Characteristics of cricket (*Gryllus bimaculatus*) chitosan and chitosan-based nanoparticles

Kyo-Sung Chae¹ · Chae-Shim Shin¹ · Weon-Sun Shin¹

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Abstract The field cricket (*Gryllus bimaculatus*) is commonly consumed as food in different parts of the world. This study was performed to characterize the chitosan extracted from crickets and to assess its potential use to the growing functional market. The degree of deacetylation (DA), Fourier-transform infrared spectra, X-ray diffraction patterns, molecular mass, scanning electron microscopy spectra, and color were measured. Cricket chitosan nanoparticles were prepared, and the optimal conditions were identified. The molecular mass of the cricket chitosan was lower than that of commercial chitosan; however, the DA, FTIR, and XRD spectra were similar. The particle size (208.27 ± 3.47 nm), zeta potential (35.72 ± 1.29 mV), and polydispersity index (PDI: 0.27 ± 0.03) of the cricket chitosan NPs were superior to the commercial. Addition of NaCl reduced the cricket chitosan NPs size up to 15.5%. This finding is a novel trial to prove the availability of the insect chitosan with a low molecular mass as an active carrier source.

Keywords Edible insect · Cricket (*Gryllus bimaculatus*) · Chitosan · Nanoparticles

Introduction

Chitosan is a linear polysaccharide consisting of randomly distributed β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine [1] obtained by partial deacetylation of chitin commonly found in the arthropod exoskeletons, such as shells of crabs and shrimps or insects, in fungal cell walls and in yeast [2, 3]. Chitosan is a natural, nontoxic, biocompatible, biologically functional, ecofriendly, bioadhesive, and biodegradable polysaccharide, and it can be derived by partial deacetylation of chitin [1, 4]. Because of the remarkable biological characteristics such as natural antioxidants [5], antimicrobial [6], hypocholesterolemic and immunostimulating [7], chitosan has been widely applied in various delivery system, pharmaceutical use, textile, and functional food [4, 8, 9] and its capability of forming polymers with ionic interactions was applied to encapsulate the bio-active compounds as a carrier.

As an emerging resource, the chitin present in some insects has been focused, and the use of these organisms as a new alternative chitin source has been suggested. Insect chitin occurs in the alpha form [10, 11] and appears to have comparable physicochemical properties to the crustacea such as shrimp shell or crab shell [11]. One difference however appears to be generally lower molecular mass of insect chitosan compared to shrimp chitosan. Insect chitosan seems to have low molecular mass ranging from 2.6 kDa for Colorado potato beetle [10] to 501 kDa of blowfly chitosan [8]. It is also shown that very short chito-oligomer (polymerization degree of 4.5) can be prepared by further treatment of cicada chitosan [12].

These efforts to find a novel source of chitosan can be explained for the growing market of the functional materials in both in vivo and in vitro delivery system.

✉ Weon-Sun Shin
hime@hanyang.ac.kr

¹ Department of Food and Nutrition, College of Human Ecology, Hanyang University, Seoul 04763, Republic of Korea

Encapsulation system prepared with various hydrocolloids and bio-carbohydrate have been widely used to protect and deliver the sensitive bioactive compounds to the target place. Through these system can increase bioavailability of an active material and the controlled-release, and even to mask specific odors and flavors [13]. Encapsulation of compounds such as microcapsules, beads, and microemulsions is commonly used in the food and pharmaceutical industries [14], and a nanoencapsulation system has been increasingly used as carriers in the delivery system [15]. In previous researches, many trials to make the nanoparticles have been conducted through ionic gelation technique using the cationic amino group of chitosan and various anions [16, 17]. However, any trial to make nanobeads or nanoparticles with cricket chitosan has not been conducted yet, and their optical properties have not been investigated. In this study, cricket (*Gryllus bimaculatus*) as a novel source was used to extract chitin and chitosan. Consecutively, the molecular characters of the extracted chitosan were qualitatively identified, and then the optimal condition of nanobeads formulation was investigated by an ionic gelation technique.

Materials and methods

Materials

Dried crickets were provided from Cricket Farm (Hwasung, Korea) and the commercial chitosan (degree of deacetylation 75–85%, Mw 50,000–190,000, catalog no. 448869) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). For the extraction of chitosan from the cricket (*G. bimaculatus*), oxalic acid was purchased from Shinyo Pure Chemistry Co. (Tokyo, Japan), and ammonium persulfate (APS) and sodium hydroxide (NaOH) were purchased from Samchun Pure Chemical Co. (Seoul, Korea). For the chitosan NP preparation, TPP was purchased from Sigma-Aldrich Chemical Co.. Acetic acid was purchased from Junsei Chemical Co. (Tokyo, Japan) and NaCl from Samchun Pure Chemical Co.. Distilled water was used in all physicochemical studies of the cricket chitosan.

Extraction of chitosan from the cricket

Extraction of chitosan from the cricket (*G. bimaculatus*) was performed according to a modified method of Ai et al. [18]. The dried crickets were ground into a fine powder in a grinder, and the cricket samples were washed with distilled water and dried at 60 °C. In order to remove the cricket

proteins, 40 g of cricket powder was immersed in 400 mL of 1 M NaOH in shaking water bath (Sejong Scientific Co., Ansan, Korea) at 95 °C and 130 rpm for 6 h. The reaction mixture was then filtered through a 100 mesh sieve, and the residue was washed with distilled water until pH 5.5. In order to remove the color, an APS solution (50% (w/v)) was freshly prepared by dissolving 400 g of APS in 400 mL distilled water at 50 °C for 30 min. The residue was immersed in the APS solution in shaking water bath at 50 °C and 130 rpm for 6 h, filtered through a 100 mesh sieve, and washed with distilled water until pH 5.5. For the demineralization process, the residue was then immersed in oxalic acid solution with stirring for 3 h at room temperature. Upon completion of the reaction, the mixture was filtered through a 100 mesh sieve, and the cricket chitin was washed to pH 5.5 with distilled water and air oven dried at 60 °C overnight. Cricket chitosan was obtained by chitin deacetylation using a highly concentrated sodium hydroxide solution (50–67%) at 95 °C with time course while shaking at 130 rpm in a shaking water bath. The cricket chitosan was filtered, washed to pH 5.5 with distilled water, and air oven-dried at 60 °C overnight. Finally, the dry weight of chitin and chitosan content (%) was measured. The experiments were performed in triplicate.

Fourier transform infrared spectroscopy

In order to identify the IR bands characteristic of chitosan, 0.01 g of cricket (*G. bimaculatus*) chitosan and commercial chitosan were analyzed using a Nicolet iS50 FT-IR & Nicolet iS50 ATR spectrometer (Thermo Fisher Scientific Inc., Madison, WI, USA). Transmittance and absorbance values were recorded between 4000 and 400 cm⁻¹. The DA of cricket chitosan was determined according to the improved method reported by Sabnis and Block [19]. The absorbance ratio (A_{1655}/A_{3450}) was linearly correlated with the DA of the cricket chitosan.

Degree of deacetylation(%) = 97.67

$$- \left[26.486 \times \left(\frac{A_{1655}}{A_{3450}} \right) \right] \quad (1)$$

X-ray diffraction

The crystallinity of the cricket (*G. bimaculatus*) chitosan was determined by XRD. The physicochemical properties of the cricket chitosan were compared with those of commercial chitosan. XRD patterns were recorded with a D8 ADVANCE with DAVINCI (BRUKER, Karlsruhe, Germany) system. Data were collected at 40 kV, 40 mA, and

2θ with a scan angle between 5° and 45° . The crystalline index value (CrI) was measured according to the formula:

$$\text{CrI}_{110} = \left(\frac{I_{110} - I_{\text{am}}}{I_{110}} \right) \times 100 \quad (2)$$

where I_{110} was the maximum intensity at $2\theta = 20^\circ$, and I_{am} was the intensity of amorphous diffraction at $2\theta = 16^\circ$ [10].

Matrix assisted laser desorption ionization–time of flight mass spectrometry

The molecular mass was obtained using AXIMA mass spectrometer (SHIMADZU-Biotech Co., Tokyo, Japan). Measurements were recorded without baseline correction. α -Cyano-4-hydroxycinnamic acid was used as the matrix (10 mg/mL). The matrix (0.01 mL) was mixed on a steel target plate with 0.01 mL of the cricket chitosan (1 mg/mL) and allowed to air-dry at room temperature.

Color, zeta potential, and pH value

For color measurement, 0.5 g of the cricket (*G. bimaculatus*) sample and commercial chitosan were placed on a transparent Petri dish. The color of the samples was measured using a colorimeter (Minolta CR-400, Minolta Co., Ltd., Osaka, Japan), which was standardized with a calibration white plate ($L^* = 96.04$, $a^* = 0.20$, $b^* = 2.01$). Each sample was individually measured in triplicate and parameters were recorded as L^* , a^* , and b^* .

In order to measure the pH value and the zeta potential, 10 mL of commercial and cricket (*G. bimaculatus*) chitosan were dissolved in 0.2% acetic acid aqueous solution at a concentration of 2.0 mg/mL. The zeta potential was determined using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK), operated at $25 \pm 1^\circ\text{C}$ with multiple narrow modes. The pH values were measured using a pH meter (S220, Mettler-Toledo International Inc., Greifensee, Switzerland).

Preparation of Nanoparticle with cricket chitosan

Chitosan NPs were prepared by ionic gelation technique using chitosan and TPP, following previously reported procedures [20]. Cricket (*G. bimaculatus*) and commercial chitosan were dissolved in 0.2% acetic acid aqueous solution at a concentration of 1.44 mg/mL. To remove the residues of insoluble particles, centrifugation ($2795 \times g$, 4°C , 15 min) was used. Under magnetic stirring at room temperature, 2, 4, and 6 mL of TPP aqueous solutions were added to 10 mL of the chitosan solutions using a Pasteur pipette at a 1 mL/min flow rate. The reaction was carried

out for 10 min. The particle size, zeta potential, and PDI of cricket and commercial chitosan were measured.

Physical characterization of nanoparticles

The particle size, zeta potential, and PDI of cricket and commercial chitosan were determined using Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK), operated at $25 \pm 1^\circ\text{C}$ with multiple narrow modes [21–23].

NaCl addition on nanoparticles

A monovalent salt (NaCl) at a concentration of 0.5 mg/mL was used to dissolve the chitosan prior to ionic gelation with the negatively charged TPP ions. Commercial and cricket chitosan were dissolved in a 0.2% acetic acid aqueous solution at a concentration of 1.44 mg/mL. To remove residues of insoluble particles, centrifugation ($2795 \times g$, 4°C , 15 min) was used. Under magnetic stirring (400 rpm) at room temperature, 1, 3, and 5 mL of NaCl aqueous solutions at a concentration of 0.5 mg/mL were added to 10 mL of the chitosan solutions using a pasteur pipette. After 5 min, TPP aqueous solutions (0.6 mg/mL) were added to the chitosan/NaCl solutions under magnetic stirring (800 rpm). The reaction was carried out for 10 min. The particle size, zeta potential, and PDI of commercial and cricket (*G. bimaculatus*) chitosan were measured.

Acetic acid concentration on nanoparticles

In order to measure the effect of the concentration of acetic acid on the formation of chitosan-TPP NPs, three acetic acid concentrations (0.1, 0.2, and 1%) were used for the preparation of the chitosan solutions. Cricket (*G. bimaculatus*) and commercial chitosan (10 mL, 1.44 mg/mL) were dissolved in 0.1, 0.2, and 1% acetic acid aqueous solutions. In order to remove the residues of insoluble particles, centrifugation ($2795 \times g$, 4°C , 15 min) was used. The particle size, zeta potential, and PDI of the chitosan/TPP NPs were compared.

Statistical analysis

All experiments were performed in at least triplicate and expressed as mean $\pm$ standard deviation (SD) values. Statistical analyses were carried out using the Statistical Package for the Social Science (SPSS, Version 21.0, IBM Co., Armonk, NY, USA) with the analysis of variance (ANOVA) by Duncan's multiple range test and Student's *t*-test at $p < 0.05$.

Results and Discussion

Yield of chitin and chitosan

This analysis revealed that chitin constitutes around 5.1% of the dry weight of cricket. From 40 g of dried cricket powder as a raw material, around 2.04 g of chitin was extracted and the chitosan yield was 41.75%. The dry weight of the chitin content has been previously reported to be 18% in *Periplaneta americana* [23], 36.6% in cicada sloughs [24], 15% in *Holotrichia parallela* [11] and 20% in *Bombyx mori* and in silkworm pupa exuviae [25]. Moreover, the chitin content of some Orthoptera species was reported to be between 5.3 and 8.9% [26]. In this study, the dry weight of the chitin of the cricket, *G. bimaculatus* was found to be similar to that of Orthoptera species [26], but lower than those of other insects. The chitin content in crustacean shells, used for commercial chitin, ranged between 7 and 40%, depending on the species [27]. Thus, if the yield could be improved, the cricket (*G. bimaculatus*) would be an attractive option as an alternative chitin and chitosan source for commercial applications. Practically, the extract yield is the most important point for the industrial application at the point of the cost and time. Indeed, the body mass of insect is small compared to the shrimp or crab shell, and even the density of chitin may be apparently less than that of the Crustacea. These factors can be a basic hurdle to increase the extraction yield, but if the each filtering step could be modified under vacuum, the extraction yield should be improved.

Analysis of Fourier transform infrared spectroscopy

FTIR is a powerful analytical method that has been widely used to identify specific functional groups within a compound [8]. Figure 1A, B showed the FTIR spectra of the cricket (*G. bimaculatus*) chitosan and of commercial chitosan. These spectra were found to be quite similar in wavelength and transmittance intensity, and were also similar to other insect chitosan studies reported by Song et al. [8] and Kaya et al. [10]. Chitosan from the cricket (*G. bimaculatus*) exhibited strong bands at 3355 and 3292 cm^{-1} , corresponding to the stretching vibration of O–H and to the extension vibration of N–H, respectively, and commercial chitosan showed these bands at 3354 and 3290 cm^{-1} . The FTIR spectra of the cricket (*G. bimaculatus*) and commercial chitosan showed bands at 2872 and 2871 cm^{-1} , respectively, corresponding to the (CH_2) vibration in the CH_2OH group. As expected, a clear weakening of the bands at 1652 cm^{-1} (C=O) of the NHCOCH_3 group (Amide I band) was observed, indicating successful deacetylation. Moreover, the characteristic N–H

bending vibration of the NHCOCH_3 group (Amide II band) was detected at 1591 cm^{-1} . The Amide I and Amide II band were observed at 1650 and at 1590 cm^{-1} , respectively [28, 29]. The bands at 1417, 1374, and 1315 cm^{-1} were attributed to the C–H stretching vibrations, and that at 262 cm^{-1} to the O–H bending vibration. The C–O stretching vibration in the C–O–C glycosidic linkage, in the secondary OH group, and in the primary OH group were observed at 1058, 1023, and 992 cm^{-1} . In addition, the band at 893 cm^{-1} was attributed to the C–H out-of-plane vibration. In summary, the FTIR spectra of the chitosan from the cricket (*G. bimaculatus*) chitosan and of commercial chitosan showed considerable similarity.

Degree of deacetylation

The DA of the chitosan extracted from the cricket (*G. bimaculatus*) was calculated using FTIR. Table 1 indicates the DA of chitosan as a function of reaction time and NaOH concentration (%). When the extracted chitosan was reacted in 50% of NaOH solution for 9 h, it was deacetylated up to 66.54%. However, when treated with 55% of NaOH solution, the DA drastically increased 75.18 and 77.63% after 9 and 12 h, respectively. In addition, the treatment with 67% NaOH for 9 h deacetylated the cricket chitosan up to 84.98% maximally, but the yield was dramatically lowered. In a previous work, Mohammed et al. [29] studied the effect of the NaOH concentration and reaction time on the deacetylation process. They showed that as the NaOH concentration and reaction time increased, the DA of cricket (*G. bimaculatus*) chitosan significantly increased, but the chitosan yield gradually decreased. The DA of the insects species, Colorado potato beetle and blowfly, are 71% [10] and 88.2% [8], respectively.

Analysis of X-ray diffraction

The chitosan extracted from the cricket, *G. bimaculatus*, was examined with XRD. Peaks were observed at 10.50° and 20.07° for the chitosan extracted from cricket, and at 10.33° and 19.90° for commercial chitosan. As shown in Fig. 1C, D, XRD patterns of cricket (*G. bimaculatus*) and commercial chitosan were quite similar. In previous studies, XRD peaks of the chitosan of Colorado potato beetle have been reported at 9.38° and 20.4° for adults and at 9.7° and 20.2° for larvae [10]. In addition, in this study, XRD analysis of chitosan showed two peaks similar to those derived from organisms such as shrimp, crayfish, and crab [30–32]. The CrI values of the chitosan from the cricket (*G. bimaculatus*) at $2\theta = 20^\circ$, maximum intensity ≈ 57.875 ; at $2\theta = 16^\circ$, the intensity of amorphous diffraction

Fig. 1 FTIR spectra of the chitosan obtained from the cricket, *G. bimaculatus* (A) and of commercial chitosan (B); X-ray diffraction patterns (XRD) of the cricket, *G. bimaculatus* chitosan (C) and of commercial chitosan (D)

Table 1 Degree of deacetylation of chitosan as a function of reaction time and NaOH concentration

Reaction time (h)	NaOH concentration (%)		
	50	55	67
1	56.47 ^a	—	—
2	57.72	—	—
3	57.92	—	—
6	66.94	—	—
9	66.54	75.18	84.98
15	—	77.63	—

^aDegree of deacetylation (%) value

≈ 24.583) and of commercial chitosan were calculated to be quite similar (57.52 and 58.92%, respectively). Al Sagheer et al. [32] noted that the CrI values of the chitosans

obtained from cuttlefish, squid pens, shrimp, and crab shells ranged between 36 and 71%. Thus, the CrI value of the chitosan from the cricket (*G. bimaculatus*) was moderate in comparison to the values obtained from other living organisms.

Analysis of Matrix assisted laser desorption ionization–time-of-flight mass spectrometry

As one of the important factors affecting the physicochemical and biological properties of chitosan, the molecular mass of the cricket (*G. bimaculatus*) chitosan was determined using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS). The lower molecular mass limit of MALDI-TOF-MS was around 500–550 Da due to signals arising from molecular,

fragment, and adduct ions of the matrix [33]. As shown in Fig. 2, the molecular mass of the chitosan from the cricket (*G. bimaculatus*) was found to be below 8302 Da. A strong signal was observed in the range of 550–1500, and a weak signal was detected in the wide range of 1511.46–8302.92. The strong peak indicated that the product was mainly composed of chitooligosaccharides (COS; (GlcN)_n and (GlcNAc)_n) with a degree of polymerization (DP) of 2–8. On the other hand, the weak peak represented the molecular weight of the chitosan polymer. In a previous work, MALDI-TOF MS analysis of *N,N,N*-trimethylated chitosan (TMC) revealed a molecular weight in the range of about 1000–9000 Da, similar to that of the chitosan extracted from the cricket [34]. The results showed that the Mw of the cricket (*G. bimaculatus*) chitosan was lower than that (50–190 kDa) of commercial chitosan [35]. The molecular mass of other species were as follows: Colorado potato beetle 2.722 kDa for adults and 2.676 kDa for larvae [10]; blowfly 501 kDa [8]; housefly 426 kDa [18]; and *Metapenaeus stebbingi* shells 2.20 kDa [30].

Numerous studies have reported that the Mw of chitosan could greatly affect its biological properties. Namely, a low-molecular chitosan in the range of 5000–10,000 Da showed a strong bactericidal and superior biological activities as compared with high-molecular chitosan [36]. Prasertsung et al. [36] reported that very-low-molecular chitosan, COS (Mw < 10 kDa) are a more favorable material for the growth and osteogenic differentiation of adipose-derived and bone marrow-derived stem cells, as compared with a high molecular chitosan. Thus, the cricket (*G. bimaculatus*)

chitosan with a low molecular mass could serve as a potential material for biomedical applications. Through this investigation, the findings clearly showed that the molecule mass was below 8,032 Da, and mainly distributed in the range of 560–1,284 Da with a highly similar molecular structure to the commercial chitosan.

Color, zeta potential, and pH value

Color is described using the CIE *L** (darkness to lightness), *a** (greenness to redness), and *b** values (blueness to yellowness). The *L**, *a**, and *b** values of cricket (*G. bimaculatus*) and commercial chitosan were shown in Table 2. Two chitosan samples exhibited different results. The cricket chitosan has significantly lighter in color than the raw cricket powder, but less light than the commercial. In contrast, the *a** value of cricket chitosan was higher than that of commercial chitosan. In previous studies, the *L**, *a**, and *b** values of the chitosan extracted from crawfish were reported to be 80.91, 1.82, and 28.86 respectively [37]. Moreover, the chitosan extracted from *Metapenaeus stebbingi* shells showed *L**, *a**, and *b** values of 83.14, −0.12, and 13.58, respectively, whereas chitosan from crab shells presented values of 58.6–59.1, 1.1–1.8, 14.0–15.4, respectively [30, 38]. Therefore, it is suggested that these differences described above could be derived from the original color intensity of insects used as a raw material as well as the extraction methods.

Table 2 also showed the zeta potential and pH values of cricket (*G. bimaculatus*) and commercial chitosan. The two

Fig. 2 MALDI-TOF MS spectrum of the cricket, *G. bimaculatus* chitosan; Strong peak signal (A); weak peak signal (B)

Table 2 Color, zeta potential and pH value of the cricket, *G. bimaculatus*, chitosan and of commercial chitosan

Sample	<i>L</i> *	<i>a</i> *	<i>b</i> *	Zeta potential (mV)	pH
Cricket powder	38.13 ± 0.06 ^c	2.77 ± 0.08 ^a	6.38 ± 0.03 ^c	–	–
Extracted chitosan	72.16 ± 0.12 ^b	1.96 ± 0.06 ^b	11.83 ± 0.04 ^b	43.58 ± 1.28	3.84 ± 0.01
Commercial chitosan	77.92 ± 0.05 ^a	0.51 ± 0.06 ^c	13.06 ± 0.05 ^a	47.17 ± 3.03	3.88 ± 0.01*

^{a–c}Means with different superscripts in the same column are significantly different at $p < 0.05$

*Indicates a significant difference between two groups ($p < 0.05$)

Each value is expressed as mean ± SD (n = 3)

chitosans were strongly cationic charged and, their zeta potential was quite similar. The pH value of the chitosan cricket was found to be about 3.88. Usually, a chitosan aqueous solution has a pH of 3.5–4.5. Under the same conditions, solutions of the cricket (*G. bimaculatus*) and commercial chitosan proved to be acidic.

Physical characteristics of cricket chitosan nanoparticles

Physical properties of the cricket (*G. bimaculatus*) chitosan, such as particle size, zeta potential, and PDI, were measured using dynamic light scattering (DLS) analysis. Table 3 showed the physical characteristics of nanoparticles (NPs) of cricket (*G. bimaculatus*) (A) and commercial chitosan (B) NPs. Compared with commercial chitosan, cricket (*G. bimaculatus*) chitosan has smaller particle size. Moreover, as the TPP volume increased, the particle size of cricket (*G. bimaculatus*) and commercial chitosan NPs increased. The size of the two chitosan NPs ranged between 174 and 245 nm and between 196 and 332 nm, respectively. The zeta potential of the two chitosan revealed a strong cationic charge, and ranged between 30.02 and 36.12 mV and between 33.46 and 38.14 mV, respectively. In general, the polydispersity index (PDI) values ranging 0–0.3 indicated a homogeneous nanosuspension, but the higher values were related to a less homogeneous particle size distribution. As presented in Table 3, the PDI values of cricket (*G. bimaculatus*) and commercial chitosan were in the range 0.26–0.29 and 0.32–0.34, respectively. Thus, cricket (*G. bimaculatus*) chitosan NPs had a more stable particle size distribution than commercial chitosan NPs. As described above, formulating the nanoparticles with a low-molecular weight chitosan extracted from cricket (*G. bimaculatus*) is a novel trial. Fortunately, relatively low molecular-mass chitosan formed the more stable nanoparticles than the commercially available chitosan. However, the network porosity and density in chitosan nanoparticle depending upon the molecular weight need to be checked further.

Effect of NaCl addition on nanoparticles

Various volumes (1, 3, and 5 mL) of NaCl at a concentration of 0.5 mg/mL were used to dissolve the cricket (*G. bimaculatus*) chitosan prior to ionic gelation with the negatively charged TPP ions. Antoniou et al. [39] reported that NaCl at a concentration of 0.5 mg/mL provided the optimal solution ionic composition, which decreased the chitosan NP size by 25% and afforded a zeta potential of about 18.5 mV and a PDI value of about 0.35. However, the effect of the volume used has not been studied yet. Table 3 showed the effect of the addition of NaCl (0.5 mg/mL) on the cricket (*G. bimaculatus*) chitosan particle size and PDI value. Upon addition of NaCl, the size of the cricket (*G. bimaculatus*) chitosan NPs decreased up to 15.5%. Pothakamury and Barbosa-Cánovas [13] reported that reduction of NPs' size increased the efficiency of absorption and the bioavailability. Our finding showed that the PDI values were in the range 0.26–0.30. Thus, the PDI values of cricket (*G. bimaculatus*) had stable particle size distribution at NaCl volumes of 1, 3, and 5 mL. Moreover, the zeta potential indicated a strong cationic charge and ranged between 33.32 and 34.04 mV. Although the decrease in the NP size was lower than that reported by Antoniou, Liu [39], the zeta potential and the PDI values revealed a stronger cationic charge and led to a narrow particle size distribution.

Effect of acetic acid concentration on nanoparticles

In order to verify whether the size reduction was affected by the acetic acid concentration, three acetic acid solutions with concentrations of 0.1, 0.2, and 1% were used to prepare 10 mL of chitosan solutions (0.144 mg/mL). This study revealed as the acetic acid concentration increased, the cricket (*G. bimaculatus*) chitosan NPs' size decreased (257, 208 and 166 nm, respectively (Table 3). The PDI values were found to range between 0.26 and 0.27, and the zeta potential revealed a strong cationic charge and ranged between 35.72 and 39.17 mV. Fan et al. [1] reported that acetic acid interacts with the amine groups and the

Table 3 Particle size, zeta potential, and PDI of cricket, *G. bimaculatus* chitosan (a) and of commercial chitosan (b) nanoparticles (NPs); effect of NaCl addition (c) and of the acetic acid concentration (d) on cricket, *G. bimaculatus* chitosan particle size and PDI value

(a)				(b)			
TPP (V/V) (mL)	Particle size (nm)	Zeta potential (mV)	PDI	TPP (V/V) (mL)	Particle size (nm)	Zeta potential (mV)	PDI
2	245.22 ± 0.67 ^a	36.12 ± 4.60 ^a	0.26 ± 0.00 ^b	2	331.97 ± 2.30 ^a	38.14 ± 0.82 ^a	0.33 ± 0.01 ^a
4	208.27 ± 3.47 ^b	35.72 ± 1.29 ^b	0.27 ± 0.03 ^{ab}	4	249.71 ± 5.70 ^b	37.75 ± 0.89 ^a	0.34 ± 0.04 ^a
6	174.30 ± 2.79 ^c	30.02 ± 1.13 ^b	0.29 ± 0.01 ^a	6	196.35 ± 0.51 ^c	33.46 ± 0.33 ^b	0.32 ± 0.01 ^a
(c)				(d)			
NaCl (V/V) (mL)	Particle size (nm)	Zeta potential (mV)	PDI	Acetic acid (%)	Particle size (nm)	Zeta potential (mV)	PDI
0	208.27 ± 3.47 ^a	35.72 ± 1.29 ^a	0.27 ± 0.03 ^{ab}	0.1	256.89 ± 5.11 ^a	39.17 ± 0.62 ^a	0.26 ± 0.0 ^a
1	195.16 ± 3.25 ^b	33.32 ± 1.75 ^a	0.26 ± 0.01 ^b	0.2	208.27 ± 3.47 ^a	35.72 ± 1.29 ^b	0.27 ± 0.03 ^{ab}
3	193.35 ± 1.43 ^b	34.04 ± 3.79 ^a	0.27 ± 0.01 ^b	1	166.36 ± 2.35	37.98 ± 0.60 ^a	0.27 ± 0.0 ^a
5	176.09 ± 3.92 ^c	34.92 ± 1.08 ^a	0.30 ± 0.0 ^a	-	-	-	-

^{a-c}Means with different superscripts in the same column are significantly different at $p < 0.05$. Each value is expressed as mean ± SE (n = 3)

increased acetic acid concentration indirectly increased the ionic strength; thus, the increased shielding effect of counter-ions (CH_3COO^-) lowers the amount of cross-linking in the chitosan molecules that can be accessed by the TPP anions. These findings revealed that the positive-charged cricket (*G. bimaculatus*) chitosan formed the smaller beads and more homogeneous phase than the commercial. Even, addition of acetic acid and NaCl reduced the size of cricket chitosan nanoparticles, contributing the phase stability in the dispersion. As a novel resource, cricket chitosan could be applied to an emerging functional market with either an intact form or an active polymer for nanocapsules.

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