Alkali or acid induced changes in structure, moisture absorption ability and deacetylating reaction of β-chitin extracted from jumbo squid (Dosidicus gigas) pens

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Alkali or acid induced changes in structure, moisture absorption ability and deacetylating reaction of β-chitin extracted from jumbo squid (*Dosidicus gigas*) pens

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Abstract

Alkali or acid-induced structural modifications in β-chitin from squid **(*Dosidicus gigas,*** d'Orbigny, 1835) pens and its moisture absorption ability (MAA) and deacetylating reaction were investigated and compared with α-chitin from shrimp shells. β-chitin was converted into α-form after 3 h in 40% NaOH or 1-3 h in 40% HCl solution, and obtained α-chitin from NaOH treatment had higher MAA than that of native α-chitin due to polymorphic destructions. In contrast, induced α-chitin from acid treatment of β-chitin had little polymorphic modifications, showing no significant change ($P>0.05$) in MAA. β-chitin was more susceptible to alkali deacetylation than α-chitin, and required lower concentration of NaOH and shorter reaction time. These results demonstrated that alkali or acid treated β-chitin remained high susceptibility toward solvents, which in turn resulted in good biological activity of β-chitosan for being used as natural antioxidant and antimicrobial substance or employed to make edible coatings and films for various food applications.

Keywords: α-chitin, β-chitin, jumbo squid pens, structural modification, crystallinity, moisture absorption ability, deacetylating reaction

1. Introduction

Chitosan, the derivative form of chitin, has been extensively studied for its antioxidant and antimicrobial functionalities and film forming capability, and demonstrated a great potential for a wide range of food applications as a natural functional substance (Huang, Zhao, Hu, Mao, & Mei, 2011; Shimojoh, Masai, & Kurita, 1996; Lin, Chen, & Peng, 2009; Sukmark, Rachtanapun, & Rachtanapun, 2011; Jung & Zhao, 2012 and 2013; Kim, No, & Prinyawiwatkul, 2007)

There are two forms of chitin, α- and β-chitin. They are distinguished in respect to the unique structural characteristics (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994; Kurita, Tomita, Tada, Ishii, Nishimura, & Shimoda, 1993; Lamarque, Viton, & Domard, 2004). Crystallites of α-chitin is tight-packed with inter-sheet hydrogen bonds formed between the antiparallel sheets, whereas that of β-chitin has loose arrangements due to much weaker intermolecular hydrogen bonds by the parallel manner of the polymeric sheets. Moreover, the crystal region (crystallinity) of the semi-crystalline α-chitin is larger than that of β-chitin (Lima & Airoldi, 2004). These structural differences directly impact their physicochemical properties, in which β-chitin presents much higher solubility and reactivity in alkali solutions during the deacetylation process than that of α-chitin (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994; Kurita, Tomita, Tada, Ishii, Nishimura, & Shimoda, 1993). In addition, extraction of β-chitin from squid pens has shown advantages of unneeded demineralization and decoloration processes in comparison with extracting α-chitin from shrimp and crab shells due to the negligible amount of mineral and carotenoid in the squid pens (Chandumpai, Singhpibulporn, Faroongsarng, & Sornprasit, 2004; Lavall, Assis, & Campana-Filho, 2007; Tolaimate, Desbrires, Rhazi, Alagui, Vincendon, & Vottero, 2000).

Chemical treatments using acid and alkali have been commonly employed to produce chitin and chitosan. It was generally believed that the demineralization and/or deproteinization processes using lower concentrations of acid and alkali and lower temperatures than those applied in the deacetylation process do not cause significant changes in the molecular weight (Mw) and degree of deacetylation (DDA) of chitin (Rhazi, Desbrières, Tolaimate, Alagui, & Vottero, 2000). However, several studies have found that the chemical treatments alter the structural properties of chitin due to swelling, dissociation of hydrogen bonds, and rearrangements of polymeric chains, and the different forms of chitin responded differently (Feng, Liu, & Hu, 2004; Li, Revol, & Marchessault, 1999; Liu, Liu, Pan, & Wu, 2008; Noishiki, Takami, Nishiyama, Wada, Okada, & Kuga, 2003; Saito, Putaux, Okano, Gaill, & Chanzy, 1997). Feng et al. (2004) and Liu et al. (2008) reported that alkali treatment of α-chitin weakens inter-sheet hydrogen bonds and decreases crystallinity index (CI) along with the polymorphic modifications as alkali concentration increased. The conversion phenomenon between β- and α-chitin during alkali treatment was also observed by Saito et al. (1997), Li et al. (1999), and Noishiki et al. (2003), in which β-chitin was converted into α-chitin forming inter-sheet hydrogen bonds between C=O and O-H in C_6, similar to the mercerization induced from cellulose I to cellulose II. According to Li et al. (1999), alkali or acid caused swelling of crystallites and destruction of the original lateral order, resulted in the rearrangement of those polymeric chains, thus conversion of chitin from one form to another. Hence, alkali or acid-induced β-chitin conversion to α-chitin with the presence of strong inter-sheet hydrogen bonds might be a concern for preparing chitosan since it may cause the loss of the original functional properties of β-chitin, especially the high reactivity and susceptibility toward solvents. Moreover, it is unclear how the alkali or acid-induced conversion exactly impact their polymorphic

84 structures, in turn the physicochemical properties of resulted chitin, and how the converted form
85 of chitin similar or different from its native form. Such information is critical to fully understand
86 the demineralization, deproteinization, and deacetylation processes in α- and β-chitin, the
87 essential steps in preparing α- and β-chitosan, as well as the functional differences between α-
88 and β- chitosan. Based on our best knowledge, no previous study has systematically reported
89 these conversion phenomena in respect to the polymorphic modifications.
90 Several previous studies have demonstrated that the functional properties of chitin and
91 chitosan depend on their originated marine sources and species (Jang, Kong, Jeong, Lee, & Nah,
92 2004; Rhazi, Desbrières, Tolaimate, Alagui, & Vottero, 2000). The jumbo squid (*Dosidicus*
93 *gigas*, d'Orbigny, 1835) pens are a newly employed source of β-chitin (Jung and Zhao, 2011 and
94 2012), and have shown some unique properties different from those mostly used β-chitin
95 extracted from *Loligo* species (Chandumpai, Singhpibulporn, Faroongsarng, & Sornprasit, 2004;
96 Lavall, Assis, & Campana-Filho, 2007; Tolaimate, Desbrieres, Rhazi, & Alagui, 2003; Tolaimate,
97 Desbrires, Rhazi, Alagui, Vincendon, & Vottero, 2000). Therefore, the objectives of this study
98 were to investigate the alkali and acid-induced polymorphic modifications in β-chitin extracted
99 from jumbo squid pens and α-chitin from shrimp shells, and to study the changes in the moisture
100 absorption ability of resulted α- and β-chitin in comparison with their native form. Moreover, the
101 deacetylating reactions of α- and β-chitin under various alkali treatments were also investigated
102 to study the impact of the structural modifications on the deacetylating process.
103
104 **2. Materials and Methods**
105 **2.1. Materials**

Dried jumbo squid (*Dosidicus gigas*, d'Orbigny, 1835) pens was donated by Dosidicus LLC (WA, USA) and α-chitin from shrimp shells were purchased from Sigma-Aldrich (NJ, USA). *N*, *N*-dimethylacetamide (DMAc), lithium chloride, NaOH, HCl, and acetic acid were purchased from Sigma-Aldrich Co. LLC (MO, USA), J.T. Baker Chemical Co. (NJ, USA), Macron Chemicals (PA, USA), EMD (NJ, USA), and Fisher Scientific (NJ, USA), respectively.

2.2. Preparation of Chitin

Samples were ground into about 18 meshes (1.0 mm, Glenmills Inc., USA). Squid pens were deprotenized once by 5% NaOH for 3 d at room temperature, washed with distilled water to reach the neutral pH, and then dried at 40 °C in an oven (Precision Scientific Inc., USA) for 24 h. Each form of the chitin was treated in 40% HCl or NaOH for 1-4 h under a given condition for studying not only the conversion phenomena from β-chitin to α-chitin, but also the polymorphic properties including crystal characteristics and CI as described below. The applied conditions for acid or alkali treatments on chitin were selected based upon the previous studies of investigating the conversion phenomenon of β-chitin (Noishiki, Takami, Nishiyama, Wada, Okada, & Kuga, 2003; Saito, Putaux, Okano, Gaill, & Chanzy, 1997). For deacetylation process, different concentrations of NaOH (40 or 50%), temperatures (60 or 90 ºC), and reaction times (2, 4, or 6 h) were applied, following our previous study (Jung and Zhao, 2011).

2.3. Viscosity-Average Mw

The viscosity-average Mw of α- and β-chitin were determined by using the Ubbelohde Dilution Viscometer (Cannon instrument Co., USA) with a capillary size of 0.58 nm. Approximate 100 mg of chitin was dissolved in 10 mL of the mixture solution of *N*, *N*-

dimethylacetamide (DMAc) containing 5% lithium chloride. The intrinsic viscosity was measured by the intercept between the Huggins (reduced viscosity, Ƞ sp/C~C) and Kraemer (relative viscosity, Ƞ rel/C ~C) plots when the concentration was 0 (Mao, Shuai, Unger, Simon, Bi, & Kissel, 2004). The viscosity-average Mw of chitosan was calculated by Mark-Houwink-Sakurada (MHS) equation: $[Ƞ] = K (M_W)^a$, where K and a were the constants, $K=2.1 \times 10^{-4}$ and a = 0.88 (Terbojevich, Cosani, & Muzzarelli, 1996); and [Ƞ] was the intrinsic viscosity obtained from the two plots, Huggins and Kraemer.

2.4. Proximate Composition Analysis

Moisture contents of chitin samples were determined by the percentage weight loss of the samples after drying in a forced-air oven at 100 ºC for 24 h. Ash contents were analyzed following AOAC method (1884). Protein contents were measured by Lowry method using bovine serum albumin standards (Walker, Waterborg, & Matthews, 1996). The Lowry method is sensitive to low protein concentrations (5 – 100 µg/mL).

2.5. Determination of Degree of Deacetylation (DDA)

In this study, two different methods were employed to determine DDA: a chemical assay by the colloidal titration method and Fourier-Transform Infrared (FT-IR) spectroscopic analysis. For the chemical assay (Chang, Tsai, Lee, & Fu, 1997), a 50 mg of deacetylated chitosan (0.5%, w/w) was dissolved in 10 mL of 5% (v/v) lithium chloride solution, transferred into a flask, and then diluted up to 30 mL with distilled water. After adding 100 µL of toluidine blue indicator, the solution was titrated by the 1/400 potassium polyvinyl sulfate (PPVS) till the solution color changed from blue to violet. DDA was calculated as:

DDA (%) = (X/161) / (X/161) + (Y/203) (1)

Where X (the weight of D-glucosamine residue, g) was calculated as "1/400*1/1000*F*161*V", F was the factor of 1/400 PVS, and V was the volume (mL) of consumed PPVS; Y (the weight of N-acetyl-D-glucosamine residue, g) was calculated as "0.5 * 1/100 – X"; 161 and 203 in equation (1) was the molecular weight of D-glucosamine and N-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose), respectively.

2.6. Moisture Absorption Ability

Functional groups including NH_2 of C_2 or OH of C_3 and C_5 in *N*-acetyl-D-glucosamine or D-glucosamine monomers can trap water penetrating into crystallites of chitin by forming hydrogen bonds. Moreover, these functional groups can be closely related to the crystal properties or crystallinity index (CI) in the polymorphic structure of chitin as water can access easily to the loose-packed crystallites of β-chitin, compared with the rigid crystallites of α-chitin (Oh & Nam, 2012).

Powdered chitin was conditioned in a P_2O_5 added desiccator for 24 h to remove residual moisture (Chen, Du, Wu, & Xiao, 2002), and then placed in a self-assembled chamber at 25 ºC and 80% RH for 40 h. The moisture absorption ability was calculated as the percentage of weight gain of dried samples after 40 h using equation (2):

$$\text{Moisture absorption ability (\%)} = \frac{Wegith\ of\ samples\ after\ 40\ h\ (g) - intial\ weight\ of\ chitin\ (g)}{Initial\ weight\ of\ chitin\ (g)} \text{x}100 \quad (2)$$

2.7. A Fourier-Transform Infrared (FT-IR) Spectroscopic Analysis

A single bound attenuated total reflection (ATR)-FTIR spectrometer (PerkinElmer, USA) was operated by Omnic 7.4 software (Thermo Fisher Inc. USA) under the operating conditions

of 32 scans at a 4 cm^{-1} resolution and referenced against air. All spectra were recorded as the absorption mode. DDA was determined by FT-IR using the method by Sabnis and Block (1997) as expressed as (3):

$$\text{Degree of deacetylation (DDA, \%)} = 97.67 - [26.486 \times (\frac{A_{1655}}{A_{3450}})] \quad (3)$$

Where A_{1655} and A_{3450} were the absorbance at 1655 cm^{-1} indicating the amide I band (a measure of *N*-acetyl group contents) and the absorbance at 3450 cm^{-1} indicating the hydroxyl groups as the reference, respectively.

The partial FT-IR spectra (1300-1800 cm^{-1}) were reported to distinguish the two forms of chitin and the inter-sheet hydrogen bonds. The band around ~1700 cm^{-1} attributed to the stretching vibration of C=O in amide (Liu, Liu, Pan, & Wu, 2008), which could be split by the inter-sheet hydrogen bond with neighboring O-H of C_6 in α-chitin, whereas β-chitin was shifted to a single peak indicating no inter-sheet hydrogen bonds and much weaker intermolecular hydrogen bonds (Cárdenas, Cabrera, Taboada, & Miranda, 2004).

2.8. X-Ray Diffraction (XRD)

X-ray diffraction patterns were recorded using a XRG 3100 x-ray diffractometer (Philips, U.S.) with a Cu Kα (1.54 Å) at a voltage of 40 kV and a current of 30 mA. A typical scan range was from 5º to 40º (2θ) at scanning speed of 0.025º/sec.

The CI was determined by equation (4):

$$\text{Crystallinity (CI, \%)} = \frac{I_{110} - I_{am}}{I_{110}} \times 100 \quad (4)$$

Where I_{110} was the maximum intensity of the (110) plane at 2θ = ~19º and I_{am} was the intensity of the amorphous regions at 2θ = ~12.6º (Focher, Beltrame, Naggi, & Torri, 1990; Focher, Naggi, Torri, Cosani, & Terbojevich, 1992).

Among various types of crystal lattices found in the polymeric structure of chitin including 020, 110, 120, 101, or 130 planes, the *d*-spacing and apparent crystal size (D_{ap}) of (020) and (110) planes were reported as both were appeared in the native and the processed α- and β-chitin. The *d*-spacing was computed using Bragg's law (5) (Feng, Liu, & Hu, 2004):

$$d\ (\text{Å}) = \frac{\lambda}{2\sin\theta} \qquad (5)$$

Where *d* was plane spacing; λ was 1.54 Å, wavelength of Cu Kα radiation; and θ was one-half angle of reflections.

The apparent crystal size (D_{ap}) was calculated with the aid of Scherrer equation (6) (Focher, Beltrame, Naggi, & Torri, 1990; Klug & Alexander, 1969):

$$D_{ap}\ (\text{Å}) = \frac{k\lambda}{\beta_0 \cos\theta} \qquad (6)$$

Where β_0 (in radians) was the half-width of the reflection; k was a constant indicating the crystallite perfection with a value of 0.9; λ was 1.54 Å, the wavelength of Cu Kα radiation; and θ was one-half angle of reflections.

2.9. Experimental Design and Statistical Analysis

Native (non-treated) and processed (acid- or alkali-treated) α- and β-chitin samples were tested using a completely randomized design (CRD). Moisture, protein, and ash contents of native chitin, and the moisture absorption ability of processed chitin were all determined in duplicate, and data were analyzed for statistical significance via least significant difference (LSD) post hoc testing as appropriate using statistical software (SAS v9.2, The SAS Institute, USA). Results were considered to be significantly different if $P<0.05$.

3. Results and Discussion

3.1. Proximate Composition and Polymeric Structure of β-Chitin Extracted from Jumbo Squid Pens in Comparison with α-Chitin

The proximate compositions of α- and β-chitin are reported in Table 1. Moisture content of β-chitin extracted from jumbo squid pens was significantly ($P<0.05$) higher than that of the commercial α-chitin from shrimp shells. This might be because the crystallites of β-chitin are less tight due to much weaker intermolecular hydrogen bonds than that of α-chitin, thus moisture accessed easily to crystallites of β-chitin and was more able to form hydrogen bonds with NH_2 or OH. Similarly, Kurita et al. (1993) reported higher retention of absorbed water in β-chitin than that in α-chitin. Mw of β-chitin was almost as twice higher as that of α-chitin at similar DDA (Table 1). According to Tolaimate et al. (2003), Mw of β-chitin was 2-3 times higher than that of α-chitin at the same DDA, which was consistent with our result. Protein and ash contents in both α- and β-chitin were negligible, thus no further deprotenization and demineralization procedures were applied on samples used in this study. The ash (mineral) content of β-chitin was below 1% prior to acid treatment (so-called demineralization), lower than that reported in the previous study on *Loligo vulgaris* (1.7%) (Tolaimate, Desbrieres, Rhazi, & Alagui, 2003).

Crystal property of β-chitin was distinguished from that of α-chitin based upon partial FT-IR spectra (Figs. 1A and 1B). The C=O band in amide (~1700 cm^{-1} indicated by dash lines) was split by inter-sheet hydrogen bonds in α-chitin due to antiparallel manner between the polymeric sheets, whereas β-chitin was shifted to a single peak without inter-sheet hydrogen bonds and much weaker intermolecular hydrogen bonds due to the parallel manner, similar to the previous finding by Cardenas et al. (2004). XRD patterns of α- and β-chitin were in the range of 5-40 º (2θ) (Fig. 2). Five crystalline planes (020, 110, 120, 101, and 130) at reflections of 9.4, 12.9, 19.4, 21.0, 23.8, and 26.5 were observed in α-chitin, whereas only two crystalline planes (020

and 110) at reflections of 8.9 and 19.7 were appeared in β-chitin (Figs. 2A and 2B). Moreover, the peaks in α-chitin were sharper than those in β-chitin, indicating that crystal structure of α-chitin was more rigid and stable than that of β-chitin (Figs. 2A and 2B). Table 2 shows CI, relative intensities (RI, %), *d*-spacing, and D_{ap} of each crystal plane (020 and 110) commonly appeared in both α- and β-chitin. CI of β-chitin was ~8% lower than that of α-chitin similar to XRD patterns showing lower intensities and broader shapes of the peaks in β-chitin. The *d*-spacing of (020) plane was relatively larger in β-chitin, indicating that space distances between aligned polymeric chains were wider than those of α-chitin. Furthermore, D_{ap} of (020) and (110) planes in β-chitin were smaller than those of α-chitin. Hence, β-chitin extracted from jumbo squid pens had loose crystallites and lower CI, thus higher reactivity toward solvents, more swelling, and higher solubility than α-chitin. These results were similar to the previous findings on β-chitin extracted from *Ommastrephes bartramii* and *Loligo* species (Chandumpai, Singhpibulporn, Faroongsarng, & Sornprasit, 2004; Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994; Lamarque, Chaussard, & Domard, 2007; Lavall, Assis, & Campana-Filho, 2007).

3.2. Alkali or Acid Induced Conversion between α- and β-Chitin

Alkali or acid induced conversion of chitin from β-form to α-form was compared with the mercerization of cellulose where the parallel chains of cellulose I were converted into the antiparallel manner of cellulose II (Nishimura & Sarko, 1987; Okano & Sarko, 1984). The different forms of chitin showing antiparallel or parallel manner could be distinguished by FT-IR spectra in C=O band depending on the presence of inter-sheet hydrogen bonds between C=O and O-H in C_6.

Fig. 1 represents the partial FT-IR spectra (~1300-1800 cm^{-1}) of alkali or acid treated α- and β-chitin for 1-4 h. In alkali treated α-chitin (Fig. 1A), the split peak in C=O band shifted to a single peak after 2 h treatment due to the dissociation of inter-sheet hydrogen bonds. Similarly, Feng et al. (2004) observed the dissociation of hydrogen bonds along with the polymorphic changes in α-chitin after alkali-freezing treatment. Liu et al. (2008) reported similar FT-IR spectra of α-chitin after 40% alkali treatment for 4 h, shown β-chitin with a single peak in C=O band. According to Li et al. (1999), however, α-chitin processed in 40% NaOH at 3 ºC for 3 h remained its native form with strong hydrogen bonds due to the favorable packing nature of the crystallites. In alkali processed β-chitin (Fig. 1B), C=O band was slightly split at 1 and 2 h, and clear split was appeared at 3 h indicating the conversion β-chitin into α-chitin, but shifted to a single peak at 4 h. Therefore, the conversion phenomenon in β-chitin depended on the reaction time (1-4 h). According to Noishiki et al. (2003), the conversion was occurred in 30% NaOH after 1 h. In acid processed chitin, C=O band in α-chitin was all split at 1-4 h indicating that α-form remained with the presence of inter-sheet hydrogen bonds unlike alkali treatments (Fig. 1C), whereas the conversion from β-form into α-form was appeared at 1-3 h (Fig. 1D). A single peak of C=O band in β-chitin was observed at 4 h similar to the alkali treatment (Fig. 1D). According to Saito et al. (1994), the conversion from β-form to α-form was appeared by 7-8 N HCl treatment for 30 min. Hence, acid was able to induce the conversion of β-chitin to α-chitin after 1-3 h treatment, whereas α-chitin still presented the antiparallel polymeric sheets with inter-sheet hydrogen bonds after 1-4 h acid treatment.

3.3. Alkali or Acid Induced Structural Changes in α- and β-Chitin

Alkali or acid induced conversion in α- and β-chitin may impact their structural properties, such as crystal properties and CI of the polymorphic chitin. Hence, the structural properties in alkali or acid treated α- and β-chitin after various reaction times were analyzed for interpreting how the conversion phenomenon may impact the structural changes.

XRD patterns of the alkali or acid treated α- and β-chitin are illustrated in Fig. 2. For alkali treatment, the intensities of the peaks were decreased in both α- and β-chitin and the peaks of (020) and (110) planes of β-chitin were shifted, meaning the destruction of crystallite by alkali treatment. After 2-3 h alkali treatment, α-chitin displayed sharp peaks with high intensities at ~32º and the peaks of (130) plane at ~26º was also observed in alkali treated β-chitin (Figs. 2A and 2B), assuming that alkali induced the formation of some crystallites. However, alkali treated β-chitin showing the conversion into α-form exerted much less rigid crystallites and lower CI, presenting broader peaks and lower intensities in comparison with native α-chitin or β-chitin. For acid treated samples (Figs. 2C and 2D), the intensities of the peaks were not decreased in α-chitin, showing higher intensities and sharper peaks in (020), (110), and (130) planes, whereas β-chitin had shifted peaks to lower angles in (020) and (110) planes at 3-4 h, interpreting that crystallites of acid treated β-chitin were destroyed and the space distance in each crystal plane became larger. Hence, the α-chitin converted from β-chitin by acid treatment had less rigid crystallites than that of the native α-chitin from shrimp shells.

Table 2 reports CI (%), RI (%), *d*-spacing (Å), and D_{ap} (Å) of (020) and (110) planes in alkali or acid treated α- and β-chitin, respectively. In alkali treatment, CI of α-chitin was more decreased than that of β-chitin. RI of (020) and (110) planes in α-chitin was lower than those in β-chitin after 1-2 h treatment. Moreover, D_{ap} of (110) plane was also decreased at 3-4 h in α-chitin. These results were consistent with the XRD patterns showing lower intensities in alkali

311 treated α-chitin due to the destruction of crystallites and the FT-IR spectra presenting that inter-
312 sheet hydrogen bonds of α-chitin were dissociated by alkali treatments for 1-4 h. In contrast, D_{ap}
313 of (020) and (110) planes increased in β-chitin, compared with native β-chitin, but RI of (110)
314 plane decreased at 3-4 h where the conversion into α-form was appeared. Hence, alkali processed
315 β-chitin remained loose crystal structure with lower CI similar to the XRD patterns representing
316 decreased intensities and shifting of the peaks. In acid treatment, CI of α-chitin slightly
317 decreased but no large changes in *d*-spacing and D_{ap}, indicating that there was no massive
318 destruction of crystallites, whereas *d*-spacing and D_{ap} of β-chitin were similar to those of native
319 β-chitin along with higher *d*-spacing of (020) plane indicating larger space distances between the
320 polymeric chains. Hence, crystallites of acid treated β-chitin were less rigid than the native or
321 acid treated α-chitin.
322 In summary, alkali treated α- and β-chitin exerted considerable destruction of crystallites and
323 lower CI than their native chitin, and α-chitin converted from β-chitin as a result of alkali
324 treatment had loose-packed crystallites and lower CI than native α-chitin. In contrast, acid had
325 relatively less impact on the structural properties of α- and β-chitin.
326
327 **3.4. Moisture Absorption Ability of Alkali or Acid Treated Chitin in Relation to their**
328 **Structural Properties**
329 Moisture absorption ability (MAA, %) of the native and alkali or acid treated α- and β-chitin
330 is reported in Table 3. The native β-chitin had significantly (P<0.05) higher MAA (~7.0%) than
331 that of the native α-chitin (~-0.8%). This result was consistent with the previous studies reporting
332 higher reactivity, swelling, and retention ability of absorbed water in β-chitin in comparison with

α-chitin (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994; Kurita, Tomita, Tada, Ishii, Nishimura, & Shimoda, 1993; Lamarque, Viton, & Domard, 2004).

In alkali treatment, MAA of α-chitin significantly ($P<0.05$) increased after 1 h, and that of β-chitin was increased after 1-2 h and had the highest MAA at 3-4 h with MAA of ~23-27%, which was significantly ($P<0.05$) higher than those of α-chitin at 3-4 h. Similarly, Kurita et al. (1993) found that the crystal structure of β-chitin is destroyed easily by high concentration of alkali treatment than that of α-chitin, showing much higher hygroscopicity and the retention of the absorbed water in β-chitosan than that in α-chitosan. Likewise, Wada and Saito (2001) reported that β-chitin readily expanded along the b-axis direction (no inter-sheet hydrogen bonds between stacked sheets) by heat and water was easily swollen along the b-axis direction. Based upon the XRD patterns in this study, the intensities of the peaks were decreased in alkali treated α- and β-chitin, but the peak of (110) plane was shifted to lower angles in alkali treated β-chitin at 2-4 h, indicating the destruction of crystallites. Moreover, RI of alkali treated β-chitin at 3-4 h was lower than that of native α-and β-chitin. In spite of the conversion of β-chitin into α-chitin at 3 h, MAA was significantly ($P<0.05$) higher probably due to the destruction of crystallites and the lower CI than those of the native α- and β-chitin. Hence, moisture absorption ability in alkali treated α- and β-chitin was significantly ($P<0.05$) higher than that of native α- and β- chitin due to the destruction of crystallites. The α-chitin converted from β-chitin exerted significantly ($P<0.05$) higher moisture absorption ability than that of the native α-chitin, assuming that its reactivity and swelling ability were higher than native α-chitin even with the presence of inter-sheet hydrogen bonds within the crystallites.

In acid treatment, moisture absorption ability of α-chitin was significantly ($P<0.05$) increased at 1 h, whereas that of β-chitin remained similar to that of the native chitin. Increase of the

moisture absorption ability of acid treated α-chitin at 1 h was probably due to the decreased CI in comparison with native α-chitin. In contrast, there was no extensive decrease of CI in acid treated β-chitin in comparison with native β-chitin and the conversion into α-form was appeared at 1-3 h, but the peaks of (020) and (110) planes were shifted to lower angles indicating the destruction of crystallites. Hence, moisture absorption ability of acid treated β-chitin was not significantly changed ($P>0.05$) by the complex structural modification. Compared with alkali treatment, acid induced less polymorphic changes in α- and β-chitin.

In summary, α-chitin converted from β-chitin showed enhanced moisture absorption ability in comparison with the native α- and β-chitin due to the polymorphic destruction, remaining higher swelling susceptibility of native β-chitin toward solutions by alkali treatments. This finding demonstrated that α-chitin originated from β-chitin is more susceptible toward deacetylation and depolymerization in comparison with the native α- and β-chitin.

3.5. Deacetylation of α- and β-Chitin under Various Alkali Treatments

DDA of α- and β-chitin subjected to various alkali treatments are shown in Table 4. DDA of α-chitosan was ~40-89% and that of β-chitosan was ~63-92% under the same deacetylating treatment conditions, exhibiting relatively higher DDA in β-chitosan. Different deacetylating treatments were required for obtaining same DDA of α- and β-chitosan, in which for obtaining ~60% DDA, 40% NaOH at 90 ºC for 6 h and 40% NaOH at 60ºC for 2 h were applied for α- and β-chitosan, respectively, while for obtaining ~75% DDA, α- and β-chitin were deacetylated by 50% NaOH at 60 ºC for 6 h and 50% NaOH at 60 ºC for 2 h, respectively. Hence, relatively milder deacetylation treatments with lower temperature or shorter reaction time were required to extract 60 and 75% DDA of β-chitosan than those required for obtaining α-chitosan. This result

379 may be interpreted by the different polymorphic structure between α- and β-chitin, in which α-
380 chitin with strong inter-sheet hydrogen bonds in tight-packed crystal structures with higher CI
381 was less reactive toward alkali treatment during the deacetylation process than β-chitin did,
382 consistent with previous finding showing higher reactivity and swelling ability of β-chitin in
383 alkali solutions than that of α-chitin (Chandumpai, Singhpibulporn, Faroongsarng, & Sornprasit,
384 2004; Kurita, Tomita, Tada, Ishii, Nishimura, & Shimoda, 1993; Lamarque, Viton, & Domard,
385 2004; Tolaimate, Desbrieres, Rhazi, & Alagui, 2003). Hence, producing β-chitosan from squid
386 pens resulted in low production cost by using lower concentrations of reagents and shorter
387 reactions times than that for α-chitin.
388
389 **4. Conclusions**
390 In comparison with α-chitin from shrimp shells, β-chitin from jumbo squid pens had loose
391 arrangements of polymeric chains, thus lower CI, which led to the higher moisture absorption
392 ability than that of α-chitin. β-chitin could be converted into α-form after 3 h or 1-3 h treatment
393 in alkali or acid solution, respectively. Alkali treatment resulted in polymorphic destructions in
394 both α- and β-chitin, exhibiting higher moisture absorption ability than their native form.
395 Moreover, moisture absorption ability of the α-chitin converted from β-chitin was significantly
396 ($P<0.05$) higher than that of the native α-chitin due to the destruction of crystallites and decrease
397 of CI as a result of alkali treatment. Acid treated α-chitin retained its inter-sheet hydrogen bonds,
398 but its moisture absorption ability was significantly ($P<0.05$) increased after 1 h treatment in
399 comparison with the native α-chitin due to reduced CI. Acid induced less destruction of
400 crystallites in β-chitin than alkali showing no significant change ($P>0.05$) in its moisture
401 absorption ability. Therefore, the exact impact of alkali and acid treatment on the structural

property and moisture absorption ability of chitin depended on the form of chitin and the reaction time, and alkali treated β-chitin was able to retain its higher reactivity even after converted into α-form. In addition, the mild deacetylating treatment was required for β-chitin than that for α-chitin when preparing similar DDA of β- and α-chitosan. These results implicated that producing β-chitosan from squid pens can be more cost effective owning to β-chitin's loose crystallites and high reactivity toward solvent, and β-chitosan may have more active biological activity than α-chitosan at the same Mw and DDA, which are critical for its applications in various food products as a natural antioxidant and antimicrobial agent and edible film and coating forming material. These functionalities of β-chitosan and its food applications are currently studied at the authors' laboratory.

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