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Physicochemical characterization of chitin and chitosan from crab shells

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ABSTRACT

Crab chitosan was prepared by alkaline *N*-deacetylation of crab chitin for 60, 90 and 120 min and the yields were 30.0-32.2% with that of chitosan C120 being the highest. The degree of *N*-deacetylation of chitosans (83.3–93.3%) increased but the average molecular weight (483–526 kDa) decreased with the prolonged reaction time. Crab chitosans showed lower lightness and WI values than purified chitin, chitosans CC and CS but higher than crude chitin. With the prolonged reaction time, the nitrogen (8.9–9.5%), carbon (42.2–45.2%) and hydrogen contents (7.9–8.6%) in chitosans prepared consistently increased whereas N/C ratios remained the same (0.21). Crab chitosans prepared showed a melting endothermic peak at 152.3–159.2 °C. Three chitosans showed similar microfibrillar crystalline structure and two crystalline reflections at 2θ = 8.8–9.0° and 18.9–19.1°. Overall, the characteristics of three crab chitosans were unique and differed from those of chitosan CC and CS as evidenced by the element analysis, differential scanning calorimetry, scanning electron microscopy and X-ray diffraction patterns.

1. Introduction

Chitin, found in the shell of crustaceans, the cuticles of insects, and the cell walls of fungi, is the second abundant biopolymer in the nature (Knorr, 1984). Structurally, chitin is a straight-chain polymer composed of β-1,4-*N*-acetylglucosamine and classified into α-, β- and γ-chitin (Cabib, 1981; Cabib, Bowers, Sburlati, & Silverman, 1988). Chitosan derived by partial *N*-deacetylation of chitin is also a straight-chain polymer of glucosamine and *N*-acetylglucosamine (Muzzarelli, Rochetti, Stanic, & Weckx, 1997). α-Chitin has a structure of antiparallel chains whereas β-chitin has intrasheet hydrogen-bonding by parallel chains (Minke & Blackwell, 1978; Jang, Kong, Jeong, Lee, & Nah, 2004). However, γ-chitin has a parallel and antiparallel structure, which is a combination of α-chitin and β-chitin (Jang et al., 2004).

Because chitin possesses many beneficially biological properties such as biocompatibility, biodegradability, hemostatic activity, and woundhealing property, much attention has been paid to its biomedical applications (Farkas, 1990; Fleet & Phaff, 1981). Chitosan has been used as a dietary supplement due to its effective lipid binding and hypocholesterolemic properties and as a film-forming agent (Shahidi, Arachchi, & Jeon, 1999). Chitin, chitosan and their derivatives can be used as an antimicrobial, emulsifying, thickening and stabilizing agent in food industry (Shahidi et al., 1999).

Chitin is always made from crustaceans and therefore; crab shell is a source of chitin and chitosan. The objective of this study was to purify crab chitin from commercial crab chitin using acid and alkaline treatments followed by decolorization with potassium permanganate and to prepare chitosan therefrom by further *N*-deacetylation treatment with concentrated sodium hydroxide solution. The yields, degrees of *N*-deacetylation (DD), molecular weights (M_w) and color characteristics of various chitosan products were determined. The physicochemical properties of chitosans prepared were then studied using element analysis, differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and X-ray diffraction patterns.

2. Experimental

2.1. Materials

Crude crab chitin, crab chitosan, potassium bromide, potassium permanganate, sodium hypochlorite and dextrans M_W 2 000 000, 670 000, 482 000, 270 000, 181 000 and 40 000 were the products of Sigma Chemical Co. (St. Louis, MO). Commercial crab α-chitosan form the snow crab (*Chionoecetes opilio)* was obtained from Dalian City, Liaonin, China. Sodium hydroxide was obtained from Wako Pure Chemical Co. (Osaka, Japan). Ethanol (95% pure) was supplied by Taiwan Tobacco & Wine Monopoly Bureau (Taipei). Other reagents were of analytical grade.

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2.2. Purification of crab chitin

Crude chitin from crab shells (~200 g each) was ground using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany) to obtain coarse power (65 mesh) and treated with the method of Kurita, Tomita, Tada, Nishimura, and Ishii (1993) with some modification. The powder of crab chitin was treated with 1 N HCl solution at room temperature for 6 h to remove minerals and then treated with aqueous sodium hydroxide solution at the ratio of 1:10 (w/v) at 100 °C for 3 h to remove protein. The mixture was filtered and washed with deionized water to neutral. For the purpose of decolorization, the precipitate thus obtained was treated further with 1% potassium permanganate solution for 1 h, and then reacted with 1% oxalic acid solution for 1 h. (Chang, 1982). Following decolorization, the precipitate was washed with deionized water to neutral. After freeze drying, the product obtained was designated as purified crab chitin.

2.3. Preparation of crab chitosan

For the purpose of *N*-deacetylation, 1 g of purified crab chitin was treated with 30 ml of 40% sodium hydroxide solution at 105 °C for 60, 90 and 120 min, respectively. After filtration, washing to neutral with deionized water and freeze drying, the corresponding chitosans obtained with various DD were designated as chitosans C60, C90 and C120. The yields of various precipitated chitosans were also determined.

2.4. Measurement of degree of N-deacetylation

The DD of crab chitosan samples, including chitosans C60, C90, C120, and crab chitosan from China (CC) and crab chitosan from Sigma (CS), was determined using an Equinox 55 infrared spectrometer (Bruker Optics Inc., Billerica, MA). An aliquot of chitosan samples was mixed with potassium bromide (1:1000) and compressed into pellets. The absorbances at 1655 and 3450 cm^{-1} were used to calculate the DD according to the following equation (Baxter, Dillon, Taylor, & Roberts, 1992; Muzzarelli et al., 1997):

$$DD(\%) = 100 - (A_{1655}/A_{3450}) \times 115$$

2.5. Determination of molecular weight

Molecular weights of crab chitosan samples were estimated using gel permeation chromatography (GPC) (Leung, Fung, & Choy, 1997). The GPC equipment consisted of a Pharmacia Biotech LC column (2.6 × 100 cm) packed with Sephacryl S-500 HR and a Pharmacia Biotech P-1 pump. Chitosan solutions (2 mg/ml) were applied to the column. The flow rate was maintained at 0.25 ml/min and 0.2 M acetic acid/0.1 M sodium acetate was used as the eluent. The eluate from the chromatographic system was collected by a fraction collector, 5 ml per tube. The standards used were dextrans M_W 2 000 000, 670 000, 482 000, 270 000, 181 000 and 40 000. The standard solutions and chitosan samples were detected by measuring the absorbance of the eluate at 488 nm using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.6. Color measurement

The reflective surface color of powder of crab chitins and chitosans was measured using a Σ80 Color Measuring System (Nippon Denshoku Inc., Tokyo, Japan) and L^*, a^* and b^* values were recorded. A standard white plate ($X = 91.98$, $Y = 93.97$ and $Z = 110.41$) was used to standardize the instrument. Each sample was individually measured in triplicate. Whiteness index (WI) was calculated based on the following equation (Hsu, Chen, Weng, & Tseng, 2003):

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.7. Elemental analysis and protein assay

A Heraeus CHN-OS Rapid Elemental Analyzer (Heraeus, Germany) was used to determine the contents of carbon, hydrogen and nitrogen elements in crab chitins and chitosans. Protein concentrations were determined by measuring the absorbance at 280 nm as described in Stoscheck (1990).

2.8. Thermal analysis

Differential scanning calorimetry was conducted with a Setaram DSC121 (Setaram Co., France). A proportion of 100–140 mg of sample dispersions was put into a stainless crucible (Setaram) with an aluminum O-ring and hermetically sealed by a sample-encapsulating press. The samples were heated from 30 to 350 °C at 5 °C/min. An empty crucible was applied as a reference. Indium was used to calibrate the instrument. Enthalpy (ΔH in J/g dry weight), and onset (T_o), peak (T_p) and completion (T_c) temperatures, were computed automatically.

2.9. X-ray diffraction

The wide-angle X-ray diffraction (WAXD) analysis was applied to detect the crystallinity of chitins and chitosans prepared and their patterns were recorded using a Rigaku III diffractometer (Rigaku Corp., Japan) with Cu radiation (40 kV, 30 mA). Data were collected at a scan rate of 1°/min with the scan angle from 2° to 40°.

2.10. Scanning electron microscopy

The samples were fixed on an SEM sample holder, dried by a critical point dryer (LADD 28000), and coated with a thin gold layer of 3 mm by a sputter coater (JBS E5150) for conductivity. A Topcon ABT-150s scanning electron microscope (Topcon Corp., Tokyo, Japan) was used to examine the microstructure of chitins and chitosans.

2.11. Statistical analysis

Each preparation and measurement was conducted in triplicate. The experimental data were subjected to an analysis of variance (ANOVA) for a completely random design (CRD) using a Statistical Analysis System (SAS Institute, Inc., 2000). Duncan's multiple range tests were used to determine the difference among means at the level of 0.05.

3. Results and discussion

3.1. Preparation of crab chitosans

Crude crab chitin was purified using acid and alkaline treatments followed by decolorization with potassium permanganate and the yield of purified chitin was 63.3 ± 0.1%. After *N*-deacetylation, the yields of crab chitosans were in the range of 30.0–32.2% (Table 1). However, the yield of chitosan C120 was higher than those of chitosans C60 and C90. The DD of chitosans increased with the prolonged reaction time and reached 93.3% at 120 min. With *N*-deacetylation for 90 min, the DD of crab chitosan C90 was higher than those of chitosans CC and CS. The average M_W of chitosans C60, C90 and C120 were in the range of 483–526 kDa and decreased with the prolonged reaction time. Obviously, with regard to the average M_W, chitosan CC was the lowest while chitosan CS was the highest.

Table 1
Product parameters of crab chitosans

Sample	Yield (%)	DD (%)	M_W (kDa)
Chitosan C60	30.5 ± 0.3 B[a]	83.3 ± 0.6 E	526 ± 7 B
Chitosan C90	30.0 ± 0.8 B	88.4 ± 0.3 B	513 ± 4 C
Chitosan C120	32.2 ± 0.7 A	93.3 ± 0.4 A	483 ± 7 D
Chitosan CC	–	87.8 ± 0.3 C	213 ± 5 E
Chitosan CS	–	85.2 ± 0.4 D	549 ± 4 A

[a] Each value is expressed as mean ± standard error (n = 3). Means with different small letters within a column are significantly different (P < 0.05).

Yen and Mau (2004) purified the same crab chitin using acid and alkaline treatments followed by decolorization with ethanol and got a similar yield of 64.4%. Yen and Mau (2006) prepared fungal chitin from shiitake stipes using alkaline treatments followed by decolorization with ethanol or potassium permanganate and obtained yields of 36.7% or 25.1%, respectively. It seems that with different decolorization methods, the yields of crab chitin were similar whereas those of fungal chitin were different. However, with different raw materials and different decolorization methods, the yields of crab chitin were apparently higher than those of fungal chitin, which might be a complex of polysaccharide and chitin (Yen & Mau, 2006).

Yen and Mau (2007a) also used 40% sodium hydroxide for *N*-deacetylation of fungal chitin and found that the yields of fungal chitosans C60, C90 and C120 were 23.7, 21.8 and 24.0%, respectively. The DD of chitosans C60, C90 and C120 was 78.1, 86.3 and 90.2% and the average M_W was 437, 403 and 383 kDa, respectively (Yen & Mau, 2007a). Apparently, crab chitosan showed higher yield, DD and M_W than fungal chitosan from shiitake stipes. In addition, as the reaction time prolonged, the DD increased whereas the average M_W decreased. These are in general agreement with the findings of Chen, Wang, and Ou (2004), Chang, Lin, and Chen (2003), Rogovina, Akopova, and Vikhoreva (1998) and Tsaih and Chen (2003). It seems that the reaction time remarkably influenced the yield, DD and the average M_W of chitosan. It is well-known that DD and M_W values affect the physicochemical and functional properties of chitosan considerately (Berth, Dautzenberg, & Peter, 1998; Chang et al., 2003; Teng, Khor, Tan, Lim, & Tan, 2001; Tsaih & Chen, 2003).

3.2. Color characteristics of crab chitosans

Purified crab chitin showed lighter and whiter than crude chitin as evidenced by its higher L^* and WI values (Table 2). Yen and Mau (2004) found that the L^*, a^*, b^* and WI values of purified crab chitin from ethanol decolorization were 50.3, 1.0, 18.5 and 47.0, respectively. Obviously, decolorization with potassium permanganate showed lighter and whiter appearance than decolorization with ethanol. Generally, *N*-deacetylation using alkaline treatment made crab chitosan beige to brown color. Apparently, the longer the reaction time, the worse the color of crab chitosans was found. Crab chitosans showed lower lightness and WI values than purified chitin, chitosans CC and CS but higher than crude chitin. With regard to a^* value, chitin and chitosan CC showed the lowest redness (both 0.3) whereas chitosan CS showed the highest (2.2). With regard to b^* value, crude chitin showed the highest yellowness (16.8) and purified chitin and crab chitosans showed higher yellowness (14.0–15.4) whereas chitosans CC and CS showed the lowest (10.1–11.4). It seems that chitosan prepared showed slight red and noticeably yellow color.

Table 2
Color characteristics of chitins and chitosans from crab shells

Sample	L^*	a^*	b^*	WI[a]
Chitin	55.4 ± <0.1 F[b]	0.3 ± <0.1 F	16.8 ± 0.1 A	43.9 ± 0.1 G
Purified chitin	62.4 ± 0.1 B	1.1 ± <0.1 E	14.7 ± 0.2 C	59.6 ± 0.1 B
Chitosan C60	59.0 ± 0.1 D	1.4 ± <0.1 C	14.6 ± <0.1 C	56.5 ± <0.1 E
Chitosan C90	59.1 ± 0.1 D	1.8 ± <0.1 B	14.0 ± 0.1 D	56.7 ± 0.1 D
Chitosan C120	58.6 ± <0.1 E	1.1 ± <0.1 D	15.4 ± <0.1 B	55.8 ± <0.1 F
Chitosan CC	63.4 ± <0.1 A	0.3 ± 0.1 F	10.1 ± <0.1 F	62.1 ± 0.1 A
Chitosan CS	60.2 ± <0.1 C	2.2 ± <0.1 A	11.4 ± <0.1 E	58.6 ± <0.1 C

[a] WI (whiteness index) = $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.
[b] Each value is expressed as mean ± SE (n = 3). Means with different letters within a column are significantly different (P < 0.05).

3.3. Elemental analysis and protein assay of crab chitosans

After purification, the nitrogen, carbon and hydrogen contents as well as N/C ratio in purified chitin significantly increased (Table 3). Similarly, after *N*-deacetylation, crab chitosans C60, C90 and C120 were high in their nitrogen, carbon and hydrogen contents as well as N/C ratios. With the prolonged reaction time, the nitrogen, carbon and hydrogen contents in chitosans prepared consistently increased whereas N/C ratios remained the same. The nitrogen of crab chitin was mainly distributed in protein and chitin. After alkaline treatment, the protein was removed and no protein was found in chitin as indicated by no absorbance at 280 nm (Layne, 1957; Stoscheck, 1990).

After conversion of the nitrogen content to the chitin content by a theoretical factor of 203/14, which is the molecular weight ratio of a monomer ($C_8H_{13}O_5N$ = 203) to nitrogen (N = 14) in the chitin (general formula $[C_8H_{13}O_5N]_n$), the chitin contents in crude and purified crab chitin were 77.1 and 90.0%, respectively. It seems that acid and alkaline treatments followed by decolorization with potassium permanganate were an effective method in improving the purity of purified chitin. However, purified chitin contained some components other than chitin.

After conversion of the nitrogen content to the chitosan content by a theoretical factor of 161/14, which is the molecular weight ratio of a monomer ($C_6H_{11}O_4N$ = 161) to nitrogen (N = 14) in the

Table 3
The elemental analysis of chitin from crab shells

Sample	Content (%)			N/C
	N	C	H	
Chitin	5.3 ± 0.1 E[a]	41.1 ± 0.1 G	7.1 ± 0.1 E	0.13 ± <0.01 C
Purified chitin	6.2 ± 0.1 D	44.4 ± 0.3 B	7.5 ± <0.1 D	0.14 ± <0.01 B
Chitosan C60	8.9 ± 0.1 C	42.2 ± 0.2 E	7.9 ± 0.2 C	0.21 ± <0.01 A
Chitosan C90	9.1 ± 0.2 B	43.2 ± 0.1 C	8.4 ± 0.1 B	0.21 ± <0.01 A
Chitosan C120	9.5 ± 0.2 A	45.2 ± 0.2 A	8.6 ± 0.3 A	0.21 ± <0.01 A
Chitosan CC	8.9 ± 0.1 C	42.8 ± 0.3 D	8.2 ± 0.2 C	0.21 ± <0.01 A
Chitosan CS	8.8 ± <0.1 C	41.8 ± 0.1 F	8.1 ± 0.2 C	0.21 ± <0.01 A

[a] Each value is expressed as mean ± SE (n = 3). Means with different small letters within a column are significantly different (P < 0.05).

Table 4
Thermal properties of chitosans from crab shells

Sample	Endotherm (°C)			
	T_o[a]	Tp[a]	T_c[a]	ΔH[a] (J/g)
Chitosan C60	126.4 ± 0.5 B[b]	152.3 ± 0.4 C	220.5 ± 0.3 C	111.0 ± 0.1 C
Chitosan C90	125.5 ± 0.1 C	158.2 ± 0.6 A	226.2 ± 0.2 A	116.6 ± 0.1 B
Chitosan C120	124.7 ± 0.3 D	159.2 ± 0.6 A	224.4 ± 0.5 B	125.2 ± 0.1 A
Chitosan CC	116.7 ± 0.1 E	138.9 ± 0.4 D	181.3 ± 0.2 E	47.5 ± 0.1 E
Chitosan CS	133.7 ± 0.2 A	153. 8 ± 0.6 B	182.5 ± 0.1 D	55.7 ± 0.1 D

[a] T_o, onset temperature; T_p, peak temperature; T_c, completion temperature; ΔH (J/g dry weight), peak enthalpy.
[b] Each value is expressed as mean ± SE (n = 3). Means with different small letters within a column are significantly different (P < 0.05).

chitosan (general formula $[C_6H_{11}O_4N]_n$), the chitosan contents, namely the purities were 102, 105, 109, 102 and 101% for chitosans C60, C90, C120, CC and CS, respectively. After *N*-deacetylation, the product with a nitrogen content of more than 7.0% was considered as chitosan (Muzzarelli & Rocchetti, 1985). After calculation, it seems that the purity for the product to be considered as chitosan was 80.5%. Accordingly, all crab chitosans were nearly pure chitosans. For the purity of crab chitosan products, the reaction time of 60 min was sufficient.

3.4. Thermal properties of crab chitosans

The thermal properties of fungal chitin and chitosans have been studied using DSC from 30 to 350 °C. Crab chitosans C60, C90 and C120 showed a melting endothermic peak at 152.3–159.2 °C and their onset and completion temperatures were 124.7–126.4 and 220.5–226.2 °C, respectively (Table 4). The onset, peak and completion temperatures of chitosan CC were all lower than those of crab chitosans prepared whereas chitosan CS had a higher onset

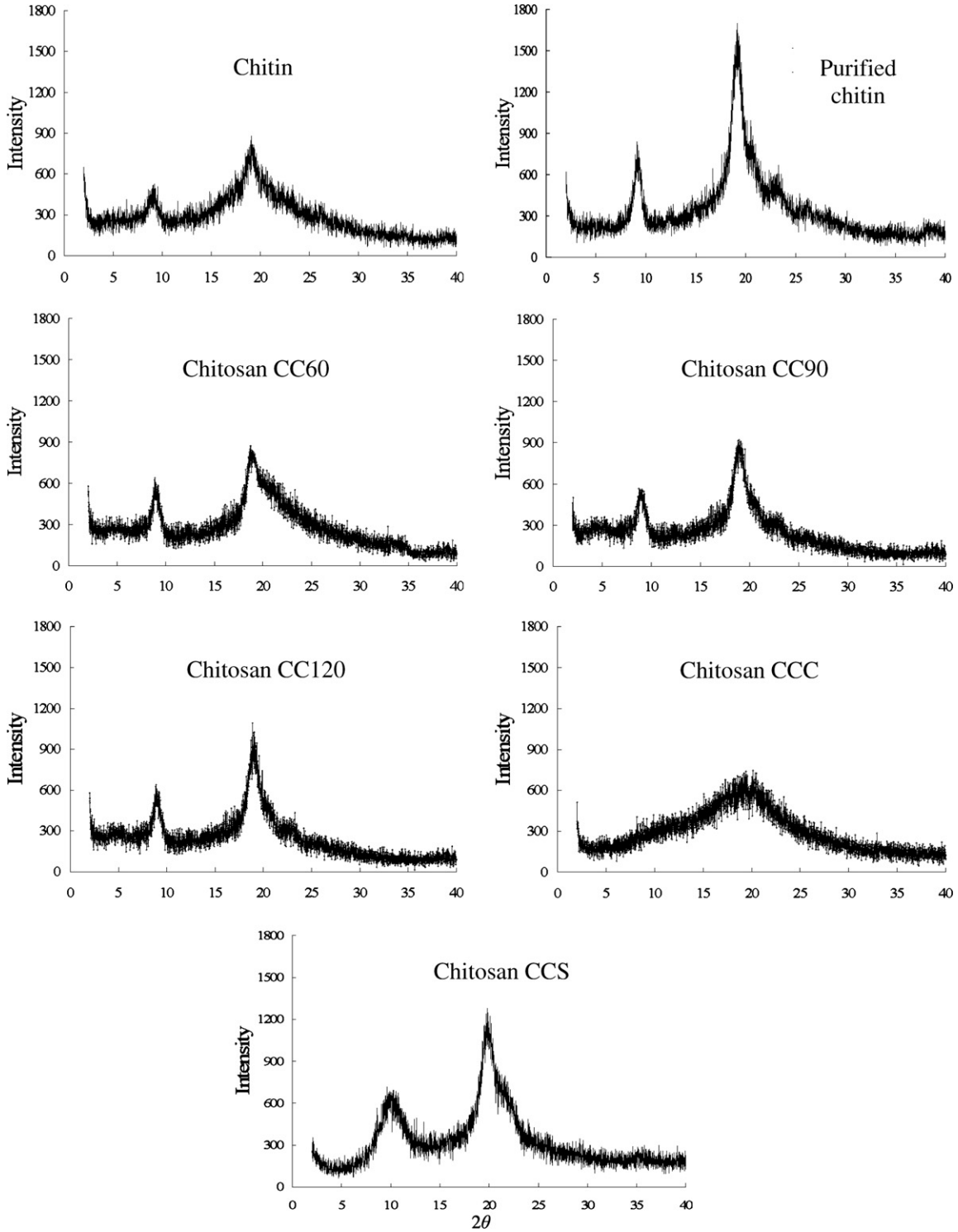

Fig. 1. X-ray diffraction patterns of chitins and chitosans from crab shells.

temperature, a comparable peak temperature and a lower completion temperature. With the prolonged reaction time, the onset temperature decreased and the peak temperature increased. In addition, the temperature ranges from T_o to T_c were in the descending order: chitosans prepared (94.1–100.7 °C) > chitosan CC (64.7 °C) > chitosan CS (48.8 °C).

The melting and dissociation enthalpy (ΔH) of chitosans prepared (111.0–125.2 J/g) increased with the prolonged reaction time and was much higher than those of chitosans CC and CS (47.5 and 55.7 J/g, respectively). Generally, chitosan is a biopolymer and high thermal energy is needed for dissociation of its structure, i.e., thermal decomposition (Bershtein & Egorov, 1994). The

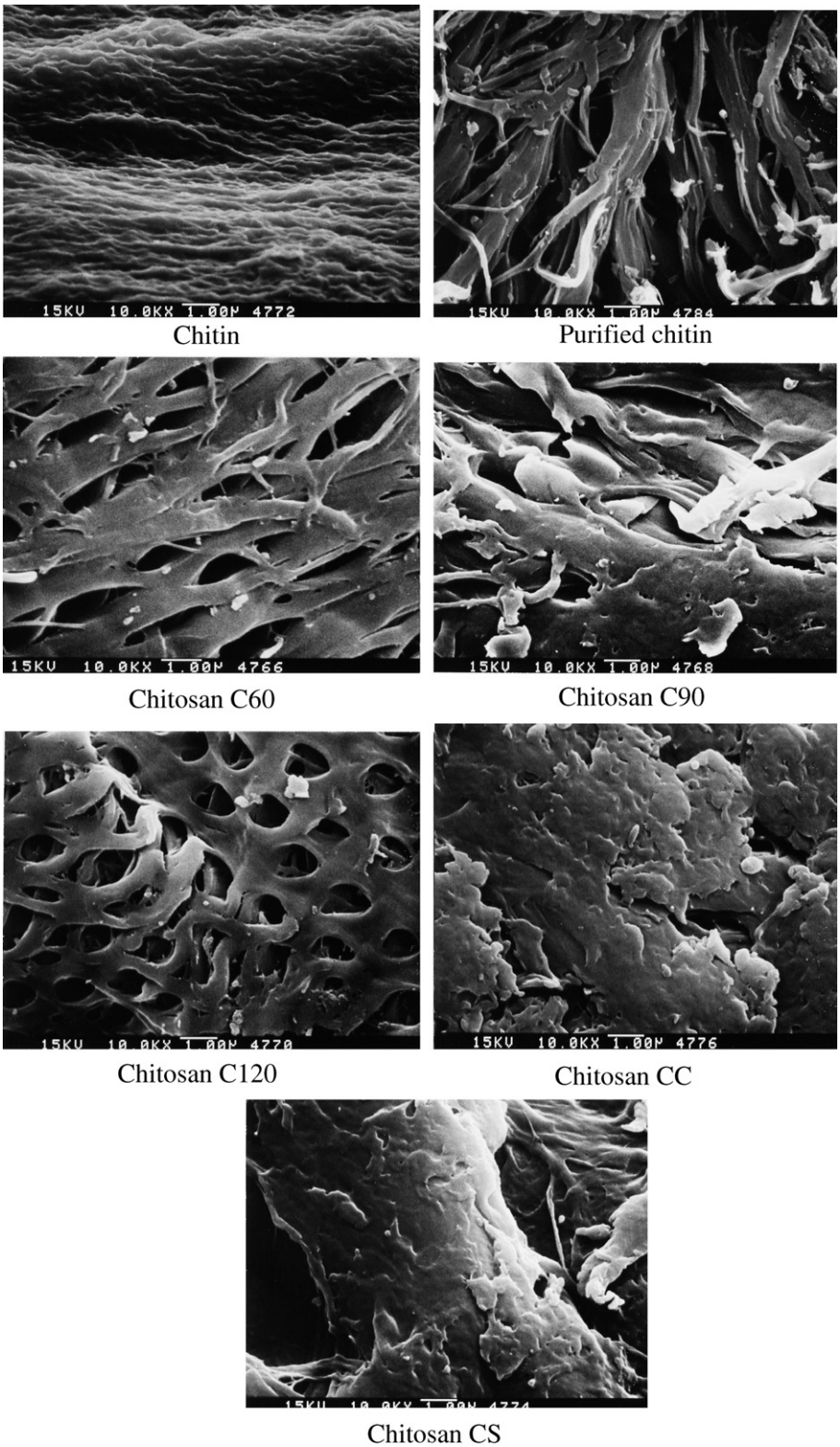

Fig. 2. SEM photographs of chitins and chitosans form crab shells at 5000× magnification. The measurement bar = 1 μm.

amount of peak enthalpy correlated with the compactness of supra-molecular chitin structure (Prashanth, Kittur, & Tharanathan, 2002). In other words, the higher the peak enthalpy, and the denser the crystallinity would be. Crab chitosans prepared showed denser crystallinity than chitosans CC and CS as evidenced by their higher ΔH. Crude chitin for chitosan preparations and chitosan CS were the products of Sigma Chemical Co. However, as compared to that used in this study, the method for chitosan CS preparation was considerably different due to the fact that chitosan CS produced showed lower ΔH and crystallinity.

Prashanth et al. (2002) also found that the endothermic peak temperature and ΔH of shrimp chitosan were 143.8–148.5 °C and 183.5–216.1 J/g, respectively. Yen and Mau (2007a) showed that the endothermic peak temperature and ΔH of fungal chitosans were 143.6–149.3 °C and 68.2–79.9 J/g, respectively. The endothermic peak temperature of shrimp and fungal chitosans were lower than those of crab chitosans prepared. However, the ΔH of shrimp chitin was much greater than those of crab chitosans, which in turn were higher than those of fungal chitosans. The discrepancy in the peak temperature and ΔH might be due to the diversity of chitin fiber aggregation in nature remained after *N*-deacetylation and different preparation methods used. Fungal chitin is classified as γ-chitin, which is a combination of α-chitin (chitins from crab and shrimp) and β-chitin (chitin from squid) and less dense in crystallinity of chitin structure (Jang et al., 2004). The discrepancies in the thermal analysis of these three types of chitosans could partially be attributed to their different intersheet or intrasheet hydrogen-bonding systems. However, the higher ΔH in crab chitosan revealed that it was less susceptible to collapse during the melting and dissociation process.

3.5. X-ray diffraction of crab chitosans

The WAXD pattern of crab chitins exhibited its characteristic crystalline peaks at $2\theta = 9.1$–$9.2°$ and 19.1–19.2° (Fig. 1). After purification, the purified chitin showed denser crystalline structure as evidenced by its sharper peaks. Chitosans C60, C90 and C120 showed two crystalline reflections at 8.8–9.0° and 18.9–19.1°, which were sharper with the prolonged reaction time. However, peaks of chitosans C60, C90 and C120 were lower than those of purified chitin but still sharper than those of crude chitin. It seems that purification and *N*-deacetylation did not affect the natural crystallinity of crab chitin and chitosan. The WAXD pattern of chitosan CS showed two crystalline peaks at $2\theta = 10.1°$ and 19.8° whereas that of chitosan CC showed only one crystalline peak at $2\theta = 19.4°$. Apparently, chitosan CC was not prepared from the same crab as other chitin and chitosans.

Yen and Mau (2004) purified the same crab chitin using a different purification method and found that the WAXD pattern of purified chitin showed two crystalline peaks at $2\theta = 9.3°$ and 19.1°. Yen and Mau (2007b) found that fungal chitin (γ-chitin) showed two crystalline reflections at 5.4–5.6° and 19.3–19.6°. Jang et al. (2004) found that crystalline peaks were 9.6, 19.6, 21.1 and 23.7° for α-chitin, 9.1 and 20.3° for β-chitin, and 9.6 and 19.8° for γ-chitin. Similarly, Cardenas, Carbrera, Taboada, and Miranda (2004) reported that WAXD patterns of α-chitin (chitins from shrimp, lobster, prawn and king crab) and β-chitin (chitin from squid) showed their major characteristic peak at 19.2–19.3° and 18.8°, respectively. Also, β-chitin from squid pen exhibited crystalline peaks at 9.8° and 19.3° (Kim, Kim, & Lee, 1996). It was obvious that three types of chitins had a consistent major peak of ~19° in their crystallinity structure.

Yen and Mau (2007a) found that fungal chitosan showed two crystalline reflections at 9.7° and 19.9°. Prashanth et al. (2002) found that the WAXD patterns of shrimp chitosan showed two major characteristic peaks at $2\theta = 9.9$–10.7 and 19.8–20.7°. Similarly, the two characteristic crystalline peaks with slightly fluctuated diffraction angles found in the WAXD patterns indicated that two types of α- and γ-chitosans exhibited comparable degree of crystallinity and had two consistent peaks of 9–10° and 19–20°.

3.6. Scanning electron microscopy of crab chitosans

Under electron microscopic examination, purified chitin showed distinctly arranged microfibrillar crystalline structure in SEM, more noticeable than crude chitin (Fig. 2). After *N*-deacetylation, chitosans C60, C90 and C120 showed similar microfibrillar structure. However, chitosans CC and CS did not exhibit apparent microfibrillar structure but showed layers of crumbling flakes.

Yen and Mau (2004) found that purified crab chitin from the same source using decolorization with ethanol also exhibited similar microfibrillar structure. Chan, Chen, and Yuan (2001) reported that fungal chitin showed microfibrillar crystalline structure in SEM. On the contrary, Yen and Mau (2007b) found that fungal chitin from shiitake stipes showed the aggregated flakes with dense and firm structure and without porosity. Also, fungal chitosans did not show the microfibrillar structure in SEM (Yen & Mau, 2007a). Usually, crude shiitake stipes were compact cell walls composed of the complex of polysaccharides and chitin. Therefore, fungal chitin could not show fibrillar or filamentous structure due to high amount of glycans present in crude chitin. The discrepancies in the observed crystallinity structure between fungal and crab chitins might also be attributed to their different intersheet or intrasheet hydrogen-bonding systems.

4. Conclusion

After *N*-deacetylation of purified chitin, the yields of crab chitosans were in the range of 30.0–32.2% with that of chitosan C120 being the highest. The DD of chitosans increased but the average M_W decreased with the prolonged reaction time. Crab chitosans showed lower lightness and WI values than purified chitin, chitosans CC and CS but higher than crude chitin. With the prolonged reaction time, the nitrogen, carbon and hydrogen contents in chitosans prepared consistently increased whereas N/C ratios remained the same. For the purity of crab chitosan products based on the nitrogen content, the reaction time of 60 min was sufficient.

Crab chitosans C60, C90 and C120 showed a melting endothermic peak at 152.3–159.2 °C. The melting and dissociation enthalpy (ΔH) of chitosans prepared (111.0–125.2 J/g) increased with the prolonged reaction time. Chitosans C60, C90 and C120 showed two crystalline reflections at $2\theta = 8.8$–$9.0°$ and 18.9–19.1°, which were sharper with the prolonged reaction time. Under electron microscopic examination, chitosans C60, C90 and C120 showed similar microfibrillar crystalline structure. Generally, three chitosans prepared showed similar physicochemical characteristics as evidenced by the element analysis, DSC, SEM and WAXD patterns. However, their characteristics were relatively different from chitosan CC and CS.

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