Original article

Effect of storage at 0 °C on mantle proteins and functional properties of jumbo squid

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Summary Effect of iced storage of jumbo squid mantle with fin on gelling capacity and changes in protein fractions and functional properties of jumbo squid mantle protein during storage at 0 °C were assessed. Most values of texture variables in gels did not significantly change during storage. On average, they were: strength 65.07 ± 4.71 N; elasticity 68.14 ± 5.3%, fracturability 52.97 ± 1.28 N and cohesiveness 36.6 ± 0.1%. Protein solubility increased more than 40%. Whippability increased during storage for 16 days at 0 °C (81–162%), as did foam stability (73–94%). Results suggest that iced squid mantle protein is a suitable ingredient for food products where these functional properties are desirable. Muscle fibres of squid mantle undergo various changes during storage. At 0 °C, they are disrupted, whereas at −20 °C, they aggregate and develop empty spaces in the tissue.

Keywords Emulsion, frozen storage, iced storage, jumbo squid, solubility, squid muscle fibre, squid proteins, thermal gelling, whippability.

Introduction

Jumbo squid *Dosidicus gigas* (D'Orbigny 1835) ranges from southern California, USA, to southern Chile. In the Gulf of California, this species migrates seasonally within the Guaymas Basin (Markaida *et al*., 2005). Main fishery localities within the Guaymas Basin are Guaymas in the East and Santa Rosalía in the West. The fishery along the Pacific coast of Mexico began in 1972 (INP, 1999). Initially, catches were destined for Asian markets as a frozen product. Recently, new markets have opened for jumbo squid, mainly in the United States and European Union (INP, 1999), which demand investigation of forthcoming products. To give added value to jumbo squid, new foodstuffs derived from it must be proposed, along with suitable postmortem management of meat product.

Chemical composition of squid muscle may vary because of age and season (Ezquerra-Brauer *et al*., 2002). Muscle proteins include myofibrillar, sarcoplasmic and stroma fractions. Myofibrillar proteins may account for 75–85% of total protein, mainly composed of myosin (heavy chain 220 kDa), actin (45 kDa) and paramyosin (111 kDa). Sarcoplasmic proteins account for 10–15% of the total protein and show high proteolytic activity (Ezquerra-Brauer *et al*., 2002; Gómez-Guillén *et al*., 2003). Stroma proteins constitute approximately 11% of the protein (Morales *et al*., 2000). Jumbo squid is a source of nutritious and functional protein that can be processed as an ingredient for food supplements. Surimi-based products have gradually become attractive as an alternative product for under-utilised species, such as jumbo squid. Because some negative effects of the various types of proteases in muscle affects functional properties during cooking (Ebina *et al*., 1995; Ayensa *et al*., 2002) or unwanted texture changes during storage (Ueng & Chow, 1998; Ando *et al*., 1999), some technological improvements are needed to improve squid-based products for the market.

There are examples of deterioration of functionality of seafood caused by endogenous enzymes, which is also the case for some squid species that lose their gel-forming capacity during heat activation of proteases. Jumbo squid has a marked decrease in rigidity of gels because of a high rate of hydrolysis of muscle proteins, hydrophobic interactions within myofibrillar proteins and melting of connective tissue (Gómez-Guillén *et al*., 2002, 2003). However, in those experiments, frozen

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mantle with no data about postmortem handling was used. To our knowledge, there is no report on functional properties of freshly caught squid. We evaluate several aspects of preparation for the market: (i) effect of iced storage (0 °C) on gel-forming capacity of squid mantle fin; (ii) the functional properties of emulsion forming, solubility and whippability of homogenates of mantle when stored under iced conditions (0 °C) and (iii) determine the arrangement of jumbo squid mantle muscle fibres stored at iced and freezing conditions.

Materials and methods

Squid samples

Experiments were conducted using jumbo squid specimens that were caught from Gaymas Basin. Sampling sites (Guaymas, Sonora or Santa Rosalía, Baja California Sur) are located on the coast of the Gulf of California. The specimens had on average a mantle length of 50 cm. Mantles for histology were first processed on the fishing vessel at time zero, as described below. On landing, all mantles were eviscerated and washed with fresh water. The mantles were wrapped in plastic bags and kept between beds of ice until reaching the laboratory within 16 h after catch. Samples from specimens were prepared, as described below, for chemical composition analysis using accepted methods (AOAC, 1995).

Effect of iced storage on gel-forming capacity of squid mantle and fin

A homogeneous batter was prepared from a pool of mantle pieces and fins from ten specimens to avoid differences caused by size or age. Pieces of skinned mantles and fins with skin were placed in a kitchen blender and ground for 30 s at high speed. During preparation of samples, temperature was kept at 2–4 °C. This batter was packed in plastic bags in 400-g batches and stored at 0 °C for up to 16 days. Triplicate samples were stored for 0, 2, 4, 6, 9, 12 or 16 days and then analysed for gel-forming capacity.

A 'sol' was prepared by adding and manually mixing 2.5% NaCl (to a final concentration of 0.43 M) to obtain a batter from these specimens. No adjustments for moisture content was done to any 'sol'. Aliquots of 40 g of 'sol' were placed in 50-mL beakers (approximately 35 mm inner diameter), which were placed inside polyethylene bags, vacuum-sealed, and placed at 0 °C until used. 'Sols' were incubated in a water bath for 30 min at 90 °C, followed by an overnight ice bath treatment. Gels prepared in this way were used for texture profile analysis (TPA) and folding test.

The folding test was performed using round slices of gel (30 mm diameter × 3 mm thick). The evaluation was on a five-point scale (Suzuki, 1981; Ayensa *et al.*, 2002), where: 5 corresponds to a gel that does not break when folded in quadrants; 4 where no breakage occurs when folded in half; 3 when gel gradually breaks at the first fold; 2 when a break at the first fold and 1 when gel disruption when gently pressed by a finger. Two slices of each gel sample were evaluated for the folding test.

A double-bite TPA was performed with a universal testing machine (Instron model 1132, series 125, FTC, Sterling, VA, USA) as described by Bourne (1978) with slight modifications. Briefly, a 78-mm flat-faced cylinder (24.52 N, load 50 kg, 0.10 m min^{-1} crosshead speed) was used to compress standard-sized pieces of squid gel (2.8 cm high × 3 cm diameter) to 25% of its original height. For TPA, a force vs. time plot was generated to describe sensations during food biting and chewing. TPA allows imitation of sensations during food biting and chewing. For consumer acceptance of a food, these parameters should remain high and constant. Texture attributes, such as fracturability (force necessary to break the gel), strength (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 analysed.

Effect of iced storage on protein fraction profile of squid mantle proteins

On preliminary experiments using mantle-fin with skin, it was found that the high content of connective tissue caused that sampling was not homogeneous causing artefacts. So, skinless and finless mantles were grouped in three units (replicates) and the batter from each group was prepared, as described above. Samples were analysed for protein fractionation, solubility, whippability and emulsion-forming capacity for changes during iced storage (0 °C) for 0, 2, 4, 6, 9, 12 and 16 days.

Preliminary experiments were conducted to evaluate the solubility of jumbo squid proteins as affected by ionic strength by using saltwater solutions. Ionic strength (i) is the same numerical value as NaCl molarity. Those results (data not shown) were used to optimise protein fractionation. After that, squid-mantle protein fractions were separated as follows: squid batter was mixed with ten volumes of distilled water, stirred at 2–3 °C for 30 min, and centrifuged at 12 000 × $\boldsymbol{g}$ for 20 min. The supernatant fraction containing non-protein nitrogen and sarcoplasmic proteins were stored at 0 °C for further separation. The sediment containing the myofibrillar fraction and stroma were mixed with ten volumes of 1 M NaCl solution at 2–3 °C for 30 min and filtered through a plastic mesh. The process was repeated twice. Non-protein nitrogen and sarcoplasmic proteins in the supernatant were separated by adding TCA (5% final volume) followed by centrifugation at

3000 ***g*** for 5 min. The sarcoplasmic fraction was recovered by adding two volumes of 0.1 N NaOH to the sediment. Protein concentration in each of the fractions and non-protein nitrogen content was determined with the Lowry method (Lowry *et al.*, 1951). The final insoluble stroma fraction was determined with the Kjeldahl method (AOAC, 1995).

Squid protein samples from the myofibrillar and sarcoplasmic fractions and those from the bulk of the proteins were analysed by SDS-PAGE (Laemmli, 1970). Samples containing 50 μg protein were mixed with a 2X load buffer: 0.125 M Tris-HCl, 4% SDS, 20% (v/v) glycerol, 0.2 M dithiothreitol (DTT), 0.02% bromophenol blue at pH 6.8. This solution heated for 5 min in a boiling water bath. Samples were analysed in a vertical electrophoresis unit (Hoefer, Inc., model SE 260, San Francisco, CA, USA), using a homogeneous 10% polyacrylamide gel for separating protein bands. Electrophoresis was conducted at a constant current (13 mA per gel). The separated protein bands were stained with a solution of 40% methanol, 7% acetic acid and 0.5% Coomassie Brilliant Blue R-250. One molecular mass calibration kit was used (Kit 17-0446-01 for 53–220 kDa, GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). After 2 h, the excess stain was removed with a solution containing 40% methanol and 7% acetic acid. Proteins used as markers and their molecular weight (kDa) were myosin, 220; α2-macroglobulin, 170; β-galactosidase, 116; transferrin, 76 and glutamate dehydrogenase, 53.

Effect of iced storage on functional properties of squid mantle proteins

Protein solubility of the stored mantle was determined as follows: a sample of batter was dispersed in NaCl solutions of 0.05, 0.3 and 0.5 ionic strengths to a final protein concentration of l0 g L^{-1} (Kjeldahl method, AOAC, 1995). After mixing thoroughly for 3 min and setting for 30 min at room temperature, the mixture was centrifuged at 12 000 ***g*** for 20 min. Solubility was evaluated by measuring the content of protein in the supernatant by the Lowry method (Lowry *et al.*, 1951). Solubility is expressed as percentage of soluble protein vs. total protein in the sample.

Whippability and foam stability were measured using the Rudin method (Wilde & Clark, 1996) with a slight modification. Briefly, a 100-mL mixture of distilled water and squid, with a final protein content of 3%, was homogenised in a kitchen blender at high speed for 1 min. The resulting foamy slurry was poured into a graduated cylinder and the foam and liquid volumes were recorded. Whippability was expressed as a percentage of increased volume. Foaming stability was expressed as the volume of foam remaining after allowing the sample to rest at room temperature for 60 min.

Emulsifying capacity was determined by slightly modifying methods described in Swift *et al.* (1961 in Hill, 1996). In brief, squid batter was mixed with distilled water to a final protein concentration of 0.3%; to this protein suspension, sunflower seed oil (1:6) was added. The mixture was homogenised for 30 s at high speed at room temperature with a propeller impeller and, immediately afterwards, centrifuged at 2000 ***g*** at 20 °C for 5 min. The volume of the emulsion was measured for each sample. Emulsifying capacity was calculated as the ratio of volumes between the emulsion formed and the initial mixture. The emulsion stability of each sample was determined by heating the emulsion at 80 °C for 30 min. The emulsion volume was measured after heating. The *emulsion stability* was expressed as a percentage of the remaining emulsifying capacity after the heating period.

Effect of iced and frozen storage on squid mantle tissue

Time zero samples (1 × 1×1 cm) were cut from the ventral zone of ten squid mantles at time of death and processed. In the laboratory, the rest of mantles were then sliced into two equal portions and placed in plastic bags; one half mantle was stored at 0 °C and the other at −20 °C. After 7 and 30 storage days, mantles kept at 0 and −20 °C were cut into 1 × 1×1 cm cubes for microscopic examination of three cut sections: X (longitudinal), Y (lateral) and Z (transversal).

Samples corresponding to each storage condition were placed in histology cases and processed (Howard & Smith, 1983). In brief, samples were fixed by immersion in Davidson solution [11% glycerine, 22% formaldehyde, 34% ethyl alcohol, 33% filtered seawater, and acetic acid (1:8)] for 48 h and then dehydrated in increasing concentrations (from 70% to 100% ethanol in 10% increments). After 1 h in each ethanol concentration, the samples were rinsed with an ethanol–xylene (1:1) solution, followed by 100% xylene. The samples were embedded in paraffin and cut into 4 μm sections. Samples were stained with Mayer's acid haematoxylin solution (Howard & Smith, 1983). Samples were examined, photographed and analysed with an image analysing light microscope system (Olympus BX50 microscope, Center Valley, PA, USA; CoolSNAP™ digital camera, Media Cybernetics, Inc., Bethesda, MD, USA, and Image Pro Plus™ 5.4.29 software, Media Cybernetics, Inc., Bethesda, MD, USA).

Statistical analysis

A one-way ANOVA, coupled with Tukey's HSD procedure was used in all experiments to determine significant differences among treatments; $P < 0.05$ was considered significant. The STATGRAPHICS PLUS for Windows® v. 5.0 software performed these operations.

Results

Chemical analysis

Average moisture of mantle-with-fin was 83.5 ± 0.22%; protein content was 79.1 ± 0.33% (dry weight basis). Mantle (without fin) contained 81.5 ± 0.06% moisture; on a dry weight basis, contents were: protein (80.83 ± 3.6%), ash (6.82 ± 0.15%), lipids (0.96 ± 0.006%) and energy (219.73 ± 1.38 J g^{-1}).

Effect of iced storage on gel-forming capacity of squid mantle and fin

Using the Suzuki scale, gels from samples stored at 0 °C for 0–12 days had a folding value of 5. Gels from samples stored 16 days broke gradually on the first fold (scale 3). TPA measurements showed some changes during storage ($P < 0.05$; Fig. 1). In this experiment, 75% gel compression was sufficient to produce fracturability, which, on average was 52.97 ± 1.28 N. Maximum values for strength, cohesiveness and elasticity were 65.07 N, 36.6%, and 68.14%, respectively.

Effect of iced storage on protein fraction profile of squid mantle proteins

Protein fractionation affected during storage

At the beginning of storage, the different fractions of protein were: myofibrillar (84.85%), sarcoplasmic (9.61%) and stroma (2.62%), and non-protein nitrogen was 2.96%. Protein fractions significantly changed during storage time (Fig. 2).

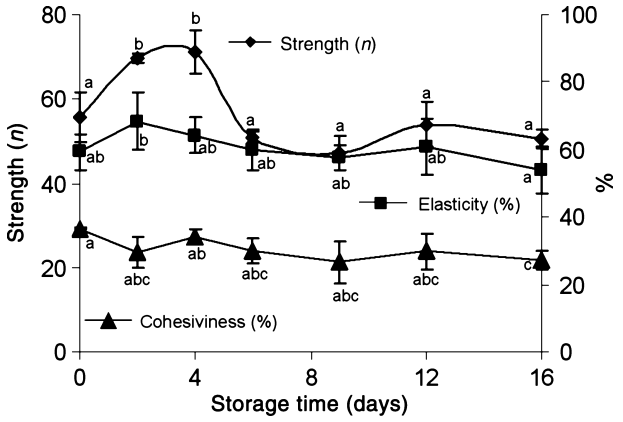

Figure 1 Change in texture profile parameters for gels prepared with homogenised squid stored in an ice bath (0 °C). Gel strength is given in N, elasticity and cohesiveness are given in %. Values represent the mean; bars indicate SD; different letters indicate significant differences ($P < 0.05$).

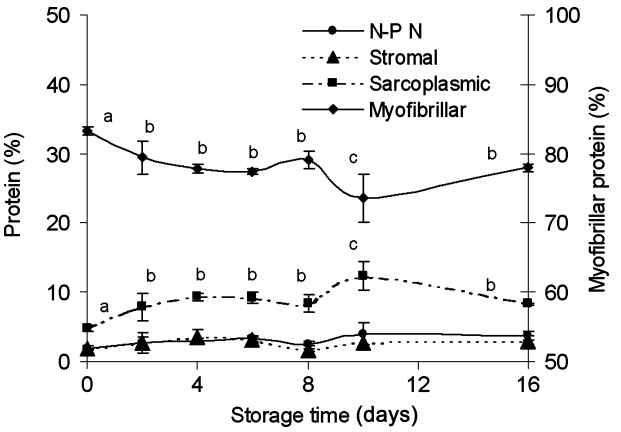

Figure 2 Effect of storage time at 0 °C on myofibrillar, sarcoplasmic and stromal proteins and non-protein-nitrogen (N-P N) fractions. Values represent the mean of at least three replicates ($n = 3$); bars indicate SD; different letters indicate significant differences ($P < 0.05$).

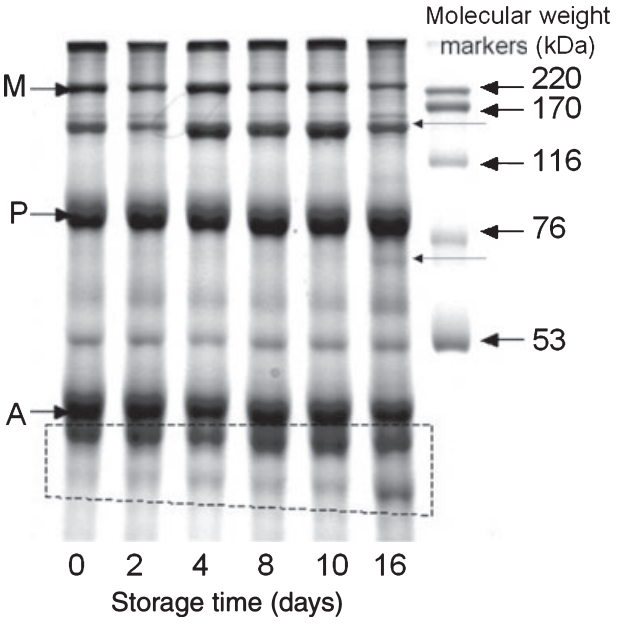

Figure 3 Effect of storage time on squid proteins. MWM = molecular weight markers (in kDa), lane numbers correspond to days of storage. M = myosin (220 kDa), P = paramyosin (approximately 111 kDa), A = actin (approximately 45 kDa). Dashed box includes proteins that changed over time.

SDS-PAGE

With electrophoresis of total (bulk) proteins (Fig. 3) from squid mantle, we obtained heavy myosin chains (220 kDa), paramyosin (111 kDa) and actin (approximately 45 kDa). Proteins did not degrade after storage for 16 days at 0 °C. However, during extraction of myofibrillar proteins, heavy myosin was not observed in

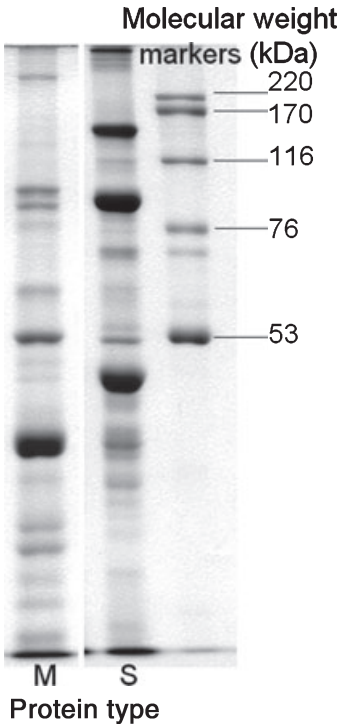

Figure 4 Myofibrillar (M) and sarcoplasmic (S) protein fractions of squid mantle muscle obtained by changing ionic strength. MWM = molecular weight markers (in kDa).

electrophoresis (Fig. 4), whereas paramyosin and actin were present and without apparent degradation. Figure 4 also shows proteins from myofibrillar (soluble at $i = 1$) and sarcoplasmic (water-soluble) fractions. The protein bands of the fractions were the same for all replicates.

Effect of iced storage on functional properties of squid mantle proteins

For squid mantle batter stored at 0 °C, protein solubility was significantly affected by length of storage, increasing up to 40% (Fig. 5). Whippability varied significantly (81–162%) during storage (Fig. 6). Maximum stability of foam was 94% at day 16. Emulsifying capacity was significantly affected by storage time, increasing over 16 days of iced storage from 23% to 33% and its stability increased from 59% to 88% (Fig. 7).

Effect of iced and frozen storage on squid mantle tissue

Histological analysis showed that muscles of recently caught jumbo squid had a continuous and homogeneous pattern of muscle fibres; however, after 7 days of iced storage (0 °C), fibres were separated and disrupted.

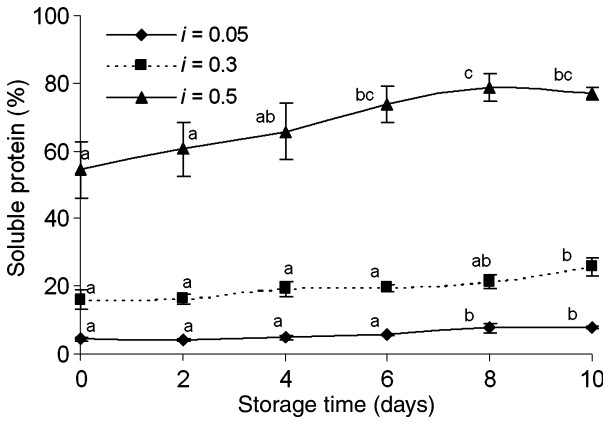

Figure 5 Effect of length of storage in ice bath (0 °C) on solubility of squid mantle proteins at three ionic strengths (0.05, 0.3 and 0.5). Values represent the mean of at least three replicates ($n = 3$); bars indicate SD; different letters indicate significant differences ($P < 0.05$).

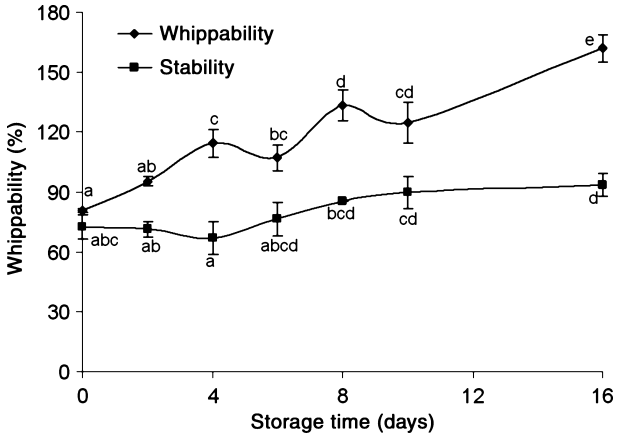

Figure 6 Effect of length of storage in ice bath (0 °C) on whippability and foam stability of squid mantle proteins. Values represent the mean of at least three replicates ($n = 3$); bars indicate SD; different letters indicate significant differences ($P < 0.05$).

Under frozen storage (−20 °C), fibres contracted, leading to spaces with a circular/spherical shape (Fig. 8). Mean values for area covered with muscle fibres show some changes, although differences were not significant. Areas of longitudinal and radial fibres were not different ($P < 0.05$), whereas circumferential fibres showed differences between the other fibres under both storage conditions (Table 1).

Discussion

One useful functional property of jumbo squid proteins is gel-forming capacity. In contrast to previous work where the postmortem condition of the squid was not

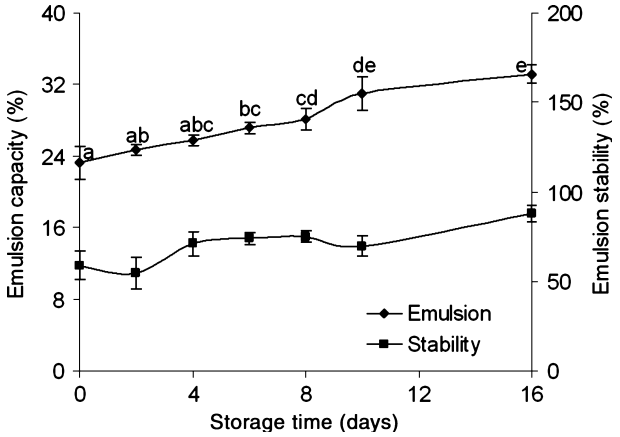

Figure 7 Effect of length of storage in ice bath (0 °C) on emulsion capacity and emulsion stability of squid mantle proteins. Values represent the mean of at least three replicates ($n = 3$); bars indicate SD; different letters indicate significant differences ($P < 0.05$). Emulsion stability did not show significant differences ($P \geq 0.05$).

specified (Gómez-Guillén *et al.*, 1997), we demonstrated that jumbo squid protein has higher gel-forming capacity, folding score and TPA under specific condition. Additionally, we included fin and skin, which could contribute to better gel-forming capacity. Fin and skin have large amounts of collagen, which could act as a structural support for protein aggregation. Thermal gelling probably was enhanced by the rapid increase in temperature from ice bath conditions to 90 °C, whereas other authors used a gradual change, usually increasing temperature about 1 °C min^{-1} (Ayensa *et al.*, 2002; Gómez-Guillén *et al.*, 2002). Studies of frozen storage on gel-forming capacity in comparison with iced (0 °C) storage are needed to recommend the most satisfactory storage conditions to use and maintain this property.

Increase in the sarcoplasmic fraction and decrease in the myofibrillar fraction, suggest that myofibrillar protein is altered during storage and/or extraction. These changes in protein solubility could be related to degradation by proteolysis of myofibrillar proteins, yielding low-molecular weight proteins, which might be more soluble in water than their native form (Lin & Park, 1996). Electrophoresis (Fig. 3) suggests that the bulk homogenate suffered negligible changes in proteins during storage, which was particularly evident in proteins < 53 kDa. However, it appears that the major effect occurred during the extraction process and myosin was hydrolysed. This is suggested from the bands near 150, 111 (slightly above paramyosin) and 76 kDa. The two largest protein bands could be heavy and light meromyosin chains. Others have reported that squid myosin is an unstable protein, compared with other squid proteins, such as paramyosin and actin (Konno *et al.*, 2003).

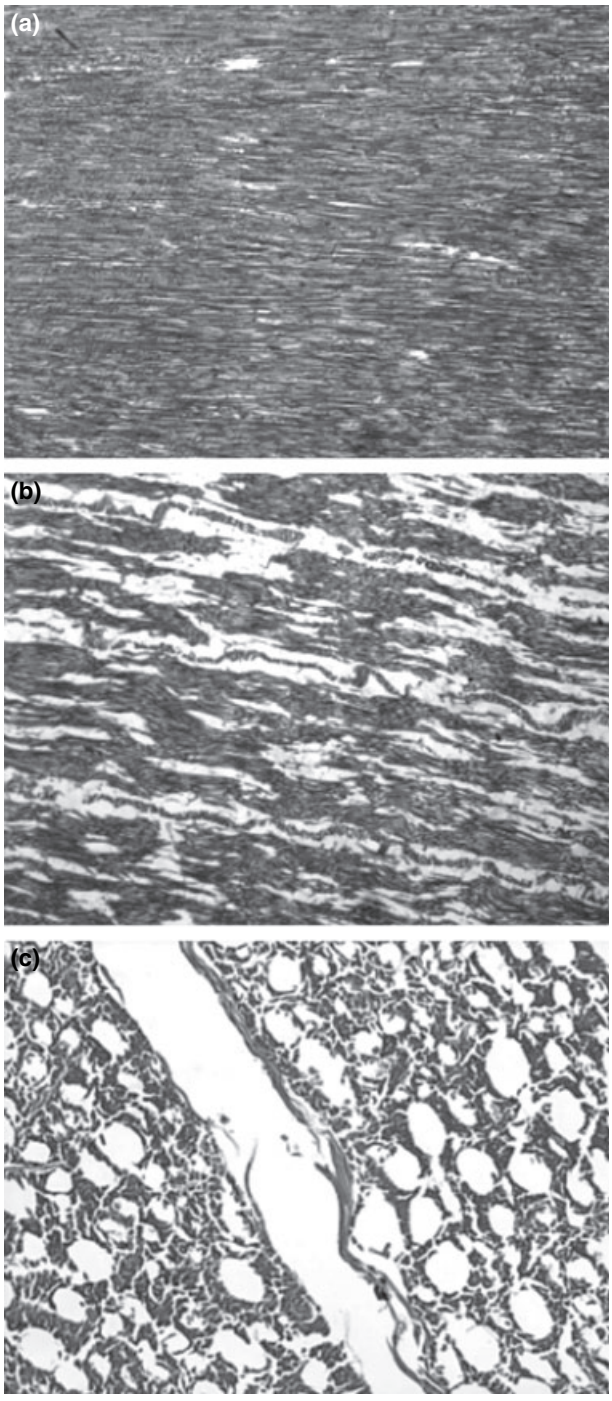

Figure 8 Effect of storage on squid muscle fibres. (a) Recently caught (< 1 h); (b) storage in ice bath (0 °C) for 7 days; (c) storage at −20 °C for 30 days. Magnification is 10×.

Although there were changes in the protein fractions, functional properties of this low-cost resource remain useful. Protein solubility is one of the most important

Table 1 Area of fibre coverage

Condition	Fibre	Fibre area (%)
Fresh match (approximately 1 h)	Longitudinal	72.31 ± 8.61 a
	Radial	68.02 ± 10.11 a
	Circumferential	55.39 ± 14.81 b
Ice stored	Longitudinal	71.50 ± 9.45 a
	Radial	70.09 ± 6.57 a
	Circumferential	60.32 ± 13.59 b
Frozen stored	Longitudinal	62.55 ± 11.49 a
	Radial	62.83 ± 13.69 a
	Circumferential	56.09 ± 11.14 b

Values represent the mean ± SD; different letters indicate significant differences ($P < 0.05$).

functional properties in food systems. In this case, more than 75% of the protein was soluble at 0.5 ionic strength, where most of the myofibrillar proteins became soluble. Changes in protein solubility may reflect some denaturing processes during storage, involving interactions between proteins and water-protein, usually manifested as endogenous proteolysis that results in increasing the sarcoplasmic fraction, whereas decreasing the myofibrillar fraction as studied according to Lin & Park (1996; Fig. 2).

Whippability increased after storage of squid mantle homogenate, and foam stability increased with increased storage time. This suggests that changes in myofibrillar and sarcoplasmic proteins enhanced these functional properties. Increased whippability of squid proteins (162%) is lesser than egg albumin (240% foam-forming capacity and 24% foam stability); however, foam stability of squid proteins is three to four times greater than albumin (Bickerstaff, 2005). These results encourage further research on using squid protein as a food additive for aerated systems, such as meringues. Although emulsion-forming capacity and emulsion stability tended to increase with storage time, the values for this functional property never exceed 40%.

Changes in muscle fibre patterns occurred during storage. Mantle stored in an ice bath was affected in a way that is similar to that in *Loligo bleekert* (Ando *et al*., 1999), where detachment of muscle fibres was probably caused by endogenous enzyme activity. At 0 °C, we measured very low proteolytic activity in jumbo squid (data not shown), which suggested that the contribution of endogenous proteolysis to fibre detachment in *D. gigas* muscle is not considerable. During frozen or chilled storage, muscle proteins are susceptible to protein–protein and protein–water interactions that lead to fibre rupture during storage. As observed in fish, myosin partially unfolded during storage and exposure to hydrophobic residues, where protein interactions and aggregation was promoted (Ramírez *et al*., 2000; Foucat *et al*., 2001). These results suggest that protein interactions are the major cause of fibre detachment, which may be the basis for softening of mantle tissue and flabbiness after several days of iced storage.

In frozen samples, fibre aggregations were similar to those found in *L. edulis* (L. Ligo), where protein aggregation was affected by temperature changes (Ueng & Chow, 1998). Empty, elliptically shaped spaces between aggregated fibres might be caused by small water aggregations during the freezing process. In fish muscle, changes in fibre patterns during frozen storage were related to conformational changes in muscle protein, protein–water interactions and mobility of water in the muscle tissue (Ramírez *et al*., 2000; Foucat *et al*., 2001; Herrero *et al*., 2005). Aggregation of fibres seems to be the cause of toughening of jumbo squid mantle during frozen storage and chewiness after thawing.

Conclusions

Jumbo squid is a rich source of high quality protein. We demonstrated that fresh jumbo squid proteins provide high thermal gelling capacity. Protein fractions showed some changes during storage at 0 °C; these changes are more strongly related to the protein extraction process than storage conditions. We recommend more studies of processing conditions. Mean values of functional properties (emulsifying, solubility and whippability) of jumbo squid showed increased when stored at 0 °C. These results are promising that jumbo squid proteins are useful as a food ingredient because their solubility and gel-forming and foam-forming capacity are excellent. Storage temperature is an important contributor to tissue changes, as shown by histological analysis. If not properly handled, fresh jumbo squid is flabby, and frozen jumbo squid becomes chewy.

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