

RESEARCH NOTE

Characteristics of Films Based on Chitosans Isolated from *Todarodes pacificus*

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Abstract Chitosans were obtained with varying deacetylation times using the β -chitin isolated from *Todarodes pacificus*, and their deacetylation degrees and average molecular weights were determined. Films prepared with the squid chitosans were characterized by estimating their tensile strengths, percent elongations, water vapor permeabilities, degree of swelling, and temperatures of glass transition and thermal decomposition. The results suggest that the squid chitosan films were comparable to common crustacean chitosan films in regard of mechanical, moisture barrier, and thermal properties, although further, multilateral investigations are necessary to make a more definitive conclusion.

Keywords: chitosan film, *Todarodes pacificus*, tensile strength, water vapor permeability, glass transition

Introduction

Chitosan films and coatings have been extensively investigated as barriers for food protection and preservation because of their excellent film forming properties, high miscibility with various natural or synthetic polymers, diverse functionalities, easy accessibility, nontoxicity, edibility, biodegradability, and biological compatibility (1-6). Chitosan, a polysaccharide, is the deacetylated derivative of chitin, a homopolymer of β -(1 \rightarrow 4)-linked *N*-acetyl-D-glucosamine, which is the most abundant biopolymer next to cellulose (6). Chitosan has been derived mostly from the chitin of α -crystallographic structure because α -chitin is easily and cheaply obtainable from the exoskeleton of crabs, lobsters, and shrimps (2, 5). The chitin of β -crystallographic structure, isolated from squid pens, has become another important chitosan precursor, because considerable amounts of squid pens are now produced as a waste product of seafood industries and chitosan can be obtained under relatively mild processing conditions (2, 7, 8).

A few studies have been reported on the properties of squid chitosan. Kurita *et al.* (7) showed that hygroscopicity and water retention capacity of a tested squid chitosan were higher than those of shrimp chitosan. Shimojoh *et al.* (9) reported that squid chitosan was more degradable in alkali/acid treatments and aggregated bovine serum albumin more efficiently than the two tested crab chitosans. Kim *et al.* (10) and Choi *et al.* (11) investigated several rheological and physicochemical properties of the squid chitosans obtained from *Todarodes pacificus* and *Ommastrephes bartrami*. However, little information has so far been published on the properties of the films based on squid chitosan.

The objective of this study was to investigate the characteristics of the films prepared with squid chitosans

isolated from *Todarodes pacificus*, in order to determine potential applications of squid chitosan film. Tensile strength (TS), percent elongation (%E), water vapor permeability (WVP), degree of swelling (DS), and temperatures of glass transition (T_g) and thermal decomposition (T_d) were determined.

Materials and Methods

Isolation of squid chitosan The pens of *Todarodes pacificus* obtained from a local seafood company were washed with water, dried at 40°C for 12 hr, cut into small pieces, pulverized to 300 μ m, and then stored at -18°C before the experiments. Demineralization was not conducted because of the low mineral content (< 0.7%) in the pens. Deproteinization was performed by heating 3 L of 1 N sodium hydroxide solution containing 200 g of the pen powder at 80-85°C for 4 hr with agitation. The remaining solid was washed with deionized water until neutral, filtered with 75 μ m sieve, and dried at 40°C for 48 hr to obtain β -chitin powder.

Deacetylation of β -chitin was performed according to the modified method of Kurita *et al.* (7) and Shimojoh *et al.* (9). A suspension of 90 g of β -chitin powder in 1.5 L of 40% (w/w) sodium hydroxide solution was prepared and heated under agitation using a coiled reflux condenser at 115°C for 30 min. The remaining solid was washed with deionized water until neutral and dried at 40°C for 48 hr to obtain squid chitosan powder (SC₃₀). The same method, but with suspension heating for 60 and 90 min, was used for two other types of squid chitosans, SC₆₀ and SC₉₀, respectively.

Degree of deacetylation and molecular weight The degree of deacetylation (DD) of the squid chitosan was determined using an IR spectroscopic method (12). The absorbance ratio of A_{1550}/A_{2878} was obtained using an FTIR spectrophotometer (Model IFS-88, Bruker Optics Inc., Karlsruhe, Germany). Triplicate measurements were conducted for each sample. The average molecular weight

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(M_a) was determined using a viscometric method (13). The intrinsic viscosity of a solution of chitosan in 0.1 M acetic acid/0.2 M sodium chloride was estimated using an Ubbelohde capillary viscometer (0.5 mm diameter) at 25°C, and M_a was calculated using the following Mark-Houwink relationship:

$$[\eta] = KM_a^\alpha \quad (1)$$

where $[\eta]$ = intrinsic viscosity, $K = 1.81 \times 10^{-3}$, and $\alpha = 0.93$.

Film preparation Squid chitosan was dissolved in a 2% (v/v) aqueous acetic acid solution at a concentration of 1% (w/w) with mild heating and stirring. The chitosan solution was cooled to room temperature, cast on a rectangular plastic frame ($20 \times 10 \times 0.3 \text{ cm}^3$), and dried at 40°C overnight. The prepared film was conditioned in a desiccator at 53% (RH, saturated magnesium nitrate solution) and $25 \pm 1^\circ\text{C}$ for more than 48 hr. The thickness of the film was measured at ten locations using a hand-held digital micrometer, and the average film thickness was obtained.

Tensile strength and percent elongation at break TS and %E were evaluated at $25 \pm 1^\circ\text{C}$ using a rheometer (Model COMPAC-100, Sun Scientific Co., Tokyo, Japan) according to the ASTM standard method D882-95a (14). Initial grip separation and cross-head speed were set to 50 mm and 5 mm/min, respectively. Ten film specimens, $6.5 \times 1.5 \text{ cm}$ strips, were tested and the measured values were averaged. TS and %E were calculated using the following relationships:

$$TS = \frac{F_m}{A} \quad (2)$$

where F_m = maximum load at break and A = cross-sectional area of the film strip.

$$\%E = \left(\frac{\Delta L}{L_0}\right) \times 100 \quad (3)$$

where ΔL = elongation at the moment of rupture and L_0 = initial length of specimen.

Water vapor permeability WVP was determined using a gravimetric method according to the ASTM standard method E96-95 (15). Three testing dishes of 6.2 cm inner diameter, made of a water vapor-impermeable plastic, were prepared. Each dish was filled with anhydrous calcium chloride (0% RH), and sealed with a film specimen. The exposed film area was 30.2 cm^2 and the air gap between the specimen and the desiccant was 13 mm. The three dish assemblies were placed in a desiccator at 53% RH and $25 \pm 1^\circ\text{C}$, and weighed periodically for 12 hr. WVP values in $\text{g/m}^2\cdot\text{s}\cdot\text{Pa}$ were calculated from the weight increase at steady state using the following permeation equation and averaged.

$$WVP = \left(\frac{L}{A\Delta P}\right) \left(\frac{dw}{dt}\right) \quad (4)$$

where L = film thickness (m), A = exposed film area (m^2), ΔP = difference of water vapor pressure between inside and outside of dish (Pa), w = weight of dish assembly (g),

and t = time (s).

Degree of swelling DS was determined according to Singh and Ray (16). Five film specimens were immersed in 0.1 M phosphate buffer solution (pH 7.4) for 24 hr at 37°C to equilibrium, taken out of the solution, and then placed between two pieces of filter paper to remove excess solution. The swollen specimens were weighed and dried at 120°C for 24 hr to obtain their dry weights. DS values (%) of the five specimens were calculated using the following equation and averaged.

$$DS = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \quad (5)$$

where W_s = weight of swollen film and W_d = weight of dried film.

Glass transition and thermal decomposition temperatures T_g and T_d were determined using a differential scanning calorimeter (Model DSC 2910, TA Instruments, Inc., New Castle, DE, USA) according to Sakurai *et al.* (4). About 5 mg of film specimen was cut into small pieces and placed into a hermetically sealed DSC sample pan. Both sample and empty reference pans were heated to 190°C, cooled to 25°C, and then heated again up to 300°C. The rate of heating and cooling scan was set to 10°C/min. Five replicates were conducted. T_g was identified in the DSC thermograms obtained in the second heating run as the temperature at the onset of a baseline step caused by the change of heat capacity (4). The details on the method of identification are provided in Hatakeyama and Quinn (17). T_d was identified in the same thermograms as the onset temperature of a sharp exothermic peak (4).

Results and Discussion

The squid chitin obtained in the current study had DD of 38%, which is relatively high compared to the 10 to 17% values commonly found for α -chitin. Choi *et al.* (11) reported a similar DD value of 33.2% for squid chitin. The authors mentioned that this relatively high value might be because β -chitin is more susceptible to deacetylation than α -chitin during the deproteination step due to its weaker intermolecular interactions. Figure 1 shows that in the present process most deacetylation occurred during the initial 30 min heating (DD = 66%), and that DD values reached 70 and 73% after 60 and 90 min, respectively. Further deacetylation was not conducted because the chitosan color changed from white to dark yellow. SC_{30} did not undergo further investigations, because it was not fully dissolved in the acidic solutions used in the current study. The undissolved colloidal chitosan particles gave the SC_{30} film a rugged surface which was easily torn. M_a values of SC_{60} and SC_{90} were estimated to be 1.01×10^6 and $9.55 \times 10^5 \text{ g/mol}$, respectively. The decrease of M_a with deacetylation time was also reported by Tolaimate *et al.* (5) for squid chitosan. The films prepared with SC_{60} and SC_{90} were transparent and flexible, of average thickness $30 \pm 4.6 \text{ mm}$.

Tensile strength and elongation TS and %E of the two

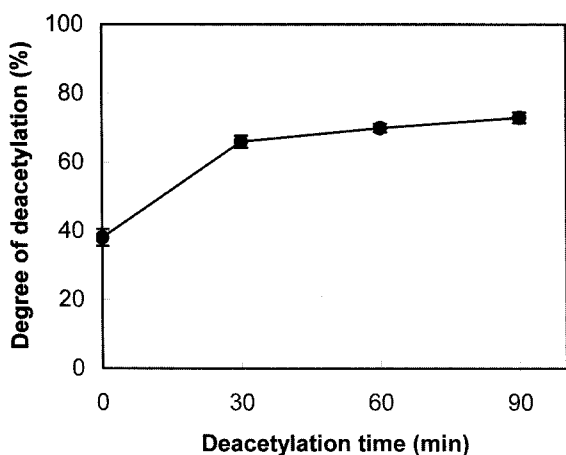


Fig. 1. Degree of deacetylation of squid chitosan with respect to deacetylation time.

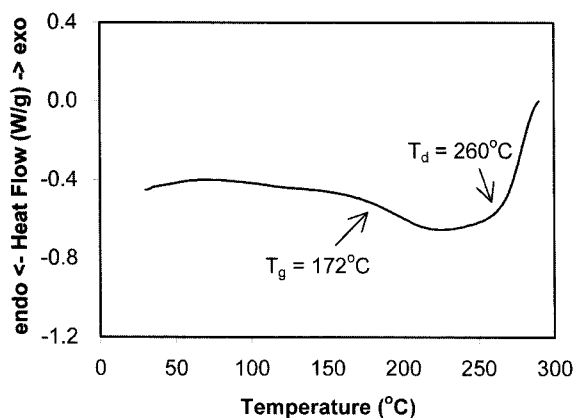


Fig. 2. DSC thermogram of squid chitosan (SC₉₀) film in the second heating run. T_g and T_d are glass transition and thermal decomposition temperatures, respectively.

squid chitosan films were between 24 and 29 MPa and between 6 and 11%, respectively (Table 1). Previously reported TS and %E values of crustacean chitosan films obtained under similar experimental conditions ranged from 3 to 27 MPa and from 3 to 84%, respectively (1, 16, 18). Simple comparison of each parameter, although variations in chitosan properties such as DD and M_n must be considered, suggests that the mechanical properties of the squid chitosan films are as excellent as those of the crustacean chitosan films. SC₉₀-film showed slightly higher TS than SC₆₀-film, although the difference was not significant ($p > 0.05$ by Student's *t*-test), and lower %E (Table 1), indicating that it is a bit stronger and tougher than SC₆₀-film. SC₉₀ had a lower DD value, indicating that it has fewer *N*-acetyl groups, and a smaller molecular size than SC₆₀. Therefore, it could probably form a more compact but less extensible film network.

Water vapor permeability and degree of swelling WVP values of the two squid chitosan films ranged between 1.7×10^{-11} and 2.0×10^{-11} g/m·s·Pa (Table 1), which were smaller than the literature values ($9\text{--}360 \times 10^{-11}$ g/m·s·Pa) of crustacean chitosan films tested under very similar

Table 1. Characteristics of squid chitosan films

Characteristics	SC ₆₀ -film	SC ₉₀ -film
TS (MPa) ¹⁾	24.50 ± 2.90 ^a	28.55 ± 4.92 ^a
%E (%) ²⁾	10.3 ± 2.2 ^a	6.1 ± 1.4 ^b
WVP ($\times 10^{-11}$ g/m·s·Pa) ³⁾	1.75 ± 0.04 ^a	1.93 ± 0.02 ^b
DS (%) ⁴⁾	151.0 ± 18.2 ^a	180.4 ± 12.7 ^b
T_g (°C) ⁵⁾	178 ± 2 ^a	172 ± 3 ^b
T_d (°C) ⁶⁾	260 ± 4 ^a	260 ± 2 ^a

All values are means of *n*-replicates ± SD. SC₆₀- and SC₉₀-films were prepared with 60 and 90 min-deacetylated squid chitosans, respectively.

^{a,b}Different superscript letters within a row indicate significant differences ($p \leq 0.05$) by Student's *t*-test.

¹⁾TS: tensile strength at break ($n = 10$)

²⁾%E: percent elongation at break ($n = 10$)

³⁾WVP: water vapor permeability ($n = 3$)

⁴⁾DS: degree of swelling ($n = 5$)

⁵⁾ T_g : glass transition temperature ($n = 5$)

⁶⁾ T_d : thermal decomposition temperature ($n = 5$)

conditions (1, 18). The measured DS values of the two films, ranging between 150 and 181% (Table 1), were in the range of literature values (110–185%; 16, 19). This simple comparison implies that the squid chitosan films could impede moisture penetration as effectively as crustacean chitosan films. WVP and DS of SC₉₀-film were 1.1- and 1.2-fold larger than those of SC₆₀-film, respectively (Table 1), indicating that SC₉₀-film is a bit poorer moisture barrier than SC₆₀-film. It is known that chitosan becomes more hydrophilic with increasing DD, which suggests that SC₉₀, with its higher DD, is more hydrophilic than SC₆₀ and is therefore able to form a more water-swellaible film network allowing greater moisture penetration.

Glass transition and thermal decomposition temperatures

Two cycles of heating and cooling runs were adopted in the current DSC measurement to eliminate the effect of absorbed moisture. Sakurai *et al.* (4) showed that this method yielded a very reliable T_g value, which was in good agreement with the value estimated from dynamic mechanical analysis (DMA), for a crustacean chitosan film. As shown in Fig. 2, an endothermic step and an exothermic peak were observed in the thermograms of both SC₉₀- and SC₆₀-films. The estimated T_g values of the two chitosan films, 172 and 178°C (Table 1), respectively, were markedly lower than the 203°C reported by Sakurai *et al.* (4) for a crustacean chitosan film. This simple comparison of the values suggests that the squid chitosan molecules might be more flexible than the crustacean chitosan molecules, possibly because squid β -chitin is known to be more flexible and reactive than crustacean α -chitin due to its weaker intermolecular interactions (2, 8). However a more detailed study is necessary to reach a definitive conclusion.

T_g of SC₉₀-film was about 6°C lower than that of SC₆₀-film (Table 1). Sakurai *et al.* (4) suggested that chitosan's lower T_g compared to that of chitin is because chitosan has fewer *N*-acetyl groups and is thus more flexible. On this basis, the lower T_g of SC₉₀-film is probably because SC₉₀ is more flexible than SC₆₀ because it has fewer *N*-acetyl groups and the average molecular size is smaller. T_d of both squid chitosan films was estimated to be 260°C

(Table 1), which is in agreement with previously reported values of crustacean chitosan films of between 250 and 273.5°C, when measured by the current method and by a single heating DSC measurement, respectively (4, 16). Currently, only limited information is available on the thermal relaxation properties of both squid and crustacean chitosans, and further investigations are therefore necessary.

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