010). Chitosan with different viscosity-average molecular weight was selected, and deionized water with pH 6.0 was added to prepare a series of solutions with different concentrations of 300-20μ g/mL. 0.5 mL of each solution was added to 5 mL of liquid medium, and 50 μL of bacterial solution (10^5-10^6 CFU/mL) was added. The bacterial and fungal sample tubes were placed in an air shaker at 37 °C and 28 °C respectively, and cultured at 180 rpm for 24 h. The concentration when there was no visible substance in the tube under the microscope was the MIC of chitosan.

The molecular weight range of chitosan with strong antibacterial activity was determined according to the antibacterial activity and the minimum inhibitory concentration of chitosan with different molecular weights, chitosan with strong antibacterial activity was obtained for the study of emulsification property.

2.3. Evaluation of emulsification property of chitosan with molecular weight range of strong antibacterial activity

2.3.1. Determination of emulsification property of chitosan with molecular weight range of strong antibacterial activity

Referencing literature method of emulsification performance detection (Guo B et al., 2020), mix 5% soybean oil with 95% solution of chitosan (0.1% and 0.4% (w/v) with 0.1M acetic acid), total of 20 ml, high speed cutting at 12000 rpm for 3 min, followed by high pressure homogenization at 80 MPa for 3 times, to produce emulsion, detection the stability time of the emulsion. Adopting particle size analyzer, the particle size of the droplets in chitosan emulsion is determined. The emulsion effect of oil-water system of chitosan with strong antibacterial activity and the chitosan with different molecular weight is compared. According to the comparative results, the emulsification effect of chitosan with strong antibacterial activity at different concentrations (0.1、0.5、1.0、1.5 g/L) are tested.

2.3.2. Investigation of emulsification stability of chitosan with molecular weight range of strong antibacterial activity

Referencing literature method of emulsification performance detection (Guo B et al., 2020), to make appropriate modifications, the emulsion stability of chitosan with strong antibacterial activity under different pH, different ionic strength (different NaCl concentration) and different temperature are inspected. Referencing literature method of evaluation emulsion stability (Fang Zhang, Xiong, et al., 2021), the emulsion stability is investigated by calculation: CI (%) = HS/HE × 100%. In the formula, CI: Creaming Index; HS: Height of serum layer formed at the test tube bottom; HE: Total emulsion height in the test tube (mix 5% soybean oil with 95% solution of chitosan, to form creamy emulsion, total of 20 ml).

2.4. Determination of viscosity and evaluation of viscosity stability within the range of emulsification concentration of chitosan with strong antibacterial activity

According to the detection results of the emulsification property of chitosan with strong antibacterial activity, the viscosity of chitosan with stronger antibacterial activity is compared with chitosan with other molecular weight within the range of concentrations to achieve stable emulsifying. The relative viscosity is detected by rotating viscometer, according to the detection results, the viscosity of chitosan with strong antibacterial activity and chitosan with other molecular weight influence on emulsification is explored, and compared stability of viscosity of chitosan with different molecular weights.

2.4.1. Determination of viscosity

According to the results of antibacterial activity test of chitosan, different molecular weight chitosan (Mv: 48.5, 126, 284, 728 kDa) with strong or weak antibacterial activity were selected for the preparation of chitosan solutions with concentrations of 0.1 g/L, 0.5 g/L, 1.0 g/L and 1.5 g/L with 0.1 M acetic acid, and shear viscosity was measured by rotating viscometer No.3 rotor at room temperature(20 °C).

2.4.2. Evaluation of viscosity stability

For the evaluation of viscosity stability of 126 kDa chitosan with strong antibacterial activity and 48.5 kDa and 284 kDa chitosan, and the difference in viscosity stability was compared. The shear viscosity of various samples was measured by rotating viscometer No.3 rotor. The chitosan with three molecular weights dissolved in 0.1M acetic acid to prepare solution with concentration of 1.00 g/L and 0.50 g/L respectively, and the pH was adjusted at room temperature(20 °C), the viscosity were detected, and the viscosity were detected under different temperature (pH 6.0), and the sodium chloride was added with different concentration (w/v) respectively to improve ion strength, the viscosity were detected at room temperature(20 °C)and pH 6.0.

2.5. Experiment of antibacterial effect of chitosan with strong antibacterial activity under simulated emulsification conditions

2.5.1. Test of antibacterial activity of chitosan with strong antibacterial activity under simulated emulsification conditions

According to the results of the antibacterial activity test of 2.2.3, three chitosan samples with strong antibacterial activity (Mv = 52.5, 126, 145 kDa) were selected and dissolved in 0.1 M CH_3COOH to prepare a solution with a concentration of 1.0 g/L, and deionized water was added to stir evenly to simulate emulsion of chitosan. according to the suitable emulsification concentration of 2.3.1, chitosan emulsion was prepared with concentrations of 0.125, 0.25, 0.5 and 0.75 g/L, respectively. The pH was adjusted to 6.0, autoclaved at 121 °C for 20 min, cooled and mixed with 1.0 mL bacterial solution, and high speed cutting

at 12000 rpm for 3 min with 3 times, forming emulsion, as simulated emulsification. The antibacterial rate was measured after simulated emulsification 20 min, the antibacterial activity of chitosan at different concentrations was investigated.

According to above test method, three chitosan samples (Mv = 52.5, 126, 145 kDa) were dissolved in 0.1 MCH_3COOH to prepare solution of 0.5 g/L (bacteria) and 1.0 g/L (fungi). The pH was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5 respectively, and high speed cutting at 12000 rpm for 3 min with 3 times, forming emulsion, as simulated emulsification. The antibacterial rate was measured after simulated emulsification 20 min. The relationship between the antibacterial activity of chitosan with pH of emulsion was investigated.

According to above test method, three chitosan samples (M_v = 52.5 kDa, 126 kDa, 145 kDa) were prepared 0.1 M acetic acid aqueous solution of 0.5 g/L (bacteria) and 1.0 g/L (fungi) respectively, and high speed cutting at 12000 rpm for 3 min with 3 times, forming emulsion, as simulated emulsification. The pH was adjusted to 6.0, and the antibacterial rate was measured after simulated emulsification 5 min, 10 min, 15 min, 20 min, 25 min, and 30 min, the antibacterial activity at different time of emulsifying was investigated.

2.5.2. Detection of chitosan charge and distribution shape under simulated emulsification conditions to explore correlation with antibacterial activity

According to the results of chitosan antibacterial activity test under simulated emulsification conditions of 2.5.1, chitosan solution with concentration of 1.0 g/L was prepared in 0.1 M CH_3COOH, and then diluted with deionized water to 0.25, 0.5 g/L. Then the charge quantity of chitosan with molecular weights of 48.5 kDa, 126 kDa and 728 kDa under the pH of strong antibacterial activity was detected in emulsion, and the distribution shape of chitosan in emulsion was detected under the pH of stronger and weaker after emulsifying for 20 min. According to the detection results, combined with the antibacterial mechanism of chitosan reported in the literature, the correlation between chitosan with different molecular as emulsifier and antibacterial activity was explored.

2.6. Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and $p < 0.05$ was considered significant differences.

3. Results and discussion

3.1. Structural characterization of low molecular weight chitosan

3.1.1. Fourier transform infrared spectroscopy (FTIR) characterization results

The results of FTIR characterization of chitosan samples with molecular weights of 728 kDa (a), 102 kDa (b) and 48.5 kDa (c) were presented in Fig. 1.

The chitosan samples (a), (b) and (c) have a broad peak at 3400 cm^{-1}, which is attributed to the multiple absorption peak broadened by the overlapping absorption peaks of O-H and N-H, the peak at wavenumber 2800 cm^{-1} represent the stretching vibration absorption peak of C-H in the alkyl group (Acosta Ferreira S et al., 2020) The decrease of the molecular weight of degraded chitosan resulted in the decrease of the hydrogen bonds, which further caused the intensity change and position shift of the absorption peak. The absorption bands at 1650 cm^{-1} and 1450 cm^{-1} assigned to C=O stretching vibration and N-H bending vibration of the amide, respectively (HBarbosa HFG et al., 2019). The lower molecular weight of degraded chitosan resulted in the reduction of amide absorption. The band located in 899 cm^{-1} correspond to the characteristic absorption peak of β-pyran glycosidic bondv (Fan Y et al., 2021). The weakening and shift of this band were caused by decrease in the number of pyran rings in the molecular structure of degraded chitosan. Compared with sample (a), the increasement of the peak intensity produced by the-C-O stretching vibration in the pyran ring of sample (b) at 1010 cm^{-1} was due to the breakage of the glycosidic bond in the molecule, as reported in literature (Fu RR et al., 2017). Reducing the molecular weight of chitosan helped the C-O stretching vibration in the pyran ring, enhancing the peak intensity (Hakima E K et al., 2026). Literature reported that the FTIR spectra of the prepared low molecular weight chitosan were similar to this experiment (Farias BS et al., 2019).

It was shown in Fig. 1 that the infrared spectrum of the molecular weight 102 kDa and 48.5 kDa chitosan samples were similar to the raw chitosan, indicating that the pyran ring in the chitosan molecule was not destroyed after acid degradation, only the intensity and position of the absorption peak had slightly changed.

3.1.2. X-ray diffraction detection

The oligosaccharides with the molecular weight of 728 kDa, 102 kDa and 48.5 kDa were characterized by XRD, and the results were shown in

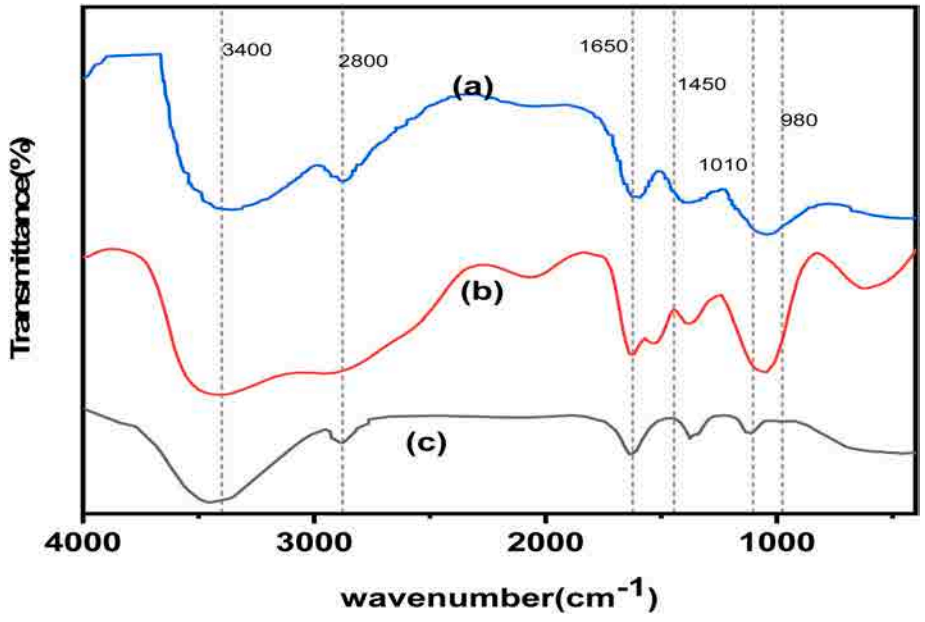

Fig. 1. FTIR spectra of three different molecular weight chitosans. (a): 728 kDa, (b): 102 kDa, (c): 48.5 kDa.

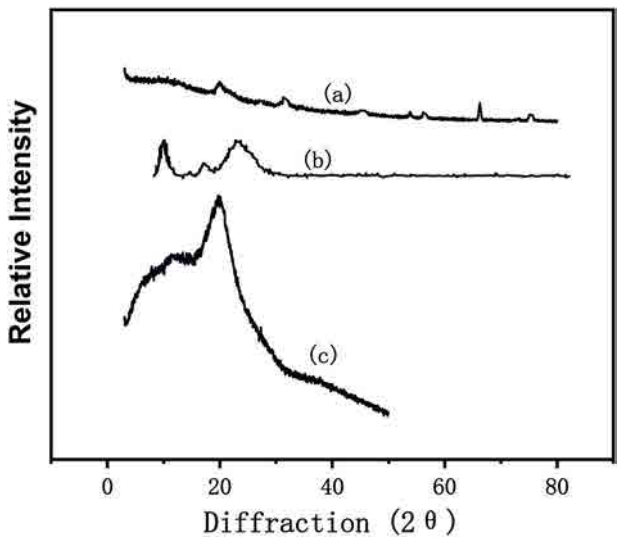

Fig. 2. X-ray diffraction pattern of low molecular weight chitosan. (a): 48.5 kDa, (b): 102 kDa, (c): 728 kDa.

Fig. 2.

In accordance with the X-ray diffraction pattern Fig. 2 (c), there were two crystal diffraction peaks (11.7° and 24.3°) in the range of 2θ (10 ~ 30°) with sharp peaks and high peak intensity, which were generally consistent with the characteristic peaks of untreated chitosan reported by Yan (Yan J et al., 2020). As shown in Fig. 2 (b), the degraded low molecular weight chitosan (102 kDa) still had two main characteristic peaks at 2θ of 11.7° and 24.3° with sharpe peak shape, which was generally consistent with original chitosan characteristic peak (Li J et al., 2008). However, the peak intensity of the degraded sample was significantly weakened, possibly due to a decrease in the number of hydrogen bonds in chitosan molecule and the destruction of some crystal structures (Farias BS et al., 2019). For the spectrum of (a), the degradation was greater than that of 102 kDa sample (b), and the decrease of peak intensity was more obvious, living only one diffraction peak that was wide and weak at around 24.3°. The peaks appeared after 40° may be caused by a significant decrease in molecular weight and intermolecular hydrogen bonding interactions. The molecular weight of chitosan decreased, the crystalline region was significantly reduced, and only the main characteristic peaks appeared, peaks at high 2θ were weakened (Farias BS et al., 2019), Similar to the results of this experiment. Literature reported that the preparation process caused change of chitosan molecular weight, and peaks at high 2θ also changed, similar to the weakening of peaks at high 2θ in degraded chitosan (Cheng. J. et al., 2020).

3.1.3. ^1H NMR detection

Chitosan with the molecular weight of 102 kDa and 48.5 kDa were characterized by ^1H NMR, and the results were displayed in Fig. 3.

As shown in Fig. 3, the single peak at 1.97 ppm is the H signal on the CH_3 linked to C=O in the molecule of 102 kDa and 48.5 kDa chitosan samples, the $\delta = 3.09$ ppm was attributed to the H signal on the NH_2 which linked to C_2, the H atoms on C_3, C_4, C_5 and C_6 in chitosan molecules are in a lower field (δ 3.67 ppm and $\delta = 3.83$ ppm), the single peak at 4.79 ppm was assigned to the H atom on C_1 (Yan J et al., 2020). The results of NMR illustrated that the peak shape and H atom shift of the samples after acid degradation were similar to those in literatures (Li Z et al., 2015; Pestov A et al., 2015), indicating that the molecular skeleton of the sample was not affected during the degradation process, which was consistent with the results of FTIR.

Fig. 3b shown enhanced peak intensity compared to (a), indicating that the degradation of chitosan had reduced the molecular weight and exposed more functional groups, enhanced the peak intensity, which was similar to the ^1H NMR spectrum of low molecular weight chitosan reported in the literature (Farias BS et al., 2019). The literature (Cai Z et al., 2026) reported that the variation in molecular weight of chitosan prepared by different process caused the change of ^1H NMR spectrum intensity, which was similar to the results of this experiment.

3.2. The antibacterial activity test of chitosan with different molecular weight

3.2.1. Detection of molecular weight range of chitosan with strong antibacterial activity

According to the 2.2.1 experimental method, a series of different molecular weight chitosan was obtained by measuring the degradation time of dilute hydrochloric acid at different times, and the molecular weight Mv can be calculated from the intrinsic viscosity. The raw chitosan can be degraded by dilute hydrochloric acid to obtain a series of chitosan with different molecular weights, which is consistent with the experimental results obtained by Prasertsung (Prasertsung I et al., 2012) using plasma solution to degrade chitosan. The antibacterial activity of each molecular weight chitosan against the experimental strains was determined according to the experimental method of 2.2.3.1.

For the two types of bacteria *E.coli* and *S.aureus* as well as the fungus *C.albicans*, the antibacterial activity was significantly different with different molecular weight ranges of chitosan, indicating that the antibacterial activity of chitosan with higher and lower molecular weights decreased. Chitosan with an antibacterial rate of more than 90% for *E. coli*, *S.aureus* and *C.albicans* was defined as strong antibacterial activity, the molecular weight range was 52.5 ~ 167 kDa.

The above results indicated that the antibacterial activity of chitosan had a corresponding window range with its molecular weight, and the antibacterial activity of chitosan with the same molecular weight against bacteria is better than that of fungi. It was found that the molecular weight of chitosan was in the range of 55 ~ 155 kDa, it had strong

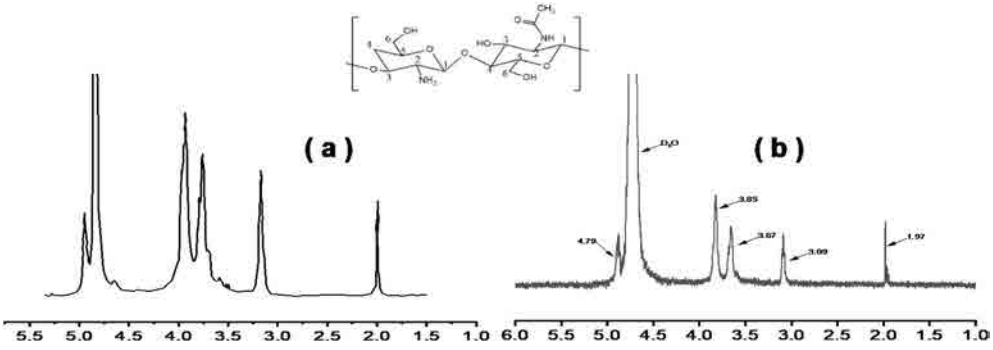

Fig. 3. ^1H NMR spectrum and chemical structure of 102 kDa and 48.5 kDa chitosan samples.

antibacterial effect (Liu N et al., 2006), which was basically consistent with the results of this experiment.

It was reported by Bano (Bano I et al., 2014) that the antibacterial activity of degraded chitosan increased with the decrease of molecular weight, while the antibacterial activity of chitosan against *C.albicans* was better in the range of 79.5 ~ 128.5 kDa (). The above reports were similar to the molecular weight range of chitosan with strong antibacterial activity obtained in this study.

3.2.2. Detection of MIC of chitosan with different molecular weight

Chitosan with molecular weight of 167, 126, 52.5 kDa and 728 kDa were selected to determine MIC against three test microorganisms according to the method of 2.2.3.2, the experimental results were shown in Table 1.

It was demonstrated in Table 1 that the antibacterial activity of chitosan with 52.5 ~ 167 kDa molecular weight was better than that of chitosan with high molecular weight for fungi and bacteria. Compared with the antibacterial activity of chitosan with medium molecular weight reported by Bernabe (Bernabe P et al., 2020), the antibacterial activity of chitosan with low molecular weight(52.5 ~ 167 kDa) prepared in this study was stronger. Comparison to the MIC of the food preservatives ε-polylysine and Nisin against *S. aureus* and *E. coli* by literature reported (Li Q et al., 2022; Liu J H et al., 2018), chitosan with low molecular weight (52.5 ~ 167 kDa) had a lower MIC against *S. aureus* and a similar MIC against *E. coli*, demonstrating that the antibacterial effect reached the level required for food preservation.

It can be seen from Table 1 that the antibacterial activity of three chitosan with low molecular weight(52.5 ~ 167 kDa) against fungi was significantly lower than that of bacteria. Reported by Sliva (Silva GLG et al., 2018), the electrostatic interaction between the cell membrane of *C.albicans* and chitosan was weaker than that of bacteria, so the antibacterial activity of chitosan against *C.albicans* decreased. However, the antibacterial activity of three chitosan with low molecular weight against *E.coli* and *S.aureus* was also different, which was due to the different cell wall composition and structure of *E.coli* (G-) and *S.aureus* (G +) (Rejane C Goy et al., 2009).

According to the above experimental results, chitosan with molecular weight of 52.5 ~ 167 kDa showed strong antibacterial activity. Therefore, the study of emulsification property of chitosan with strong antibacterial adopted chitosan with molecular weight of 52.5 ~ 167 kDa.

3.3. Determination of the emulsification performance of chitosan with strong antibacterial activity

According to the emulsification detection method in 2.3.1, chitosan with molecular weight of 126 kDa and 728 kDa were selected for comparison of emulsification performance, and the results are shown in Fig. 4a.

Fig. 4a shown, the 1.0 and 4.0 g/L concentration solutions of the two kinds chitosan had significant emulsification effects, and the emulsion was stable at 1 h, 4 h, 24 h, and 7 days. It shown that the emulsification performance of chitosan with strong antibacterial activity was similar to chitosan with high molecular weight. Compared with the emulsification performance of corn starch reported in the literature (Guo, B et al., 2020), chitosan had obvious advantages in oil-water emulsification. The literature (Zhang F et al., 2021) reported that the emulsification effect composite casein with low molecular weight chitosan can improved, indicating that chitosan with low molecular weight had obvious emulsifying effect.

According to the above experimental results, the oil-water emulsified particle size of 0.1% concentration of chitosan with molecular weight 38.0, 126, 284, 728 kDa was compared, and the results were shown in Fig. 4b. It can be seen from Fig. 4b that the droplet size and uniformity of emulsion with 126 kDa chitosan were better than emulsion with 38.0, 284, and 728 kDa chitosan, indicating that chitosan with strong antibacterial activity had advantage in oil-water emulsification effect compared with other chitosan with molecular weight. Compared with the emulsion particle size formed oil-in-water emulsion by arabic gum, pectin, soybean isolate protein, casein reported in the literature (Liu J et al., 2021), the particle size of oil-in-water emulsion of chitosan with strong antibacterial activity was significantly smaller.

The above emulsification performance results shown that chitosan with strong antibacterial activity was feasible as an emulsifier and had practical application value. Therefore, according to the molecular weight range of chitosan with strong antibacterial activity, chitosan with 38.0、126、284 kDa molecular weight were selected, based on the experimental results in Fig. 4a, the emulsion was prepared with concentration of chitosan of 0.1, 0.5, 0.0, 1.5 g/L, and the emulsification effect was compared. Fig. 4a shown that the emulsion was stable for 7 days, so the emulsification detection method in 2.3.1 was modified, mix 5% soybean oil with 95% solution of chitosan, total of 20 ml, high speed cutting at 12000 rpm for 3 min with 3 times, forming emulsion, for quicker comparison of emulsification effects, the results were shown in Fig. 5.

Fig. 5 shown, chitosan with a molecular weight of 38.0 kDa at concentration of 0.1 and 0.5 g/L could not reach stable emulsification, and the stability of the emulsion at concentration of 1.0 and 1.5 g/L was lower, indicating that chitosan with molecular weight of 38.0 kDa had no practical application value as an emulsifier, chitosan with molecular weight of 126 and 284 kDa at concentration of 0.1 g/L can achieve stable emulsification, and the emulsification effect was enhanced with the increase of concentration, experimental results were consistent with the result of literature research(Li & Xia, 2011). The experimental result shown that chitosan with strong antibacterial activity can play an emulsifying role at lower concentration, and its emulsification performance similar to chitosan with high molecular weight.

Literature(Li & Xia, 2011) reported the emulsification performance of chitosan with molecular weight of 299 kDa, which was similar to the experimental results of chitosan with molecular weight of 284 kDa in this study, but the literature did not study the emulsification performance of chitosan with lower molecular weight. The experimental results of this study showed that chitosan with strong antibacterial activity had the same emulsification performance as chitosan with high weight. If the molecular weight of chitosan was further reduced, the emulsification performance of chitosan would also decrease, the emulsification test results of chitosan with molecular weight of 38.0 kDa had confirmed this phenomenon.

3.4. The emulsification stability of chitosan with strong antibacterial activity

According to the Investigation method of emulsification stability in 2.3.2, based on the result of different concentrations of chitosan emulsification test, selected chitosan with molecular weight of 52.5、126 and 284 kDa, the emulsification stability was inspected with 0.5 and 1.0 g/L concentration of chitosan emulsion. Adjusted the pH value of the emulsion system of chitosan with 0.1M NaOH, the stability of emulsion was investigated at different pH, the emulsion system of chitosan was kept at different temperatures for 30 min to investigate stability, and addition of NaCl into emulsion system of chitosan, the stability of emulsion was investigated at different ionic strength. The stability of emulsion system of chitosan was expressed by the emulsification index

Table 1
MIC of different molecular weight chitosan on the test strains.

Test Strains	MIC(μ g/mL)			
	728 kDa	167 kDa	126 kDa	52.5 kDa
E. coli	40	30	30	25
S. aureus	40	15	20	25
C. albicans	300	150	150	150

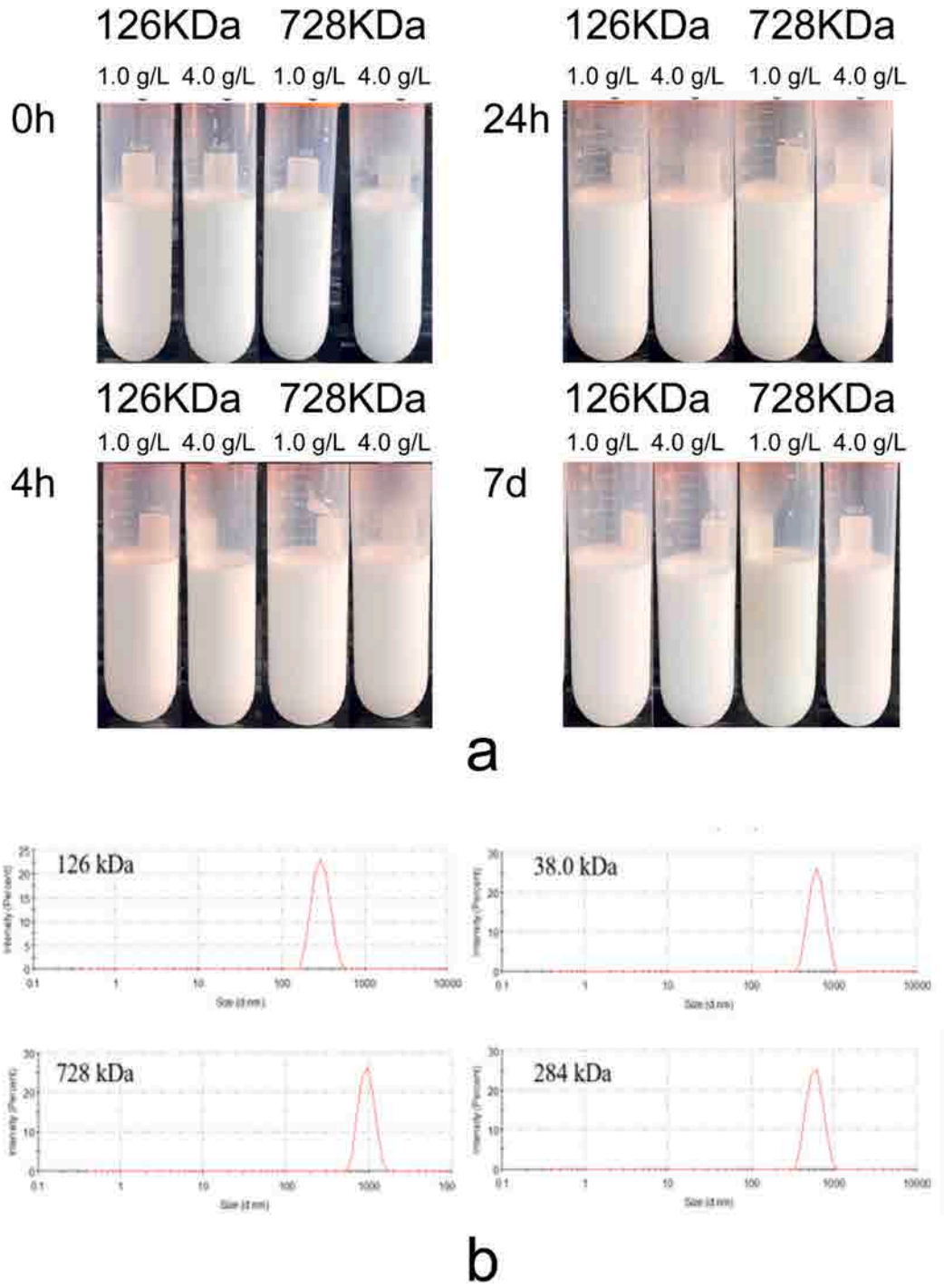

Fig. 4. Emulsifying performance of chitosan with molecular weights of 126 kDa and 728 kDa(a); particle sizes of oil-water emulsion with 0.1% chitosan with 126 kDa, 38.0 kDa, 728 kDa and 284 kDa(b).

(CI%), and compared the emulsion stability under different conditions. The results were shown in Table 2.

Table 2 shown that the emulsification of chitosan with molecular weights of 52.5, 126, and 24 kDa were relatively stable under the pH, temperature, and ionic strength of the experiment. When pH was lower than 5.0, temperature was higher than 45 °C, and NaCl concentration was greater than 2.5% (w/v %), the emulsion stability of chitosan was affected. The experimental results were similar to the influence of pH, ionic strength and temperature on emulsification stability of chitosan microparticles applied to Pickering emulsions reported in the literature (William, W M et al., 2016). Depending on the pH, ionic strength and preservation temperature of the food, the conditions of emulsion stability of chitosan with strong antibacterial activity were highly correlated.

The emulsification index shown in Table 2 could distinguish the slight differences in emulsion stability between 52.5 kDa chitosan and126, 284 kDa chitosan, especially for the emulsion with chitosan concentration of 0.50 g/L. But, the emulsification stability of chitosan with molecular weight of 52.5 kDa was superior to that of corn starch reported in literature (Guo, B et al., 2020). The viscosity of chitosan solution was obviously greater than that of starch, protein, arabic gum and other macromolecule emulsifiers (Eduardo MF et al., 2014), so the

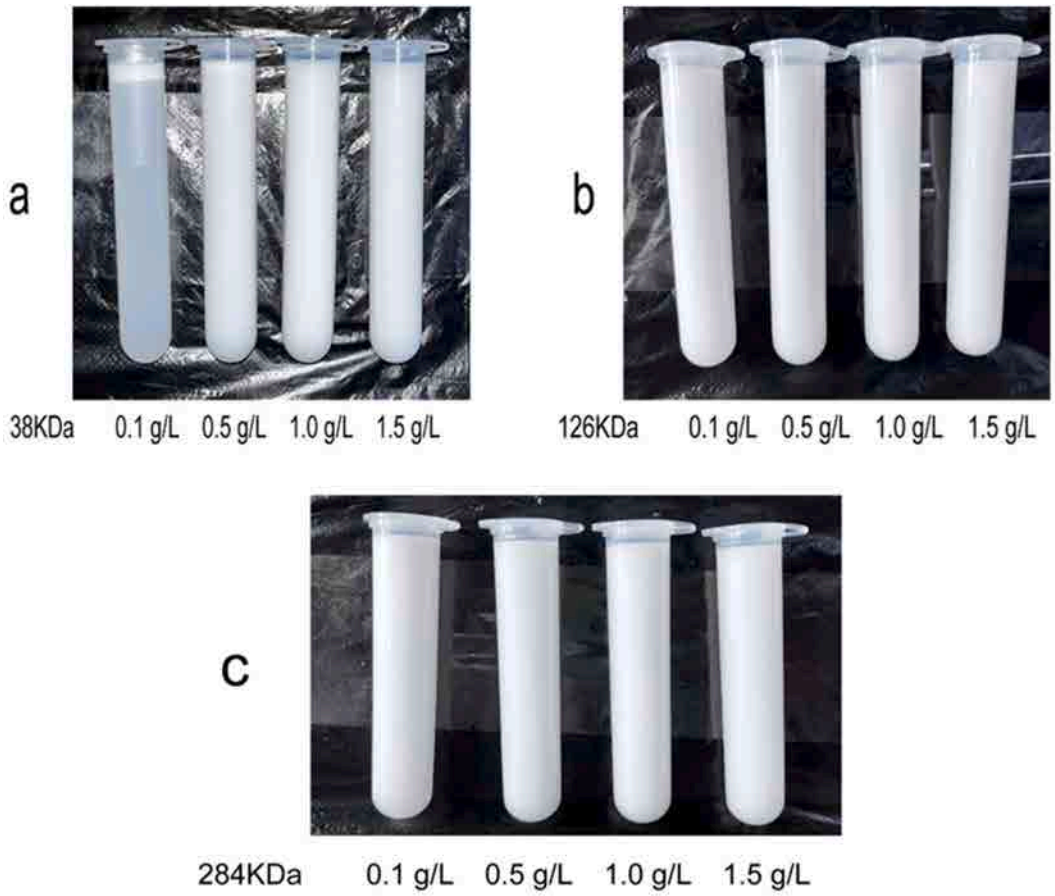

Fig. 5. Emulsification effect of chitosan with different molecular weights (a: 38 kDa; b: 126 kDa; c: 284 kDa; chitosan concentrations: 0.1, 0.5, 1.0, and 1.5 g/L).

Table 2
Emulsification index (CI%) of three molecular weight chitosan under different pH, temperatures, and ionic strengths.

MolecularWeight(kDa)		52.5		126		284	
Concentration (g/L)		0.50	1.00	0.50	1.00	0.50	1.00
different pH (20 °C, NaCl: 0 w/v %)	4.5	10.7%	10.1%	10.2%	9.9%	10.4%	9.6%
	5.0	9.7%	9.4%	9.1%	8.6%	9.0%	8.3%
	5.5	9.6%	9.1%	9.0%	8.2%	8.9%	8.1%
	6.0	9.4%	8.8%	8.5%	7.6%	8.4%	7.6%
	6.5	8.5%	7.9%	7.7%	6.4%	7.9%	6.6%
different temperature (°C) (pH6.0, NaCl: 0 w/v %)	15	7.3%	7.1%	6.9%	6.3%	7.0%	5.9%
	25	8.9%	8.4%	7.5%	6.9%	7.5%	6.3%
	35	9.2%	8.9%	8.4%	7.9%	8.5%	7.3%
	45	10.1%	9.7%	9.2%	8.7%	9.2%	8.5%
	55	11.2%	10.6%	9.7%	9.2%	9.7%	8.9%
different ion strength (NaCl w/v %) (20 °C, pH6.0)	1.0	8.5%	8.2%	7.8%	6.3%	7.3%	6.4%
	1.5	9.0%	8.5%	8.3%	6.9%	7.7%	6.6%
	2.0	9.9%	9.5%	9.1%	8.3%	8.5%	7.3%
	2.5	10.3%	9.9%	9.9%	9.3%	9.8%	8.5%
	3.0	11.5%	10.7%	10.8%	10.2%	10.7%	9.7%

emulsification system of lower concentration of chitosan with molecular weight of 52.5 kDa still had extensive application value.

Table 2 shown that the emulsion stability of 126 kDa chitosan was consistent with 284 kDa chitosan, compared with the emulsion stability of chitosan composite with casein reported in the literature (Zhang F et al., 2021), it had obvious advantages, and reflected stronger emulsification ability.

Literature (Li & Xia, 2011) reported that chitosan with molecular weight of 299 kDa had better emulsification performance than chitosan with molecular weight of more than 600 kDa. The experimental results of this study showed that chitosan with molecular weight of 126, 284 kDa had better emulsification performance than chitosan with lower molecular weight. This indicated that chitosan had a molecular weight window for its emulsification performance, and both too high and too low weight chitosan had reduced emulsification performance.

3.5. Determination and stability evaluation of viscosity of chitosan with strong antibacterial activity

Literature (Liu J et al., 2021) reported that the cutting viscosity reflected the basic property as an emulsifier, and both too high and too low viscosities were not conducive emulsification. Literature (Zhang F et al.,

2021) studied shown that the viscosity of chitosan was the main factor of emulsion stability of chitosan composite with casein. The viscosity of solution was determined by shear force with rotary viscometer, which could reflect the shear viscosity of chitosan solution. So the viscosity of chitosan solution (Mv: 48.5, 126, 284, 728 kDa) with concentration of emulsification effect test was detected according to the experimental method of 2.4.1, the determination results were shown in Table 3.

As shown in Table 3, the shear viscosity of chitosan solution increased with the increase of the corresponding molecular weight of chitosan, and the concentration of chitosan solution shown correspond to its shear viscosity, consistent with the results of the study reported in the literature (Yue W., 2014). Within the concentration range of the experiment, the viscosity of 48.5 kDa chitosan was significantly lower than that of 126 kDa and 284 kDa chitosan. Too low viscosity was not conducive to emulsification (Soares LS et al., 2019), which corresponds to the weak emulsification performance of chitosan with lower molecular weight as in this study.

The suitable viscosity range of emulsification system of food was 60 - 140 mPa s (Guo YS et al., 2022; Klinkesorn U., 2013), and this viscosity range corresponded to the results shown in Table 3, where chitosan with molecular weight of 126、284 kDa and solution concentration of 0.1-1.5 g/L, as well as molecular weight of 782 kDa and solution concentration of 0.1-0.5 g/L, which indicated 0.1-1.5 g/L solution of chitosan with strong antibacterial activity had suitable viscosity for food emulsification. To this end, the viscosity stability of chitosan with 48.5, 126, 284 kDa was further evaluated.

According to the experimental methods in 2.4.2, the results of chitosan viscosity with molecular weights of 48.5 kDa, 126 kDa, and 284 kDa in different pH(4.5、5.0、5.5、6.0、6.5、7.0、7.5), temperatures(10、20、30、40、50、60 、70 °C), and different sodium chloride concentration(0.5、1.0、1.5、2.0、2.5、3.0、3.5 w/v%)(ion strength)are determined. Calculated the coefficient of variation (CV) of the viscosity (mPa·s) of solution of chitosan with each molecular weight under different conditions (CV% = standard deviation/mean × 100%), the stability of viscosity was expressed by the coefficient of variation (CV%). The results were shown in Table 4.

According to the suitable viscosity of chitosan for emulsification mentioned in reference (Guo YS et al., 2022) as well as the results in Table 3, the results of Table 4 showed that stability of viscosity of chitosan with 126 kDa at concentration of 0.50-1.00 g/L was higher compared to the chitosan with 284 kDa and 48.5 kDa. Refer to the research results of literature (Zhang F et al., 2021), the experimental results of Table 4 indicated that chitosan with strong antibacterial activity could form relatively stable emulsion system. Literature(William, W M et al., 2016) selection of chitosan with molecular weight of 50-190 kDa was carried out for the study of emulsification stability in Pickering emulsions, which was similar to the results of this experiment that chitosan with strong antibacterial activity was relatively stable in emulsification.

Table 4
The CV of viscosity of chitosan with48.5 kDa, 126 kDa, and 284 kDa at different pH, temperature, and ion strength.

Molecular Weight (kDa)	48.5		126		284	
Concentration (g/L)	1.00	0.50	1.00	0.50	1.00	0.50
CV% of different pH (NaCl 0 w/v%, 20 °C)	12.7%	10.6%	1.19%	1.67%	8.57%	2.13%
CV% of different temperature (pH6.0, NaCl 0 w/v%)	1.48%	3.44%	0.43%	0.83%	1.91%	1.90%
CV% of different ion strength (pH6.0, 20 °C)	2.69%	5.00%	0.43%	0.62%	1.55%	1.85%

3.6. *The antibacterial experiment of simulated emulsification for chitosan with strong antibacterial activity*

In order to explore the chitosan with strong antibacterial activity reflected the antibacterial activity in emulsification, the antibacterial activity test of chitosan simulated emulsification in aqueous solution was carried out, on the basis of the conditions related to the emulsification test of chitosan, and discussed the correlation between simulated emulsification and antibacterial activity.

3.6.1. *Antibacterial test of chitosan simulated emulsification*

According to the chitosan with strong antibacterial activity of molecular weight range, the samples with strong antibacterial activity (M_v = 145, 126, 52.5 kDa) were selected. According to the experimental method of 2.5.1, the experimental results were shown as follows Table 5.

The antibacterial activity of chitosan with strong antibacterial activity with different concentrations for simulated emulsification against *E.coli, S.aureus* and *C.albicans* was demonstrated in Table 5. For the three test strains, the antibacterial activity of chitosan with three molecular weights increased with the increase of concentration., which was beneficial to its adsorption on the surface of microbial cells and enhanced the antibacterial effect (Bingjun Q et al., 2015). The emulsification effect also increased with the increase of chitosan concentration, based on the results of the experiment in Fig. 5.

For *E.coli* and *S.aureus*, the antibacterial rate increased rapidly from 0.125 to 0.5 g/L to 0.75 g/L, and the antibacterial rate was higher than 90%, which was similar to antibacterial effect of food preservative ε-polylysine (Zhou X J et al., 2023). The antibacterial rate of chitosan with three molecular weights at 1.0 g/L concentration against *C.albicans* was more than 90%. Because cell wall structure and physiological characteristics of fungi were different from bacteria, difference in antibacterial activity of chitosan against bacteria and fungi, similar to the food preservative nisin (Gong F et al., 2018).

Table 3
The shear viscosity of chitosan solution with different molecular weight.

concentration(g/L) / viscosity(mPa·s) / M_v (kDa)	0.10	0.50	1.00	1.50
48.5	8	32	40	48
126	60	80	120	120
284	84	100	144	144
728	112	140	300	480

Table 5
Antibacterial activity of chitosan with strong antibacterial activity for simulated emulsification under different concentration, pH, emulsification time.

MolecularWeight(kDa)		52.5			126			145		
Strains antibacterial rate (%)		*E.coli*	*S.aureus*	*C.albicans*	*E.coli*	*S.aureus*	*C.albicans*	*E.coli*	*S.aureus*	*C.albicans*
Concentration (g/L) (pH6.0, emulsification 20mins)	0.125	38.7 ± 2.2	37.5 ± 2.3	26.8 ± 2.5	43.1 ± 2.1	51.7 ± 1.8	34.6 ± 2.2	78.3 ± 2.6	79.2 ± 2.3	42.4 ± 1.7
	0.25	69.7 ± 2.0	70.8 ± 1.9	35.2 ± 1.7	96.3 ± 2.3	94.7 ± 2.5	56.4 ± 1.9	95.8 ± 2.2	94.3 ± 2.0	78.6 ± 1.8
	0.50	93.4 ± 2.2	86.8 ± 1.7	55.7 ± 2.1	96.8 ± 2.4	95.6 ± 2.1	69.3 ± 1.8	96.7 ± 2.3	94.8 ± 2.5	92.7 ± 2.1
	0.75	93.6 ± 2.4	91.2 ± 2.3	85.6 ± 1.9	98.3 ± 2.2	96.4 ± 1.8	88.4 ± 1.5	98.9 ± 2.5	96.2 ± 2.3	94.8 ± 1.9
	1.0	95.7 ± 1.6	94.3 ± 2.1	88.4 ± 2.2	98.4 ± 1.9	96.5 ± 2.3	90.2 ± 2.1	98.5 ± 1.9	96.7 ± 2.0	95.1 ± 2.2
pH (concentration 0.5 g/L (bacteria) and 1.0 g/L (fungi), emulsification 20mins)	4.5	94.2 ± 1.8	83.7 ± 1.8	83.1 ± 2.2	94.2 ± 2.1	95.9 ± 2.4	87.6 ± 1.9	98.4 ± 2.3	94.8 ± 1.9	96.3 ± 2.0
	5.0	95.5 ± 1.9	84.8 ± 2.2	86.9 ± 1.7	99.6 ± 2.5	96.4 ± 1.9	88.3 ± 2.3	99.3 ± 2.2	94.±2.3	98.2 ± 2.4
	5.5	95.3 ± 2.4	86.2 ± 1.9	90.7 ± 2.1	99.7 ± 2.4	92.6 ± 2.3	92.5 ± 2.0	99.4 ± 2.3	95.1 ± 2.0	98.7 ± 1.9
	6.0	98.4 ± 2.5	90.7 ± 2.2	91.8 ± 1.8	99.8 ± 2.0	99.9 ± 2.4	94.3 ± 2.1	99.8 ± 2.4	96.6 ± 1.8	99.1 ± 2.3
	6.5	92.3 ± 1.7	81.5 ± 2.1	88.3 ± 1.9	98.1 ± 2.4	94.2 ± 2.2	88.7 ± 2.0	96.3 ± 2.3	91.8 ± 2.1	96.2 ± 2.5
emulsification time (min) (concentration 0.5 g/L (bacteria) and 1.0 g/L (fungi), pH6.0)	5	57.2 ± 2.1	25.3 ± 1.7	17.6 ± 1.6	71.8 ± 2.2	43.7 ± 1.8	20.1 ± 1.7	58.2 ± 2.0	61.8 ± 2.3	22.6 ± 16
	10	60.6 ± 1.9	69.2 ± 2.0	21.3 ± 1.5	74.9 ± 1.8	73.9 ± 2.3	35.2 ± 1.8	62.4 ± 2.2	73.2 ± 2.4	48.3 ± 2.0
	15	84.4 ± 2.3	81.3 ± 1.7	80.1 ± 2.0	86.4 ± 2.2	91.2 ± 1.9	88.7 ± 2.3	83.5 ± 2.1	84.7 ± 2.0	81.6 ± 1.8
	20	89.5 ± 2.2	83.4 ± 1.9	81.7 ± 2.3	91.5 ± 2.1	93.1 ± 2.3	90.6 ± 2.0	90.2 ± 1.8	90.4 ± 2.1	83.5 ± 2.2
	25	91.6 ± 2.1	85.7 ± 2.0	87.4 ± 2.2	94.7 ± 2.3	94.2 ± 2.0	91.2 ± 1.9	91.8 ± 2.1	91.5 ± 1.8	89.1 ± 1.7
	30	93.1 ± 2.3	88.1 ± 2.1	90.2 ± 1.9	95.2 ± 2.2	93.8 ± 2.1	98.5 ± 2.3	94.6 ± 2.0	93.2 ± 2.2	93.3 ± 2.1

As shown in Table 5, the antibacterial activity of chitosan with strong antibacterial activity gradually increased with the increase of emulsion pH from 4.5 to 6.0, and the antibacterial activity was the strongest at pH 6.0, the antibacterial activity decreased at pH 6.5, indicating that pH of the emulsification system had important impact on the antibacterial activity of chitosan. It was reported in the literature (Chen RH and Hua HD, 1996) that the viscosity of chitosan solution at pH 4.5-6.5 gradually increased, indicated that the antibacterial activity of chitosan related to viscosity of solution.

The pKa value of chitosan is about 6.5, inducing the protonation for NH_2 in chitosan molecule to form NH^{3+} in acidic medium (Li J H et al., 2016), which can be adsorbed on the cell surface and played an antibacterial role. In the near neutral medium, a part of NH^{3+} will be neutralized, to result in decreased antibacterial activity of chitosan (Bingjun Q et al., 2015). The above reports were consistent with the experimental results of this study.

It can be seen from Table 5 that the antibacterial rate of chitosan with strong antibacterial activity to the three test bacteria increased with the increase of emulsification time. By 30 min, the antibacterial rate of chitosan with three molecular weights against three test bacteria was basically stable, indicating that the antibacterial activity of chitosan with three molecular weights was basically fully reflected when the emulsification time was 30 min. It has been reported in the literature that with the prolongation of action time, more and more amino positive charges are adsorbed on the surface of microorganisms, so that chitosan can exert stronger antibacterial effect (Wang X et al., 2021), which is consistent with the experimental results of this study. The above results demonstrate that when chitosan was added as emulsifier, the antibacterial activity of chitosan can be fully reflected over time.

The mechanism of the antibacterial effect of chitosan in Fig. 5 was similar to the mechanism of ε-polylysine to disrupt microbial cell walls, and the mechanism of nisin to exert antibacterial effects by attacking the microbial cell walls reported in the literature (Chang S L et al., 2022; Li Q et al., 2022). The emulsified chitosan was fully dispersed, which facilitated the attack on microbial cell walls and improved the antibacterial effect.

Literature reported that nisin was used for food preservation, with concentrations of 0.5 mg/mL inhibiting 5.5 × 105 CFU/mL *E. coli* and 1 × 106 CFU/mL *S. aureus*, the antibacterial rates were lower than that of chitosan at the same concentration against *E. coli* and *S. aureus*, as shown in Table 5 (Li Q et al., 2022; Zhao G et al., 2023). Zhao P et al. reported that 1.0 mg/mL ε-polylysine inhibited *E. coli* and *S. aureus* at 1×10^5 CFU/mL with antibacterial rates of 63.7 ± 3.4% and 73.7 ± 3.7% respectively, prepared nanoparticles to enhance the antibacterial effect, increasing the antibacterial rates against *E. coli* and *S. aureus* to 79.3 ± 6.7% and 95.6 ± 5.3% respectively, for application in food preservation (Zhao P et al., 2024).

Compared with the antimicrobial effects of Nisin and ε-polylysine reported in the literature, emulsified chitosan had the potential as a food preservative.

3.6.2. The detection for the charge and distribution shape of chitosan under antibacterial conditions of simulated emulsification and discussion of antibacterial mechanism

According to the experimental method of 2.5.2, chitosan samples (Mv = 48.5 kDa, 126 kDa, 728 kDa) with strong or weak antibacterial activity were selected to prepare 1.0 g/L solution, diluted with water to 0.5 g/L to simulate emulsification, and the pH was adjusted to 6.0, to detect the charge. The results were shown in Fig. 6.

It was depicted in Fig. 6 that the chitosan with 48.5 kDa, 126 kDa, 728 kDa all had high positive charge, the positive charge of 728 kDa chitosan was higher than that of 126 kDa and 48.5 kDa chitosan, consistent with the literature reported that chitosan with higher molecular weight had higher positive charge (Li JH et al., 2016). Chitosan with positive charge was beneficial to contact with microbial cells to exert antibacterial effect, but the antibacterial effect was not proportional to the amount of positive of chitosan (Wang X., 2021). The viscosity of chitosan with low molecular weight was reduction, which enhanced permeability was conducive to antibacterial (Minh NC et al., 2018; Wang WQ et al., 2020). The antibacterial activity of 728 kDa

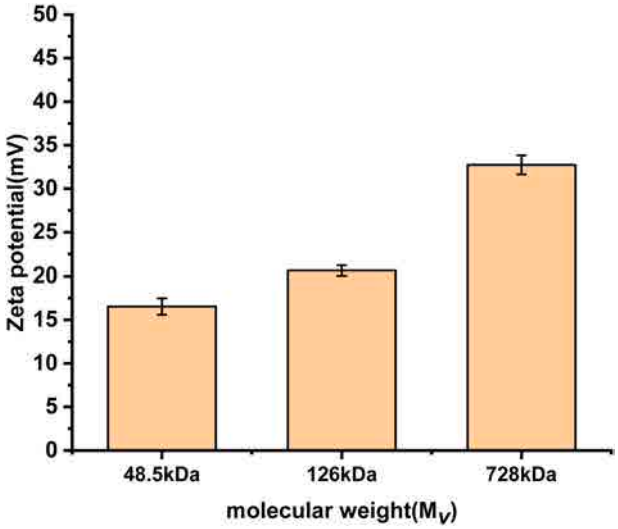

Fig. 6. Zeta potential of chitosan with 48.5 kDa, 126 kDa, 728 kDa.

chitosan was lower than that of chitosan with 55~155 kDa (Prasertsung I et al., 2012). Therefore, comparison of the charge detection results in Fig. 4 with the antibacterial effects of chitosan with the corresponding molecular weight could certify the antibacterial mechanism of chitosan reported in the above literature.

According to the results of antibacterial activity test of simulated emulsification of chitosan, chitosan (Mv: 48.5 kDa, 126 kDa, 728 kDa) were selected to prepare 0.25 g/L and 0.5 g/L solutions at pH 6.0 and 6.5, respectively. By detecting the average particle size of chitosan for simulated emulsification after 20 min, the distribution shape of chitosan in the solution was investigated. The results of particle size distribution were shown in Table 6.

It can be seen from the results of Table 6 that the distribution particle size of 126 kDa chitosan was significantly smaller than that of 48.5 and 728 kDa chitosan with 0.25 g/L and 0.50 g/L at pH 6.0. It was compared the results of Table 5 that the antibacterial activity of chitosan increased rapidly when the concentration reached 0.25-0.50 g/L, and the antibacterial activity was the strongest at pH 6.0, and the antibacterial activity reached higher after emulsifying for 20 min. Thus, the particle size distribution of chitosan emulsion determined under the above conditions could clearly reflect the main factors of the stronger antibacterial activity of 126 kDa chitosan. The particle size distribution of the chitosan emulsion increased at pH 6.5 shown in Table 6, which was consistent with decrease in the antibacterial activity of chitosan at pH 6.5 shown in Table 5.

The antibacterial mechanism of chitosan was the action on cell wall of bacteria (Bingjun Q et al., 2015; Li J H et al., 2016; Rejane C Goy et al., 2009). The destruction of microbial cells for the chitosan with smaller molecular weight resulted the strong antibacterial activity (Liu H et al., 2004). However, the distribution shape of chitosan was critical for the action on cell wall and the destruction on cells. The antibacterial activity of chitosan nanoparticles was significantly improved, and the small particle size had stronger antibacterial activity, indicating that the particle size distribution of chitosan with different molecular weights in solution was an important factor for antibacterial activity (Pan CL et al., 2020). Literature reported that the preparation of nisin nanoparticles enhanced antibacterial efficacy, and the preparation of ε-polylysine nanoparticles facilitated to disrupt cell walls and improved antibacterial efficacy, with a mechanism similar to the results of this experiment (Li Q et al., 2022; Liu J H et al., 2018).

The molecular weight of 48.5 kDa was lower than 126 kDa, but the antibacterial activity was weaker, which was mainly caused by the difference in particle size distribution in the emulsion. The above experimental results had not been reported in similar studies.

The literature(Isobe N et al., 2020) reported that the emulsification performance of Arabic gum were closely related to its molecular structure, and the emulsification performance was improved with smaller molecular structure. Therefore, chitosan with strong antibacterial activity distributed to smaller particle size in emulsion, which was also conducive to improving the emulsification effect.

4. Conclusion

In this research, a series chitosan with different molecular weight were prepared by dilute hydrochloric acid degradation of chitosan. Characterized by FTIR, XRD and ^{1}H NMR, there was no change on the pyran ring structure of chitosan molecule after dilute hydrochloric acid degradation.

By measuring the antibacterial activity and minimum inhibitory concentration of chitosan with different molecular weights to *E.coli, S. aureus* and *C.albicans*, it was determined that the molecular weight range of chitosan with strong antibacterial activity was 52.5-167 kDa. The emulsification performance was determined and the emulsifying stability was evaluated for chitosan with strong antibacterial activity. The emulsification effect of 126 kDa chitosan with strong antibacterial activity was similar to that of 728 kDa chitosan, and the particle size of the oil drop in the emulsion was smaller than that of 38.0, 284, and 728 kDa chitosan. The emulsification stability of 126 kDa chitosan was similar to that of 284 kDa chitosan, and higher than that of 52.5 kDa chitosan at different pH, temperature and ionic strength. Within the emulsification concentration range, 126 kDa and 284 kDa chitosan solutions exhibited suitable viscosity for food emulsification system, and the viscosity stability of 126 kDa chitosan was higher under different pH, temperature and ionic strength. The literature (Li & Xia, 2011)reported that chitosan with molecular weight above 600 kDa exhibited reduced emulsification performance, the results of this study shown chitosan with molecular weight below 50 kDa had weaker emulsification effects, indicating that chitosan as an emulsifier had a window of molecular weight.

Table 6
Average particle size of chitosan with 48.5, 126, 728 kDa at different concentrations.

Molecular Weight(kDa)	Concentration(g/L)	Z-ave(nm)	
		pH 6.0	pH 6.5
48.5	0.25	329 ± 5.8	346 ± 6.4
	0.50	359 ± 1.6	354 ± 1.7
126	0.25	240 ± 2.0	497 ± 3.4
	0.50	278 ± 2.4	456 ± 9.9
728	0.25	396 ± 6.2	556 ± 4.2
	0.50	566 ± 1.1	553 ± 5.1

Within the concentration range of the emulsification test, the chitosan with strong antibacterial activity simulated emulsification was closely related to the antibacterial effect. The chitosan concentration 0.5-1.0 g/L, pH6.0 and emulsified for 30min could show significant antibacterial activity. The morphology distribution of chitosan with strong antibacterial activity in simulated emulsification was very beneficial to antibacterial. The results of this study suggested that chitosan with strong antibacterial activity could be reliably used as an emulsifier, and could exhibit significant antibacterial in emulsification systems. The results of this study provided new strategies for the emulsification application of chitosan in the food field.

CRediT authorship contribution statement

Junqing Qian: Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Junlan Yin:** Writing – original draft, Investigation, Formal analysis, Data curation. **Peilong Ma:** Writing – original draft, Methodology, Data curation. **Chenghong Mo:** Writing – original draft, Validation, Methodology. **Yan Chen:** Writing – original draft, Validation, Methodology. **Hui Guo:** Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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