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Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*

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Abstract

β-chitin and its chitosan from the pens of *Loligo lessoniana* and *Loligo formosana* has been isolated, prepared, and physico-chemically characterized to demonstrate a potential chitin source. Without deminerization due to negligible ash content, only deproteinization was used in the chitin isolation with an yield of 35–38%, without significant difference either between the two species or the collection seasons. Reducing step not only saves production cost but also obviates acid pollutant. Mild alkaline deacetylation with various time periods was employed in the chitosan preparation. Optical rotation and thermal transition of chitin from both species suggested the weak intermolecular forces compared with shrimp chitin. The results of nitrogen contents indicate the effectiveness of the deproteinization method used. The samples were categorized as a Class III: moderate hygroscopicity. Traces of elements presented in pens markedly decreases but are incapable to be got rid of within the step of chitin–chitosan preparation. In addition, a small amount of cadmium, as the contamination, was detected in the samples from *L. formosana*.

Keywords: Squid pens; *Loligo lessoniana*; *Loligo formosana*; β-Chitin; Chitosan

1. Introduction

Chitin is a linear polymer of *N*-acetyl-D-glucosamine linked by β(1–4) glycosidic bond. It is recognized to be the second most abundant biopolymer on earth, next to cellulose. It occurs primarily as a structural component in the exoskeleton of crustaceans, insects, in the pens of squids; it is also found to lesser extents in other animals, plants, fungi and bacteria (Muzzarelli & Jeuniaux, 1976). Chemically, chitin is regarded as a fairly intractable material since it is insoluble in most ordinary solvents such as water, alcohols, acetone, hexane, dilute acids, and dilute and concentrated alkalines. Hence, this restricts the scope of applications for chitin. However, deacetylation of chitin with concentrated strong alkaline produces poly-D-glucosamine or chitosan, which has a high density of amino group, and is soluble in weakly acidic solvents such as acetic acid or formic acid. It appears that the physico-chemical properties of chitin and chitosan are widely different, which are governed by three principal factors, i.e. source of raw material, molecular weight and degree of deacetylation (Bough, Satter, Wu, & Perkin, 1978; Brine & Austin, 1981).

Apart from chitosan, the other number of chitin derivatives have been synthesized in order to modify their chemical and physical properties appropriate for the specific purpose in various applications. These applications include biomedicine, food technology, paper technology, cosmetics, and wastewater treatment. It is noteworthy, however, that most of the chitin and its derivatives studied so far are

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obtained from crustacean, particularly prawn and crab shells (Brine & Austin, 1981; Muzzarelli & Jeuniaux, 1976). Although it has been shown that squid pens comprises of chitin about 35% of the dry weight (Brine & Austin, 1981), detailed information from this source is very limited. A low accessibility of squid pen is probably a critical barrier. Interestingly Muzzarelli and Jeuniaux (1976) reviewed that the chitin from crustacean shells has a different structure and properties from that the pens of mollusks. The former type has the so-called α-structure where the polymer chains are arranged in an anti-parallel fashion with strong inter-molecular hydrogen bonding. In comparison, chitin from the pens of mollusks has the so-called β-structure that is characterized as a loose-packing parallel fashion with weak inter-molecular interactions. There have been little published information on β-chitin, mostly studied by Kurita et al. (Kurita, Kaji, & Nishiyama, 2000; Kurita et al., 1993a,b, 1994). The authors used the pens from the squid, *Ommastrephes bartrami* as their starting material. They concluded that β-chitin exhibited a higher solubility in various media solvents and a greater reactivity for deacetylation and chemical modification than α-chitin.

The aim of the study is to clarify and compare the physico-chemical properties of chitin and chitosan prepared from two species of squid pen which are available in considerable amounts as a by-product of the frozen industry in Thailand. In addition to the previously reported parameters, some other properties such as optical activity, thermal transition, trace elements and heavy metal contents were elucidated. The effects of duration of deacetylation reaction that is thought to be a principal factor in determining the properties of chitosan were evaluated. The consistency in properties of product prepared under identical condition as well as samples collected from different seasons were also investigated. Some comparisons have also been made between chitin and chitosan from the two species of squid pen and those from prawn shell (*Penaeus monodon*).

2. Experimental section

2.1. Collection and preparation of squid pen powder

Squid pens of the two species, *Loligo lessoniana* and *Loligo formosana*, were freshly collected from Tepitak Frozen Co. Ltd, Pattani. To investigate the consistency in properties, the samples were collected during summer (April) and rainy (January) seasons. They were washed several times with tap water and dried at 60 °C overnight in forced air oven. The dried samples were pulverized with Waring blender, then ground to a powder with a cutting mill and passed through a 0.75 mm-sieve. Prawn shells (*P. monodon*) were freshly collected from Kiang Huat Sea Gull Trading Frozen Food Co., Ltd, Songkhla, Thailand. Only the abdominal portions were used to minimize the lipid and protein contents in the raw material. The shell was processed to a powder as described for the squid pen samples.

2.2. Preparation of chitin from squid pen

Preliminary studies in this laboratory indicated that squid pen from both species contained only a very small amount of ash (approximately 0.03%); the normal demineralization step was therefore omitted from isolation scheme of the chitin (Sornprasit, 1997). Deproteinization was carried out by slowly adding the sample powder to 1.0 M NaOH solution to obtain a ratio of solid to alkaline solution of 1:13 (w/v). The temperature of the reaction mixture was maintained at 50 °C with constant stirring for 5 h. Chitin product was filtered through four layers of gauze with the aid of vacuum pump and washed with de-ionized distilled water until the pH became neutral. It was dehydrated twice with methanol, and once with acetone, transferred to glass tray, and dried overnight at 50 °C in forced air oven. To accurately determine the percent yield, it was weighed immediately after the sample was cooled in the desiccator. The dried chitin was kept in a well-stoppered polyethylene bottle for preparation of chitosan and for further studies. To evaluate the difference between the species as well as from the season of collecting the samples, each sample was separately prepared in duplicates.

2.3. Preparation of chitosan from squid chitin

A preliminary study on the preparation of chitosan was carried out by slowly adding the dried chitin powder into a three-necked boiling flask containing a solution of 50% (w/v) NaOH to obtain a ratio of solid to alkaline solution of 1:15 (w/v). The temperature of reaction mixture was maintained at 100 °C and refluxed under nitrogen atmosphere. Under these conditions, the color of product changed from off-white to light brown after 2 h-treatment. Moreover, the chitosan powder aggregated and formed clumps after 4 h of deacetylation. Accordingly, in the main preparation of chitosan a lower temperature, as low as 60 °C, was used for the deacetylation of the squid pen chitin.

To investigate the effects of deacetylation duration on the properties of the chitosan, a slurry sample was taken from the reaction mixture at 2, 4, 6, and 8 h from the start. At each interval, the sample was filtered and a portion of residue was taken while the remaining was further refluxed with essentially the same solid to alkaline solution ratio as used initially. The chitosan product was washed, dehydrated, dried, weighed and stored as for the chitin sample.

2.4. Preparation of chitin and chitosan from prawn shells

The shell powder was de-mineralized with 1.0 M HCl (3.0%, w/v) at room temperature with constant stirring for 1.5 h, using a ratio of solid to acid solution of 15:190 (w/v). The decalcified product was washed, dehydrated, dried,

weighed and stored as for chitin. It was then deproteinized and deacetylated as described for the squid pens. These were performed in order to obtain comparable properties of samples.

2.5. Chemical analyses

Nitrogen contents in chitin and chitosan were determined using semi-micro Kjeldahl method (AOAC, 1984). Ash contents were analyzed as essential procedure as described in AOAC (1984). But the samples were in a furnace at 800 °C for 8 h. After the dried samples were digested with perchloric acid, trace elements including, Cd, Cu, Fe and Pb in the acid solution were analyzed by atomic absorption spectroscopy (AAS: G.B.C., Australia), while As, Ca, Mg and Hg were analyzed by induced couple plasma emission spectroscopy (ICPS: Plasma 1000 Polarimeter, Polax-D). Four replicate determinations were performed for each sample.

2.6. Determination of molecular weight, degree of deacetylation of chitosan and optical activity and thermal transition of chitin

The average molecular weights (M_v) of the chitosan samples were determined using viscoscopic method previously described (Bronswijk, 1975). The samples were dissolved in 0.17 M (1%, v/v) acetic acid, filtered through a sinter glass (ASTM No. 40–60, C), and viscosity of the solutions was measured with Ubbelohde capillary viscometer at 25 ± 1 °C. Then, M_v was calculated from Mark–Houwink equation:

$$[\eta] = K_m M_v^a$$

where $[\eta]$, and K_m and a are intrinsic viscosity, and viscometric constants, respectively. The values of K_m and a are taken from what was previously determined with identical setting as 8.93×10^{-4} and 0.71, respectively (Lee, 1974). On the basis of this procedure it has been demonstrated that an essential agreement of the calculated M_v for a commercial chitosan (Fluka: the value of 810,535 Da) was met compared with that denoted by maunfacturer ($M_r = 750{,}000$).

The degree of deacetylation of chitosan was determined following the spectroscopic method given by Muzzarelli (1985). The samples were dissolved in 0.01 M acetic acid solution. The first derivative spectra of the sample solutions were read with UV–visible spectrophotometer (UV-160, Shimazu, Japan) at the crossing point of 0.01–0.03 M acetic acid. Then, the degree of deacetylation was determined from the calibration curve of *N*-acetylglucosamine within a range of 5.0–40.0 mg/l.

The optical activity of chitin samples dissolved in dimethylacetamide containing 5% lithium chloride was determined following the procedure described by Austin, Brine, Castle, and Zikakis (1981). An optical activity was analyzed with Polarimeter (Podex D; Atago, Japan). Thermal transitions were studied by differential scanning calorimetry (DSC7, Perkin Elmer Co. Ltd, USA) running from 250 to 550 °C with a heating rate of 10 °C/min.

2.7. Hygroscopic activity

To investigate the moisture readsorption property of the chitin and chitosan from the two species of squid pens, an accurate weights of approximately 3-g sample was transferred in known weight beaker. They were dried at 100–105 °C in forced air oven until a constant weight was reached, allowed to stand at room temperature in desiccator, and their initial weight were determined immediately after cooling. The dried samples were covered with gauze and stood in an ambient moist environment. Weights were daily monitored until equilibrium was reached; percent moisture readsorption of each sample was then calculated with respect to its initial dried weight. Four replicate determinations were carried out for each sample.

3. Results

The contents of ash and traces of elements found in pens, chitin, and chitosan of *L. lessoniana* and *L. formosana* are tabulated in Tables 1 and 2, respectively. Whereas, the percentage of the yields for chitin and chitosan prepared from the pens of both species compared to those from the prawn species (*P. monodon*) are shown in Table 3. Table 4 shows some physical properties of individual chitin obtained from *L. lessoniana*, *L. formosana*, and *P. monodon* including optical rotations and thermal behaviors. Thermal transition of chitin of α- and β-forms obtained from squid pens and prawn shells, respectively, are also illustrated in Fig. 1.

The viscosity–average molecular weights of chitosan corresponding to different deacetylation times are listed in Table 5. Whereas, Fig. 2 shows how the degree of deacetylation of the chitosan from chitin of both squid species changed with deacetylation time. To demonstrate the effectiveness of deproteinization process prior to deacetylation, the nitrogen content of chitosan was plotted against its degrees of deacetylation and shown in Fig. 3. In addition, Fig. 4 illustrates moisture sorption profiles of squid pens and their chitin as well as 4- and 8-h deacetylated chitosans.

4. Discussion

4.1. Preparation of chitin and chitosan

Tables 1 and 2 indicate that a very small amount of ash (0.025, 0.042%) was found in the pen of the two species,

Table 1
The contents of ash and trace elements in the samples prepared from *L. lessoniana*

Sample	Ash (%)	Ca (ppm) mean (SD)	Mg (ppm) mean (SD)	Cu (ppm) mean (SD)	Fe (ppm) mean (SD)	Cd (ppm) mean (SD)
Squid pens[a]	0.025 (0.01)	17.73 (3.32)	3.30 (0.80)	0.93 (0.28)	7.74 (0.50)	Nd
Chitin[b]	Nd	3.25 (1.57)	2.43 (0.34)	Nd	3.14 (1.13)	Nd
Chitosan[b]						
2-h	Nd	3.19 (2.03)	1.54 (0.73)	Nd	2.59 (1.11)	Nd
4-h	Nd	2.79 (1.43)	1.21 (0.57)	Nd	1.69 (0.95)	Nd
6-h	Nd	3.74 (0.35)	1.06 (0.36)	Nd	Nd	Nd
8-h	Nd	3.39 (1.50)	0.67 (0.32)	Nd	Nd	Nd

As there were no significant differences with season of collection, the values are pooled data. As, Hg and Pb were all below the detection limits. Nd, none detected (below detection limits). Detection limits (ppm) for ICPS: Ca, 0.001; Mg, 0.001; As, 0.02; and Hg, 0.02. Detection limits (ppm) for AAS: Cd, 0.0004; Cu, 0.001; Fe, 0.005; and Pb, 0.01.

[a] The data are based on four replications.

[b] The data are based on eight replications. The number of hours refers to the time for alkali deacetylation using 50% NaOH at 60 °C.

Table 2
The contents of ash and trace elements in samples prepared from *L. formosana*

Sample	Ash (%)	Ca (ppm)	Mg (ppm)	Cu (ppm)	Fe (ppm)	Cd (ppm)
Squid pens[a]	0.04±0.01	24.2±5.8	5.5±1.0	16.2±5.7	17.2±2.8	8.1±0.2
Chitin[b]	Nd	6.6±3.5	2.5±0.3	8.5±2.0	4.7±0.4	2.8±0.2
Chitosan[b]						
2-h	Nd	3.2±2.1	2.1±0.7	6.9±2.2	2.4±1.1	2.9±0.2
4-h	Nd	3.2±1.6	1.8±0.8	5.3±1.3	2.3±0.9	2.3±0.1
6-h	Nd	2.5±1.6	1.5±0.6	3.2±0.6	1.7±1.1	1.1±0.2
8-h	Nd	1.6±1.3	1.2±0.5	2.8±0.4	Nd	0.3±0.1

As there were no significant differences with season of collection, the values are mean±SD of pooled data. As, Hg and Pb were all below the detection limits. Nd, None detected (below detection limits). Detection limits (ppm) for ICPS: Ca, 0.001; Mg, 0.001; As, 0.02; and Hg, 0.02. Detection limits (ppm) for AAS: Cd, 0.0004; Cu, 0.001; Fe, 0.005; and Pb, 0.01.

[a] The data are based on four replications.

[b] The data are based on eight replications. The number of hours refers to the time for alkaline deacetylation using 50% NaOH at 60 °C.

while the content could not be detected in their chitin and chitosan products. This confirms that it was not necessary to include a specific demineralization step when isolating chitin from the squid pens. Omitting this step represents not only reduction in time and production costs, but would also reduce any acid pollutant from a commercial production line.

However, Tables 1 and 2 show that some elements could be detected when the AAS and ICPS methods were used. Higher contents of some analyzed elements were found in the pen from *L. formosana* than those from *L. lessoniana*, but these markedly decreased in the chitin isolation step. During the deacetylation process, the contents fell slightly but still remained at the end of the process. In comparison, the levels of Mg and Ca in both the raw material and the isolated chitin in this study were substantially lower than those reported for squid species, *O. bartrami* (Kurita et al., 1993a). Since the As, Hg, and Pb contents were below the detectable level, they are not included in the tables. In addition, it should be noted that small amounts of Cd were detected in samples from *L. formosana* (8 ppm in the pens, 0.3 ppm in the final chitosan). This cadmium contamination should be cautioned during raw material collection.

Table 3 shows that there were no significant difference in percent yield of chitin and chitosan both within species and between two seasons. The chitin yield of approximately 35–38% was comparable to that of 35–40% from another squid species (*O. bartrami*) reported by Kurita et al. (1993a). In comparison, it is markedly higher than that from prawn shell (*P. monodon*).

Table 3
Percent yield of chitin and chitosan prepared from the pens of the squid species, *L. lessoniana* and *L. formosana* and the prawn species (*P.monodon*)

Species	Percent yield		
	Chitin[a]	Chitosan[b]	Chitosan[c]
L. lessoniana	36.06	27.59	77.55
L. formosana	36.55	28.21	77.21
P. monodon[d]	22.18	17.35	78.23

As there were no significantly different in seasonal variable, the presented values are pooled data and are mean of four replications.

[a] The percent yield of chitin was calculated on the basis of the weight of dried squid pen powders.

[b] The value was calculated on the basis of the weight of dried squid pen powders.

[c] The value was calculated on the basis of the dried chitin weight.

[d] The preparation of chitin from *P. monodon* was different from that of *Loligo* species, where the demineralization of latter was omitted. A higher temperature (126 °C) and longer deacetylation period (8 h) are utilized for preparation of chitosan from *P. monodon* chitin.

Table 4
Optical rotation and thermal characteristics of chitin under study

Species	Specific rotation[a] $[\alpha]_D^{25}$		Thermal behavior[b]		
	Initial	7-day	T_g, SD (°C)	ΔC_p, SD (J/g °C)	T_{deg}, SD (°C)
L. lessoniana	−96.57	−96.57			
L. formosana	−96.58	−96.58	355.63, 9.40	10.22, 4.04	449.62, 35.58
P. monodon	+98.82	−98.82	412.18, 2.22	11.59, 2.87	452.71, 8.14

[a] The specific rotation of chitin dissolved in dimethylacetamide with 5% $LiCl_2$, after standing at 25 °C for 7 days. The value was the same after 14 days-storage.
[b] The data were obtained from DSC running from 50 to 550 °C at 15 °C/min. Each of the data was the mean, and standard deviation of three replications. The thermal characteristics of chitin from the two *Loligo* species were not significantly different (data not shown). The table presents glass transition temperature (T_g) with its heat capacity (ΔC_p) and degradation temperature (T_{deg}).

4.2. Characterization of squid chitin

Table 4 shows that the β-chitin from both the *Loligo* species exhibited similar optical activities. Initially, the solution of squid pen chitin was levorotatory whereas that of prawn shell was dextrorotatory. After 7 days of storage, the α-chitin solution became to levorotatory and then remained stable at this value thereafter, whereas no change was observed for the β-chitin. It has previously been demonstrated that the optical rotation of chitin from various sources capable of being deacetylated with mild and harsh alkali conditions were levorotatory and dextrorotatory, respectively, with similar optical rotatory behavior to that observed here (Austin et al., 1981). It is deduced that the chiroptical activity arises not only from the chiral carbon atoms but also from its conformation as a polymer in solution that may be related to its crystallographic structure prior to dissolution. The polymer–solvent interaction of levorotatory characteristics may be stable and entropic favorable. The β-chitin apparently exhibits only weak intermolecular forces in the solid state, whereby the polymer might more readily interact with the solvent and almost immediately achieve its stable state. In contrast, the α-form of chitin has strong intermolecular forces in the solid state, so that when dissolved in the solvent its conformation might require a period of time for further molecular rearrangement to approach the same thermodynamically stable state as from β-chitin with levorotatory optical rotation.

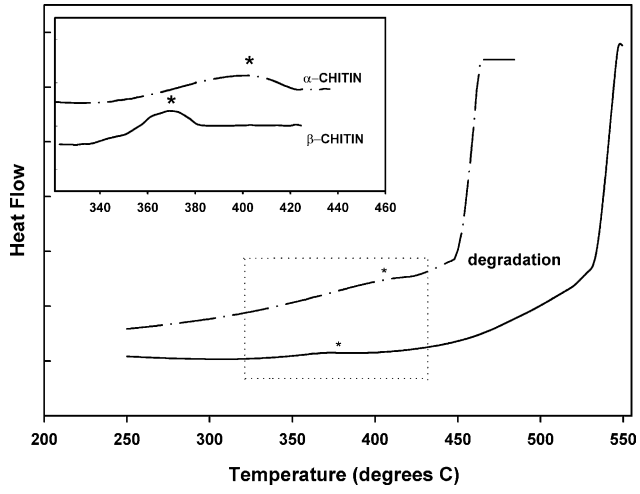

Fig. 1. Thermal transition of chitin from prawn shell (α-chitin) and squid pens under study (β-chitin). The inset of the figure is the magnification of the area specified by the dotted box. The glass transition temperature of each of the endotherms is labeled as an asterisk.

Fig. 1 illustrates the thermal transitions of chitin in the α- and β-forms. Only one glass transition was observed before the samples underwent degradation. Most of the previous work had difficulty in determining the glass transition temperature (T_g). Some authors have not observed any T_g (Peesan, Rujiravanit, & Supaphol, 2003; Kittur, Prashanth, Sankar, & Tharanathan, 2002) for it has been

Table 5
The viscosity–average molecular weight (M_v), intrinsic viscosity [η], of chitosan with corresponded deacetylation time

Species	Deacetylation time (h)	Intrinsic viscosity [η] (dl/gm)	M_v (Da×10[6a]; summer, rainy)
L. lessoniana			
	2	84.75±1.30	10.24±0.22 (9.86, 10.62)
	4	82.62±0.94	9.84±0.14 (9.65, 10.03)
	6	80.38±0.75	9.50±0.12 (9.32, 9.69)
	8	78.00±1.15	9.11±0.19 (8.78, 9.44)
L. formosana			
	2	84.75±0.25	10.24±0.04 (10.28, 10.20)
	4	83.38±0.31	10.00±0.05 (10.07, 9.94)
	6	81.50±0.61	9.69±0.10 (9.86, 8.98)
	8	76.00±0.54	8.78±0.09 (8.90, 8.66)
Commercial	–		0.76±0.22[b]
			0.95[c]
			0.54±0.05[d]

As there were no significant differences with season of collection, the values are mean±SD of pooled data.
[a] The data were determined from four replications, each two from summer and rainy seasons (means of two samples from seasonal variability were seen in the parentheses), respectively.
[b] M_v of shrimp chitosan from Kurita et al. (2000).
[c] M_v of shrimp chitosan from Kurita et al. (1993a) (no SD values given).
[d] Weight-average MW of chitosan from Dungeness crab from Lee (1974). The standard deviation was obtained from the data of different deacetylation times where the authors claimed that the numbers were not statistically significantly different.

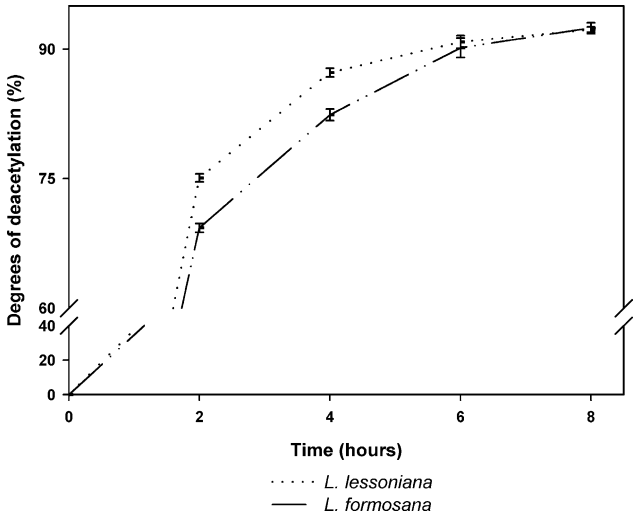

Fig. 2. Degrees of deacetylation–time profiles of chitosan produced from the pens of *L. lessoniana* and *L. formosana*.

predicted that the T_g of chitin as well as chitosan is hidden the temperature range of decomposition (T_{deg}) (Jiang et al., 1996). Fortunately, the chitin in this study exhibited an order of magnitude higher molecular weight of its deacetylated product compared with the chitin as well as the commercial chitosans previously reported (Table 5). The polymer may be able to withstand thermal agitation so its T_{deg} occurs at a higher temperature allowing its transition T_g to be revealed.

As seen in Table 4, the T_g of β-chitin occurred at a temperature considerably lower than that of α-chitin, whereas T_{deg} appeared in the comparable temperature. The results suggest that the chains of chitin of the β-form may be arranged in a looser fashion compared to that of α-form, allowing polymer movement at lower temperatures. This thermal behavior of the different forms of chitin is consistent with the optical properties previously discussed.

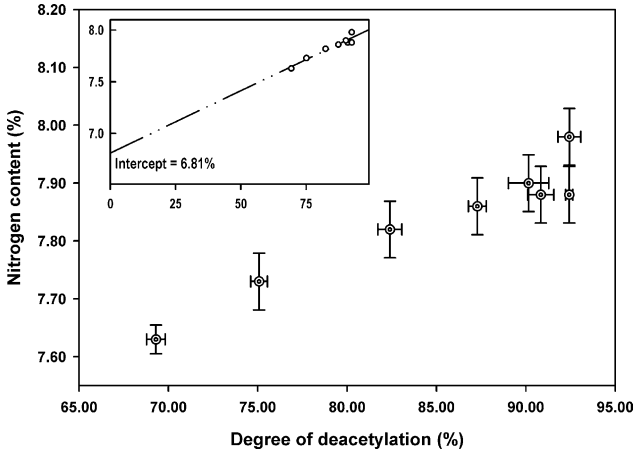

Fig. 3. The nitrogen content of chitosan plotted against its degrees of deacetylation. The inset of the figure illustrates the linear regression line of the data coordinates.

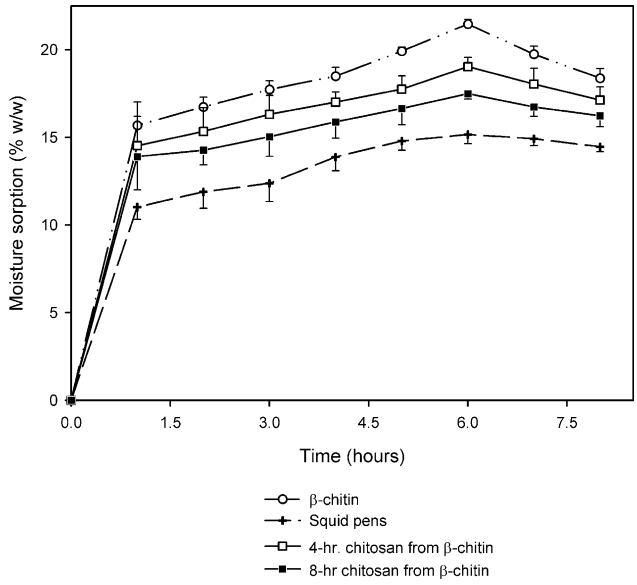

Fig. 4. Moisture sorption kinetic profiles of squid pens and their chitin as well as 4- and 8-h deacetylated chitosans.

4.3. Characterization of chitosan derived from squid chitin

There were no significant differences in the molecular weight either between the season of collection of the samples or between the two squid species (Table 5). Furthermore, the viscosity–average molecular weight of 10.24×10^6 Da observed after the 2 h deacetylation period, gradually but significantly decreased to that of $8.78–9.11\times10^6$ Da after the 8 h deacetylation period. Similar effects have been reported previously for β-chitin from the pens of *Loligo vulgaris* species (Tolaimate, Desbrieres, Rhazi, Vincendon, & Vottero, 2000). By contrast, commercial chitosan derived from shrimp chitin (Kittur et al., 2002; Zhang & Neau, 2001) and Dungeness crab chitin (Ottoy, Varum, & Smidsrod, 1996) exhibited far lower molecular weights of $7.6–9.5\times10^5$ and $5.2–5.9\times10^5$ Da, respectively. The latter authors also reported that the molecular weight of the samples did not significantly vary with the deacetylation time, suggesting that there had been no polymer cleavage during the deacetylation process in their case. It seems that chitin undergoes degradation because of the high concentration of reagents, high temperature, and prolonged reaction time required for completing deacetylation (Hasegawa, Isogai, & Onabe, 1994; Tolaimate et al., 2000). The authors who worked with Dungeness crab chitin also suggested that low temperature and oxygen-free conditions used in their study might have protected their chitin from degradation (Ottoy et al., 1996).

The nitrogen contents did not vary significantly within either the squid species or the season from which they were collected. For example, the mean nitrogen contents (±standard deviation) of the 6 h deacetylated chitosan from the chitin of *L. lessoniana* and of *L. formosana* were

7.88 ± 0.05 and 7.90 ± 0.05%, respectively. The values for other samples also varied within the similar ranges. The β-chitin isolated from both squid species exhibited nitrogen contents of 6.30% (95% confidence interval ± 0.29%). The values obtained were within the established specification range of 6–7% (Muzzarelli, 1985).

It was demonstrated that the degrees of deacetylation of samples from *L. lessoniana* were slightly higher within the 4 h deacetylation period (Fig. 2). The values were then comparable after 6 h period.

The nitrogen contents of chitin and chitosans from both squid species are plotted against the corresponding degrees of deacetylation in Fig. 3. A linear relationship should be expected if the nitrogen is only from the amine groups in the polysaccharide chain (that is, not from any residual protein). The linear regression line (an inset of Fig. 3) had an intercept (standard error) of 6.81% (0.12%) and a slope (standard error) of 0.0121 (0.0014), with a correlation coefficient of 0.9226. The nitrogen contents of fully acetylated chitin and its fully deacetylated product based on their molecular formulas are 6.98 and 7.82%, respectively (The Merck Index, 1989). The expected slope should then be 0.0134. A *t*-statistic test to compare the parameters from regression and the 'theoretical' ones shows no significant differences, with *p*-values of 0.53 and 0.38 for the intercept and the slope, respectively. This confirms that the nitrogen content was solely from the chitin–chitosan polymer backbone, or in other words, the deproteinization process prior to deacetylation was fully effective.

4.4. Hygroscopicity of chitin and chitosan from squid pens

Fig. 4 demonstrates that the chitin and the chitosans from squid pens under study adsorbed a considerable amount of moisture, causing the weight to increase by 10–20% when placed in an ambient moist environment. The chitosans adsorbed a slightly less amount of moisture than the chitin. The greater the deacetylation, the lesser the moisture adsorption. Kurita et al. (1993a) reported that the equilibrium moisture content of squid chitin under 93% relative humidity was significantly higher than that of shrimp chitin. The hygroscopicity of β-chitin may not only be attributable to the looser molecular arrangement as previously discussed (Kurita et al., 1993a) but also to the acetyl groups present in the polymer. An attempt has been previously made to classify the hygroscopicity of materials especially for those used in pharmaceuticals (Callahan, Cleary, Kaplan, Kensler, & Nash, 1982). When applied to the chitin and chitosan under study, the samples were classified as 'Class III: moderately hygroscopic', where moisture essentially increases below 80% relative humidity and increases less than 50% after storage for 1 week above 80% relative humidity.

5. Conclusion

The yields, chiroptical activity, behavior under thermal stress, viscosity–average molecular weight of its deacetylated products, and nitrogen contents, for chitin isolated from *L. lessoniana* and those from *L. formosana* were not significantly different. Neither was season. There were traces of heavy metal (especially Cd) present in pens that markedly decreased but were incapable to be got rid of within the step of chitin–chitosan preparation. Thus, the contamination of health-hazardous heavy metals should be of concern in raw material selection. The chiroptical activity and thermal behavior of the squid pen chitin demonstrated a weaker intermolecular interaction in the native polymer arrangement (referred to as the β-form) compared to that of chitin from prawn shell (referred to as the α-form). The pens of both *Loligo* species had a negligible ash content so that the normal demineralization step of chitin isolation could be omitted. In addition, the deproteinization step utilized in this study was evidently so effective that the nitrogen content involved in the following deacetylation step was solely from the polysaccharide. In terms of moisture adsorption, chitin and chitosan from squid pens may be classified as moderately hygroscopic materials. The moisture adsorption may be important when it comes to the processing and the applications of chitin and chitosan.

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