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Effect of freezing on protein denaturation and gelling capacity of jumbo squid (*Dosidicus gigas*) mantle muscle

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ABSTRACT

The effect of freezing storage on protein denaturation and gelling capacity of jumbo squid muscle was evaluated. Squid mantle muscle samples were evaluated in both, ground (G) and whole presentations (W), and in a control consisting of ground muscle (C). G and W samples were submitted to two freezing storage periods (−20 °C), 1 day (GF-1, WF-1) and 30 d (GF-30, WF-30). Proximate composition, pH, surface hydrophobicity (SoANS) and differential scanning calorimetry (DSC) were evaluated. Additionally folding test, texture profile analysis and water holding capacity were monitored in gels prepared with the different samples. The quality of gels was not affected by any treatment. Neither freezing, grinding or storage period had a significant effect on the above parameters, suggesting that functional properties of the squid mantle muscle are highly stable. DSC did not reveal any denaturation, also adducing high stability of muscle proteins. SoANS revealed that freezing, grinding and time, had an effect on protein properties; however, these influences were apparently not significantly enough to have a further effect on the gel quality. Finally, gelling capacity of muscle was not affected by any factor. Considering these results, squid muscle may be considered as a useful resource either fresh or frozen.

1. Introduction

The world demand for food products has continuously increased during the last decades; in this context, efforts focused on improving the production, quality and distribution of food are essential. For instance, seafood produced by fisheries and aquaculture contributes with 15–20% of average animal protein consumption to 2.9 billion people worldwide (Martinez-Porchas & Martinez-Cordova, 2012). Several marine species are captured and/or produced to cope with such demand; however not all are properly utilized.

The jumbo squid (*Dosidicus gigas*) is considered a sub-valuated species despite its high protein quality and abundance in the East Pacific. The species has historically reached low prices in the international market (Salinas-Zavala, Coop, Rebollo, Hernández, Verdugo, Angulo, Quintana, & Morales, 2003). Thus, strategies aimed to improve the profitability of exploiting this resource are needed. In particular, one of the most successful strategies is promoting its consumption throughout the development of value-added products. Recent evidence has shown that the consumption of squid value-added products has increased in the last years (Luna-Raya, Aragón Noriega, & Salinas-Zavala, 2009; Luna-Raya, Urciaga-García, Salinas-Zavala, Cisneros-Mata, & Beltrán-Morales, 2006).

Considering that jumbo squid muscle has properties such as low fat content, high quality proteins, mild flavor, and white color, it has been a useful raw material for developing several value-added products. These products include protein concentrates (Cortés-Ruiz, Pacheco-Aguilar, Lugo-Sanchez, Carvallo-Ruiz, & Garcia-Sanchez, 2008; Dihort-García et al., 2011), imitation of restructured fish fillets (Campo-Deaño, Tovar, & Borderías, 2010), emulsified-gel products (Félix-Armenta et al., 2009) and protein hydrolysates (De La Fuente-Betancourt, García-Carreño, & Toro, 2008). For most of these products preserving the gelling capacity of the muscle is essential, even after freezing–thawing processes.

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However, without the use of cryoprotectants most muscles lose functionality when they are frozen, due to protein denaturation and/or aggregation (Goeller et al., 2004; Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2012; MacDonald, Lanier, Swaisgood, & Hamman, 1997).

Previous research at our laboratory has provided evidence to hypothesize that the gelling capacity of the jumbo squid muscle is maintained even after frozen storage without the use of cryoprotectants. If the above could be confirmed, the squid muscle would be positioned as a priority over other muscle types to be used as a raw material for value-added products. However, scarce studies have been performed to evaluate the effect of freezing on the functional properties of squid muscle (Gómez-Guillén, Martínez Alvarez, & Montero, 2003; Pérez-Mateos, Montero, & Gómez-Guillén 2002). Therefore, the aim of the present research was to evaluate the effect of freezing storage on protein denaturation and gelling capacity of the jumbo squid (*D. gigas*) mantle muscle.

2. Materials and methods

Jumbo squid was captured at Guaymas, Sonora immediately stored in ice and transported to the laboratory under 24 h post-capture. In our laboratory, the mantle was removed from viscera and washed thoroughly with distilled water. Then, mantles were weighted (0.5–08 kg) and measured (33–42 cm). Finally, the skin was removed and mantle cut into 3 cm pieces. Cut mantle was divided into three groups or treatments.

2.1. Treatments

The first treatment called "ground-frozen" (GF), consisted of grinding the squid mantle muscle and storing it at −20 °C. Thus, squid pieces were ground using a mill (HOBAR, Model 52) through a 0.9 cm sieve. For the second treatment (whole-frozen: WF), the pieces of squid mantle (3 cm) were not ground but were also stored at −20 °C. A third treatment was considered as control (C) and the squid pieces were grounded, but the product was not submitted to the above freezing process.

The GF and WF treatments were submitted to two storage periods, 1 day (GF-1 & WF-1) and 30 days (GF-30 & WF-30). After storage, the samples were thawed at 4 °C during 24 h. Thereafter, a portion of the thawed samples was considered to evaluate their proximate composition (moisture and protein), pH, surface hydrophobicity (SoANS) and differential scanning calorimetry (DSC), whereas the rest of the samples were used to perform heat-set gel preparation (GF-1, WF-1, GF-30, WF-30 and C). All samples were flat-packed in polyethylene bags of 1 kg each.

2.2. pH

The pH was measured in ground muscle at 25 °C using a Corning digital pH meter Model 240 (Corning Inc., Corning, NY) in a homogenate of 2 g of sample with 18 mL of distilled water as recommended by Martin (1992).

2.3. Gel preparation

Regarding heat-set gel preparation, sols for each sample (GF, WF and C) were prepared by adding 2.5 g NaCl/100 g of protein system at short intervals in a Model DLC-8 Plus Cuisinart Food Processor (Cuisinart Inc., Greenwich, CT). Crushed ice was added to adjust the final moisture of sols to 80 g/100 g of sample. Mixing was continued until the sol's temperature reached 5 °C. The pH of the sol was adjusted to neutrality with sodium bicarbonate (Na_2HCO_3). Each sol was packed into a Petri dish (1 cm height) and vacuum sealed in moisture/vapor-proof film bags (Cryovac Corp., Duncan, SC) with a Super Vac Smith vacuum machine (Smith Equipment Co., Clifton, NJ). Each sol was heat-set in a water bath at 90 °C/30 min. Thereafter, heat-set gels were immediately chilled to 5–10 °C in an ice–water mixture and held overnight at 2–4 °C prior to functional evaluation. Finally gels prepared with the different samples were submitted to different analyses such as: folding test, texture profile analysis (TPA) and water holding capacity (WHC).

2.4. Protein and moisture contents of gels

Evaluations of protein and moisture contents were determined by following the methodologies recommended by the AOAC (2000).

2.5. Quality of gels

Folding test, texture profile analysis (TPA) and water holding capacity (WHC) were monitored in gels prepared with the different samples of squid muscle to evaluate their quality.

For the folding test of gels, the methodology proposed by Tanikawa, Motohiro & Akiba (1985) was followed, considering a gel sample of 3 mm × 30 mm. 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; 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). Regarding to texture profile analysis (TPA) of squid samples an Instron texturometer (Texture Lab Pro, Food Technology Corp. Sterling, VA) was used. TPA was performed according to the technique described by Veland & Torrissen (1999) using a load cell (100 N); samples were transversely cut at 1 cm. To estimate the TPA, the maximum effort required to cut the sample (hardness) was registered; in particular, compression forces at 75 and 90% (double-bite analysis) of the original gel sample height were used to compute compression hardness, elasticity and cohesiveness.

Water holding capacity (WHC) was estimated by the method proposed by Jiang, Ho, & Lee (1985) with modifications. Each sample was weighted and placed into a centrifuge tube; the samples were centrifuged at 28,000 × g and 4 °C for 20 min. After centrifugation, samples were removed with tweezers and dried with paper towel to record the final weight. WHC was expressed as g of water held per g of protein in the sample.

2.6. Protein aggregation-denaturation analyses

In order to measure the effect of freezing over the protein aggregation-denaturation of samples, surface hydrophobicity and differential scanning calorimetry were conducted. Surface hydrophobicity (SoANS) was determined using the method proposed by Li-Chan, Nakai & Wood (1985) with minor modifications (Alizadeh-Pasdar & Li-Chan, 2000), using a hydrophobic fluorescence probe 1-anilino-8-naphtalene-sulphonate (ANS). Samples were homogenized during 2 min with 4.0 mL of cold buffer (pH 7.0) containing 0.1 mol/L NaF, 0.01 mol/L Na_2HPO_4 and 0.01 mol/L Na_2HPO_4. Afterwards, the homogenates were submitted to a Branson Ultrasonic Cleaner (Model 2510R-DTH; Branson Ultrasonic Corp., Danbury, CT, USA) for 2 h at 0 °C, to decrease the particle size. The homogenates were diluted with the above buffer until a concentration of 1.5 mg mL^{-1} was achieved. The protein content in the diluted solution was estimated by the bicinconinic acid method (BCA™ Protein Assay Kit) (PIERCE 2005). The diluted homogenate was serially