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Physicochemical and functional properties of chitosans prepared from shells of crabs harvested in three different years

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ABSTRACT

Selected physicochemical and functional properties of chitosans prepared from shells (designated S-04, S-05, and S-07, respectively) of crabs harvested in three different years (2004, 2005, and 2007) were evaluated. S-07 contained higher protein (27.39%) and chitin (20.62%) but lower ash (49.47%) contents compared with those from S-04 (22.42%, 17.35%, and 51.07%) and S-05 (24.94%, 18.20%, and 51.67%). Physicochemical characteristics of chitosans somewhat differed depending on the harvest year of crabs. Degree of deacetylation (89.01%) of chitosan from S-04 was higher than that from S-05 (84.11%) and S-07 (82.49%). Chitosan from S-04 had comparable L^* (76.41) but lower a^* (−0.16) and b^* (13.70) values than chitosans from S-05 (76.94, −0.04, and 15.66) and S-07 (74.92, 0.26, and 14.45). The highest water- (555%) and dye-binding (65.5%) capacities and DPPH radical scavenging activity (18.1%) were observed with chitosan from S-04, having the lowest viscosity (200 mPa s) and the highest degree of deacetylation.

1. Introduction

Chitosan is biocompatible, non-antigenic, non-toxic, and biofunctional (Hirano et al., 1990; Li, Dunn, Grandmaison, & Goosen, 1992). During the past several decades, chitosan has attracted significant interest over a broad range of scientific areas, including uses in biomedical, food, and various chemical industries (Knorr, 1984, 1991; Li et al., 1992; Muzzarelli, 1977; No, Meyers, Prinyawiwatkul, & Xu, 2007; Sandford & Hutchings, 1987). Specifically, chitosan has been known for its versatile biological activities such as antimicrobial (Andres, Giraud, Gerente, & Le Cloirec, 2007; Fernandez-Saiz, Lagaron, & Ocio, 2009; No, Kim, Lee, Park, & Prinyawiwatkul, 2006; No, Park, Lee, & Meyers, 2002), antitumor (Tokoro et al., 1988), and hypocholesterolemic functions (Sugano, Yoshida, Hashimoto, Enomoto, & Hirano, 1992). Chitosan has also been documented to possess several distinctive properties for use in water and fat uptake, emulsification (Knorr, 1982), dye binding (Knorr, 1983), and gelation (Vorlop & Klein, 1981).

Furthermore, chitosan has been documented to possess a film-forming property for use as edible films or coatings (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Jeon, Kamil, & Shahidi, 2002; Nadarajah, Prinyawiwatkul, No, Sathivel, & Xu, 2006). Blending of chitosan with natural or synthetic polymers such as pectin, gelatin, or polyvinyl alcohol appears to be a promising approach for producing polymers for specific uses (Arvanitoyannis, 1999; Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997; Arvanitoyannis, Nakayama, & Aiba, 1998; Yao et al., 1996). Chemical modifications have also been occasionally suggested as a means to improve the properties of chitosan (Mourya & Inamdar, 2008).

A major source of crustacean that is processed into chitosan in Korea is red crab (*Chionoecetes opilio*). However, the annual production of red crab decreased since 1995 (Coh, Lee, Kim, & Park, 2003). The catch in 2007 year was approximately 25,388 metric tons (MMAF, 2008); this presently available quantity of shell from crab may not be sufficient to support a high demand of commercial chitosan industries in Korea. Perhaps one way to alleviate shortage of crustacean shells due to greater demand of chitosan in the future is to collect crab shells, if in excess and not utilized in some years, and to store them for later use for chitosan production.

Crustacean shell mainly consists of 30–40% protein, 30–50% ash, and 20–30% chitin (Johnson & Peniston, 1982). These chemical compositions vary with species and with season (Green & Mattick, 1979). Thus, chemical composition of crab shell may differ with crab harvest year. Earlier studies (Cho, No, & Meyers, 1998; No & Meyers, 1997) revealed that physicochemical characteristics of chitosan influence its functional properties, which differ with crustacean species and preparation methods. However, very few attempts have been made to compare physicochemical and functional properties of chitosans prepared from crab shells harvested in different years. Possible varying physicochemical and functional properties of these chitosans due to different harvest years should be monitored in order to effectively utilize chitosan products for particular usage.

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The objectives of the present research were to compare chemical composition of shells of crabs harvested in three different years, and to compare some physicochemical and functional properties of chitosans prepared from stored shells.

2. Materials and methods

2.1. Materials

Dried crab (*C. opilio*) leg shells, prepared from crabs immediately right after harvest, were obtained from Keumho Chemical (Seoul, Korea) in July 2004, January 2005, and September 2007. Upon receiving, the shell was ground through a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ, USA) with a 2-mm mesh screen; sifted with 20 (0.841 mm) and 40 mesh (0.425 mm) sieves using a portable sieve shaker (JISICO, Seoul, Korea) and placed in opaque plastic bottles. The ground shells were stored at ambient temperature until December 2007 prior to chitosan production, resulting in 3.5 years, 3 years and 3 months storage time, respectively. The ground shell of 0.841–0.425 mm particle size was used throughout this research to obtain reproducible and consistent results.

The dye used for evaluation of binding capacity was FD&C Red No. 40 {disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid}. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Production of chitosan

The production of chitosan involved the demineralization, deproteinization, deacetylation, and decoloration steps (Youn, No, & Prinyawiwatkul, 2007). The ground crab leg shell was demineralized with 1 N HCl for 30 min at ambient temperature with a solid/solvent ratio of 1:15 (w/v). Following the demineralization step, the demineralized shell was collected on a 100-mesh sieve, washed to neutrality in running tap water, rinsed with deionized water, and filtered to remove excess moisture. The deproteinization step was accomplished by treating the demineralized shell with 3% NaOH for 15 min at 15 psi/121 °C and a solid/solvent ratio of 1:10 (w/v). The residue was then washed, filtered as mentioned above, and dried at 60 °C for 4 h in a forced-air oven. The deacetylation step was achieved by treating chitin under conditions of 15 psi/121 °C with 45% NaOH for 30 min and a solid/solvent ratio of 1:10 (w/v). For the decoloration step, the resulting chitosan was collected, washed as mentioned above, and dried by sun drying (approximately at 23 °C) for 4 h (Youn et al., 2007).

2.3. Chemical composition of crab shell

The moisture content was determined using a halogen moisture analyzer (HG53, Mettler Toledo, Greifensee, Switzerland). Ash was determined by a standard AOAC method 942.05 (AOAC, 1990). Fat was determined using a Soxhlet extractor (Sox 416, Gerhardt, Germany). Nitrogen was determined using an elemental analyzer (EA 1110, CE Instruments, Rodano-Milan, Italy). Crude protein was calculated by multiplying nitrogen content of the sample by 6.25. Chitin was determined by the method of Black and Schwartz (1950). All analyses were performed in triplicate.

2.4. Physicochemical properties of chitosans

Degree of deacetylation was determined in triplicate according to a colloid titration method (Tôei & Kohara, 1976) using N/400 potassium polyvinyl sulfate ($f = 1.00$, Wako Pure Chemical Ind., Osaka, Japan). Viscosity was determined in triplicate with a Brookfield viscometer, model LVDV-II+ (Brookfield Engineering Labs., Stoughton, MA, USA). Chitosan solution was prepared in 1% (v/v) acetic acid at a 0.5% (w/v) concentration on a moisture-free basis. Viscosity measurements were made using a small sample adapter at a shear rate of 5.28 s^{-1} in the solution (8 mL) at 25 ± 0.3 °C, and reported in mPa s. Color measurements were measured with a portable Minolta Chroma Meter CR-200 (Minolta Camera Co., Ltd., Osaka, Japan) using illuminant C, and reported as L^* (lightness), a^* (+ for redness and − for greenness) and b^* (yellowness). Three measurements were made at different locations on each sample. The IR spectra of chitosans were obtained with a JASCO FTIR spectrometer (4100, Tokyo, Japan) using KBr pellets. Each chitosan sample (particle size less than 100 mesh) was mixed with KBr to form a homogeneous mixture for the FTIR measurements.

2.5. Water-binding capacity of chitosans

Water-binding capacity (WBC) of chitosan was measured in triplicate using a modified method of Wang and Kinsella (1976). Water absorption was initially carried out by weighing a centrifuge tube (50 mL) containing 0.5 g of sample, adding 10 mL of water, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with intermittent shaking for 5 s every 10 min and centrifuged at 3200 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC was calculated as follows: WBC (%) = [water bound (g)/sample weight (g)] × 100.

2.6. Dye-binding capacity of chitosans

Dye solution was prepared by dissolving dye in deionized water at a concentration of 250 mg/L. For the standard curve determination, the maximum absorbance of the aqueous dye solutions containing 2.5–20 mg of dye/L was measured with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Tokyo, Japan) using deionized water as a blank.

Dyeing of chitosan was achieved by shaking 0.2 g of chitosan and 10 mL of aqueous dye solution (containing 2.5 mg of dye) in horizontally positioned screw-capped polypropylene conical tubes at 20 °C for 1 h using a shaker (100 rpm; MMS-3010, Tokyo Rikakikai Co., Japan). After settling of the dyed chitosan particles, the supernatant was withdrawn with a pipet and filtered through a glass filtering Gooch crucible (2G-3) using a glass microfiber filter paper (Whatman, 47 mm). The dyed chitosan was then repeatedly washed with deionized water and filtered until the filtrate was clear. The dye concentration of the combined filtrate was determined spectrophotometrically. The amount of dye bound to chitosan was determined by calculating differences in concentrations between the initial dye solution and the combined filtrate. Dye-binding capacity (DBC) was expressed as % adsorption (Cho et al., 1998). Triplicate measurements were performed.

2.7. DPPH radical scavenging activity of chitosans

DPPH radical scavenging activity of chitosan was determined in triplicate by the method of Blois (1958) with some modifications. 0.4 mL of chitosan solution (1.0% in 1.0% acetic acid) was added to 3 mL of 0.1 mM DPPH radical methanolic solution. The reaction mixture was shaken vigorously and stored in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm using a spectrophotometer (Ultraspec® 1000, Pharmacia Biotech Co., Cambridge, England). The free radical scavenging activity was calculated by the following equation:

$$\text{Scavenging activity (\%)} = [1-(\text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}})] \times 100.$$

2.8. Statistical analysis

All experiments were carried out in triplicate, and means ± standard deviations were reported. All data were analyzed using ANOVA. Means of the main effects were separated by Duncan's multiple-range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) software package.

3. Results and discussion

3.1. Chemical composition of crab shell

Chemical compositions of crab shell harvested in different years are shown in Table 1. Compared with the shells obtained from 2004 and 2005 harvest year (designated S-04 and S-05, respectively), the shell from 2007 harvest year (designated S-07) contained higher protein and chitin but lower ash contents. There were no significant differences in ash and chitin contents between S-04 and S-05. The level of fat was negligible (⩽0.01) for all shells.

The crab shells used in our study contained 22.42–27.39% protein, 49.47–51.67% ash, and 17.35–20.62% chitin. No, Lee, Park, and Meyers (2003) reported similar protein (24.3%), much lower ash (43.8%) and much higher chitin (24.4%) contents, on a dry basis, of shell from the same crab species (*Chionoecetes opilio*) harvested in 2002. Results from this study and No et al. (2003) confirmed that chemical compositions of shell of crabs vary with harvest year.

3.2. Physicochemical properties of chitosans

Physicochemical characteristics of chitosans prepared from S-04, S-05, and S-07 are shown in Table 2. Results indicate that deacetylation of chitosan could be achieved more effectively with S-04 than with S-05 and S-07. In comparison of viscosity of chitosan, the lowest viscosity was obtained from S-04, followed by from S-05 and S-07. Chitosan prepared from S-04 had comparable L^* value and lower a^* and b^* values than chitosans from S-05 and S-07. These data indicate that the physicochemical characteristics of chitosan products, especially viscosity, may differ depending on the crab harvest year and/or storage time. In general, the S-04 sample, having longer storage time (3.5 years), yielded chitosan with higher degree of deacetylation, lower viscosity, and less red (lower a^* value); this may be due to partial depolymerization of crab shell and oxidative destruction of carotenoids present in crab shell during a prolonged storage period at room temperature. However, the IR absorption spectra (Fig. 1) of the chitosans prepared from these three crab shells showed that the position and absorption intensity of the bands were comparable and typical of chitosan (Paulino, Simionato, Garcia, & Nozaki, 2006; Youn, No, Kim, & Prinyawiwatkul, 2008), irrespective of crab harvest year.

The main component of carotenoid fraction in crustacean exoskeleton is astaxanthin (No, Meyers, & Lee, 1989). The oxidative degradation of carbon–carbon double bonds present in astaxanthin causes decoloration of carotenoids (Henry et al., 2000). Therefore, partial oxidative destruction of carotenoids in crab shell during a prolonged storage may have resulted in more effective decoloration of chitosan from the S-04. In our present studies, some significant differences in color L^*, a^*, and b^* values were observed among three chitosans by the instrumental measurement. However, these differences were not easily discerned by visual observation.

Table 1
Chemical composition[a] of crab shell harvested in three different years.

Composition (%)	Harvest year		
	2004	2005	2007
Crude protein	22.42 ± 0.85a	24.94 ± 1.17b	27.39 ± 0.64c
Fat	<0.01	<0.01	<0.01
Ash	51.07 ± 0.31b	51.67 ± 0.46b	49.47 ± 0.23a
Chitin	17.35 ± 0.95a	18.20 ± 1.61a	20.62 ± 0.29b

[a] Mean ± standard deviation of triplicate determinations, on a dry basis. Means with different lowercase letters (a–c) within each row indicate significant difference ($P < 0.05$).

Table 2
Physicochemical and functional properties[a] of chitosans prepared from shells of crabs harvested in three different years.

Properties	Harvest year		
	2004	2005	2007
Deacetylation (%)	89.01 ± 1.22b	84.11 ± 0.40a	82.49 ± 1.40a
Viscosity (mPa s)	200 ± 5a	529 ± 7b	669 ± 16c
Color value L^*	76.41 ± 0.93a,b	76.94 ± 0.35b	74.92 ± 1.01a
a^*	−0.16 ± 0.02a	−0.04 ± 0.04b	0.26 ± 0.06c
b^*	13.70 ± 0.25a	15.66 ± 0.23c	14.45 ± 0.32b
Water-binding capacity (%)	555 ± 4b	491 ± 4a	496 ± 1a
Dye-binding capacity (%)[b]	65.5 ± 1.3b	39.9 ± 0.5a	42.0 ± 1.6a
DPPH radical scavenging activity (%)[c]	18.1 ± 0.1c	14.0 ± 0.2b	12.5 ± 0.2a

[a] Mean ± standard deviation of triplicate determinations, on a dry basis. Means with different lowercase letters (a–c) within each row indicate significant difference ($P < 0.05$).
[b] At 2.5 mg of dye concentration/0.2 g of sample.
[c] At 1% chitosan concentration in 1% acetic acid.

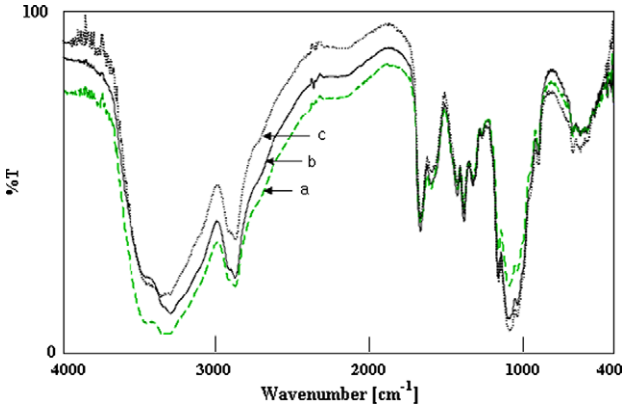

Fig. 1. The FTIR spectra of chitosans prepared from shells of crabs harvested in 2004 (a), 2005 (b), and 2007 (c).

3.3. Water- and dye-binding capacity of chitosans

Chitosan prepared from S-04 exhibited higher water-binding capacity (WBC) and dye-binding capacity (DBC) compared with those prepared from S-05 and S-07 (Table 2). There were no significant differences in WBC and DBC between the latter two chitosans.

Several researchers have reported differences in WBC and DBC (for FD&C Red No. 40) among various chitosans. For example, Cho et al. (1998) observed that WBC and DBC of five commercial chitosan products ranged from 458% to 805% and 35.2% to 85.6%, respectively. WBC and DBC of six commercial chitosan products observed by No, Lee, and Meyers (2000) were in the range of 355–611% and 21.3–100%, respectively. The corresponding WBC (491–555%) and DBC (39.9–65.5%) of chitosans observed in our study were comparable to those of commercial chitosans reported by Cho et al. (1998) and No et al. (2000); these researchers also observed that neither WBC nor DBC were significantly correlated with viscosity and DD of chitosan. In our present study, the highest WBC and DBC were obtained with chitosan (from S-04) having the lowest viscosity and the highest DD.

3.4. DPPH radical scavenging activity of chitosans

The highest DPPH radical scavenging activity of chitosan was obtained from S-04, followed by from S-05 and S-07 (Table 2). The scavenging activity of chitosan may be due to the reaction between free radicals and protonated amino groups (Castagnino et al., 2008; Xie, Xu, & Liu, 2001).

Previous investigators have revealed that the DPPH radical scavenging activity of chitosan increased with decreasing molecular weight (M_W) (Chien, Sheu, Huang, & Su, 2007; Cho, No, & Prinyawiwatkul, 2008; Kim & Thomas, 2007; Yen, Tseng, Li, & Mau, 2007). According to Kim and Thomas (2007), the higher M_W chitosan (120 kDa) would have lower mobility than the lower M_W chitosan (30 kDa). Consequently, this would increase the possibility of inter- and intra-molecular bonding of the high M_W chitosan molecules, and, thus, the chance of exposure of their amine groups might be restricted. This may explain our findings in that scavenging activity of chitosan increased with decreased viscosity (Table 2). Among three chitosans tested, chitosan from S-04 exhibited the lowest viscosity (200 mPa s), followed by those from S-05 (529 mPa s) and S-07 (669 mPa s). Thus, chitosan prepared from crab shell with longer storage time (S-04) may exhibit a better antioxidant activity due mainly to the lower viscosity or M_W.

4. Conclusion

This study demonstrated that chemical composition of crab shells and selected physicochemical and functional properties of chitosans prepared from these shells somewhat differed due to crab harvest year (July 2004, January 2005, and September 2007) and/or storage duration of shell. Chitosan prepared from S-04 showed higher degree of deacetylation (89.01%), lower viscosity (200 mPa s), and less red (lower a^* value; −0.16) than those prepared from S-05 (84.11%, 529 mPa s, and −0.04) and S-07 (82.49%, 669 mPa s, and 0.26, respectively). Furthermore, chitosan prepared from S-04 exhibited higher water-binding capacity (555%), dye-binding capacity (65.5%), and DPPH radical scavenging activity (18.1%) than those from S-05 (491%, 39.9%, and 14.0%) and S-07 (496%, 42.0%, and 12.5%, respectively). This indicates that crab shell stored for a certain period of time may be advantageous for chitosan production with improved functionality for particular usage. Further studies are needed to focus on crab shells collected in different seasons to ascertain whether the storage of shell is substantially detrimental or not to the chitosan quality.

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