Original paper

Obtaining a Protein Concentrate from Squid Mantle (*Dosidicus gigas*) by Direct Isoelectric Precipitation and Evaluation of its Gelling Capacity

Murrieta-Martínez CLAUDIA¹, Ocano-Higuera VICTOR M¹, Ramírez-Wong BENJAMIN², Torres-Arreola WILFRIDO², Ruiz-Cruz SAUL³ and Márquez-Ríos ENRIQUE^{2*}

¹Departamento de Ciencias Químico Biológicas. Universidad de Sonora, Encinas y Rosales s/n. Hermosillo, 83000, Sonora, México.

²Departamento de Investigación y Posgrado en Alimentos. Universidad de Sonora, Encinas y Rosales s/n. Hermosillo, 83000, Sonora, México.

³Departamento de Biotecnología y Ciencias Alimentarias. Instituto Tecnológico de Sonora, 5 de febrero 818 sur. Obregón, 85000, Sonora, México.

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This study investigated protein recovery from squid mantle (*Dosidicus gigas*) using the following two methods: a conventional method (CM; water washing) and direct isoelectric precipitation (IP). The IP treatment offered a better yield. Protein fractionation showed a myofibrillar protein content of 64.83 ± 0.02 and $55.47 \pm 0.05\%$ for CM and IP, respectively. Differential scanning calorimetry showed differences in the myosin denaturation temperature with values of 37.27 ± 0.74 and $40.93 \pm 0.41^\circ\text{C}$ for the CM and IP treatments, respectively. Moreover, Surface hydrophobicity showed major hydrophobicity with the CM treatment. The total sulfhydryl content was higher in IP than in CM, but a decrease in sol-gel transition was more evident in CM than in IP. The hardness of the gel obtained from IP was higher than that of CM with values of $1695.66 \pm 177.82_{\text{g-f}}$ and $668.33 \pm 21.19_{\text{g-f}}$, respectively. Direct isoelectric precipitation of protein from squid mantle generated a better yield and better functional protein compared to the conventional method.

Keyword: Jumbo squid, protein concentrate, functionality, gelling properties.

Introduction

Currently, the giant squid (*Dosidicus gigas*) fishery is one of the most important in Northwest Mexico. The main reasons for the growing commercial appeal of giant squid mainly lie in the abundance of the species, low cost, lean muscle, white muscle, no spines, no scales, high yields and high nutritional content (Moreno *et al.*, 2012). However, despite the widespread availability of giant squid, it is underutilized due mainly to problems associated with the unpleasant taste of its muscle (acid/bitter) and high initial value of volatile nitrogen compounds, which is incorrectly associated with a reduced quality resulting in a low purchase price (Bjarnason,

1989). Therefore, a way to make this resource more profitable is to obtain protein concentrates, which can be used in the surimi industry (Gomez-Guillen *et al.*, 2002).

Cephalopod muscle, such as that of giant squid, has the potential to be used in the manufacture of protein concentrates for the preparation of seafood or other products based on gel formation (Sánchez *et al.*, 2007). Surimi preparation involves a series of wash cycles with water, which is known as the conventional method. This process results in a protein concentrate with reduced functional and technological quality, and it also results in a low yield due to high solubility of muscle protein of some species. For

*To whom correspondence should be addressed.

E-mail: emarquez@guayacan.uson.mx

this reason, it was necessary to develop alternative methods for surimi preparation. One of these methods consists of the solubilization of muscle proteins as a function of pH. Protein solubilization can be performed in acid or alkaline solutions followed by isoelectric precipitation of solubilized proteins (Huntin and Kelleher, 1999, 2000).

From the industrial point of view, the recovery process through alkaline and acid dissolution has the advantage of dissolving the majority of muscle proteins, which in some cases constitutes a yield up to 90%. In giant squid, high solubility of giant squid proteins has been obtained in both acid and alkaline solutions, but low protein recovery occurs when the protein solution is adjusted to the isoelectric point, which is attributed to inherent characteristics of giant squid proteins (Dihort-García *et al.*, 2011; Cortes-Ruiz *et al.*, 2008). Greater recovery has been obtained via alkaline solution (Dihort-García *et al.*, 2011), but the gelling property is better with an acid solution (Cortes-Ruiz *et al.*, 2008). Few investigations have been focused on functionality of proteins recovered by isoelectric precipitation without prior acid/alkaline dissolution. The present study investigated giant squid mantle protein recovery by means of direct isoelectric precipitation to maximize protein recovery, and this study also investigated the gelling ability of giant squid mantle protein and its conformational changes in sol-gel transition, which explains the gelling ability.

Materials And Methods

Sampling Jumbo squid (*D. gigas*) was harvested off the coast of Kino Bay, Mexico (28°N and 112°W) in September, 2010. Ten specimens were deheaded and degutted on site, and they were then washed with freshwater at room temperature (25°C). The mantles (experimental samples) were bagged and placed in alternating layers of ice-squid-ice in a portable cooler, and the mantles were then transported to the laboratory. The elapsed time between capture and reaching the laboratory did not exceed 12 h.

Protein Concentrates Two protein concentrates were obtained as follows: one was obtained using the conventional method (CM; mantle washed with water), and the other was obtained through direct isoelectric precipitation at pH 5.5 (IP). In both procedures, the muscle was homogenized in a ratio of 1:5 (mantle:water) with distilled water at 4°C for 10 min by employing a tissue homogenizer. To obtain a concentrate by the conventional method, the homogenate mantle was centrifuged at 15 000 x g for 15 min at 4°C using a refrigerated centrifuge (Sorvall stratos, Biofuge, Germany), and the precipitate was then resuspended in a ratio of 1:5 (mantle:water), homogenized, and centrifuged under the same conditions used above. The precipitate was regarded as the protein concentrate obtained by the conventional method (CM). To prepare the protein concentrate by means of isoelectric precipitation (IP), the pH of the homogenized mantle was adjusted to 5.5 using a 6 M HCl solution, and the solution was then centrifuged under the same conditions as used for the CM.

Color The color of sols and heat-set gels was measured by tristimulus colorimetry using a CR-400 chromameter (Konica Minolta Sensing, Inc., Tokyo, Japan). Color coordinates for degree of lightness (L), redness/greenness (+a/-a), and yellowness/blueness (+b/-b) were obtained. From the color coordinates, the whiteness index (WI) was calculated as follows: $WI = [100 - [(100 - L)^2 + a^2 + b^2]^{1/2}]$ (Lanier, 1992).

Protein Fractionation Sarcoplasmic, myofibrillar, alkali-soluble, and stroma proteins were fractionated based on their solubility following the separation scheme proposed by Hashimoto *et al.* (1979). Protein concentration in each fraction was determined by the micro-Kjeldahl method (AOAC, 2005).

Electrophoretic Profile (SDS-PAGE) The electrophoretic profile was analyzed in each protein concentrate by polyacrylamide gel electrophoresis (PAGE) using a dissociating sodium dodecyl sulfate (SDS) buffer system in a discontinuous gel (4% stacking gel and 10% separating gel) according to the method of Laemmli (1970). A Mini PROTEAN 3 Cell Multi-Casting Chamber (Bio-Rad Laboratories, Hercules, CA) was used. Electrophoretic runs were performed at room temperature (25°C) at 80 V. Thirty micrograms of protein was loaded into each lane of the gels, and a broad range molecular weight protein standard solution (BioRad Laboratories, Richmond, CA) containing myosin (200 kDa), beta-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (31 kDa) was used. After electrophoresis, the gel was stained with 0.125% (w/v) Coomassie brilliant blue R-250 in 40% (v/v) methanol and 7% (v/v) acetic acid, and the gel was destained with 50% (v/v) methanol and 10% (v/v) acetic acid.

Differential Scanning Calorimetry (DSC) A 20 mg sample was placed in a calorimetric cell, and adequate contact between the sample and the lower face of the capsule was ensured. All samples were scanned at a heating rate of 5°C/min in the range of 10–90°C in a DSC (DSC 8000, Perkin Elmer, Shelton, USA). The total enthalpy of denaturation was estimated by measuring the area under the curve (Paredi *et al.*, 1994).

Determination of Surface Hydrophobicity Protein surface hydrophobicity was measured according to the method reported by Kato and Nakai (1980) using 1-aniline-8-naftalen-sulfonate (ANS) as a probe. Protein was dissolved in a 20 mM Tris-HCl buffer (pH 7.0) containing 0.42 M NaCl to obtain different concentrations (0.0, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06%). The diluted protein (5 mL) was then mixed with 80 µL of 2 mM ANS in a 20 mM Tris-HCl buffer (pH 7.0). The fluorescence intensity of ANS/protein conjugates were measured using a luminescence spectrophotometer (LS50B, Perkin-Elmer, New Jersey) at the excitation and emission wavelengths of 389 and 470 nm, respectively. Surface hydrophobicity was calculated from the initial slope of the plot of fluorescence intensity against protein concentration and was referred to as S_0 ANS.

Heat-Set Gel Preparation Sols for each protein system were

prepared by adding 2.5 g of NaCl/100 g of protein system at short intervals in a Plus Cuisinart Food Processor (Model DLC-8, Cuisinart Inc., Greenwich, CT). The pH was adjusted to neutrality with sodium bicarbonate (Na_2CO_3). To 96.1 g of IP, 1.4 mL of water and 2.5 g of NaCl (a total of 100 g) were added; while to CM only 2.5 g of NaCl was added (97.5 g of CM + 2.5 g of NaCl = 100 g), to obtain moisture and protein contents of 86.0 and 11.5%, respectively. Each sol was packed into a Petri dish (1 cm height) and vacuum sealed in moisture/vapor-proof film bags (Cryovac Corp., Duncan, SC) with a Smith Supravac Vacuum Machine (Smith Equipment Co., Clifton, NJ). 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 maintained at 2–4°C overnight prior to functional evaluation.

Gel-Forming Ability (GFA) Gels were tempered for 60 min at room temperature (25°C) prior to analysis. The GFA was evaluated in terms of the folding test (FT) and texture profile analysis (TPA). The FT was carried out as described by Tanikawa *et al.* (1985). The test was conducted by folding a 3.0 mm by 30.0 mm (diameter) slice of heat-set gel between the thumb and index finger. Results were based upon the degree of cracking occurring along the folds as follows: grade AA or 5, extremely elastic gel (no cracks on folding into quarters); grade A or 4, moderately elastic gel (no crack on folding in half but cracks on folding into quarters); grade B or 3, slightly elastic gel (some cracks on folding in half); grade C or 2, non-elastic gel (breaks into pieces on folding in half); and grade D or 1, poor gel (breaks into pieces with finger pressure). The TPA was conducted on cylinder-shaped samples of uniform dimensions (1 cm diameter and 1 cm height) obtained from each gel using a sharp-edged plastic tube. Texture was measured using a TA-XT2 Plus Texturometer (Food Technology Corp., Sterling, VA) with a 3.8 cm diameter compression plunge attached to a 100 N load cell. Compression forces at 75% of the original gel sample height were used to compute compression hardness, gel strength, fracture, cohesiveness and elasticity.

Water-Holding Capacity (WHC) The WHC was measured using the methodology reported by Cheng *et al.* (1979). A 5 g portion of each gel was centrifuged in a refrigerated centrifuge at 3000 × g for 20 min at 4°C. (Sorvall, Stratos, Model Biofugue). The WHC was expressed as “water retained”, which was the sample weight loss percentage with regard to its initial weight.

Determination of Total and Reactive Sulfhydryls Total sulfhydryl (TSH) content was measured according to the method reported by Patrick and Swaisgood (1976). To 1 mL of protein solution (2%), 1 mL of 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) in 20 mM Tris-HCl buffer (pH 7.0) was added followed by the addition of 1.44 g of urea. After incubation at 40°C for 25 min, the absorbance at 412 nm was measured using a spectrophotometer (Varian, Cary 50, Walnut Creek, California). The total SH content was calculated using a molar extinction coefficient of 13,600 $\text{M}^{-1}\text{cm}^{-1}$. Reactive sulfhydryl content was

determined by mixing 5 mL of protein solution (2%) with 1 mL of 10 mM DTNB in 20 mM Tris-HCl buffer (pH 7.0). The reaction mixture was incubated at 40°C for 25 min, and the absorbance at 412 nm was measured using a spectrophotometer (Varian, Cary 50, Walnut Creek, California).

Statistical Analysis The experiment was conducted three times ($n = 3$), and each determination was performed in triplicate. Data analysis was performed using the JMP 5.0.1 statistical package. Descriptive statistics (mean and standard deviation) and one-way ANOVA were performed to determine differences among treatments. Analysis of variance was performed according to Tukey-Kramer using a 5% significance level (Cochran and Cox., 1992).

Results and Discussion

Yield In this study, preliminary tests were conducted to determine the pH at which the highest percentage of insoluble protein was obtained from squid mantle. The pH of the homogenate was 6.3 (initial) and the most suitable pH for precipitation was 5.5. Protein concentrates had 87.25 ± 0.09 and $85.02 \pm 0.12\%$ of moisture, and 12.95 ± 0.16 and $15.35 \pm 0.87\%$ of protein, for CM and IP, respectively. The yields for the conventional method and isoelectric treatment were $36.06 \pm 0.39\%$ and $47.03 \pm 0.11\%$, respectively. This recovery was similar to that reported by Cortes-Ruiz *et al.* (2008), who worked with *D. gigas* and reported a yield of 48.20% after using acid dissolution (pH 3.0) and subsequent isoelectric precipitation (pH 5.5). However, the yield in the present study was higher than that reported by Dihort-Garcia *et al.* (2011), who worked with *D. gigas* and reported a yield of 43.90% after using alkaline dissolution (pH 11.0) and subsequent isoelectric precipitation (pH 5.5). In another study, Batista *et al.* (2007) solubilized muscle proteins from *Sardina pilchardus* at alkaline pH (11.5) and precipitating the proteins at pH 5.5 to obtain a yield of 71.50%. The yield obtained in the present study was good when compared to other studies with the same species (*D. gigas*), but the yield was low compared to that from other species, which may be due to intrinsic differences in proteins of this mollusk as compared with fish muscle proteins. Another factor that can influence this difference, particularly in the protein concentrate obtained in the traditional manner, is the high solubility of the squid mantle proteins. Thus, two cycles of washing removes a high protein content, which impacts the yield.

Color The results obtained for L parameters (lightness) and whiteness index are shown in Table 1. The a and b parameters are not reported because squid muscle is not pigmented, so these parameters are generally small for squid muscle. The L parameter values were higher for sols and gels prepared by IP than those prepared with the conventional method ($p < 0.05$). Moreover, the whiteness index values were higher for sols prepared by IP compared to the conventional method, and there were no differences in the whiteness index values when comparing gels

Table 1. L* parameter and whiteness of sols and gels of IP and CM

Treatment	L		Whiteness	
	Sol	Gel	Sol	Gel
IP	68.55±0.65 ^a	82.54±0.42 ^a	67.99±0.67 ^a	81.02±0.50 ^a
CM	63.70±0.19 ^b	81.77±0.28 ^b	63.44±0.20 ^b	81.07±0.25 ^a

IP: Isoelectric point. CM: Conventional method. Data are the mean of n=3 ± standard deviation. Different superscript within each column indicates significant differences ($p < 0.05$)

made with both methods ($p \geq 0.05$).

The sols and gels L and whiteness values in the present study were higher than those reported by Cortes-Ruiz *et al.* (2008), who developed protein concentrates from *Dosidicus gigas* by acid dissolution and the conventional method. However, the lightness (L) and whiteness values in the present study were slightly lower than those reported by Dihort-Garcia *et al.* (2011), who obtained a protein concentrate by alkaline dissolution and subsequent isoelectric precipitation for the same species.

Slight differences in L and whiteness values found in the present study may be due to a different protein composition obtained in the protein concentrates. These differences may also be due to the moisture content of sols and gels. The refraction phenomenon of light due to the increased hydration and solubilization capacity of proteins may have played an important role in these parameters (Palombo and Wijngaards, 1990). However, due to inherent characteristics of giant squid muscle, lightness and whiteness values reported in sols and gels of *D. gigas* always exceed those reported for fish. Thus, protein concentrates made from squid are of excellent quality when considering the color quality (Lanier, 1992).

Fractionation of Proteins The solubility pattern was significantly different ($p < 0.05$) (Table 2) for both methods. The CM treatment was performed by washing the mantle. With this method, is expected to have a low level of soluble protein at low ionic strength; however, a high content of sarcoplasmic protein was found, which may be due to the high solubility of mantle protein from *D. gigas* (Sánchez-Alonso *et al.*, 2007). Furthermore, a high content of sarcoplasmic protein resulted from the IP treatment because a large amount of sarcoplasmic protein precipitated at pH 5.5, which explains the major yield obtained by direct isoelectric precipitation.

Soluble protein was not detected in the alkali soluble fraction, thus indicating that the possible denaturation that can occur in the extraction process was not reflected in the solubility. In other studies where the processing conditions for obtaining protein concentrates are more severe, it is common to find a fraction of alkali soluble protein (Cortés-Ruiz *et al.*, 2008; Dihort-Garcia *et al.* 2011). A high content of alkali soluble protein (insoluble protein at low and high ionic strength) can be an indicator of denaturation occurring during the extraction process, but a high content of alkali

Table 2. Fractionation of proteins in protein concentrates (g/100 g of total protein)

Fraction	CM	IP
Soluble at ionic strength 0.05	19.52±0.02 ^a	24.98±0.06 ^b
Soluble at ionic strength 0.50	64.83±0.05 ^a	55.47±0.02 ^b

IP: Isoelectric point. CM: Conventional method. Data are the mean of n=3 ± standard deviation. Different superscript within each row indicates significant differences ($p < 0.05$).

soluble protein was not present in this study.

Electrophoretic Profile The high proteolytic activity of squid muscle can cause muscle protein degradation, thereby reducing its use as a protein concentrate (Ramírez-Olivas *et al.*, 2004). Kolodziejaska *et al.* (1987) and Konno *et al.* (2003) showed that an endogenous protease in squid muscle promotes myosin degradation, thereby affecting texture characteristics. Figure 1 shows that both treatments had similar electrophoretic profiles with bands corresponding to myosin (M), heavy meromyosin (HMM), paramyosin (PM), actin (Ac), and tropomyosin (Tpm) as well as a band potentially representing troponin (Tpn). However, in the isoelectric treatment, a band with an approximate size of 75 kDa was present, and this band can be associated with light chain meromyosin (LCMM) (Kijowski, 2001).

Konno *et al.* (2003) reported that squid mantle muscle contains a metalloprotease that hydrolyzes myosin heavy chain (MHC) and light chain of meromyosin (LMM). As is shown in the electrophoretic profile, both bands appeared in the isoelectric treatment, but the LMM band was not clearly visible in the conventional method. The LMM band was also reported by Cortes-Ruiz *et al.* (2008) to be present after acid treatment, which is attributed to conformational changes due to the pH effect on the protein structure, thereby making it more susceptible to enzymatic hydrolysis. The results also suggest that the enzyme responsible for these changes is stable at the isoelectric point (pH 4.5). Moreover, the electrophoretic profile indicated a band approximately 120 kDa in both treatments, which may be associated with degradation products of myosin.

In general, the electrophoretic pattern showed no obvious differences between treatments. The lack of differences may be due to the end result being the same by precipitating primarily myofibrillar protein when adjusting the pH to 5.5 in the IP

Fig. 1. Electrophoretic profile (SDS-PAGE) of CM and IP. Lanes: 1) broad range standard; 2) CM; 3) IP. IP: Isoelectric point. CM: Conventional method. Myosin (M), Heavy meromyosin (HMM), Paramyosin (PM), Light meromyosin (LMM), Actina (AC), Tropomyosin (Tpm) y Troponin (Tpn).

treatment and when concentrating myofibrillar protein after washing away most of the sarcoplasmic protein in the traditional manufacture of surimi. However, it is noteworthy that the band corresponding to actin was more intense with the isoelectric treatment than with the CM. These differences were minimal, but they may influence the results obtained in the other analyses.

Differential Scanning Calorimetry (DSC) To perform this analysis, we used the sols of both protein concentrates (CM and IP), and the calorimetric analysis results are shown in Table 3. The CM and IP generated two endotherm transitions. The first transition for the CM treatment ($40.93 \pm 0.41^\circ\text{C}$) was attributed to myosin according to obtained values for Atlantic cod and Iota with transition values of 37.6 and 38.8°C , respectively (Riebroy *et al.*, 2008). Riebroy *et al.* (2008) also reported a denaturation temperature of $61.3\text{--}63.9^\circ\text{C}$ for actin, which was similar to the denaturation temperature detected in the second peak ($65.35 \pm 0.52^\circ\text{C}$). The first transition for the IP treatment appeared at a temperature of $37.27 \pm 0.74^\circ\text{C}$, which was attributed to myosin denaturation. Thus, the extraction procedure using direct isoelectric precipitation (IP) caused conformational changes in mantle muscle protein resulting in a minor denaturation temperature for myosin. Working with catfish, Ranghavan and Kristinsson (2007) established a margin ranging from 38 to 44°C , which was consistent with the margin reported by Togashi *et al.* (2002) for

Table 3. Denaturation temperature of proteins present in CM and IP sols

Sample	Tmax ($^\circ\text{C}$)	ΔH (J/g)
Peak 1		
CM	40.93 ± 0.41^a	0.032 ± 0.01^a
IP	37.27 ± 0.74^b	0.095 ± 0.01^b
Peak 2		
CM	65.09 ± 0.16^a	0.0150 ± 0.005^a
IP	65.35 ± 0.52^a	0.0248 ± 0.006^a

IP: Isoelectric point. CM: Conventional method. Values are the mean $\pm$ standard deviation of $n = 3$. Different superscript within each column indicates significant differences ($p < 0.05$).

Alaska pollock. Displacement from 37.27 ± 0.74 to $40.93 \pm 0.41^\circ\text{C}$ could be attributed to conformational changes caused by the extraction procedure. The previous result can be explained by a possible aggregation of myofibrillar proteins due to pH effects because at the isoelectric point, proteins tend to aggregate as a consequence of hydrophobic interactions due to reduced electrostatic repulsion (Latif *et al.*, 2009).

The second transitions were 65.09 ± 0.16 and 65.35 ± 0.52 for CM and IP, respectively. These transitions could be attributed to actin. There is little information about thermal denaturation of myofibrillar proteins from *D. gigas* muscle. However, it is known that the T_d of *D. gigas* muscle proteins is less than that of mammalian muscle, and similar results have been reported for the muscle of other squid species (Rodger *et al.*, 1984, Hastings *et al.*, 1985) and other species of mollusks and fish (Howell *et al.*, 1991; Paredi *et al.*, 1994). The present results showed that the extraction procedure did not affect the denaturation temperature of actin from jumbo squid. Hence, actin could not be responsible for the functional characteristic changes in the IP treatment.

Surface Hydrophobicity (S_{ANS}) Figure 2 shows the results for hydrophobicity. Significant differences ($p < 0.05$) between the two treatments were detected. The CM treatment showed higher hydrophobicity, which may have been due to the different proportions of protein in each system and the conformational changes induced by pH adjustment (5.5). The CM treatment is based on the removal of sarcoplasmic proteins, thereby concentrating myofibrillar and stromal proteins. In contrast, direct isoelectric precipitation at pH 5.5 not only precipitates myofibrillar protein but also a small fraction of sarcoplasmic protein. Another reason to explain this result could be the conformational changes that muscle protein underwent by means of isoelectric treatment. At this pH (5.5), proteins have minimum solubility because the charge balance is zero resulting in aggregation of proteins. When proteins are submitted to neutral pH, some proteins never reach their native conformation, so non-polar amino acids could be grouped, forming a hydrophobic nucleus surrounded by a polar residue layer in contact with water. Due to the propensity of non-

Fig. 2. Changes in surface hydrophobicity of CM and IP sols. Values are the mean $\pm$ standard deviation of $n = 3$. IP: _Isoelectric point. CM: Conventional method.

Table 4. Total and reactive sulfhydryl in sols and gels of IP and CM

Treatment	Total		Reactive	
	Sol	Gel	Sol	Gel
IP	6.83 ± 0.74^a	4.74 ± 0.51^a	3.51 ± 0.38^a	2.13 ± 0.09^a
CM	4.28 ± 0.46^b	3.24 ± 0.35^a	2.00 ± 0.21^b	0.52 ± 0.00^b

IP: _Isoelectric point. CM: Conventional method. Data are the mean of $n=3$ $\pm$ standard deviation. Different superscript within each column indicates significant differences ($p < 0.05$).

polar amino acid residues to position themselves in the interior of the protein molecules in solution, which prevents contact of these residues with the aqueous surrounding, only a portion of them could be considered as being effective in terms of hydrophobicity (Totosa *et al.*, 2002).

Total and Reactive Sulfhydryls Quantification of total (TSH) and reactive (RSH) sulfhydryls is shown in Table 4. The recovery protein of the IP treatment showed greater amount of TSH both in sols and gels ($p < 0.05$) as compared to that of the CM, which may be due to a higher ratio of sarcoplasmic/myofibrillar proteins present in the IP treatment as revealed by a high concentration of SH groups in the sarcoplasmic fraction. Similar results have been reported by Tolano-Villaverde *et al.* (2013), who studied the alkaline dissolution from giant squid mantle (*Dosidicus gigas*). These authors found a higher concentration of TSH in the mantle than in the alkaline protein concentrate, and they attributed this result to the higher content of sarcoplasmic protein in the mantle. The TSH decreased during the sol-gel transition in both treatments suggesting the formation of disulfide bonds during the heating process. Similarly, the RSH decreased during gel formation, which involves inter- or intra-molecular disulfide bond formation. These results were similar to those reported by Yongsawatdigul and Park (2003) for threadfin bream and by Benjakul *et al.* (2001) for

mackerel (*Auxis thazard*) where they noted that further oxidation of SH groups occurs with subsequent formation of disulfide bonds at high temperatures during heating. The higher content of TSH resulting from the IP than from the CM may be due to the IP gels being much harder than the CM gels.

Folding Test (FT) The folding test of gels showed values of 5 ± 0.0 (AA) and 3.66 ± 0.57 (AB) for CM and IP treatments, respectively (Table 5). The FT is a subjective evaluation, but the results were consistent with the texture profile analysis where the isoelectric gel showed fracture and the CM gel did not show fracture. Studying the same species (*D. gigas*), Cortés-Ruiz *et al.* (2008) prepared gels using protein recovered from the conventional method and from acid dissolution with FT values of 3.3 ± 1.2 and 5.0 ± 0.0 , respectively. In another study, Dihort-García *et al.* (2011) prepared gels using mantle mince from squid (*D. gigas*), and they recovered protein from the conventional method and through alkaline dissolution with FT values of 2.80 ± 0.45 and 3.40 ± 0.54 , respectively. According to the FT, a better quality gel was obtained using recovered protein with the conventional method. However, the FT has limitations in describing gel textures because it only evaluates mechanical resistance to bending. Thus, gels were evaluated through texture profile analysis (TPA).

Texture Profile Analysis The data generated in the TPA are

Table 5. Gelling capacity of IP and CM

Parameter	Gel CM	Gel IP
WHC	48.99±2.73 ^a	63.02±6.03 ^b
Folding Test	5±0.0 ^a	3.66±0.57 ^b
Hardness (g-f)	668.33±21.19 ^b	1695.66±177.82 ^a
Fracture (g-f)	Not present	1430.5±6.36 ^a
gel strength (g-f. mm)	1320.18±50.25 ^b	2581.37±223.05 ^a
% Elasticity	67.35±0.0 ^b	88.92±0.10 ^a
% Cohesiveness	31.44±0.23 ^a	34.07±0.04 ^a

IP: Isoelectric point. CM: Conventional method. WHC: Water holding capacity. Data are the mean of n=3 ± standard deviation. Different superscript within each row indicates significant differences (p < 0.05).

shown in Table 5. The gel obtained from protein recovered by the CM was softer than that obtained from IP (p < 0.05) with values of 668.33 ± 21.19_{g-f} and 1695.6 ± 177.82_{g-f}, respectively. Fracture was not detected in gels prepared with CM, but IP gels showed fracture at 1430.5 ± 6.36_{g-f}. The difference in hardness was noticeable, which was indicative of a better structured gel, thereby explaining the better WHC, strength and elasticity of gels obtained by IP compared to those obtained by CM. Cortés-Ruiz *et al.* (2008) and Dihort-Garcia *et al.* (2011) studied the acid and alkaline dissolution, respectively, of mantle protein from jumbo squid (*D. gigas*), and they reported hardness of 1849.50 ± 459.20_{g-f} and 687.66 ± 53.57_{g-f} for gels made by recovered protein from acid and alkaline solution, respectively. The hardness value for the IP gel obtained in the present study was higher than that reported by Dihort-Garcia *et al.* (2011) but slightly lower than that reported by Cortés-Ruiz *et al.* (2008). However, the gels made by Cortés-Ruiz *et al.* (2008) showed a 95% WHC and AA quality according to the folding test. It is important to mention that a softer gel is not precisely bad; however, for surimi manufacture certain degree of hardness is required.

Water-Holding Capacity (WHC) The methodology to obtain protein concentrate showed a significant effect (p < 0.05) in the water-holding capacity of the gels prepared with values of 48.99 ± 2.73% and 63.02 ± 6.03% for CM and IP gels, respectively (Table 5). Higher WHC is indicative of a better protein/water interaction inside the gel structure. The present values were higher than those reported by Dihort-Garcia *et al.* (2011), who obtained a protein concentrate from *D. gigas* through alkaline solution and subsequent isoelectric precipitation obtaining a WHC of 38.1 ± 0.1%, thereby suggesting a serious impact of the alkaline dissolution on the recovered proteins, thus affecting the WHC of the prepared gels. In contrast, Cortes-Ruiz *et al.* (2008) obtained a protein concentrate using the same species by acid dissolution and subsequent isoelectric precipitation obtaining a WHC of 95.3 ± 2.0%. The acidic/alkaline dissolutions induce protein denaturation, and such distortion may be beneficial or detrimental. This was demonstrated in the study by Cortes-Ruiz *et al.* (2008), where the acid solution was shown to provide the gel with excellent water-holding

capacity.

Direct isoelectric precipitation treatment of muscle proteins from squid mantle (*D. gigas*) generated a better protein yield compared to the conventional method. Furthermore, the proteins recovered in this manner had better functional properties than those obtained through the conventional process. Moreover, the IP method resulted in a lower degree of protein denaturation and greater integrity compared to alternative processes of acid/alkali dissolution.

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