

Functional Properties of Protein from Frozen Mantle and Fin of Jumbo Squid *Dosidicus gigas* in Function of pH and Ionic Strength

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Functional properties of protein from mantle and fin of the jumbo squid *Dosidicus gigas* were explained based on microscopic muscle fiber and protein fractions profiles as observed in SDS-PAGE. Fin has higher content of connective tissue and complex fiber arrangement, and we observed higher hardness of fin gels as expected. Myosin heavy chain (MHC) was found in sarcoplasmic, myofibril and soluble-in-alkali fractions of mantle and only in sarcoplasmic and soluble-in-alkali fractions of fin. An additive effect of salt concentration and pH affected the solubility and foaming properties. Fin and mantle proteins yielded similar results in solubility tests, but significant differences occurred for specific pH and concentrations of salt. Foaming capacity was proportional to solubility; foam stability was also affected by pH and salt concentration. Hardness and fracture strength of fin gels were significantly higher than mantle gels; gels from proteins of both tissues reached the highest level in the folding test. Structural and molecular properties, such as MHC and paramyosin solubility, arrangement of muscle fibers and the content of connective tissue were useful to explain the differences observed in these protein properties. High-strength gels can be formed from squid mantle or fin muscle. Fin displayed similar or better properties than mantle in all tests.

Key Words: functional properties, jumbo squid, protein fibers, fin, mantle, *Dosidicus gigas*

INTRODUCTION

Jumbo squid *Dosidicus gigas*, widely distributed in the Eastern Pacific (Markaida and Sosa-Nishizaki, 2003), has a life cycle of 2 years. Among harvested squid, it is the largest species (Markaida et al., 2004), but a thriving fishery is not established in Mexico (Martínez-Aguilar et al., 2006). As a potential food ingredient, squid muscle proteins contain all essential amino acids (Córdova-Murueta and García-Carreño, 2002). In Mexico, jumbo squid is an underutilized marine resource because the whole body is not always used or processed. The head has a commercial value of about US\$0.30/kg, mantle even lower and the fin has no value. For this reason, the mantle and fin are usually discarded after processing (Kristinsson and Hultin, 2003).

Because endogenous proteolytic enzymes are responsible for hydrolysis and softening of the raw material (Gildberg, 1993), endogenous enzyme activities lead to cleavage of proteins, which negatively affects its useful properties, especially gelling capacity (Gómez-Guillén et al., 1997; Konno et al., 2002; Park et al., 2005). Appropriate handling during harvesting and transport is an important step for preserving the original characteristics of processed muscle to avoid using gelation-enhancing ingredients. Thus, it is necessary to verify the integrity of muscle fibers and useful molecules, especially the myosin heavy chain (MHC) and paramyosin (PM) to assure and explain the properties of proteins from muscle sources.

A major concern in seafood processing is to achieve optimal properties of raw material during processing. For that, utility of squid proteins treated under different conditions must be studied to take advantage of the proteins in this marginally exploited species. Characterization of squid proteins from different tissues will advance the prediction of their performance during processing. This study evaluated some properties of squid mantle and fin muscle proteins under operational variables, particularly pH and ionic strength.

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MATERIALS AND METHODS

Preparation of Samples

Squid were caught in the Gulf of California in June 2005. On board, fresh, small pieces of mantle and fin were excised and prepared for later histological study. The rest of the captured squid were immediately dissected and kept on crushed ice until reaching the laboratory, where the samples were frozen at $-30\text{ }^{\circ}\text{C}$ within 12 h after capture and stored until processing. Frozen-thawed skinned mantle and fin were processed for histological study. The large pieces of mantle and fin were chopped into small pieces, and then ground in a mill (Retsch, Haan, Germany) at low speed. Homogeneous pastes of each tissue were stored at $-30\text{ }^{\circ}\text{C}$ until used in tests of solubility, foaming capacity and gelling. During the analyses, samples were kept on ice; at no time were temperatures higher than $5\text{ }^{\circ}\text{C}$.

Histology and Quantification of Connective Tissue

Fresh and frozen-thawed tissues were prepared for histological examination to compare integrity of muscle fibers before and after storage. Triplicated samples were processed according to Howard and Smith (1983), where samples are dehydrated by immersion in Davidson's solution for 48 h. The solution is replaced with increasing concentrations of ethanol. After 1 h in each solution, samples were submerged in paraffin, then, thin-sectioned ($4\text{ }\mu\text{m}$) with a rotational microtome (RM 2155, Leica Microsystems, Wetzlar, Germany) along three spatial axes and then stained using the Masson trichromic blue-staining method for muscle and connective tissue (Sheehan and Hrapchak, 1973). Sections were viewed and digitalized with an image analysis system, including an optical microscope (BX41, Olympus America, Central Valley, PA); a digital camera (Cool Snap-Pro, DVC, Austin, TX); and imaging software (Image Pro Plus 5.4, Media Cybernetics, Bethesda, MD). For evaluation of the collagen content, pixels of blue-stained tissue were counted by the threshold selection method (Otsu, 1979) and relative abundance was determined as a percent of the total, using the same software. All pictures of connective tissue were taken at $20\times$.

Methods

Protein Fraction Profiles by SDS-PAGE

Sarcoplasmic and myofibril protein fractions, soluble in alkali fractions, and stromal proteins were separately obtained from three different samples of mantle and fin, using the method of Hashimoto et al. (1979). Sarcoplasmic proteins were solubilized in low ionic strength buffer ($i=0.05$, pH 7.5, 15.6 mM Na_2HPO_4 , 3.5 mM KH_2PO_4), myofibril proteins were solubilized

in high ionic strength buffer ($i=0.5$, pH 7.5, 0.45 M de KCl, 15.6 mM de Na_2HPO_4 , 3.5 mM KH_2PO_4) and the soluble-in-alkali fraction in 0.1N NaOH. Stromal proteins were obtained as pellets after centrifugation ($12\,000\times g$) and discarded. By SDS-PAGE, equal amounts of protein concentration of each fraction was loaded in a discontinuous system, according to the method of Laemmli (1970), using 10% separating gel and 4% stacking gel. Samples were heated for 5 min in a boiling water bath. A constant current of 15 mA per gel was used for separating the samples; temperature was kept at $4\text{ }^{\circ}\text{C}$ using a bath and circulator (CH/P 2067, Forma Scientific, Marietta, OH). Gels were stained in a solution containing 0.05% Coomassie brilliant blue (R-250), 40% methanol and 7% acetic acid. Digital images of the electrophoresis gels were recorded with a scanner (Duoscan T1200, AGFA, Mortsel, Belgium) and compared for presence of MHC and PM protein bands.

Solubility

Solubility of squid protein was evaluated according to Chobert et al. (1988) with minor modifications. Homogenates containing 1% protein in water or NaCl solutions (according to required conditions) were prepared with a kitchen blender by two pulses of 15 s each. Three sets of seven suspension portions were adjusted to pH 2, 4, 5, 6, 8, 10 and 12 with 0.1M NaOH or HCl solutions. The ionic strength for each set of pH solutions was adjusted to 0, 0.2 or 0.4M NaCl. Samples were kept under these conditions for 30 min at $2-4\text{ }^{\circ}\text{C}$, using constant cold water flow and agitation. Triplicate 1-mL aliquots were used for each experimental group and centrifuged for 20 min at $12\,000\times g$ at $4\text{ }^{\circ}\text{C}$. Concentration of proteins in the supernatant was measured by the dye-protein binding method (Bradford, 1976) with a microplate spectrophotometer (VersaMaX, Molecular Devices, Sunnyvale, CA), using bovine serum albumin as the standard.

Foaming and Foam Stability

Foaming capacity and foam stability were measured by the Rudin method (Wilde and Clark, 1996), where triplicated 30-mL samples were placed in a 10 cm diameter plastic beaker and stirred for 1 min with a 3.5 cm diameter stainless steel plate agitator with a stirrer (LFE, Hamden, CT) at high speed and then poured into dry, 100-mL glass cylinders. Foaming capacity (%C) was measured immediately after agitation and foam stability (%S) was calculated after 1 h, using these equations:

$$\%C = (V_t/V_p) \times 100 \quad (1)$$

$$\%S = (V_f/V_o) \times 100, \quad (2)$$

where V_t is the total volume immediately after agitation, V_p the volume of 3% protein homogenate (30 mL), V_f the remaining foam volume 1 h after initial agitation and V_o the foam volume after agitation.

Gelling

Gels from mantle and fin were formed from 30 g of paste from the respective tissue. After thawing, the pastes were poured into 100-mL beakers and heated for 30 min at 90 °C in a reciprocal shaking bath (25 MR, Precision Scientific, Chicago, IL). Gels were stored at 0 °C in a bed of ground ice and refrigerated for 24 h. Texture profile analyses were performed to each gel with a texture analyzer (TA.XT2, Stable Micro Systems, Goldaming, Surrey, UK). The method has two cycles of compression of the samples, in which several rheological parameters are measured. We compared hardness and fracturability of six samples of squid mantle and fin. Gels were cut into 1 cm diameter, 1.5 cm height cylinders and pressed to 75% of their height. Results were analyzed with software (Texture ExpertTM, Stable Micro Systems) and expressed as hardness (N/g). The folding test was performed with round slides (3 cm diameter \times 3 mm thick) of the gels. The results are based on the 5-point scale (Suzuki, 1981): 5 = no break when folded into quadrants; 4 = no break when folded in half; 3 = gradually breaks at the first fold; 2 = breaks at first fold; and 1 = breaks when pressed by fingers.

Statistics

Data were analyzed by one-way ANOVA followed by Tukey's test of the means, using statistical software (Statgraphics Plus for Windows v. 5.0) to identify differences among treatments. For evaluation of collagen content, the non-parametric Kruskal–Wallis test was used because of the absence of normality and homogeneity of variances of data. Significant differences was set at $p < 0.05$.

RESULTS AND DISCUSSION

Structure of Muscle Fiber and Content of Connective Tissue

Muscle fibers in vertebrates and coleoid cephalopods have different structures because those in cephalopods are not supported by a rigid skeleton and are arranged in three mutually perpendicular layers that are specialized for a variety of movements and serve to generate force and provide support (Kier and Thompson, 2003). Microscopic images of frozen squid mantle and fin muscle fibers taken along the three axes were assembled to obtain 3D images (Figures 1a and 1b). Images of fresh muscle were also studied for comparison. Typical arrays of mantle and fin fibers had previously been described (Kier and Thompson, 2003). Mantle is mainly composed of circular fibers and radial fibers,

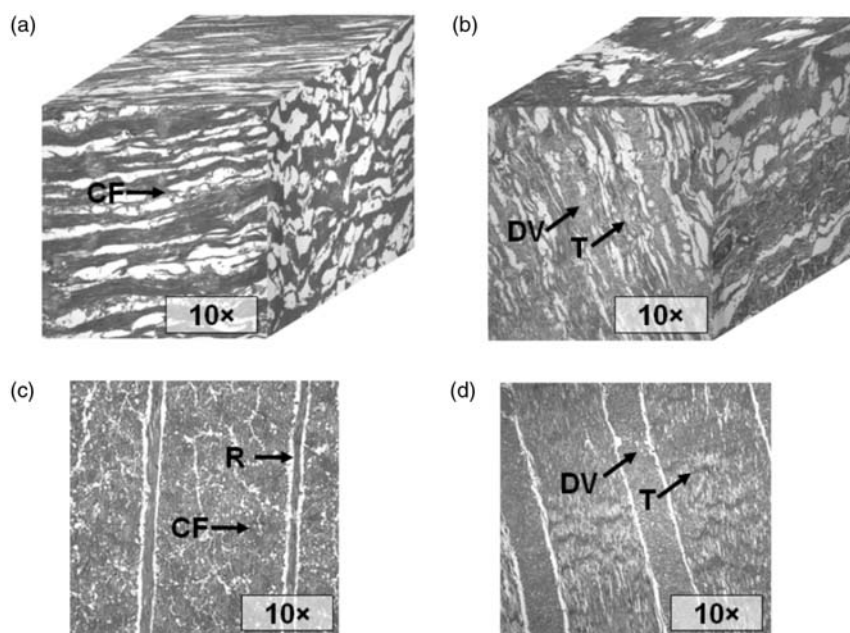


Figure 1. Muscle fibers of jumbo squid *D. gigas*. (a) frozen-thawed mantle; (b) frozen-thawed fin; (c) fresh mantle corresponding to the lateral view of frozen mantle; and (d) fresh fin corresponding to the frontal view of frozen fin. Note: CF, circular muscle fibers; R, radial fibers; T, transverse muscle; DV, dorsoventral muscle. Images were taken at 10 \times . Fresh tissue was sampled immediately after squids were caught.

which have distinct functions, such as respiration and jetting (Gosline et al., 1983). Only circular fibers were evident in frozen tissue; radial fibers were observed only in fresh samples (Figures 1a and 1c). As described by Kier and Thompson (2003), fins have a heterogeneous arrangement of tissues, consisting of a transverse muscle, dorsoventral muscle and several dimensions of connective tissue and cartilage (Figures 1b and 1d). Fins are designed to produce wavy movements that provide stability when squid swim at low speed, which requires a complex array in which myofibril proteins are attached to the connective tissue. Tissue-specific structural characteristics may contribute to the different uses observed in fin and mantle proteins.

Despite maintaining samples at low temperature after capture until freezing is completed, white or empty spaces were observed in mantle and fin samples, which probably were caused by contraction of muscle fibers and water loss. This was not observed in fresh muscle (Figures 1c and 1d). Because samples were kept at 0 °C all the time, we disregard autohydrolysis of the muscle fibers as an explanation for the empty spaces because protease activity at this temperature should be very low. Consequently, damage caused by freezing and thawing processes should not prevent functional properties of proteins; however, freezing and thawing could lead to toughness of raw muscle, as described by Ueng and Chow (1998). They found textural and histological changes of some squid muscle and damage from frozen storage related to increases of toughness.

Presence of stain reactions for collagen (Masson's trichromic staining) was quantified. The technique is based on the conversion of the image in color to an intensity level to enhance a determined dye (Otsu, 1979). The collagen fibers appear blue or green from aniline blue or light green staining. This method of quantifying collagen has been validated in many studies (Fernández et al., 2000; Cuaz-Perolin et al., 2006; Somoza et al., 2006). The Kruskal–Wallis test of collagen content demonstrated that blue-stained fin (10.84%) and mantle (7.35%) areas represent collagen (Sheehan and Hrapchak, 1973). Collagen plays an important role in thermal gelling properties of muscle (Tornberg, 2005); hence, these results are useful when discussing gel properties of squid proteins. As fin has a higher content of connective tissue and complex fiber arrangement, we expected higher hardness of gels.

Protein Fractions Determined by SDS-PAGE

Sarcoplasmic, myofibril and soluble-in-alkali protein fractions were measured by SDS-PAGE (Figure 2). Of special interest was the MHC band (M_r 220 kDa), which has a major role in applications for food (Paredi et al., 1996; Konno et al., 2002, 2003). MHC fibers were found in sarcoplasmic, myofibril and soluble-in-alkali fractions of mantle and only in sarcoplasmic and soluble-in-alkali

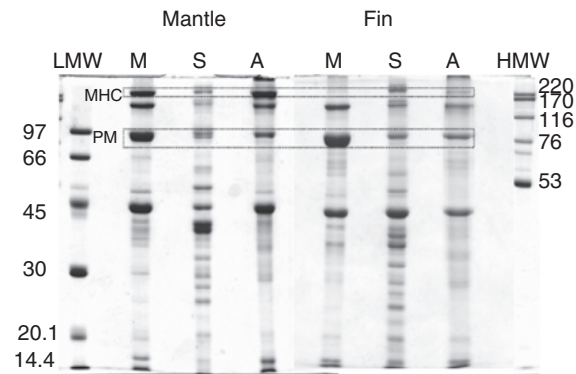


Figure 2. SDS-PAGE of protein fractions of jumbo squid *D. gigas* mantle and fin muscle.

Note: M, myofibril muscle fraction; S, sarcoplasmic muscle fraction; A, soluble in alkali muscle fraction; MHC, myosin heavy chain; PM, paramyosin; LMW, low molecular weight marker in kDa; HMW, high molecular weight marker in kDa obtained from Amersham Biosciences: 220 (myosin); 170 (α_2 -macroglobulin); 116 (β -galactosidase); 76 (transferrin); 53 (glutamic dehydrogenase); 97 (phosphorilase b); 66 (albumin); 45 (ovalbumin); 30 (carbonic anhydrase); 20.1 (trypsin inhibitor); and 14.4 (α -lactalbumin).

fractions of fin. The presence of this band in sarcoplasmic fractions indicates high solubility of squid myofibril proteins in low ionic strength buffer. This was observed in the mantle and fin sarcoplasmic protein fraction and is opposite to a general characteristic of MHC fibers, which commonly solubilizes at high ionic strength (Lin and Park, 1998). This phenomenon is of particular interest for processes in which washing in water is included, as in surimi manufacture. If water washing is applied to squid muscle for isolating myofibril proteins, the MHC fibers could be lost by solubilization. Surprisingly, only a light band of MHC fibers was found in the myofibril protein fraction of fin. Although this could result from cleavage of the molecule during processing, this hypothesis was rejected because the MHC fibers were present in the other two fractions from the same sample of fin (Figure 2). PM fibers has also been related to behavior of muscle protein in food systems because, it is known that its presence in cephalopods muscle confers them with unique properties (Fukuda et al., 2006). We found the PM band (M_r 97 kDa) in all fractions extracted, but mainly in the myofibrillar fraction, which is part of the pool of proteins extracted with high ionic strength buffer. Consequently, PM fibers could account for a part of the functional properties of muscle when salt is added.

Solubility

Additive effects of pH and salt on the solubility of mantle and fin protein were observed; protein from

mantle and fin performed in a similar way in solubility tests (Figure 3). The minimum solubility for both tissues occurred at pH 5–6 when no salt was added (Figure 3). At this mildly acidic pH, the average net charge of muscle protein mixture should be near zero; in this condition, proteins precipitate because there is no repulsion among molecules. Yongsawatdigul and Park (2004) found similar pH values for the lowest solubility point of rockfish muscle (*Sebastes paucispinis*) actomyosin.

As salt concentration increased, the pH of the lowest solubility point changed from pH 5 to pH 4 (when $i=0.2$ and 0.4). This phenomenon occurred in fin and muscle tissues (Figure 3). Borderías et al. (2003) reported pH at 4.5–5.0 for the highest precipitation of squid muscle protein at 0.5 M salt concentration. This response could be attributed to the negative charge provided by the chloride ion, which neutralizes the positive charges in the media at acid pH. A similar phenomenon occurred in rockfish treated under low and high levels of pH (Kim et al., 2005). In conditions of increasing salt concentration, chloride ions could impart negative charge to proteins surface; then it is necessary to add more protons to provide a net near-zero charge to the proteins. A significant increase in protein solubility occurred when the pH was shifted from the observed isoelectric point for any value of the ionic strengths tested (Figure 3). Yongsawatdigul and Park (2004) had reported this effect in fish muscles, attributing it to the charges imparted by the media to the surface of the proteins.

The combined effect of pH and ionic strength demonstrated that some salt concentrations lead to proteins being *salted in* or *salted out*, depending on the pH of

the medium. At near-neutral pH (pH 6–8), solubility of mantle and fin proteins increased significantly when the salt concentration was increased to 0.2 and 0.4 M (Figure 3). Under these conditions, chloride ions from the salt bind to the protein surface, conferring a net negative charge and causing repulsion among molecules. At extreme pH (2 or 12), solubility of proteins significantly decreased when the salt concentration was increased (Figure 3). Kim et al. (2005) reported similar results for solubility of rockfish sarcoplasmic protein when salt was added at low pH. Thawornchinsombut and Park (2005) described similar protein solubility of Pacific whiting muscle, with increased solubility with salt concentration at pH 7 and 10 and decreased solubility with salt concentration at pH 4. These reactions result from the net positive charge on the protein surface that bind negative chloride ions from the salt, resulting in a net charge approaching zero as salt is added and causing precipitation of proteins. The effect of salt concentration on solubility of protein from diverse sources has been described by several authors (Wagner and Añon, 1990; Anton and Gandemer, 1997; Huda et al., 2001; Kristinsson and Hultin, 2003; Ramos-Clamont et al., 2003). Our results with jumbo squid proteins agree with these studies. An interesting phenomenon occurs at pH 5 in both tissues. At this pH, protein interaction with water is low because no effect of salt concentration is observed on protein solubility.

Although the solubility curves of both tissues were of similar shape, as the variables were applied, some significant differences in solubility of mantle and fin occurs. At pH 8 and 12, mantle protein had higher solubility (Figure 3a) than fin protein (Figure 3b). The effect was more evident at salt concentrations of 0.2 and 0.4 M. Ruiz-Capillas et al. (2003) found similar results when studying solubility of mantle and arms of some cephalopods.

From our data, fin and mantle of squid could be included in many processes to improve functional or nutritional factors to take advantage of their solubility properties. These data showed how squid protein solubility is affected by operational conditions, such as pH and salt concentration; therefore, it is necessary to identify the desired final conditions of salt and acidity on protein properties to select the best protein performance in the final product.

Foaming and Foam Stability

Solubility is a requisite for useful properties of proteins (Damodaran, 1996); for example, a homogenate with highly soluble protein is expected to have high foaming capacity. The effects of pH under different ionic strengths on the foaming capacity of squid mantle and fin proteins are shown in Figures 4a and 4b. The minimum foaming capacity for mantle proteins (Figure 4a) occurred at pH 6, the lowest solubility

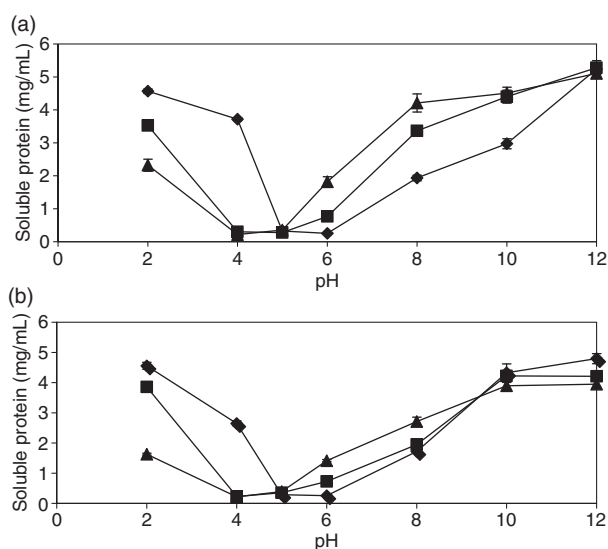


Figure 3. Effect of pH on solubility of jumbo squid *D. gigas*. (a) mantle and (b) fin protein (addition of NaCl at (◆) 0 M; (■) 0.2 M; and (▲) 0.4 M). Note: Values given are means of triplicate analyses.

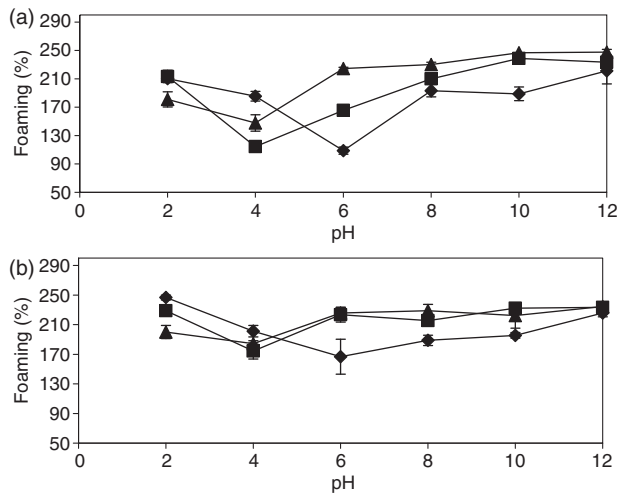


Figure 4. Effect of pH on foaming of jumbo squid *D. gigas* in (a) mantle protein and (b) fin protein at different ionic strengths (addition of NaCl at (◆) 0 M; (■) 0.2 M; and (▲) 0.4 M). Note: Values given are means of triplicate analyses.

(Figure 3a), when no salt was added to the system. When salt was added, significant increases in foam capacity occurred at pH 6. The lowest foaming occurred at pH 4, when $i=0.2$ and 0.4 (Figura 4a). For fin proteins, the lowest foaming occurred under the same conditions: pH 6 with salt concentrations of 0 and pH 4 when salt concentration were 0.2 and 0.4 M. Fin proteins always showed significantly more foaming than mantle under these conditions (Figures 4a and 4b). At 0 M NaCl, the greatest foaming occurred at pH 2 and 12. Increasing salt concentrations caused decreases in foaming at these extreme pH values, except for fin protein at pH 12, where salt concentration had no significant effect on foaming. Foaming is also an indirect way to understand structural features of proteins. This property is directly related to hydrophobicity of the protein surface. Concentrations of salt and pH levels can affect the net charge of proteins surface, so that these variables resulted in strong changes in foaming.

Foaming capacity were similar for fin and mantle at alkaline pH. With SDS-PAGE, we showed that MHC and PM are highly soluble under alkaline conditions (Figure 2) improving foaming capacity at alkaline pH. Both tissues could be used for foam-forming purposes in food products. At acidic pH, except at pH 6 when $i=0.4$, fin protein had significantly better foaming capacity, (Figures 4a and 4b). This is useful information when working conditions need to be acidic and high foaming capacity is required.

Stability of foam depends on different characteristics than those needed to form foam. Results are presented in Tables 1 and 2. Stability of 100% was achieved at alkaline pH with ionic strength at 0, 0.2 and 0.4.

Table 1. Effect of pH and ionic strength on foam stability of jumbo squid *D. gigas* mantle proteins.

pH	Addition of NaCl		
	0	0.2 M	0.4 M
2	75.68±4.73 b, Y	85.89±1.63 b, X	71.74±1.83 b, Y
4	80.12±6.54 b, X	78.23±5.78 bc, X	71.31±2.13 b, X
6	71.57±4.85 b, Y	53.64±1.99 d, Z	100±0.00 a, X
8	75.12±4.52 b, Y	100±0.00 a, X	100±0.00 a, X
10	98.33±2.35 a, X	100±0.00 a, X	100±0.00 a, X
12	73.60±1.27 b, X	72.06±1.06 c, X	75.3±1.37 b, X

Values are means of triplicate analyses. Small letters in the same column indicate significant differences ($p < 0.05$). Capital letters in the same row indicate significant differences ($p < 0.05$).

Table 2. Effect of pH and ionic strength on foam stability of jumbo squid *D. gigas* fin proteins.

pH	Addition of NaCl		
	0 M	0.2 M	0.4 M
2	73.87±0.69 bc, Y	88.057±3.27 b, X	72.77±0.25 cd, Y
4	77.86±1.26 bc, X	73.85±1.00 c, Y	75.90±0.72 bc, X, Y
6	60.91±0.90 c, Y	88.26±6.89 b, X	100±0.00 a, X
8	89.78±12.13 ab, XY	100±0.00 a, X	77.19±0.90 b, Y
10	100.00±0.00 a, X	98.56±0.02 ab, X	71.42±2.35 d, Y
12	71.96±1.93 c, X	72.47±1.06 c, X	75.39±0.56 bc, X

Values are means of triplicate analyses. Small letters in the same column indicate significant differences ($p < 0.05$). Capital letters in the same row indicate significant differences ($p < 0.05$).

For extreme alkali conditions, such as pH 12, stability decreased at all ionic strengths. Minimum stability occurred at pH 6 for mantle when salt concentrations was 0.2 and for fin at 0. Depending on the preferred characteristics of the final product, conditions that enhance or reduce foam must be understood.

Hardness and Fracturability of Gels

Gels were formed from frozen-stored homogenates of squid mantle and fin. Hardness values of gels from squid mantle and fin were 3.33 and 5.82 N/g gel, respectively. This difference was confirmed statistically. The greater quantity of connective tissue in fin is probably the basis for these results. Tornberg (2005) states that the linking properties of connective tissue play an important role in thermal behavior of proteins. Although the effect of storage on gelling properties was not measured, others report that protein properties may change during freezing or cold storage, which can enhance or reduce gelling capacity (Ueng and Chow, 1998; Kagawa et al., 2002; Benjakul et al., 2005; Ganesh et al., 2006). Our squid samples stayed frozen for 11 months. Previous investigations found higher water-holding capacity of gels

after storage at $-30\text{ }^{\circ}\text{C}$ (de la Fuente-Betancourt et al., 2009). Hence, under the frozen condition of our samples, we suggest that this contributed to the observed results.

Texture profile analysis also provided results on fracturability of gels. Fracturability is a parameter that simulates biting. Fracture strength was significantly higher for fin gels than mantle gels (1095 g_f vs. 541 g_f). These results are higher than previously reported data, where gelling additives were used (Park et al., 2005). In the folding test, both tissues performed at the highest level, folding into quarters without breaks (grade 5). This result is of special value, considering that no gelling additives or salt were added to the minced squid meat. This suggests that the gel is sufficiently rigid for a wide range of uses. During the gelling process, the pH was not shifted and the highest grade of gelling was even achieved at pH 6.3, when low solubility could hinder gelling capacity. Gómez-Guillén and Montero (1997) used gelling additives, but the results were less than grade 5. Low-quality gel-forming capacity of squid muscle were reported by Gómez-Guillén and Montero (1997), Konno et al. (2002, 2003) and Park et al. (2005), but these reports did not provide information about post-capture conditions. Our samples were obtained at the fishing ground and maintained at $0\text{ }^{\circ}\text{C}$ until reaching our laboratory. Incorrect post-capture practices may lead to increased endogenous enzymatic activity that reduces gel-forming capacity. Borderías et al. (2003), describing a method for obtaining squid protein concentrate, also found that temperature is a crucial factor for stopping lysis of protein. We believe that although muscle fiber integrity after freezing, frozen storage and thawing is not optimal (Figure 1), our acceptable results are attributable to post-capture conditions of storage ($0\text{ }^{\circ}\text{C}$ until freezing), which prevented proteolytic activity and maintained the structure of the useful MHC and PM molecules.

CONCLUSIONS

Solubility property of squid proteins was an important prerequisite for foam formation. Variable conditions of pH and concentration of salt in squid mantle and fin proteins induced changes in the protein-surface net charge that resulted in wide ranges of high and low solubility and foaming properties. High-strength gels can be formed from squid mantle or fin muscle. Fin displayed similar or better properties than mantle in all tests. We conclude that: (1) squid fin can be incorporated into squid-based food products and (2) appropriate post-capture handling helped to maintain useful properties of the muscle proteins, especially MHC proteins.

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