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Conformational changes in proteins recovered from jumbo squid (*Dosidicus gigas*) muscle through pH shift washing treatments

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ABSTRACT

Conformational and thermal–rheological properties of acidic (APC) and neutral (NPC) protein concentrates were evaluated and compared to those of squid (*Dosidicus gigas*) muscle proteins (SM). Surface hydrophobicity, sulfhydryl status, secondary structure profile, differential scanning calorimetry and oscillatory dynamic rheology were used to evaluate the effect of treatments on protein properties. Acidic condition during the washing process (APC) promoted structural and conformational changes in the protein present in the concentrate produced. These changes were enhanced during the heat setting of the corresponding sol. Results demonstrate that washing squid muscle under the proposed acidic conditions is a feasible technological alternative for squid-based surimi production improving its yield and gel-forming ability.

1. Introduction

The demand for fish protein has exceeded the supply of traditional raw materials, leading to great pressure on fish stocks (Kristinsson, Theodore, Demir, & Ingadottir, 2005). Fishery products demand has increased accordingly to the global population, but not the fish supply (Hultin & Kelleher, 2000). Thus, alternative fish protein sources such as jumbo squid (*Dosidicus gigas*; d'Orbigny, 1835) have recently been evaluated.

Jumbo squid is an endemic species from the pelagic zone of the eastern Pacific. It is the largest and most abundant squid species found from Chile up to the Northwest coast of the United States (Markaida, 2005; Nigmatullin, Nesis, & Arkhipkin, 2001). In Mexico, it is quite abundant in the Gulf of California, representing 96–99% of the total squid species annual catch and approximately 2.2% of the total country catch (CONAPESCA, 2013). Its mantle, representing 60–80% of the edible portion (Slabyj, Ramsdell, & True, 1981), has a great potential for surimi and/or functional protein concentrate production due to its low fat content and whiteness. However, as mentioned by several authors (Konno, Young-Je, Yoshioka, Shinho, & Seki, 2003; Sánchez-Alonso, Careche, & Borderías, 2007), its use for surimi production, by the conventional method, represents a hurdle due to residual protease activity that interferes with the gelation process. It is also mentioned that its protein muscular ultra-structure and conformation differ from that of common fish which also affect gelation. Moreover, application of the conventional surimi technology to this species results in a low yield due to the high water solubility of its myofibrillar protein fraction as previously mentioned by Matsumoto (1958).

Therefore, alternative processes must be developed for using this species as raw material for production of protein concentrates. It is important to correlate protein functionality with their physicochemical and structural characteristics to define better processing parameters and to understand how the process affects functionality of recovered proteins.

Based on the above, the main objective of the present study was to evaluate the changes in structural and physicochemical properties of jumbo squid (*D. gigas* d'Orbigny, 1835) mantle muscle protein induced by two different washing procedures, the so called acid protein concentrate (APC) that includes an acidic solubilization followed by the isoelectric precipitation of proteins, and the neutral protein concentrate (NPC) process that just uses water.

2. Materials and methods

2.1. Raw material

Three different sampling lots of jumbo squid were harvested off the coast of Kino Bay, Mexico (28 °N and 112 °W), from March to

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May of 2008. Specimens were beheaded and gutted on site. Immediately, mantles (experimental samples) were bagged and placed in alternated layers of ice-squid–ice inside a portable cooler and transported to the laboratory. Elapsed time between capture and reaching the laboratory did not exceed 12 h. Experimental samples were the same as those reported by Cortés-Ruiz, Pacheco-Aguilar, Lugo-Sánchez, Carvallo-Ruiz, and García-Sánchez (2008).

2.2. Protein concentrates preparation

Both protein concentrates (APC, NPC) were prepared according to Cortés-Ruiz et al. (2008): Mantles were cleaned from skin as well as connective tissue and washed in cold water (4 °C). Squid samples were minced thoroughly before being processed. For the APC, a mixture of squid–water (1:9, w/v) was homogenized at 0 °C (ice-bath) using a Tissumizer TR-10 tissue homogenizer (Tekmar Co. Cincinnati, USA). Then, the homogenate was acidified to pH 3.2 with 1 M HCl and centrifuged at 16,000×*g*/15 min/2 °C in a Model J2-21 Beckman refrigerated centrifuge (Beckman Instrument Inc. Palo Alto, USA). After centrifugation, the precipitate was discarded and the acidified protein solution (APS) recovered. To recover protein from the APS, its pH was adjusted to 5.5 to achieve the isoelectric precipitation of muscle protein (Hultin & Kelleher, 2000) with 1 M NaOH and centrifuged at 16,000×*g*/10 min/2 °C. The APC thus obtained was packed in polyethylene bags and stored on ice in a refrigerated chamber until its use on the same working day. For the NPC, a mixture of squid–water (1:5, w/v) was homogenized at 0 °C (ice-bath) using a tissue homogenizer and centrifuged four times at 16,000×*g*/15 min/2 °C, in order to remove the excess water adsorbed by the proteins. The precipitate (NPC) thus obtained (final pH 6.5), was packed in polyethylene bags and stored on ice in a refrigerated chamber until its use on the same working day.

2.3. Moisture, protein, non-protein nitrogen and pH analyses

Composition analyses (moisture, protein and non-protein nitrogen) were carried out on squid mantle muscle samples (SM), APC and NPC following the methodologies recommended by Woyewoda, Shaw, Ke, and Burns (1986). The pH was measured at 25 °C using a Corning digital pH meter Model 240 (Corning Inc., Corning, USA) in a homogenate of 2 g of sample (SM, APC, NPC) with 18 mL of distilled water as recommended by Martin (1992).

2.4. Heat-set gel preparation

Sols for each protein system (SM, NPC and APC) were prepared by adding 2.5 g NaCl/100 g of protein system at short intervals in a Model DLC-8 Plus Cuisinart Food Processor (Cuisinart Inc., Greenwich, USA). Crushed ice or sucrose were added to adjust the final moisture of sols to 80%. Mixing was continued until the sol temperature reached 5 °C. The pH of APC sol was adjusted to 6.5 as that of NPC with sodium bicarbonate (Na_2HCO_3). Each sol was packed into a Petri dish (1 cm height) and vacuum sealed in moisture/vapor-proof film bags (Cryovac Corp., Duncan, USA) with a Super Vac Smith vacuum machine (Smith Equipment Co., Clifton, USA). Each sol was heat-set in a water bath at 90 °C/30 min. Heat-set gels were immediately chilled to 5–10 °C in an ice–water mixture and held overnight at 2–4 °C prior to evaluation.

2.5. Surface hydrophobicity (SoANS)

SoANS of each sol (SM, NPC and APC) was determined by the technique described by Li-Chan, Nakai and Wood (1985) and Chan, Gill, Thompson, and Singer (1995), with minor modifications, by using the 1-anilino-8-naphtalene-sulfonate (ANS) hydrophobic fluorescence probe. Sols (1.0 g) were homogenized for 2 min with 4.0 mL of cold 0.1 M NaF, 0.01 M Na_2HPO_4 and 0.01 M NaH_2PO_4 buffer (pH 7.0). In order to decrease the particle size in samples, homogenates were kept for 2 h on an ice-cold sonicator water bath (2510R-DTH Branson Ultrasonic, Branson Ultrasonics Corp., Danbury, USA) at its standard settings (130 W, 40 kHz, 60% potency). Homogenized sample was diluted with the same buffer to reach a concentration of 1.5 mg/mL. Then, samples were serially diluted with the same buffer to protein concentrations ranging from 0.05 to 1.0 mg/mL. Protein content in diluted solutions was confirmed by the bicinchoninic acid method using the Pierce™ BCA Protein Assay Kit (Pierce Biotechnology. Rockford, USA).

After stabilizing samples at 20 °C, 7.5 μL of ANS solution (8 mM in 0.01 M phosphate buffer, pH 7) were added to 1.5 mL of the diluted sample solution. ANS–protein relative fluorescence intensity (RFI) was measured with a LS50B luminescence spectrophotometer (Perkin-Elmer de Mexico, S.A., Mexico) at wavelengths ($\lambda_{ex}/\lambda_{em}$) of 390/470 nm, respectively, with 2.5 and 5.0 nm widths on excitation and emission slits, respectively. The net RFI was obtained by subtracting the RFI of each sample measured without the probe from that measured with the probe. The initial slope (So) of the RFI against protein concentration (mg/mL), calculated by linear regression analysis, was used as an index of protein surface hydrophobicity.

2.6. Sulphydryl (—SH) content evaluation

Total sulphydryl (—SH) content of protein contained in SM, NPC, APC, soluble protein fractions and gels were determined following the methodology by Yongsawatdigul and Park (2004). For SM, NPC, APC and their respective gels, one gram samples were homogenized with 9 mL of 0.2 M Tris–HCl buffer/2% SDS, 10 mM ethylenediaminetetraacetic acid (EDTA) and 8 M urea (pH 8.0), heated at 100 °C/5 min and centrifuged at 10,000×*g*/15 min. Ten microliters of Ellman's reagent (10 mM 5,5′-dithiobis(2-nitrobenzoic acid)) were added to 1.0 mL of supernatant and subsequently heated in a water bath at 40 °C/25 min. To obtain the soluble protein fraction of SM, NPC and APC, three gram samples were homogenized by adding 27 mL of 20 mM Tris–HCl buffer/0.6 M KCl (pH 7.0) and centrifuged at 10,000×*g*/30 min/4 °C. After centrifugation a 0.25 mL aliquot of the fraction obtained was mixed with 2.5 mL of 0.2 M Tris–HCl buffer (pH 7.0) containing 2% SDS, 10 mM EDTA and 8 M urea. Then, 50 μL of Ellman's reagent were added to the blend and heated in a water bath at 40 °C/25 min. The total —SH content of all samples was determined at 412 nm by using a CARY 50 Bio UV–Visible spectrophotometer (Varian Australia Pty Ltd, Victoria, Australia) and a molar extinction coefficient of 13,600 M^{-1} cm^{-1}. Protein content in supernatants was determined by the bicinchoninic acid method.

The reactive sulphydryl (R—SH) were only determined in the soluble protein fraction of SM, NPC and APC, extracted as described in the previous paragraph. Fifty mL of Ellman's reagent were added to 2.75 mL of soluble fraction diluted (1:10) with same buffer and subsequently placed in a cold room (5 °C) for 1 h. The amount of R—SH was measured at 412 nm, using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}. Protein content in soluble fraction was determined by the bicinchoninic acid method using the Pierce™ BCA Protein Assay Kit (Pierce Biotechnology. Rockford, USA).

2.7. Secondary structure profile

Analysis was performed following the methodology proposed by Ogawa, Kanamaru, Miyashita, Tamiya, and Tsuchiya (1995), with minor modifications. Samples (1 g) of SM, NPC and APC sols were homogenized for 2 min with cold 0.01 M Na_2HPO_4 and

0.01 M NaH_2PO_4 buffer/0.1 M NaF (pH 7.0) in a 1:4 ratio (w/v). Samples were kept for 2 h on an ice-cold sonicator water bath (2510R-DTH Branson Ultrasonic, Branson Ultrasonics Corp., Danbury, USA) at its standard settings (130 W, 40 kHz, 60% potency). Then, the homogenate was diluted with the same buffer to a final protein concentration of 0.2 mg/mL. Protein content was confirmed by the bicinchoninic acid method. Samples were filtered and analyzed in a J-810–150S JASCO spectropolarimeter (JASCO Co. Tokyo, Japan) equipped with a cell container–receptor of double wall with water. Temperature was controlled at 25 °C with a NESLAB RTE-111 thermostat. A scanning of ellipticity was carried out in the range of 180–260 nm using a 0.2 cm path length cell.

Secondary structure analysis profile was also performed on proteins in the liquid drained from the SM, NPC and APC gels obtained by the methodology of Wang and Damodaran (1991), with minor modifications. Samples of gels (15 g) were centrifuged at 35,000×*g*/1 h/5 °C. Protein content in the drained liquid was determined by the bicinchoninic acid method and diluted to a protein concentration of 0.2 mg/mL with HPLC water. Secondary structure profile was determined as previously described for sols. Secondary structure content was calculated using the K2D3 algorithm (Louis-Jeune, Andrade-Navarro, & Perez-Iratxeta, 2011) at http://k2d3.ogic.ca//index.html.

2.8. Thermal stability of sols

Differential scanning calorimetry (DSC) was performed to measure the thermal stability of SM, NPC and APC sols (2.5% NaCl and 80% moisture). A differential scanning calorimeter (Perkin Elmer Diamond DSC, Shelton, USA) was calibrated for temperature and baseline using indium as the standard. Previously minced and weighed muscle samples (50 mg) were placed into iron pans and hermetically sealed. An empty sealed pan was used as reference. The samples were scanned from 20 to 90 °C at a heating rate of 5 °C/min. The enthalpy change (ΔH) for the major thermal transitions was determined by measuring the area above the transition curve, with a straight baseline constructed from the start to the end of the exotherms. The temperature at the maximum heat flow (T_{max}) and the enthalpy change (ΔH) were recorded.

2.9. Rheology

SM, NPC and APC sols (2.5% NaCl and 80% moisture) were analyzed for changes in their dynamic gelling properties due to treatments. Samples were subjected to dynamic rheological testing using a RFS II rheometer (Rheometric Scientific, Inc. Piscataway, USA) equipped with two parallel plates with a 2 mm gap. Sols (4 °C) were loaded in the space between the parallel plates, and the exposed rim was covered with a thin layer of mineral oil to prevent dehydration during heating. A minute was given before the start of measurement to allow for temperature equilibrium. Sol–gel transition was achieved by heating samples from 10 to 90 °C at a rate of 1 °C/min. Samples were continually sheared in an oscillatory mode at a fixed frequency of 1 rad/s with a maximum strain of 0.05. Changes in the storage modulus (G′) (rigidity due to elastic response of the material) were monitored throughout the gelling process.

2.10. Statistical analysis

Data was analyzed using a one-way ANOVA and Tukey–Kramer multiple comparison test when necessary. A significance level of 5% was used for all the statistics. Three replicates were carried out for each treatment. All analyses were carried out in duplicate.

3. Results and discussion

3.1. Composition and process yield

Neutral protein concentrate (NPC) retained the highest ($P < 0.05$) moisture content (89.4 ± 1.9), while moistures for APC (83.3 ± 2.2) and SM (84.3 ± 1.2) were similar ($P \geqslant 0.05$). On the other hand, APC showed the highest ($P < 0.05$) protein content (15.1 ± 2.1) while no difference ($P \geqslant 0.05$) was observed between SM (8.4 ± 0.7) and NPN (9.3 ± 1). The effect of process on the protein content of concentrates was highly noticeable for the APC as the result of the isoelectric precipitation of protein when pH was adjusted to 5.5.

As expected, washing of squid minced muscle under acidic condition (APC) showed an incremental effect in NPN removal as compared with the NPC process. NPN for SM, NPC and APC were 0.98 ± 0.10, 0.17 ± 0.01 and 0.09 ± 0.01, respectively ($P < 0.05$).

During production of protein concentrates, high yields are desirable in order to make the process commercially attractive. The proposed processes resulted in motivating yields, with the NPC process showing an average wet yield of 70%, while the APC showed a 43% ($P < 0.05$), being this last one similar to that of Kim, Park, and Choi (2003) for their proposed pH-shift process (35–45%). Regardless of difference in wet yields, similar ($P \geqslant 0.05$) solid and protein recoveries were obtained with 47 ± 4% *vs* 46 ± 6% and 76 ± 7% *vs* 76 ± 9%, for the NPC and APC treatments, respectively.

3.2. Surface hydrophobicity

Protein surface hydrophobicity index is used to determine protein molecule unfolding induced by physical treatments such as heat, freezing and pH changes (Ang & Hultin, 1989). Unfolding of protein could promote exposure of hydrophobic amino acids, thus modifying its surface hydrophobicity (Careche & Li-Chan, 1997). From the technological point of view, a change on surface hydrophobicity could have a positive effect on the gel-forming ability of protein systems (Marín-Martínez, 1993). Fig. 1 shows changes in the surface hydrophobicity of proteins present in concentrate sols as the results of treatments.

As the result of the adjustment to neutrality, APC-sols showed considerably higher ($P < 0.05$) surface hydrophobicity (3 to 4 folds)

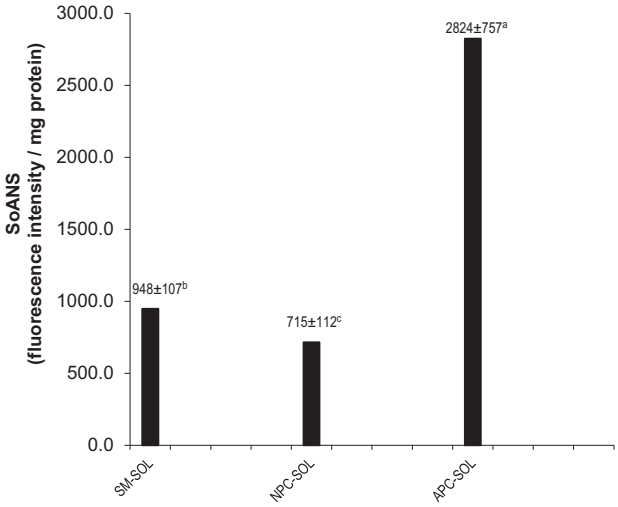

Fig. 1. Surface hydrophobicity (SoANS). SM-sol: squid muscle sol; NPC-sol: neutral protein concentrate sol; APC-sol: acidic protein concentrate sol. pH of APC sols were adjusted to neutrality.

than those from SM and NPC. Previous studies reported higher surface hydrophobicity in protein systems subjected to acid treatments compared to those under alkaline conditions (Kim, Yongsawatdigul, Park, & Thawornchinsombut, 2005; Kim et al., 2003).

As mentioned previously by Cortés-Ruiz et al. (2008), lowering the pH to 3.2 during preparation of the APC generated loss of the myosin quaternary structure, followed by an unfolding of their globular heads and separation of the myosin heavy chains by repulsion due to a positive net charge since pH of media was below the average pI of muscle proteins (5.5) (Foegeding & Lanier, 1996). Thus, washing condition under pH 3.2 induced loss of quaternary structure and conformational changes of the tertiary structure yielding an unfolded heavy chain myosin molecule with its hydrophobic residues more exposed.

Results suggested that this unfolding promotes protein–protein hydrophobic aggregation when pH was brought back to 5.2 during washing to allow protein recovery. This acidic pH also enhances electrostatic interactions in the protein via charge neutralization and might have influence on the stability of the hydrogen bonds. The changes in electrostatic interactions and hydrogen bond stability could in turn contribute to the loss of α-helix content under acidic conditions. Unfolding promoted a closer association of proteins at low pH, increasing the strength of hydrophobic interactions and disulfide bonds formation (Westphalen, Briggs, & Lonergan, 2005). Riebroy, Benjakul, Visessanguan, Erikson, and Rustad (2008) reported the formation of disulfide bonds and increased hydrophobicity in myosin during acidification. Hence, it was likely that conformational changes during acidification initiated the protein–protein interactions and, as a consequence, a better gel matrix will be formed.

The unfolding remained as the pH was adjusted to neutrality during APC sol preparation, giving higher values for hydrophobicity ($P < 0.05$) when compared with the other two processes (Fig. 1). This noticeable increment gave better gelation properties to the APC in part because more of the myosin –SH content is located in the globular head of the myosin heavy chains as reported by Cortés-Ruiz et al. (2008). Similar results were obtained by Kim et al. (2003) and Alizadeh-Pasdar and Li-Chan (2000).

3.3. Total sulphydryl (—SH) contents

Sulphydryl groups (—SH) are the most reactive moieties in protein. They can be easily oxidized to covalent disulfide bonds (—S—S—) under the presence of oxygen. With regard to the myosin molecule which is responsible for the gel-forming ability of fish muscle proteins, 68% of its total —SH groups are located in the myosin globular head (Xiong, 1997). As mentioned before, these —SH could become oxidized forming intermolecular —S—S— bonds essential for the formation of aggregated structures distinctive of gels. Total and reactive —SH in 0.6 M KCl protein extracts from SM, NPC and APC are shown in Table 1, while total —SH contents of SM, NPC, APC and their respective gels are in Table 2. Accordingly with the surface hydrophobicity data, the acidic treatment promoted unfolding, and thus a higher exposure of reactive —SH, promoting also its oxidation to —S—S— (Table 1). This is the reason why APC treatment showed lower content of total and reactive —SH. It is well documented that intermolecular —S—S— plays a significant role in the formation and rigidity of gels giving a desired texture (Benjakul, Seymour, Morrissey, & An, 1997; Gerrard, 2002; Marín-Martínez, 1993). However, results indicated that heat set gelation did not show further effect in promoting —S—S— bonds (Table 2). Previous work by the authors reported the gel forming-ability of SM, NPC and APC, where APC clearly overcame ($P < 0.05$) the other concentrates for all gel functionality parameters evaluated (Cortés-Ruiz et al., 2008).

Table 1
Total and reactive sulfhydryl contents in soluble protein fraction of SM, NPC, APC.

Treatment	Moles SH/1 × 10^5 g of protein	
	Total —SH	Reactive —SH
SM	8.6 ± 0.5[a]	5.5 ± 0.1[x]
NPC	9.4 ± 1.2[a]	5.7 ± 1.0[x]
APC	3.4 ± 1.1[b]	1.1 ± 0.4[y]

Different letters in columns denotes statistical difference ($P < 0.05$). SM: squid muscle.
NPC: neutral protein concentrate. APC: acidic protein concentrate. $n = 3$.

Table 2
Total sulfhydryl (—SH) content of proteins in SM, NPC, APC and their respective gels.

Treatment	Moles SH/1 × 10^5 g of protein[a]	
	Concentrate	Gel
SM	2.7 ± 0.9	2.9 ± 0.8
NPC	2.7 ± 1.0	2.5 ± 0.6
APC	1.6 ± 0.5	1.8 ± 0.5

[a] No statistical difference was found among samples ($P \geqslant 0.05$). SM: squid muscle. NPC: neutral protein concentrate. APC: acidic protein concentrate. $n = 3$.

3.4. Secondary structure profile

Circular dichroism spectroscopy (CD) is a useful tool to evaluate conformational changes in protein structure. CD spectra data (Fig. 2) showed representative α-helix curves mainly for the SM sol with a negative peak at 220 nm and a paired negative-positive peaks at 208–190 nm. High α-helix content is a characteristic secondary structure for actomyosin, myosin and myosin fragments (Ogawa et al., 1995).

Results indicated that the α-helix secondary structure of protein was affected by treatments. According to results, the APC sol α-helix ellipticity (5%) decreased compared to SM sol (9%) due to a transition of the secondary structure from α-helix to β-sheet (23–27%). It has been documented that β-sheets are an important conformational component of the aggregated-state secondary structure of proteins (Choi & Ma, 2007) and that during the gelation process a transformation of α-helix to β-sheet could occur. The presence of more β-sheets and fewer α-helix prior to heating would increase gel strength (Wang & Damodaran, 1991).

Additionally, dichroic UV absorption at wavelengths higher than 220 nm (Fig. 2) for APC and NPC suggested higher exposure of chromophore groups located at the globular head region of myosin due to the heretofore mentioned unfolding promoted by treatments. The strongest cross linkages involved in gelation occur at the myosin head region where most of the —SH are located. Conformational changes that occur during thermal denaturation of myosin are thought to be first initiated in that region (Jones, 1984). Results indicated that both phenomena promotes the heat setting from which a better protein–protein interaction and better gels could be obtained from the APC, as confirmed by the previous results reported by Cortés-Ruiz et al. (2008), where all the gel-forming ability parameters (hardness, fracturability, gel-strength, elasticity, and cohesiveness) were superior for this material.

3.5. Thermal stability of sols

DSC is a direct method to study thermal transitions of muscle proteins as a result of folding-unfolding processes during treatment (Paredi, Tomas, & Crupkin, 2002). The thermal stability of proteins from SM, NPC and APC sols (T_{max} and ΔH) is shown in Table 3. Two distinctive transition peaks were observed with the

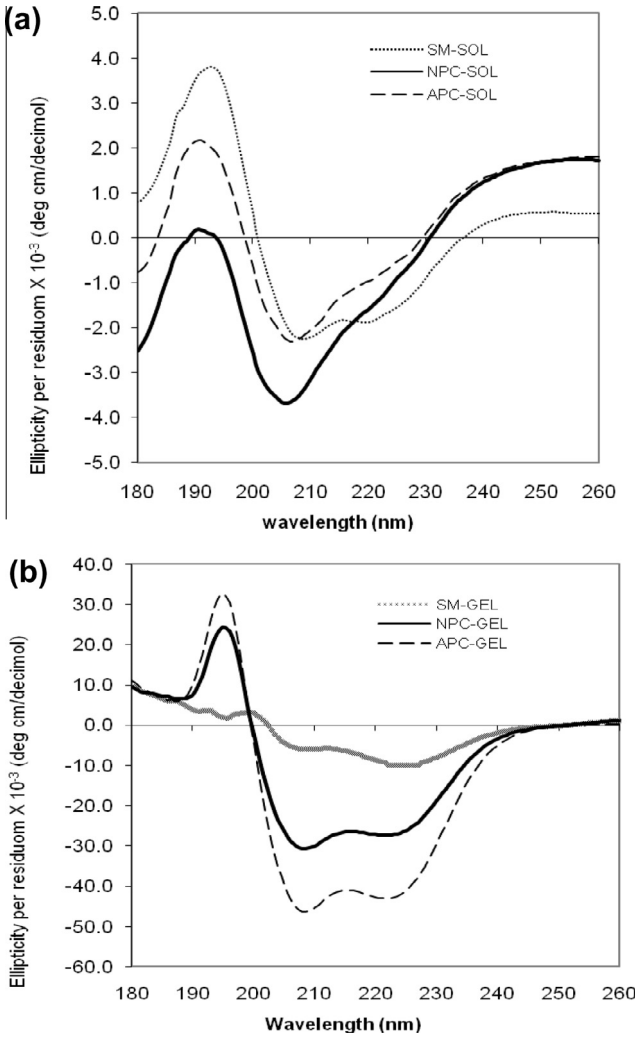

Fig. 2. Secondary structure profile in (a) homogenates with high ionic strength solutions; (b) fluid expressed at high centrifugal force from their respective gels. SM-sol: squid muscle sol; NPC-sol: neutral protein Concentrate sol; APC-sol: acidic protein concentrate sol.

first and second corresponding to myosin and actin denaturation, respectively. Acidic treatment moved transition peak of myosin towards a lower temperature, indicative of the denaturing effect of the treatment as corroborated with the lower enthalpy of denaturation (ΔH_D). Enthalpy of denaturation (endothermic peaks due to unfolding of protein) showed a similar tendency in myosin denaturation with NPC needing more energy to denature than SM, and APC showing the minimum energy. Similar results were reported by Park and Lanier (1989) studying the thermal behavior of tilapia muscle proteins. Protein in APC sols showed the highest surface hydrophobicity meaning that myosin molecules were easier to denature since partial unfolding of proteins increased sensitivity to heat denaturation, process that needed less energy. The APC-sol displayed the lowest thermal stability for myosin and actin compared to SM and NPC sols and gels.

3.6. Rheology

Oscillatory dynamic rheology (ODR) is an effective methodology for studying the sol–gel heat transition of fish proteins (Ehara, Nakagawa, Tamiya, Noguchi, & Tsuchiya, 2004; Gómez-Guillén, Hurtado, & Montero, 2002). The gel-forming mechanism of actomyosin and myosin of fish muscle has been studied based on results of observed dynamic viscoelasticity (Ehara et al., 2004). According to Kim et al. (2003), ODR can reveal the conformational change of proteins related to thermal denaturation and association. Hence, it is meaningful to compare oscillatory dynamic properties among fish proteins concentrates prepared by various methods, since the temperature at which a drop of storage modulus (G') value take place indicates dissociation of the actin-myosin complex and the helix-coil transformation of the myosin tail (Kim et al., 2003, 2005). Unlike fish species, which possess excellent heat-set gel formation properties, jumbo squid show a marked decrease in rigidity values at temperatures around 35 to 40 °C (Gómez-Guillén, Borderías, & Montero, 1997).

Data shown in Fig. 3 clearly show the effect of treatment on the ODR of concentrates. Results support the fact of how conformational changes of squid proteins (unfolding, intermolecular —S—S— formation, α helix–β sheet transition), promoted under acidic conditions during preparation of APC affected rheology of sols during its transition to gels, showing higher storage modulus values (G') than the rest of sols above 30 °C, temperature at which gel formation begins (Gómez-Guillén et al., 1997). Moreover, the APC did not show the expected decrement on G' around 35–40 °C as seen for SM and NPC as result of molecular dissociation and unfolding during heat setting.

It has been postulated that during gelation a trans-conformation of α-helix and non-periodic structures into β-sheet is involved (Wang & Damodaran, 1991). Results suggested that under the APC process conditions, these changes in secondary structures were promoted even before the gelation process started, results that can explain the greater values registered in the texture profile analysis of APC gels reported by Cortés-Ruiz et al. (2008). Results also suggest that protein interactions, such as hydrophobic and —S—S— mediated, as well as the α helix to β sheet transition were promoted under the acidic conditions of APC prior to its sol–gel transition, avoiding the decrease in G' and enhancing, on the other hand, its gelation as showed by the continuous increment of G'. As expected, NPC showed a marked decrease in rigidity values at temperatures around 30 °C.

Dynamic curves indicate that protein in APC and NPC were partially unfolded before thermal treatment. On the other hand, unfolding was minimum for SM at the beginning of the thermal

Table 3
Differential scanning calorimetry of squid mantle muscle and concentrates sols.

	Peak 1 (myosin)			Peak 2 (actin)			Peak 3 (collagen)		
	T_{Dmax} (°C)	ΔH_D (J/g)	ΔCp_D (J/g°C)	T_{max} (°C)	ΔH_D (J/g)	ΔCp_D (J/g°C)	T_{Amax} (°C)	ΔH_A (J/g)	ΔCp_A (J/g°C)
SM	39.3	0.134	0.374	70.8	0.061	0.409	84.5	−0.001	0.045
NPC	40.7	0.212	0.207	68.6	0.139	0.113	ND	ND	ND
APC	38.7	0.128	0.159	70.2	0.020	0.051	76.9	−0.005	0.061

SM = squid muscle. NPC = neutral protein concentrate sol. APC = acidic protein concentrate. T_{Dmax}: peak temperature of denaturation; ΔH_D and ΔCp_D: enthalpy and specific heat gradients of denaturation; T_{Amax}: peak temperature of aggregation; ΔH_A and ΔCp_A: enthalpy and specific heat gradients of aggregation. ND, non-detected.

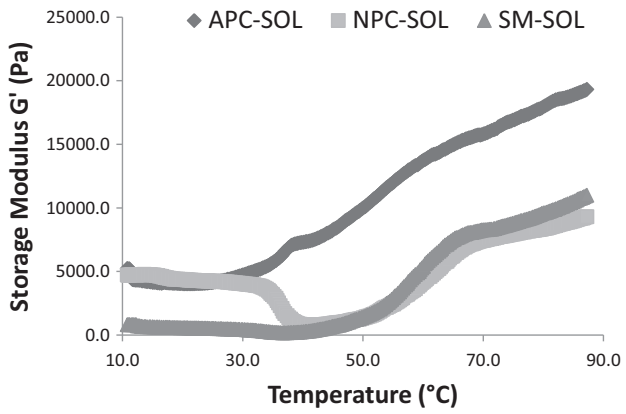

Fig. 3. Changes in storage modulus (G′) during linear heating of SM-SOL: squid muscle sol; NPC-SOL: neutral protein concentrate sol and APC-SOL: acidic protein concentrate sol.

process, only with a perceptible drop at 30 °C similar to that for NPC. Results make evident that a more complete and ordered gelation process took place for APC than for SM and NPC.

Complementary, the amount of fluid expressed from APC-gels (2.7%) was considerably less than that for NPC-gels (16%) and much less than that for SM-gel (33%) (data not shown). This result reflects and confirms a better three-dimensional network for the former as reported in the previous article by the authors (Cortés-Ruiz et al., 2008).

4. Conclusions

The squid muscle and its protein concentrates (NPC and APC) are complex protein systems with proteins of different structure and molecular weights. During the acidic precipitation–dissolution process (APC), conformational changes in proteins are promoted which proved to be highly favorable for the functional–technological quality of the protein concentrate thus obtained. According to results, to improve the quality of gels obtained from jumbo squid muscle, it is necessary to induce changes in its proteins, either by an acidic washing or other means, allowing a modification of their solubility properties, increasing the surface hydrophobicity, promoting the protein–protein aggregation and inducing transconformation of α-helix and aperiodic structures into β-sheets conformation. Results obtained represent an important advance in presenting the APC process as an alternative for surimi or surimi-type material production with high functional–technological properties.

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