Effect of Shrimp Chitin and Shrimp Chitin Hydrolysate on the State of Water and Dehydration-induced Denaturation of Lizard Fish Myofibrillar Protein

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To assess the potential utilization of shrimp waste, shrimp chitin (SC) and shrimp chitin hydrolysate (SCH) were prepared from black tiger (*Penaeus monodon*), endeavour (*Metapenaeus endeavouri*) and giant freshwater (*Macrobrachium rosenbergii*) prawns. Effect of SC and SCH on the state of water and denaturation of lizard fish (*Saurida wanieso*) myofibrillar protein (Mf) at concentrations of 5% (dry weight/wet weight) were assessed based on water activity (*A_w*) and changes in Mf Ca-ATPase activity during dehydration. The effect was compared with untreated Mf (control) and Mf containing glucose. The *A_w* of Mf for each SCH was remarkably lower than the Mf with SC and control. The Mf with SCH showed a remarkably high level of inactivated Ca-ATPase activity, followed by Mf with SC and the control. Mf with glucose showed slightly higher inactivated Ca-ATPase activity than SCH, reaching *A_w* levels of 0.65, before rapidly decreasing. These findings revealed that SCH contributed to the retardation effect on dehydration-induced denaturation of Mf by stabilizing hydrated water molecules surrounding the Mf.

Keywords: chitin, hydrolysis, fish myofibrils, water activity, Ca-ATPase activity

Introduction

Thailand exports more than 250,000 tons of shrimp products annually with black tiger prawn, a marine shrimp, accounting for 50% (Fishery Information Technology Center, 2001). Giant freshwater prawn is the biggest freshwater shrimp that has become a commercially important species of crustacean cultured extensively throughout Southeast Asia. Aquaculture of giant freshwater prawn in Thailand accounted for 4.8% (13,300 tons) of total freshwater culture in 2001 (Fishery Information Technology Center, 2001). Endeavour prawn, a marine shrimp, is imported from Australia and re-exported after processing in Thailand. The wastes from shrimp processing plants have been estimated at about 35–45% of total weight/shrimp. The considerable quantity of shell waste from processing of shrimp has become a major concern for seafood processing plants. Most of this waste is used as animal feed but these by-products are an important source of chitin.

Chitin is a natural polysaccharide that is abundantly present as a structural compound in shell of crustaceans such as crabs and shrimps (Fereidoon and Jozef, 1991; Ferrer *et al.*, 1996; Kungsuwan *et al.*, 1996; Kurita, 2001). Chitin and chitosan are insoluble in water at neutral pH (Ilyina *et al.*, 1999; Kurita, 2001). The hydrolysis of chitin or chitosan produces oligosaccharides consisting of β -(1→

4)-linked *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose: GlcNAc) or glucosamine (2-amino-2-deoxy-D-glucose: GlcN), which are currently attracting the attention of food scientists because of their chemical behaviors, physical properties and biological functions. The oligosaccharides from chitin and chitosan exhibit significant physiological functions including the induction of phytoalexins, antimicrobial activity, and immune enhancing activity (Kurita, 2001). The oligosaccharides prepared by acid hydrolysis are promising as medicines, diagnostic drugs, fungicides and food additives (Shimoda *et al.*, 1996; Weiner, 1992).

Dehydration is the oldest method for preserving food by removal of water. Unfortunately, dehydration not only vaporizes water but it also causes marked changes in meat quality by causing a decrease of water retention and solubility of protein (Migita *et al.*, 1956; Suzuki, 1971; Hanafusa, 1973), resulting in a tough, dry and fibrous fish muscle texture. Water is the main component of biological materials that plays an important role in determining their shape, structure, physical and chemical composition (Maguer, 1987). Water in meat is closely associated with functional of protein and structure (Kauzmann, 1959; Nemethy and Scheraga, 1962). Hamm (1963) has estimated that 70% of the water content of fresh meat is bound by the myofibrillar protein, 20% by the sarcoplasm and 10% by the connective tissues. Thus, the myofibrillar proteins appear to be the main water-binding

components in muscle. Myofibrillar protein constitutes 66–77% of the total protein in fish meat and plays an important role in coagulation and gel formation (Suzuki, 1981). However, the amount of water in a product is not as important to its shelf life as its water activity (A_w) is. Since the free water at the capillary layer is an important resource for enzymatic reactions and growth of microorganisms (Drapron, 1985; Jakobse, 1985; Leistner and Rodel, 1975; Richard-Molard *et al.*, 1985) it is also one of the major causes of the denaturation of protein. To reduce the mobilization of water in protein and inhibit this unwanted denaturation, many additives such as polysaccharides, phosphates, organic acids, protein hydrolysates, amino acids and sugars have been reported as effective (Romero *et al.*, 1998; Kanna *et al.*, 1972; Inada *et al.*, 1992; Migita *et al.*, 1956; Matsuda, 1971; Matsuda, 1979 a, b; Yamashita *et al.*, 2002; Zhang *et al.*, 2002). The addition of sugars can stabilize proteins from dehydration-induced denaturation (Matsuda, 1979a, b; Carpenter *et al.*, 1987, 1988). However, sugars can impart a sweet taste and can change the final product color considerably, and this has led to a search for alternative cryoprotectants (Carvajal *et al.*, 1999; Herrera and Sampedro, 2002; Park and Lanier, 1987; Sultanbawa and Li-Chan, 1999).

Decreased freshness and protein deterioration occur very quickly in lizard fish (*Saurida* spp.) kept in ice storage, and stability in frozen storage is very low (Holmes *et al.*, 1992; Nozaki *et al.*, 1978; Yasui and Lim, 1987). In the South East Asian region, most of lizard fish is used for dried fisheries products and low-quality minced meat for low-priced fishcake products. Thus, myofibrillar protein of lizard fish was selected for use in this study. Romero *et al.* (1998) and Yamashita *et al.* (2002) have reported the effects of chitin and oligosaccharides from crab and squid on the state of water and retardation of dehydration induced-denaturation of lizard fish myofibrillar protein. Therefore, the present study was designed to utilize shrimp chitin and its oligosaccharides as a natural preservative and food additive and to investigate of their effects on the state of water and dehydration-induced denaturation of lizard fish myofibrillar protein.

Materials and Methods

Materials Frozen shrimp shells of black tiger (*Penaeus monodon*), endeavour (*Metapenaeus endeavouri*) and giant freshwater (*Macrobrachium rosenbergii*) prawns were obtained from a seafood processing factory in Thailand (Hoko Fishing Co. Ltd., Samutsakorn). Fresh lizard fish (*Saurida wanieso*) was purchased from the Nagasaki wholesale fish market, Japan.

Preparation of shrimp chitin Chitin was isolated according to the method of Hackman (1954) with a slight modification. Shrimp shells were washed, dried and ground to 40 mesh. The ground sample was demineralized with 20 volumes of 2 N HCl solution at room temperature for 60 h with replacement of the HCl solution every 12 h and subsequent stirring in 20 volumes of 1 N NaOH solution at 60°C for 48 h in order to remove protein.

The NaOH solution was changed at 6, 12, 24 and 36 h. The sample was then washed with distilled water until the pH became neutral and was soaked overnight in 5 volumes of 95% ethanol in order to remove fat and color substances. After the samples were dried in a hot air oven at 60°C for 48 h, the fine flake shrimp chitin was obtained.

The degree of acetylation of shrimp chitin The degree of acetylation (DA) of chitin was determined by the method of Brugnerotto *et al.* (2001) using a Fourier Transform InfraRed spectrometer (Excalibur FTS 3000 MXN, Digilab, Tokyo, Japan) with Bio-Rad Digilab Win-IR Pro Software.

The mean molecular weight and degree of polymerization of shrimp chitin Due to a limitation of solubility, chitin samples were changed to chitosan before further analyses. Samples were de-acetylated with 50% NaOH solution for 1 h at 110°C. De-acetylation was carried out twice to ensure complete solubility. The molecular weight of chitosan samples (2 mg/ml) was estimated by gel permeation chromatography (GPC) using pullulan (MW 5,900–788,000) as a standard. Analysis was performed on an Ultrahydrogel linear column (7.8 ID × 300 mm, Waters, USA) connected in line with a Waters GPC apparatus. Spectra of chitosan samples (solid state) were recorded on a Bruker DPX-300 Nuclear Magnetic Resonance Spectrometer (NMR) at 75 MHz (25°C). The degree of polymerization of SC was calculated from the number-average molecular weight (M_n) divided by molecular weight of repeating unit.

Preparation of chitin hydrolysate The shrimp chitin hydrolysate was prepared according to the modified method of Rupley (1964). The shrimp chitin was stirred with 12 N HCl for 7 h in a water bath at 40°C. The hydrolytic reaction was terminated by adding 6 volumes of distilled water and neutralized by adding 25% NaOH solution. After salt was eliminated by desalting machine (Micro Acilyzer G3, Asahi Kasei Inc., Kanagawa, Japan), the desalted sample was subjected to Millipore ultra-filtration (Minitan, Pellicon XL, Billerica, USA) using a molecular weight (MW) cutoff at 1.0–10⁴. The filtrate was subjected to a spray dryer (IGA 32, Yamato Scientific Inc., Tokyo, Japan) and powder shrimp chitin hydrolysates were obtained.

Proximate composition and properties of shrimp chitin and shrimp chitin hydrolysate Moisture, crude protein, crude lipid and ash content of the SC and SCH were analyzed by the methods of atmospheric heat drying at 105°C (AOAC, 1984), Lowry's method (Lowry *et al.*, 1951), a Soxhlet system and heating in a furnace (600°C), respectively. Sodium chloride was determined by a salt analyzer (SAT-2A, TOA Electronics, Ltd., Japan). The amounts of SC and SCH were obtained by subtracting the corresponding crude protein, crude lipid and ash content from the total amount of the material.

The molecular weight distribution of the SCH was estimated by measuring the absorbance at 210 nm for acetyl residues derived by separation of the oligosaccharides on a Bio-Gel P-6 column (inner diameter 14 mm, length 100

mm) using N-acetylglucosamine (MW 221) as a standard. The sample was dispersed in distilled water at a concentration of 4 mg/ml, and a 2 ml sample was introduced into the column. The sample was eluted with distilled water at a flow rate of 10 ml/h. An automatic fraction collector collected 1 ml aliquots of each separated sample. The number-average molecular weight of SCH was calculated from total weight of all molecules (total peak area) distributed in each SCH divided by the total number of molecules (number of peaks). Average degree of polymerization of the SCH was calculated from the number-average molecular weight divided by the molecular weight of the standard, N-acetylglucosamine.

Preparation of fish myofibrillar protein Fish myofibrillar protein (Mf) was prepared according to the method of Katoh *et al.* (1977) with a slight modification (Nozaki *et al.*, 1991). Minced lizard fish meat was washed 3 times with 5 volumes of 0.1 M KCl–20 mM Tris-maleate buffer (pH 7.0), then homogenized with 3 volumes of same buffer and passed through nylon net (16 mm) to remove connective tissues. Subsequently, Triton X-100 at 1% final concentration was added and left stand for 30 minutes. To remove the Triton X-100 solution, the mixture was centrifuged at $1,400 \times g$ for 10 min. The residue was washed with the same buffer, stirred and centrifuged until the supernatant became clear. The precipitate was washed again with 5 volumes of cold distilled water and centrifuged at $5,000 \times g$ for 10 min. Finally, the excess water was removed by centrifugation at $27,000 \times g$ for 20 min. The obtained residue was used as Mf. The approximate components of the Mf used in the present study were 88.74% water, 10.66% crude protein, 0.14% crude lipid and 0.46% ash.

Sample preparation Shrimp chitin and their oligosaccharides were added to Mf at a level of 5% (dry wt/wet wt). The samples were then mixed and ground using a mortar and pestle for 20 min at 5°C. The mixtures were put in cellophane bags, sealed, embedded in silica gels inside desiccators and kept in a cold room with occasional replacement of the silica gels for dehydration. Further dehydration was carried out when the moisture content of samples reached approximately 10% by reducing pressure in a desiccator. Mf with glucose and without additives (control) was used in order to compare the effect of SC and SCH. All samples were treated identically.

Determination of water activity and moisture content The Mf samples were serially collected and the water activity (Aw) was measured by the indirect equilibrium vapor pressure method (Akiba, 1974) using oil manometer at 20°C. Moisture content of the samples during the dehydration process was determined using atmospheric heat drying at 105°C (AOAC, 1984).

State of water analysis Desorption isotherms were obtained by plotting values of moisture content against those of Aw. The inflection point was obtained by B.E.T. analysis (Brunauer *et al.*, 1938). The moisture content at this point was defined as the amount of monolayer sorbed water (M_1). According to Bull's report (1944), the moisture content at the inflection point of Aw, which corre-

sponds to the minimal point on (M/Aw)-Aw, was defined as the amount of multilayer sorbed water (M_2). The sorption surface area (S) of Mf in monolayer-sorbed water was calculated using the following equation:

$$S = M_1 \times S_w \times N / (M_w \times 10^3)$$

where, S=the sorption area per mg of sorbed water (m^2/mg), M_1 =the amount of monolayer sorbed water (mg/mg of dried matter), S_w =the cross-sectional area of water molecules ($10.8 \text{ \AA}^2$), N =Avogadro's number ($6.02 \times 10^{23}/\text{mol}$) and M_w =molecular weight of water (18 g/mol).

Determination of fish Myofibrillar protein Ca-ATPase activity One gram of the dehydrated Mf was taken out at different moisture content level, suspended in 30 volumes of 0.1 M KCl–20 mM Tris maleate buffer (pH 7.0) and left to stand overnight at 5°C for water restoration. It was then homogenized (Nichion-irika Kikai Seisakusho Histocolon NS-560) at 1,000 rpm for 1 min and centrifuged at $750 \times g$ for 10 min. The Mf precipitate was suspended in the same buffer. The protein concentration was determined using the biuret method (Gornall *et al.*, 1949) using bovine serum albumin (fraction V) as the standard. The Mf Ca-ATPase activity was determined according to the method of Katoh *et al.* (1977). The activity was tested at 25°C in a reaction medium composed of 100 mM KCl, 5 mM CaCl_2 , 25 mM Tris-maleate (pH 7.0), 1 mM ATP, and 200–400 $\mu\text{g}/\text{ml}$ Mf. This reaction was terminated after 5 min by adding 30% TCA solution at the final concentration of 5% and free inorganic phosphate was measured by colorimetry. Activity was indicated by μmol per min of the inorganic phosphorus (Pi) released from 1 mg of myofibrillar protein.

Results and Discussion

Proximate composition, number-average molecular weight, average degree of polymerization and degree of acetylation of shrimp chitin and shrimp chitin hydrolysate The chemical composition and properties of shrimp chitin (SC) are presented in Table 1. Sugar substrate was the major component, accounting for over 99% of all SC samples. Crude protein, crude lipid and ash accounted for less than 0.004%, 0.05–0.18% and 0.23–0.58%, respectively. This result indicated that chitin obtained in the present study has high purity. The number-average molecular weight of SC ranged from 1.32×10^5 to 1.58×10^5 . The average degree of polymerization was lowest in black tiger prawn (4.93×10^2), followed by giant freshwater prawn (6.05×10^2) and endeavour prawn (7.23×10^2). The degree of N-acetylation of SC ranged from 82.22 to 85.70%, which is in agreement with the finding of Kurita (2001) and Romero *et al.* (1998).

The chemical composition and properties of shrimp chitin hydrolysates (SCH) are presented in Table 2. Sugar substances were the main component of all SCH samples. The total amount of crude protein, crude lipid and ash contents were less than 1%. Sodium chloride was less than 0.002%. The molecular weight distribution of SCH is presented in Fig. 1. The monomer was the major component of all SCH, accounting for 76.07%, 69.55%, and 62.14% in the SCH of black tiger prawn,

Table 1. Chemical composition and properties of chitin from various shrimp species.

Composition and properties	Black tiger prawn	Endeavour prawn	Giant freshwater prawn
Sugar substrate (chitin) $\pm$ S.D. ^{*1}	99.24 $\pm$ 0.09	99.34 $\pm$ 0.15	99.72 $\pm$ 0.03
Ash $\pm$ S.D. ^{*1}	0.58 $\pm$ 0.09	0.55 $\pm$ 0.17	0.23 $\pm$ 0.02
Crude lipid $\pm$ S.D. ^{*1}	0.18 $\pm$ 0.01	0.11 $\pm$ 0.01	0.05 $\pm$ 0.01
Crude protein ^{*2}	trace	trace	trace
Number-average molecular weight (Mn)	1.08 $\times 10^5$	1.58 $\times 10^5$	1.32 $\times 10^5$
Average degree of polymerization	4.93 $\times 10^2$	7.23 $\times 10^2$	6.05 $\times 10^2$
Degree of acetylation (%) $\pm$ S.D.	84.6 $\pm$ 1.22	85.7 $\pm$ 0.24	82.22 $\pm$ 0.91

S.D. = Standard deviation (n = 5).

^{*1} g/ 100 g of dried matter.^{*2} < 0.004 g**Table 2.** Chemical composition and properties of chitin hydrolysates from various shrimp species.

Composition and properties	Black tiger prawn	Endeavour prawn	Giant freshwater prawn
Sugar substance $\pm$ S.D. ^{*1}	99.33 $\pm$ 0.04	99.13 $\pm$ 0.04	99.23 $\pm$ 0.05
Ash $\pm$ S.D. ^{*1}	0.61 $\pm$ 0.03	0.81 $\pm$ 0.03	0.70 $\pm$ 0.06
Crude lipid $\pm$ S.D. ^{*1}	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01
Crude protein ^{*2}	trace	trace	trace
NaCl ^{*3}	trace	trace	trace
Number-average molecular weight (Mn)	351	383	420
Average degree of polymerization	1.59	1.73	1.90

S.D. = Standard deviation (n = 5).

^{*1} g/ 100 g of dried matter.^{*2} < 0.004 g^{*3} < 0.002 g

endeavour prawn and giant freshwater prawn, respectively. The SCH of black tiger prawn exhibited monomer to trimer, the SCH of endeavour prawn exhibited monomer to tetramer and the SCH of giant freshwater prawn exhibited monomer to pentamer. Thus, SCH of black tiger prawn showed the lowest number-average molecular weight, while giant freshwater prawn showed the highest number-average molecular weight among the shrimp species (Table 2). The average degree of polymerization of SCH was 1.59 to 1.90.

Water activity and state of water The desorption isotherms of dehydrated Mf containing SC and SCH are presented in Fig. 2. The desorption isotherms of all samples showed a sigmoid curve with two inflection points at Aw of 0.05–0.15 and 0.5–0.7, and belonged to type B sorption isotherms as classified by Shibasaki *et al.* (1967). The curves were similar to those of Atka mackerel meat (Akiba, 1961), carp actomyosin (Nakano, 1987), fractured meat (Suzuki, 1971) and white croaker myofibrils (Nozaki *et al.*, 1991). The desorption isotherms of dehydrated Mf

containing SC from the three shrimp species were similar and exhibited few Aw values higher than those of the control irrespective of shrimp species at the same moisture content. These results indicated that SC could not immobilize water in Mf. In addition, SC might interrupt bonding between Mf and water since chitin possibly competed with water for protein binding resulted in an increase of free water. Kauzmann (1959) reported that denaturation may cause considerable changes in the ability of a protein to bind other substances.

The desorption isotherm of dehydrated Mf containing SCH for the three shrimp species showed a similar tendency, and showed lower Aw values than that of the control at the same moisture level. These results indicated that the free water in Mf containing SCH was immobilized and changed into bound water to some extent. It is revealed that after the main chain of SC was cleaved, the hydrophilic OH residues of *N*-acetyl-D-glucosamine were exposed and made interaction with water in Mf possible. Matsumoto (1980) hypothesized that the cryoprotectant

Fig. 1. Gel chromatogram on Bio-Gel P-6 of various shrimp chitin hydrolysates. Eluent: water. Flow rate: 10 ml/h. Fraction volume: 1 ml/tube. Symbol: $\bigcirc$ std. *N*-acetylglucosamine (GlcNAc), $\bullet$ oligomers. The numbers above the peaks denote the elution peaks of oligomers; (1) monomer, (2) dimer, (3) trimer, (4) tetramer, (5) pentamer.

Fig. 2. Desorption isotherms of lizard fish myofibrils at 20°C in the presence of shrimp chitin, shrimp chitin hydrolysates, glucose and control. Control consisted of myofibrils without additives. Symbols: Endeavour SC, Black tiger SC, Giant $\bigcirc$ Control, $\times$ Glucose, $\square$ Endeavour SCH, $\diamond$ Black tiger SCH, $\triangle$ Giant freshwater SCH.

molecules (sugars) may bind or associate with protein molecules at a functional group either by ionic or hydrogen bonds. Thus, each protein molecule is coated by the cryoprotectant. These results led us to conclude that SCH has the same mechanisms as sugars. The Mf containing glucose showed the lowest Aw value at all moisture content levels and this might be due to the increased amount of the hydrophilic OH residues in glucose compared to hydrolysates. The suppressive effect of sugars on protein denaturation depends on the sugar composition (Matsuda, 1979a, b; Matsuda and Arai, 1987;

Matsumoto *et al.*, 1985), such as the number of hydrophilic OH residues (Noguchi, 1992; Oozumi, Hashimoto *et al.*, 1981).

To determine the amount of bound water in the Mf, the state of water was analyzed. The monolayer sorbed water (M_1), multilayer sorbed water (M_2) and sorption surface area (S) were calculated from the desorption isotherms at 20°C of the control and the Mf containing SC, SCH and glucose results are presented in Table 3. The M_1 , M_2 , and S of the control were 7.65 g/100 g, 15.5 g/100 g, and 0.30 m²/mg, respectively. The Mf containing glucose exhibited

Table 3. Amount of monolayer, multilayer water and sorption surface area of lizard fish myofibrillar protein to which 5.0% of various shrimp chitin (SC) and shrimp chitin hydrolysate (SCH) from desorption isotherm at 20°C had been added.

Sample	Monolayer water		Multilayer water		M_2/M_1	Sorption surface area ^{*4}
	M_1 ^{*1}	Md_1 ^{*3}	M_2 ^{*2}	Md_2 ^{*3}		
Control	7.65	0.08	15.50	0.18	2.03	0.30
Glucose	11.65	0.13	22.55	0.29	1.94	0.48
Sucrose	7.27	0.08	15.89	0.19	2.19	0.28
Endeavour SC	7.30	0.08	14.45	0.17	1.98	0.28
Black tiger SC	7.46	0.08	14.69	0.17	1.97	0.29
Giant freshwater SC	7.44	0.08	14.60	0.17	1.96	0.29
Endeavour SCH	9.82	0.11	19.09	0.24	1.94	0.39
Black tiger SCH	9.22	0.10	18.50	0.23	2.01	0.37
Giant freshwater SCH	9.32	0.10	20.00	0.25	2.15	0.37

^{*1} Moisture content (g/ 100g of sample) estimated by B.E.T. analysis.

^{*2} Moisture content (g/ 100 g of sample) estimated by Bull's analysis.

^{*3} Moisture content (g/ g of dried matter).

^{*4} Sorption surface area (m²/ mg of dried matter).

the highest M_1 , M_2 , and S values (11.65 g/100 g, 22.55 g/100 g, and 0.48 m²/mg). Mf containing each kind of SC showed a slightly lower M_1 , M_2 , and S (7.3–7.46 g/100 g, 14.45–14.69 g/100 g, and 0.28–0.29 m²/mg) than those of control. Whereas, Mf containing SCH had values higher than those of the control (9.22–9.82 g/100 g, 18.5–20.0 g/100 g, and 0.37–0.39 m²/mg), respectively, but lower than those of Mf containing glucose. The increment of M_1 and M_2 in the Mf containing SCH supported the hypothesis that it exhibited a strong bonding capacity between water and SCH which can retard the effect of dehydration-induced denaturation of Mf. The Mf containing giant freshwater SCH exhibited the highest M_2 values, followed by Mf containing endeavour SCH and Mf containing black tiger SCH, respectively. The functional properties of chitin and chitosan oligomers have clearly revealed their high dependency on the degree of polymerization (Hirano and Nagano, 1989; Suzuki, 1996). Yamashita *et al.* (2002) have also reported that hydrolysates from king crab chitin (*Paralithodes camtschtica*), which exhibit the highest average degree of polymerization, showed the highest M_2 values for dehydrated lizard fish Mf. The amounts of M_1 of all Mf containing SCH were similar. The finding that the sorption surface area (S) of Mf containing SC was similar to that of the control while those of Mf containing SCH and glucose were increased supported the hypothesis that water molecules were captured by SCH or glucose. The M_2/M_1 ratio of all samples in the present study ranged from 1.94–2.19, which corresponded with M_2/M_1 ratio (1.5–2.0) for various proteins (Bull, 1944).

Changes in Myofibrillar Ca-ATPase activity The effect of SC and SCH on the dehydration-induced denaturation of Mf was assessed in terms of Mf Ca-ATPase activity. The correlation between relative Mf Ca-ATPase activity to the initial value and A_w is shown in Fig. 3. The Mf Ca-ATPase activity of the control decreased rapidly by more than 90% in the capillary condensation domain from the initial activity at A_w levels of 0.85, before gradually decreasing in the multilayer to monolayer range.

This finding suggested that the interaction between protein and water molecules was closely correlated with the degree of denaturation of Mf. Since Mf containing SC was not able to interact with water molecules surrounding Mf, its relative Ca-ATPase activity exhibited a similar tendency to that of the control. At the A_w level of 0.85, Ca-ATPase activity of Mf containing SC decreased by more than 75% of the initial value while the activity of Mf containing SCH only decreased 10–20% of the initial value. Consequently, at the multilayer to monolayer range, Ca-ATPase activity of Mf containing SC, SCH and the control gradually decreased except for Mf containing glucose that kept high rate on decrement and finally remained activity lower than that of Mf containing SCH. The giant freshwater SCH exhibited the highest inhibiting effect on the dehydration-induced denaturation of myofibrils, followed by endeavour SCH and black tiger SCH, respectively. Since water is recognized as an important functional factor concerning numerous phenomena in regard to the quality of food, these findings showed that SCH can prevent the destruction of hydrogen bonds and/or hydrophobic bonds between water molecules and protein during dehydration. Back *et al.* (1979) noted that hydrophobic interactions between pairs of hydrophobic groups are stronger in sucrose or glycerol solutions than in pure water. They concluded that this is the mechanism by which sugars may stabilize proteins to heat denaturation. The hydrolysates from shrimp chitin in this present study seem to demonstrate the same mechanism as sugars. At A_w levels of 0.02, the Ca-ATPase activity of Mf containing SCH prepared from giant freshwater prawns showed the highest remaining inactivated activity (70%), followed by that of endeavour prawn (60%), black tiger prawn (45%) and glucose (42%). These results showed a high correlation between hydrated water and degree of denaturation of Mf. The amount of bound water in Mf containing each kind of shrimp was 29.32 g/100 g in giant freshwater prawn, 28.92 g/100 g in endeavour prawn, and 27.77 g/100 g in black tiger prawn.

Fig. 3. Relationship between the water activity and relative Ca-ATPase activity of myofibrils added to shrimp chitin, shrimp chitin hydrolysates, glucose. Control consisted of myofibrils without additives. Symbols are same as in Fig. 2.

Drying proteins has many commercial advantages, such as lower shipping costs, more convenient storage, and usefulness in dry mix applications. A shift of surimi production from the frozen to the dried form could thus open many new applications for fish proteins and lead to their wider acceptance as food ingredients. However, during drying the water surrounding the protein molecules that stabilizes the intramolecular hydrogen and hydrophobic bonds that keep the molecule in its native structure is moved and this can lead to structural changes or denaturation. To prevent denaturation, water movement must be controlled and the proteins should be stabilized. Sucrose seems most effective during storage of the freeze-dried product and does not induce browning due to Maillard reactions. However, sucrose imparts a sweet taste and has a high caloric content. In a preliminary study we determined the effect of sucrose and sorbitol on the state of water of lizard fish Mf. Unfortunately sucrose and sorbitol could not decrease water activity of dehydrated Mf (data not shown). Glucose is the one sugar that has been reported to be a good anti-denaturant, but it induces Maillard reaction as well. The SCH in the present study offers advantages in the reduction of sweetness and have potential application as highly functional additives in food manufacturing. Although shrimp chitin could not be immobilized water of dehydrated Mf, it did show a suppressive effect on the dehydration-induced denaturation of Mf.

Conclusion

It was found that Mf with SCH showed almost the same tendency as results of oligosaccharides from crab and squid as reported by Yamashita *et al.* (2002). Thus, it may be concluded that chitin oligosaccharides obtained from shrimp, crab, and squid reduced water activity in Mf and also showed a suppressive effect on the dehydration-

induced denaturation of Mf. Thus, the utility of these oligosaccharides as natural food protective materials for dry or partially dry foods could stimulate more interest in food processing and preservation.

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