PROTEIN SOLUBILITY AND PRODUCTION OF GELS FROM JUMBO SQUID

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ABSTRACT

Solubility at several ionic strengths (0 to 1.0 M), pH (2 to 13) and gelling capacity of jumbo squid Dosidicus gigas *muscle proteins were evaluated. Protein recovery was >90% at pH 9–12. Autohydrolysis was evaluated and affected only sarcoplasmic proteins. Folding score was 5 on all gels. Strength was higher for thermal gels prepared from squid fin (50.2 ± 1.2 N) than that prepared from the mantle (23.4 ± 2.5 N). There was no significant difference in gel strengths from previously frozen (46.4 ± 7.5 N) and never-frozen samples (43 ± 5.5 N). Moisture, water drip and water-holding capacity were evaluated on thermal gels. There were significant differences between frozen and never-frozen samples. Results on solubility and gel forming capacity of the proteins from mantle and fin of jumbo squid suggest that these properties can provide additional value to this resource.*

PRACTICAL APPLICATIONS

Jumbo squid is a rich source of high-quality protein. Obtaining sophisticated products would be an easy way to take advantage of the high-quality proteins in jumbo squid muscle. Knowledge of the functional properties of those proteins is needed to achieve this. The use of such properties, such as gel-forming capacity, which are similar to other proteins used in the food

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industry, will give jumbo squid added value. The results described here indicate that jumbo squid proteins may be useful as a food ingredient because of their solubility and gel-forming capacity.

INTRODUCTION

The growing worldwide demand for marine protein supplies has led to the exploration of alternative sources of raw material. Research has focused on by-catch species or under-utilized marine resources. The jumbo squid (*Dosidicus gigas*) is a low-valued resource that is barely processed in Mexico and mostly exported as raw material to Asian countries (SEMARNAP 2000). Investigations related to understanding the characteristics of the squid and its protein is necessary to develop methods for protein recovery and use, hoping to make the squid fishery an economically attractive activity in Mexico. Fractions of protein from the squid mantle muscle differ from those of marine vertebrates. The stromal fraction, composed mainly of connective tissue, represents ~11% of all proteins, whereas fish muscle reaches ~2%. These differences are mostly related to the physiological needs of the jumbo squid, such as the high, energy-demanding movements needed for locomotion (Macgillivray *et al.* 1999; Kier and Curtin 2002; Kier and Thompson 2003).

Proteins of the sarcoplasmic fraction of the squid muscle represent about 15% of all proteins, and this fraction contains endogenous proteolytic activity responsible for a high rate of autohydrolysis (Sikorski and Kolodziejska 1986; Ezquerra-Brauer *et al.* 2002; Gómez-Guillén *et al.* 2003). The myofibrillar fraction constitutes ~75–85% of the proteins from squid mantle; in this fraction, myosin (220 kDa) is the most important protein, followed by actin (45 kDa) and paramyosin (111 kDa). Squid myosin has low stability and it is very sensitive to enzymatic activity (Konno *et al.* 2003). Paramyosin is particularly common in marine invertebrates and can represent 25% of myofibrillar proteins (Sikorski and Kolodziejska 1986).

Endogenous enzymatic activity has been demonstrated in mantle of several squid species (*Loligo vulgaris, Illex coindetii, Toradores eblanae* and *D. gigas*). This proteolytic activity promotes lose of texture and protein functionality during storage or processing (Ayensa *et al.* 2002; Gómez-Guillén *et al.* 2002; Ruiz-Capillas *et al.* 2003). Several enzymes (carboxydase, aminopeptidase, trypsin, chymotrypsin-like enzyme and azocaseinolytic activity) were found in jumbo squid muscle and fluctuate with season or age of the squid (Ezquerra-Brauer *et al.* 2002).

Autohydrolysis processes could be used to recover soluble protein from squid byproduct hydrolysates that were a good source of aquaculture feed ingredient, especially as a starter diet for fish larvae (Lian *et al.* 2005). Poor

functional properties caused by endogenous proteolytic activity have restricted the usefulness of jumbo squid as a food ingredient (Gómez-Guillén *et al.* 2003). If enzymatic activity is not controlled, most of the protein in muscle tissues will be degraded to small peptides that do not have the functional properties needed to be useful as food ingredients. A process should be designed to eliminate endogenous proteolytic activity; this could be achieved by differences in solubility of each protein fraction.

Solubility of the proteins is affected by pH and ionic strength, and for jumbo squid, it must be tested to propose any process of protein recovery or removal of undesirable components. Changes in pH may affect other functional properties when proposing conditions that improve a process or final product. For fish muscle proteins, adding acid or alkali improves its emulsifying properties, which are mainly correlated with an increase in surface hydrophobicity and surface-interfacial activity (Kristinsson and Hultin 2003). In other cases, protein fractionation is achieved by changes its ionic strength (Hashimoto *et al.* 1979). Likewise, a protein treatment using both methods could be a suitable option for improving functional properties (Sánchez-Alonso *et al.* 2007).

This study evaluated pH and ionic strength for recovery of protein from jumbo squid muscle by measuring endogenous enzymatic proteolysis under different conditions for preparing gel from squid mantle, fin or both that had been frozen or were never frozen.

MATERIALS AND METHODS

Squid Samples

Jumbo squid specimens with an average mantle length of 50 cm were collected at Santa Rosalía, Baja California Sur, Mexico. Specimens were killed in iced seawater on the fishing vessel and kept in an ice bath until docking. After docking, the mantle was separated, eviscerated, washed with freshwater and wrapped in plastic bags. The mantles were kept between beds of ice and transported to the laboratory within 16 h after the catch. At the laboratory, mantles were stored (−30C) until needed or kept between beds of ice (0C), depending on the requirements of the experiment.

Sample Preparation

Squid batter was prepared from a pool of mantle pieces from several individuals, after removing the skin and fins, by placing small, cut pieces from thawed mantle in a kitchen blender and processed for 30 s at high speed. During preparation, temperature was kept at 2–4C. The batter was used to prepare different squid homogenates by adding distilled cold water or one of several NaCl solutions.

Chemical Composition of Squid Mantle

Protein, moisture, ash and lipids of the batter were determined according to standard methods (AOAC International 1995). An aliquot of the batter was freeze-dried and the powder was used for preparing the amino acid profile according to methods described in Vázquez-Ortiz *et al.* (1995).

Protein Recovery via Ionic Strength

Aliquots of 1 g of the batter were used to prepare a squid homogenate by adding either distilled water or NaCl solutions at different ionic strengths to a volume of 10 mL. The ionic strengths were: 0, 0.2, 0.4, 0.6, 0.8 and 1.0 M. Each aliquot was stirred for 3 min; placed in an ice bath and stirred for 30 min, then centrifuged at 12,000× g for 20 min at 4C. The supernatants were separated and kept at 0C for further experiments. The sediments were suspended in 1 mL NaCl solution at the same concentration used in the previous step, vigorously stirred (with a vortex) for 2 min and centrifuged at 12,000× g for 20 min at 4C. Both supernatants were pooled. The final sediment was suspended in distilled water (1:1). Protein content of the supernatants and the suspended final sediments were first determined by the Lowry method (Lowry *et al.* 1951) and then analyzed by SDS-PAGE (Laemmli 1970).

Protein Recovery by pH Treatment

This experiment was conducted in two steps. As a first approach, a titration curve was obtained by adding 1 N HCl or 1 N NaOH to a new squid homogenate containing 1% protein (based on total protein quantification by the microKjeldahl method (AOAC International 1995). The second experiment was conducted with another squid homogenate containing a protein concentration of 3% (based on total protein quantification by the microKjeldahl method). This homogenate was divided into 13 portions and the pH of 12 of them was adjusted to different values (pH 2 to 13), using 1 N NaOH or 1 N HCl. The remaining portion was kept at the natural pH of squid homogenate (5.9). Each pH-treated portion was incubated at 3C under continuous stirring. Aliquots from each pH treatment were taken after 10 min incubation and centrifuged at 12,000× g for 20 min at 4C. The protein content of the supernatant of each sample was determined by the Lowry method (Lowry *et al.* 1951) and their soluble protein was analyzed by electrophoresis.

Endogenous Enzyme Activity

A squid homogenate was prepared by separately mixing batter with several buffer solutions in 1/10 ratios: 50 mM Tris-HCl at pH 7.5; 50 mM sodium phosphate at pH 7.5 and 0.1 M citric acid at pH 6.5. The proteolytic activity of

each homogenate was determined by the method described by Navarrete del Toro and García-Carreño (2002), with slight modifications by using 0.5% azocasein in Tris-HCl 50 mM at pH 7.5 as the substrate. The reaction was conducted at 25C for 10 min. Trichloroacetic acid (TCA) was added to a final concentration of 5% to stop the reaction. The sample was incubated for 10 min at 0C, centrifuged at $10,000\times$ g for 5 min at room temperature and the light absorbance was read at 366 nm using a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA). One activity unit was defined as the change in absorbance at 366 nm/minute/mg protein in the enzyme solution.

Autohydrolysis

Squid homogenate was prepared with squid batter, as described previously, by adding distilled water to achieve 3% protein content, based on total protein quantification by the microKjeldahl method (AOAC International 1995). Endogenous proteolysis was measured at 25, 40 and 50C, and at pH 5.9 (natural pH) and pH 8 (adjusted with 1 N NaOH). Each treatment of squid homogenate was incubated 6 h and sampled every hour. By SDS-PAGE, these samples were followed to assay autohydrolysis. Each sample was divided into two portions. One was directly mixed with loading buffer, boiled for 5 min and injected into the PAGE wells. The other was centrifuged at $5,000\times$ g for 10 min; the supernatant was mixed with loading buffer and boiled for 5 min and placed in the gel for quantification.

SDS-PAGE

Samples of squid protein from the assays were analyzed by SDS-PAGE (Laemmli 1970). Samples containing 50 µg protein were mixed with a load buffer containing dithiothreitol as the reducing reagent (1:1). Samples were heated for 5 min in a boiling water bath and then analyzed in a vertical electrophoresis unit (model SE 260, Hoefer, San Francisco, CA), using a homogeneous 10% polyacrylamide gel for separating protein bands. SDS-PAGE gradient gels (7.5 to 15%) were used, when indicated under a constant current (13 mA per gel). The gels were stained with a solution containing 40% methanol, 7% acetic acid and 0.5% Coomassie Brilliant Blue R-250 to reveal the bands. Calibration kits 17-0615-01 (for 14 to 97 kDa) and 17-0446-01 (for 53 to 220 kDa) were used (GE Healthcare Bio-Sciences, Piscataway, NJ). After 2 h, excess stains were removed with a solution containing 40% methanol and 7% acetic acid.

Texture Parameters of Gels from Squid Mantle and Fin

Several sols were prepared by manually mixing 2.5% NaCl (w/w to a final concentration of 0.43 M) to batters prepared from mantle and fin, with

TABLE 1.
SAMPLES USED FOR EACH THERMAL GELATION ANALYSIS

Storage	TPA	Folding test	Moisture, WHC, % drip	Color
Never frozen (fresh)	M	M	M	M
	Fs	F	F	Fs
	M-Fs	Fs	Fs	M-Fs
		M-Fs	M-Fs	
Frozen, thawed	M-Fs	M	M	n. d.
		F	F	
		Fs	Fs	
		M-Fs	M-Fs	

M, skinned mantle; F, skinned fin; Fs, fin with skin; M-Fs, mixture of skinned mantle and fin with skin; n. d., not evaluated.

and without skin, and from thawed and never-frozen samples, as indicated in Table 1. Never-frozen samples from recent catches were labeled as fresh. In Table 1, abbreviations of the tissue types used to prepare gels are described and are used in the text. No adjustment to moisture content was done to any sol. Aliquots of 40 g sol were placed in 50-mL beakers (~35 mm inner diameter), which were placed inside polyethylene bags, vacuum-sealed and stored at 0C until used. Sols were incubated for 30 min at 90C, followed by overnight storage in an ice bath (Dondero *et al.* 2002). Gels prepared in this manner were used for texture profile analysis (TPA), folding test, moisture content, water-holding capacity and water-loss-by-drip; color was also recorded. Methods of evaluation are described as follows.

A double-bite TPA was performed with a universal testing machine (model TMS-PRO, Food Technology, Sterling, VA) as described by Bourne (1978), with slight modifications. A system of two flat plates (4.4 in. by 4.3 in.; load of 1,000 N) was used to compress standard-sized pieces of squid gels (2.8 cm high × 3 cm diameter) to 25% of its original height (75% compression). For TPA, a force versus time (or distance) plot was generated that can be related to sensations during biting and chewing. Attributes of texture, such as fracturability (force necessary to break the gel), hardness (force necessary for compressing the gel to represent bite force), elasticity (capacity of the gel to recover from the first bite) and cohesiveness (gel integrity after a first and second compression) were evaluated. Four replicates were analyzed.

The folding test was performed on two round slices of gel (30 mm diameter × 3 mm thick) using a 5-point scale (Suzuki 1981 in Ayensa *et al.* 2002), where:

5: gel that does not break when folded in quadrants,
4: no breakage occurs when folded in half,

3: gel gradually breaks at the first fold,

2: break occurs at the first fold, and

1: gel disrupts when gently pressed with finger.

To determine moisture content, the gels were cut into small pieces and placed on a thermal balance (Precisa XM-60, Dieticon, Switzerland) set at 95C. This condition was maintained until the weight was constant. Moisture content was calculated as the difference between the original and the final weight.

To determine the water-holding capacity, a slice of gel was weighted and placed between two sheets of filter paper. A centrifuge tube was filled with cotton in the bottom, the gel slice placed between the paper filters was placed in the tube, and the tube was completely filled with cotton and centrifuged at $11,000 \times$ g for 30 min. After that, the centrifuged gel slice was weighted. The water-holding capacity was calculated as the difference between the original and the final weight.

To determine the water drip, measurement was made in a procedure similar to thaw drip measurements (Santos and Regenstein 1990, in Turan *et al.* 2003). The sol and the resulting gel were weighed. Differences were assumed to be water lost by dripping during processing according to the formula: % water-loss-by-drip = ([weight of sol − weight of gel]/weight of sol) × 100.

Color measurements of lightness (L^*), red-green (a^*), and yellow-blue (b^*) of gels were measured with the CIELab scale (Young and Whittle 1985; Park 1995). Measurements were done with a colorimeter (Minolta CR400, Ramsey, NJ) and standardized with respect to the white calibration plate.

Statistical Analysis

One-way ANOVA coupled with Tukey's honestly significant difference procedure was used in all experiments to determine significant differences among treatments (Statgraphics Plus for Windows v. 5.0). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Chemical Composition

Chemical composition of squid mantle batter is shown in Table 2; values are given on wet weight basis. Squid mantle is a marine resource with similar protein content (15–20%) to some fish (Huss 1995); its low lipid content (<1.0%) is desirable for meeting the demand for low-fat food products.

TABLE 2.
CHEMICAL COMPOSITION OF JUMBO SQUID MANTLE

Component	% in mantle*
Cal/100 g†	5251.5 ± 0.33
Moisture	81.5 ± 0.06
Protein	14.95 ± 0.07
Ash	1.26 ± 0.028
Lipids	0.18 ± 0.001

* Mean of four determinations ± SD.
† Dry weight basis.

Amino Acid Composition

Whole mantle proteins contain all of the essential amino acids for humans (FAO/WHO/UNU 1985; Córdova-Murueta and García-Carreño 2002), except tryptophan, which was not evaluated in the present study. Essential amino acids in protein of jumbo squid (bulk and water-soluble) are compared with the essential amino acids content of casein and recommendations of FAO/WHO. According to the recommendations, whole squid mantle is low in phenylalanine. Squid whole protein has a lower content of essential amino acids than casein, although the content of isoleucine, leucine, lysine, methionine and valine are similar in casein and whole squid. Essential amino acids in water-soluble squid proteins are lower than bulk proteins; however, by changing the pH or via ionic strength, a higher proportion of essential amino acids could be recovered in soluble form. For human nutrition, jumbo squid proteins could practically satisfy amino acid requirements, except for phenylalanine.

Protein Solubility via Ionic Strength

Mantle proteins solubilized at different ionic strengths were displayed in electrophoresis gel (Fig. 1a). Squid myosin could be soluble in low ionic strength solutions (Sikorski and Kolodziejska 1986), as shown in the electrophoresis profile; nonetheless, it appeared as a very low intensity protein band and is barely visible, compared with the nonsoluble fraction. Nonsoluble proteins suspended in water are shown in Fig. 1b. After protein extraction in a 1.0-ionic strength solution, myofibrillar proteins remained nonsoluble, suggesting that another homogenizing step is needed to completely extract all myofibrillar proteins. When extracting proteins at the ionic strength of 1.0, three phases were formed after centrifugation: (1) the supernatant contained soluble protein; (2) the sediment contained nonsoluble protein; and (3) a middle fraction showed the same protein profile as the soluble fraction, but with a viscosity that was notably higher (not measured) and had the appear-
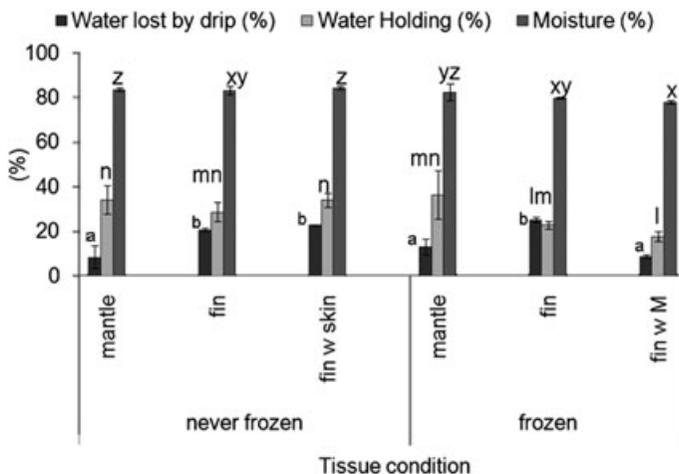
FIG. 1. EFFECT OF IONIC STRENGTH ON PROTEIN SOLUBILIZATION PROFILE
Numbers below lanes represent the ionic strength used for (a) soluble proteins and (b) nonsoluble
proteins. Lane numbers indicate ionic strength. Myosin = M; actin = A; and paramyosin = P.

ance of a gel or a colloidal system (Fig. 1a, Lane 1*). This fraction contained
the highest myofibrillar proteins. In procedures for protein separation by using
ionic strength, such as proposed by Hashimoto *et al.* (1979), the recommen-
dation is to use low (0.05) ionic strength buffer solutions to solubilize the
sarcoplasmic protein fraction, followed by extraction with higher (0.5 M) ionic
strength solutions to solubilize the myofibrillar fraction. As demonstrated here,
jumbo squid proteins can be easily separated by solubilizing sarcoplasmic
proteins with distilled water, whereas myofibrillar proteins can be easily
recovered in a suspension (not solution) with water.

Protein Solubility Using pH

To calculate a global isoelectric point involving all proteins present, a
solubility curve (pH versus soluble protein) was constructed (Fig. 2), and a
global isoelectric point was observed at pH 5–6. As can be observed in the
solubility curve and in the soluble protein profile (Fig. 3), at both pH 5 and 6,
the lowest solubility is achieved; knowledge of this will be useful in further
applications, such as protein recovery. The data for this curve can be comple-
mented with the soluble protein profile observed at other pH values (Fig. 3) to
suggest possible future uses of squid protein, for example, as an ingredient in
nutritive beverages. Protein fractions recovery can be improved with knowl-
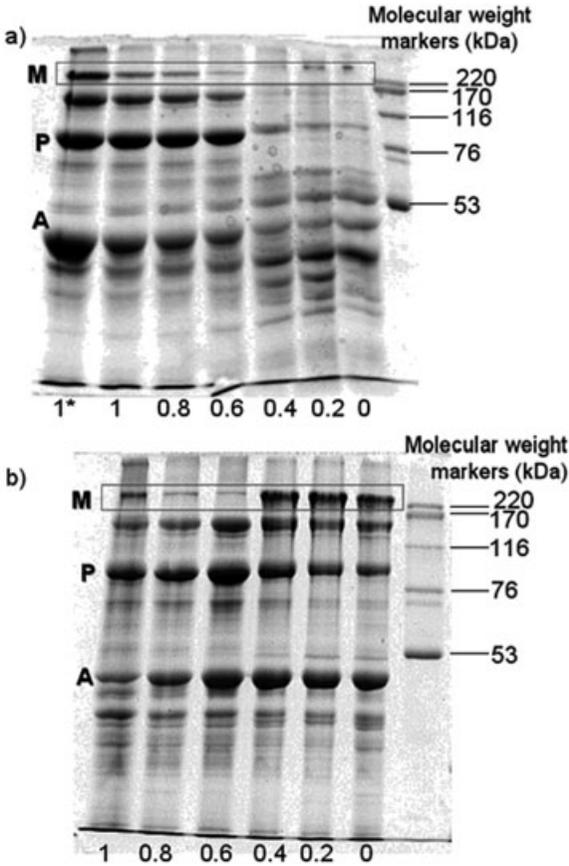edge of protein solubility at various pH values. Myosin is soluble at pH > 10

FIG. 2. SOLUBILITY CURVE AFFECTED BY pH
Values represent the mean of at least three replicates; whisker lines indicate SD and different letters
indicate significant differences at $P < 0.05$.

(Fig. 3), whereas paramyosin and actin are extracted at pH 8–9. Then, at alkaline pH, myofibrillar proteins may be separated for further use. An application of this knowledge for protein recovery is to shift the pH to separate specific protein fractions, which can be used to remove the distinctive acid-bitter flavor of squid muscle (Sánchez-Brambilia *et al.* 2004).

Endogenous Enzyme Activity

Endogenous proteolytic activity was 0.204, 0.315 and 0.317 AU/g mantle for phosphate, Tris-HCl and McIlvaine buffers, respectively. These buffers
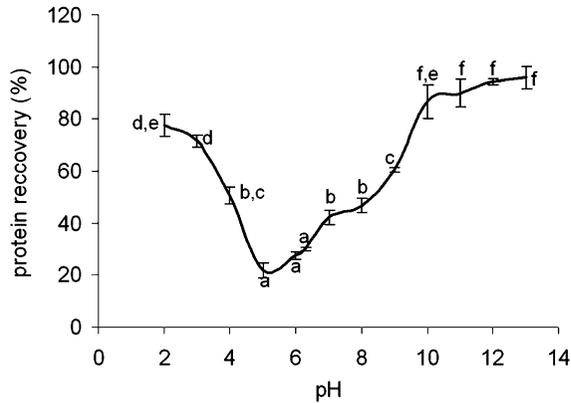
FIG. 3. PROTEINS SOLUBILIZED AT SEVERAL pH VALUES, SDS-PAGE (10%)
Lane numbers indicate pH. Myosin = M; actin = A; and paramyosin = P.

were selected, as reported before by other authors (Ayensa *et al.* 2002; Ezquerra-Brauer *et al.* 2002; Konno *et al.* 2002). Ezquerra-Brauer *et al.* (2002) used phosphate buffer to obtain similar values of enzyme activity in jumbo squid mantle. Using phosphate, Tris-HCl, and McIlvaine buffers, proteolytic activity was low in comparison with similar experiments in the pancreas where proteolytic activity of 3.268 (phosphate buffer) and 6.446 (McIlvaine buffer) AU/g tissue occurred (data not shown).

Squid proteins showed no noticeable changes after incubating at the different temperatures and pH treatments. We only present the electrophoretic results of samples incubated at pH 5.9 at 50C (Fig. 4). After incubation for 6 h, squid proteins showed low rates of autohydrolysis in all treatments. In previous reports, considerable changes in the protein profile had been demonstrated from endogenous enzymatic hydrolysis. Those reports did not mention whether the samples were taken or incubated with stirring, so the homogeneity of the sample is not clear. We assumed that the homogeneity of the aliquot could affect the electrophoresis profile. To demonstrate this, we took samples during continuous stirring to measure bulk proteins and then the supernatant after centrifugation of the sample to obtain the water-soluble proteins. The bulk protein profile observed in Fig. 4a had no noticeable changes, which was unusual especially for myosin, as reported elsewhere, as an unstable protein susceptible to enzyme activity, which is present in muscle tissues (Konno *et al.* 2003). When the water-soluble fraction of the proteins was analyzed, a change occurred within the first 2 h of incubation (Fig. 4b). These results demonstrated that only the sarcoplasmic fraction was affected by the endogenous proteolytic activity.
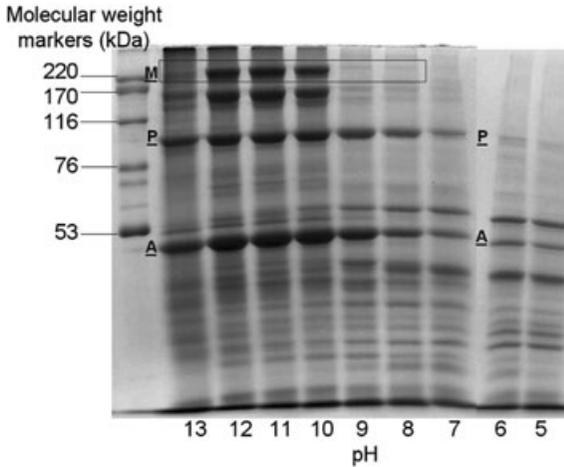
FIG. 4. ENDOGENOUS PROTEOLYSIS AT pH 5.9, 50C FOR UP TO 6 H
(A) = Squid bulk; (B) = Sarcoplasmic proteins, SDS-PAGE (7.5–15%). Numbers in lanes indicate
time of incubation. Myosin = M, actin = A and paramyosin = P.

Gel-Texture Parameters

The texture profile parameters for the double compression cycle of thermal gels, under several tissue conditions and combinations, are shown in Fig. 5. In general, jumbo squid proteins have high gelling capacity. This characteristic was noted in previous work (de la Fuente-Betancourt *et al.* 2008), where squid gels prepared with a mixture of M-Fs from never-frozen organisms, had a higher folding test score (5) and higher TPA parameters than previously reported (Gómez-Guillén *et al.* 1997, 2002, 2003).

Jumbo squid is a low-cost resource, where postmortem handling includes storage of the mantle by freezing. We had assumed that the use of frozen squid was the basis for low gelling capacity reported by others (Gómez-Guillén *et al.* 1997, 2002). In this study, we used samples frozen for approximately 1 year. They had similar gel strength as fresh samples. As described in the Materials and Methods section, the handling of the specimens after the catch was conducted to maintain maximum freshness.

Gels Containing Squid Fin

Gels containing fin muscle showed higher gel strength than gels made from mantle (Fig. 5). The highest gel strength occurs when squid fin is present, which is probably caused by the high content of collagen in the connective tissue (Sikorski and Kolodziejska 1986). This composition could act as a
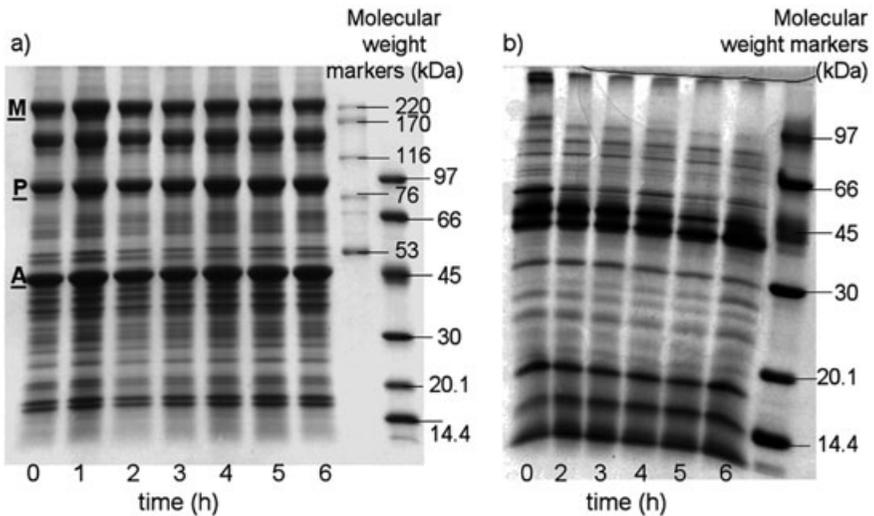
FIG. 5. TEXTURE PROFILE PARAMETERS FOR DOUBLE CYCLE COMPRESSION, WHERE
HARDNESS CORRESPONDS TO THE FIRST COMPRESSION
Values represent the mean of at least three replicates; whisker lines indicate SD; different letters
indicate significant differences at $P < 0.05$.

support for protein aggregation. The highest gel strength observed in fresh
samples was from gels made from fins mixed with mantle, compared with gels
prepared exclusively from mantle tissue (Fig. 5). Currently, squid fin is fre-
quently discarded. As demonstrated here, fin in gels is desirable because it
yields two benefits: improvement of the product and reduction of wastes. The
results show that squid fin improves the qualities of gel when mixed with
mantle.

Gel Processing

Gels prepared in this study provided better texture parameters than pre-
viously reported (Gómez-Guillén *et al.* 1997, 2002), as mentioned previously.
That difference may be an effect of the thermal change rate during gel pro-
cessing, which we considered a critical issue. Traditional assay methods for
thermal gelling (Gómez-Guillén *et al.* 1997, 2002) involved a gradual tem-
perature change (1C/min); here a rapid thermal increase was made by trans-
ferring the batter from an ice bath (~0C) to a hot water bath (90C). This
process was used because during experimental work, we observed jumbo
squid proteins aggregating quickly when temperature increased quickly.
However, this was not quantified, which will require experiments to measure
the effect of the rate of thermal change on gel-forming capacity to confirm this
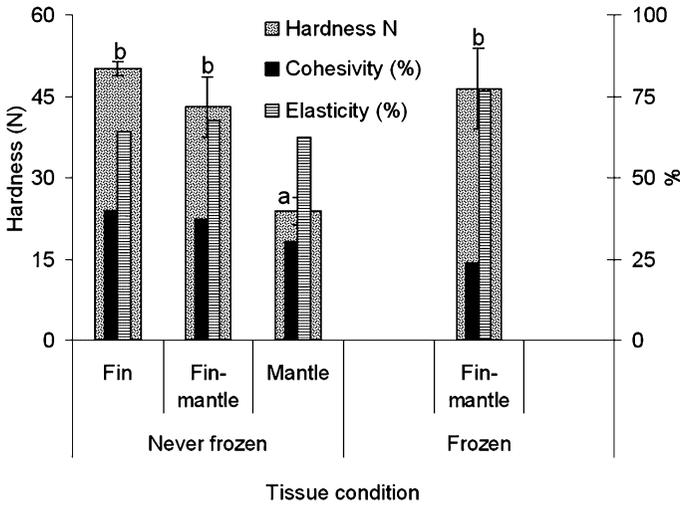observation.

FIG. 6. MOISTURE (%), WATER-HOLDING CAPACITY (%) AND WATER LOST BY DRIP (%)
OF SQUID THERMAL GELS
Values represent the mean of at least three replicates; whisker lines indicate SD; different letters
indicate significant differences at $P < 0.05$.

Moisture, Water-Holding Capacity and Dripping

The moisture of gels varied from 78.0 to 84.4% (Fig. 6). Moisture was significantly lower when using thawed Fs or M-Fs than other tested tissue conditions and combinations. There was no significant difference between the moisture content in never-frozen and frozen mantle samples; the same occurred for fin samples. This suggests that proteins aggregate to form a general structure containing similar amounts of water, even though the tissue was stored.

In most samples, the water-holding capacity (Fig. 6) is affected by the tissue condition in similar way as moisture is related (Fig. 6), whereas water loss from dripping (Fig. 6) is more related to the use of mantle or fin. When compared with texture parameters, gels containing fin from fresh samples had the greatest strength (50.2 ± 1.2 N). Although fin-based gels lost more water by dripping (20.8% for fresh samples and 25.1% for frozen samples) during the gel production (temperature treatment), these fin-based gels showed the highest water-holding capacity (71.4%). These results suggest that the three-dimensional structure was more stable in fin-based gels than in gels exclusively of mantle tissue. Mantle-only gels had the lowest gel strength ($P < 0.05$) and the lowest water-holding capacity ($P < 0.05$). When a mixed M-Fs batter was used, there was no significant difference in gel strength between fresh and

TABLE 3.
COLOR PARAMETER OF JUMBO SQUID THERMAL GELS†

Parameter	Mantle-fin (SM-Fs)	Skinned mantle (SM)	Fin with skin (Fs)
L*	77.8 ± 3.9	86.2 ± 0.8	76.2 ± 3.5
a*	2.9 ± 0.1	−2.5 ± 0.2	6.8 ± 0.01
b*	4.7 ± 1.7	4.5 ± 1.3	5.0 ± 0.3

† Mean of four determinations ± SD.

frozen products. Frozen M-Fs had higher water-holding capacity than mantle or fin alone ($P < 0.05$). Also, M-Fs gels demonstrated the highest elasticity. Fin-with-skin produced gels with slightly lower water-holding capacity than SF gels. These results suggest that the latter gels would probably show higher gel strength (not measured) than mantle gels.

Color Parameters

Color parameters ($L*$, $a*$, $b*$) of squid gels are summarized in Table 3. These results suggest that squid mantle can be used as a colorless protein, for example, in the manufacture of surimi-like products. We found a pink color when the mantle was mixed with fin-with-skin, varying from bright pink to a colorful red in gels from fins-with-skin, ideal for red-pink products, such as sausage.

CONCLUSION

Jumbo squid is a rich source of high-quality protein. Here, we demonstrated that jumbo squid mantle proteins provide gels with better texture parameters than in previous reports. Including fin or skin can enhance this capacity. Frozen-thawed squid enhanced the strength of the gel, so frozen storage of raw material is an excellent way to manage this process. Sarcoplasmic squid proteins can be removed from the myofibrillar protein fraction by water extraction followed by centrifugation. Protein recovery may be achieved by a change in pH greater than pH 10, at which level, more than 80% of the proteins are solubilized. Autohydrolysis of squid muscle proteins only affects the sarcoplasmic fraction at moderate pH and temperature. These results hold promise that jumbo squid proteins are useful as a food ingredient because of their excellent solubility and gel-forming capacity.

ACKNOWLEDGMENTS

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