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Production and functional evaluation of a protein concentrate from giant squid (*Dosidicus gigas*) by acid dissolution and isoelectric precipitation

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Abstract

A protein concentrate from giant squid (*Dosidicus gigas*) was produced under acidic conditions and its functional–technological capability evaluated in terms of its gel-forming ability, water holding capacity and colour attributes. Technological functionality of the concentrate was compared with that of squid muscle and a neutral concentrate. Protein–protein aggregates insoluble at high ionic strength ($I = 0.5$ M), were detected in the acidic concentrate as result of processing with no preclusion of its gel-forming ability during the sol-to-gel thermal transition. Even though washing under acidic condition promoted autolysis of the myosin heavy chain, the acidic concentrate displayed an outstanding ability to gel giving samples with a gel strength of 455 and 1160 g cm at 75% and 90% compression respectively, and an AA folding test grade indicative of high gel strength, elasticity, and cohesiveness. The process proved to be a good alternative for obtaining a functional protein concentrate from giant squid muscle.

Keywords: Giant squid; Surimi; Functional protein concentrates; Acidic dissolution; Gel-forming ability

1. Introduction

Giant squid (*Dosidicus gigas*) is an abundant resource in the Mexican pacific coast but with low commercial value. It has been an unstable fishery due to its little attractiveness that results from non-competitive prices. In 1997 the fishery registered a peak production of 121,016 tonnes, whereas in 1998 it was only 26,682 tonnes. By the year 2002, the capture recovered again to 115,954 tonnes (SAGARPA-CONAPESCA, 2003). Its catch and commercialization does not represent a main activity to the involved sector, due to problems associated with an unpleasant flavour of its muscle (sour–bitter) and a high initial level of volatile nitrogen compounds (N-total volatile basis) incorrectly associated with reduced sanitary quality. The world demand for squid meat is high and continues increasing, mainly as value-added products, reason by which the economic importance of this fishery has increased in the last years (Bjarnason, 1989).

In contrast with the amount of studies carried out on the functional–technological properties of fish muscle and fish protein concentrates, there are scarce studies with invertebrate muscle (Ehara, Nakagawa, Tamiya, Noguchi, & Tsuchiya, 2004). It has been reported that giant squid mantle muscle has a reduced ability to thermal gelling, yielding weak and brittle gels with low water holding capacity (Pérez-Mateos, Montero, & Gómez-Guillén, 2002). This behavior has been attributed to a high proteolytic activity in mantle leading to the degradation of muscle proteins, thus reducing its technological use in the elaboration of functional protein concentrates (Ramírez-Olivas, Rouzaud-Sánchez, Haard, Pacheco-Aguilar, & Esquerra-Breuer, 2004). Kolodziejska, Sikorski, and Sadowska (1987)

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and Konno, Young-Je, Yoshioka, Shinho, and Seki (2003) have demonstrated that an endogenous squid muscle protease promotes myosin degradation and suggested that this proteolysis could affect its meat texture quality.

Giant squid has the potential to be used for the production of protein concentrates (surimi or surimi-like) to further prepare seafood analogues and other new products that require a given gel-forming ability from its protein content (Sánchez-Alonso, Careche, & Borderías, 2007). However, the application of the traditional surimi process to squid results in a concentrate with reduced functional–technological quality and low yield due to the high water solubility of the muscle protein (Matsumoto, 1958). Thus, it is necessary to develop technological procedures for the improvement or adjustment of the process to guarantee the stability of the concentrate and explore its potential use as functional ingredient by the food industry. Lately, an alternate methodology to the traditional surimi process that consist in the recovering of muscle proteins through their acidic dissolution and isoelectric precipitation was developed by Hultin and Kelleher (1999). The process results in a greater protein recovery with functionality similar to the one obtained with the traditional surimi process. The main objective of this research was to apply this alternative process for the production of a functional protein concentrate from giant squid mantle, to evaluate its functional–technological capabilities in terms of its gel-forming ability, water holding capacity and colour attributes and to prove that the elaboration of a functional protein concentrate from giant squid is a feasible alternative for its industrialization and commercialization. Additionally, the effect of process on recovery yield of solids and protein and changes in the solubilization pattern of muscle protein was also evaluated.

2. Materials and methods

2.1. Raw material

Three different sampling lots of giant squid (*D. gigas*) were harvested off the coast of Kino Bay, Mexico (28°N and 112°W), from March to May of 2006. Specimens were de-headed and degutted on site and the mantles with fins (experimental samples) bagged and placed in alternated layers of ice-squid-ice in a portable cooler and transported to the laboratory. Elapsed time between capture and reaching the laboratory did not exceed 12 h.

2.2. Proximate composition

Analyses were carried out in squid muscle (SM) and concentrates (APC: acidic protein concentrate; NPC: neutral protein concentrate). Protein, non-protein nitrogen (NPN), lipid, moisture and ash were determined following the methodologies recommended by Woyewoda, Shaw, Ke, and Burns (1986).

2.3. pH

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

2.4. Colour

Sols and heat-set gels colour of SM, APC and NPC, was measured by tri-stimulus colorimetry using a Chroma Meter CR-400 (Konica Minolta Sensing, Inc. Tokyo, Japan). Colour coordinates for degree of lightness (L), redness/greenness ($+a/-a$), and yellowness/blueness ($+b/-b$) were obtained. From them, the whiteness index (WI) as $[100 - [(100 - L)^2 + a^2 + b^2]^{1/2}]$ was calculated (Lanier, 1992).

2.5. Acidic protein concentrate (APC)

The experimental samples (ES) were water washed and minced thoroughly before subjected to the processes. A mixture of ES–water (1:9, w/v) was homogenized at 0 °C (ice-bath) using a tissue homogenizer. The homogenate was acidified to pH 3.2 with 1 M HCl and centrifuged at 16,000g/15 min/2 °C in a Beckman refrigerated centrifuge Model J2-21 (Beckman Instrument Inc. Palo Alto, CA). After centrifugation the precipitate was discarded and the acidified protein solution (APS) recovered. To recover protein from the APS, its pH was adjusted to 5.5 to achieve the isoelectric precipitation of muscle protein (Hultin & Kelleher, 1999) with 1 M NaOH and centrifuged at 16,000g/10 min/2 °C. The APC thus obtained was packed in polyethylene bags and stored on ice in a refrigerated chamber until its use on the same working day.

2.6. Neutral protein concentrate (NPC)

A mixture of ES–water (1:5, w/v) was homogenized at 0 °C (ice-bath) using a tissue homogenizer and centrifuged four times at 16,000g/15 min/2 °C in a Beckman refrigerated centrifuge Model J2-21 (Beckman Instrument Inc. Palo Alto, CA), in order, to remove the excess water adsorbed by the proteins. The NPC thus obtained (final pH 6.5), was packed in polyethylene bags and stored on ice in a refrigerated chamber until its use on the same working day.

2.7. Protein solubility (PS)

Analyses were carried out in SM, NPC and APC, following the methodology described by Camou and Sebranek (1991), with minor modifications. An aliquot of 3.5 g of sample was mixed with 35 mL of a buffered saline solution (pH 8.3) containing 0.56 M NaCl, 17.8 mM sodium tripolyphosphate ($Na_5P_3O_{10}$) and 5 mM sodium azide (NaN_3). Sample container was placed in an ice-bath.

The mixture was homogenized for 1 min in a Tissumizer tissue homogenizer Model TR-10 (Tekmar Co., Cincinnati, OH, USA), avoiding foaming by fitting the mouth of the sample container with parafilm. The homogenate was centrifuged at 12,800*g*/1 h/2 °C. Protein concentration in the supernatant was determined by the Biuret method (Gornall, Bardawill, & David, 1949). Solubility was expressed as g of solubilized protein/g protein in the sample.

2.8. SDS-PAGE electrophoresis

Polyacrylamide gel electrophoresis (PAGE), using both a dissociating sodium dodecyl sulphate (SDS) and mercaptoethanol-SDS buffer system in a discontinuous gel (4% stacking gel and 10% running gel) were run according to Laemmli (1970). A Mini PROTEAN® 3 Cell Multi-Casting Chamber (Bio-Rad Laboratories, Hércules, CA) was used. SM, APC and NPC were analysed for their electrophoretic protein patterns. Samples were submitted to the dissociating system containing 8 M urea, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 10 mM ethylenediaminetetraacetic acid (EDTA), 0.01% NaN_3 and 0.6 M KCl. Electrophoretic runs were performed at 18–22 °C for 2.5 h at 80 V. A broad-range-molecular weight protein standard solution (BioRad Laboratories, Richmond, CA) containing myosin (200 kDa), beta-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (31 kDa) was used. After electrophoresis, gel was stained with 0.125% (w/v) Coomassie brilliant blue R-250 in 40% (v/v) methanol and 7% (v/v) acetic acid and de-stained with 50% (v/v) methanol and 10% (v/v) acetic acid.

2.9. Heat-set gel preparation

Sols for each protein system (SM, NPC and APC) 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 and/or sucrose were added to adjust the final moisture of sols to 80%. Mixing was continued until the sol temperature reached 5 °C. The pH of APC 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. Heat-set gels were immediately chilled to 5–10 °C in a ice–water mixture and held overnight at 2–4 °C prior to functional evaluation.

2.10. Gel-forming ability (GFA)

SM, APC and NPC gels were tempered for 60 min to ambient temperature (25 °C) prior to analysis. The GFA was evaluated in terms of the folding test (FT) and the texture profile analysis (TPA). FT was carried out as described by Tanikawa, Motohiro, and Akiba (1985). The test was conducted by folding a 3.0 mm thick by 30.0 mm diameter slice of heat-set gel between the thumb and index finger. Results were based upon the degree of cracking occurring along the folds as follows: Grade AA or 5 = extremely elastic gel (no cracks on folding into quarters); grade A or 4 = moderately elastic gel (no crack on folding in half; cracks on folding into quarters); grade B or 3 = slightly elastic gel (some cracks on folding in half); grade C or 2 = non-elastic gel (breaks into pieces on folding in half); and grade D or 1 = poor gel (breaks into pieces with finger pressure). The test was carried out applying the procedure to six slices/gel/replicate.

The TPA was conducted on cylinder-shaped samples of uniform dimensions (1 cm diameter and 1 cm height) obtained from each gel using a sharp-edged plastic tube. Texture was measured in a Texture Lab Pro Texturometer (Food Technology Corp., Sterling, VA) with the 3.8 cm diameter compression plunge attached to a 100 N load cell. Compression forces at 75% and 90% (double bite analysis) of the original gel sample height were used to compute compression hardness, gel strength, fracture, cohesiveness and elasticity. Results were reported as described by Abbott (1972). For each textural parameter, 10 samples/gel/replicate, were evaluated.

2.11. Water holding capacity (WHC)

It was evaluated by the technique outlined by Jiang, Ho, and Lee (1985). A portion of 5 g of each gel was centrifuged at 3000*g*/20 min/4 °C. WHC was expressed as percentage of water hold related to amount of water present in the sample before centrifugation (WHC-1) and g of water held per g of protein in the gel (WHC-2).

2.12. Protein fractions

An aliquot of 20 g of sample was homogenized with 200 mL of a phosphate buffer (pH 7.5), $I = 0.05$ (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4). The homogenized sample was centrifuged at 5000*g*/15 min/2 °C. Precipitate was mixed again with 200 mL of a phosphate buffer, and was repeated homogenized and centrifuged. The two supernatants were mixed and then trichloroacetic acid (TCA) was added until a final concentration of 5%, in order to generate a new precipitate (soluble proteins fraction in $I = 0.05$), and a supernatant (non-protein nitrogen compounds fraction). Initial precipitate was homogenized with 200 mL of phosphate KCl buffer (pH 7.5), $I = 0.5$ (0.45 M KCl, 15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4), avoiding foaming and centrifuged at 5000*g*/15 min/2 °C (the last two steps were repeated one more time). Supernatants were considered as soluble proteins fraction in $I = 0.5$, while the precipitated was submitted overnight to exhaustive extraction with 100 mL of 0.1 N NaOH with continuous shaking. The mixture was centrifuged at 5000*g*/15 min/2 °C. The supernatant obtained was the alkali soluble proteins

fraction and the final residuum was considered as stromal protein fraction (Hashimoto, Watabe, & Kono, 1979). Protein fractions and non-protein nitrogen compounds were analysed through determination of nitrogen by Micro-Kjeldahl technique (Woyewoda et al., 1986).

2.13. Statistical analysis

The data were analysed using one-way ANOVA and Tukey-Kramer multiple comparison test when necessary with a significance level of 5%. Three replicates were carried out for each treatment. All analyses were carried out in duplicate.

3. Results and discussion

A 60% yield was obtained after the de-heading and degutting of giant squid. Mantle and fins represented the 53%. Slabyj, Ramsdell, and True (1981) reported yields for the edible portion of squid (mantle, fins and tentacles) ranging from 60% to 80% depending on species, weight and size. Compared to the yields obtained from most fishes, the giant squid resource represents a major advantage as a raw material for the fish processing industry. The major constituents of the giant squid mantle were: moisture $83.7 \pm 0.9\%$, crude protein $14.3 \pm 0.6\%$, ash $1.2 \pm 0.1\%$ and lipid $0.9 \pm 0.4\%$. Similar composition has been reported by Hernandez-Andres, Guillen, Montero, and Perez-Mateos (2005) and Sánchez-Alonso et al. (2007).

Recent studies had demonstrated that giant squid is a convenient raw material for surimi making because of its leanness and whiteness (Gómez-Guillén, Martinez-Alvarez, & Montero, 2003; Sánchez-Alonso et al., 2007). Nevertheless, some reserve must be considered for its use due to the extreme solubility of the mantle proteins at low ionic strengths (Matsumoto, 1958) and to the intense endogenous protease activity present in muscle (Sánchez-Alonso et al., 2007) that would impact yields if the traditional surimi protocol is applied.

The chemical composition of giant squid muscle (SM) and the two protein concentrates prepared in this study named neutral protein concentrate (NPC, control) and acidic protein concentrate (APC, experimental), is shown in Table 1. Giant squid mantle muscle contains almost 1% of NPN giving a net protein content of 8.9%. As expected, the applied washing procedures significantly reduced ($p < 0.05$) the NPN content in both PC. The effect of process on the removal of lipids, ash and NPN, along with the concentration of protein especially for the APC is clearly observed. Solids and protein recoveries indicated a 48.2% (±6.0) of solids and 77.2% (±7.0) of protein for the APC and 49.7% (±4.7) and 76.8% (±7.8) for the NPC.

Table 2
Protein fractions of giant squid mantle muscle and concentrates (g/100 g total protein)

Protein fraction	SM	NPC	APC
Soluble in ionic strength 0.05	34.7 ± 3.3^{a}	24.3 ± 5.1^{b}	18.4 ± 1.9^{b}
Soluble in ionic strength 0.50	57.1 ± 2.2^{a}	69.5 ± 6.1^{b}	21.1 ± 5.1^{c}
Alkali soluble	2.4 ± 0.6^{a}	2.1 ± 0.7^{a}	53.6 ± 6.2^{b}
Non-soluble	5.7 ± 1.8^{a}	4.1 ± 1.5^{a}	6.9 ± 0.9^{a}

Figures are the means and standard deviation of $n = 3$. Figures in the same row with the same superscript are equal ($p > 0.05$). SM: squid muscle. APC: acidic protein concentrate. NPC: neutral protein concentrate.

Quantitative differences in the protein fraction profile were observed for the APC as compared with SM and NPC (Table 2). Only 21.1% of its total protein was soluble in $I = 0.5$, whereas 53.6% was alkali soluble protein, indicating the presence of non-soluble, non-covalent myofibrillar protein aggregates formed during the isoelectric precipitation.

Data in Table 2 indicated that those aggregates remained insoluble at $I = 0.5$ even after pH adjustment at 7.5. Similar results were reported by Cortés-Ruiz, Pacheco-Aguilar, García-Sánchez, and Lugo-Sánchez (2001), applying the same process to bristly sardine (*Opisthonema libertate*). It is well known that changes in pH alter protein structure resulting in conformational modification and changes in surface hydrophobicity affecting functionality (Haard, 1992). Data for APC specially suggest that the relationship of protein solubility with the others functional properties evaluated should be done with care. Even though functionality of muscle and surimi proteins is commonly evaluated in terms of their solubility properties (Hultin, Feng, & Stanley, 1995), sometimes is difficult to conclude about their functionality based only on this attribute, thus it is not always correct to assert that proteins should have high solubility in order for them to express a high degree of others functional properties (Borderías & Montero, 1988).

Myosin heavy chain (MHC) and actin (AC) were two major components noticeable in the protein pattern for SM, NPC and APC. A component supposed to be paramy-

Table 1
Yields and chemical composition of giant squid mantle muscle and protein concentrates (%)

System	Yield	Moisture	Protein	Lipid	Ash	NPN
SM	100	83.7 ± 0.9^{b}	8.9 ± 0.5^{b}	0.9 ± 0.4^{a}	1.1 ± 0.1^{a}	0.9 ± 0.0^{a}
NPC	75.4 ± 3.7^{a}	89.2 ± 1.9^{a}	9.1 ± 1.6^{b}	0.8 ± 0.5^{a}	0.3 ± 0.1^{b}	0.2 ± 0.0^{b}
APC	43.1 ± 5.8^{b}	81.8 ± 1.3^{b}	16.0 ± 1.7^{a}	0.7 ± 0.5^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0^{c}

Figures are means and standard deviation of $n = 3$. Figures in the same column with the same superscript are equal ($p > 0.05$). SM: squid muscle. APC: acidic protein concentrate. NPC: neutral protein concentrate.

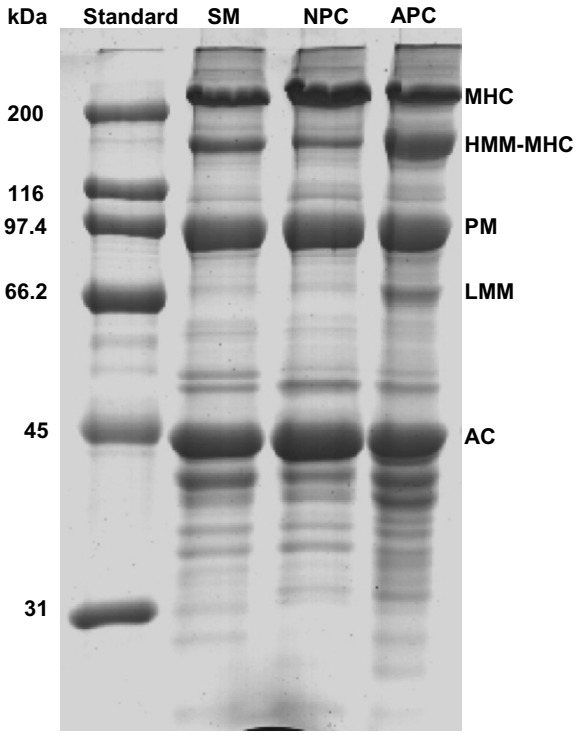

Fig. 1. Electrophoretic protein pattern of giant squid muscle (SM) and concentrates (NPC and APC). MHC: myosin heavy chain; HMM–MHC: heavy meromyosin–myosin heavy chain; PM: paramyosin; LMM: light meromyosin; AC: Actin.

osin (PM) was also detected (Fig. 1). It is known that squid muscle contains proteolytic enzymes that degrade myosin molecule into shorter fragments. Konno et al. (2003) reported that squid mantle muscle contains a metalloprotease that cleaves myosin into HMM and light meromyosin (LMM). The electrophoretic profiles indicated the appearance of heavy meromyosin myosin heavy chain (HMM–MHC) with a molecular weight (MW) of approximately 150 kDa due to autolysis of MHC along with LMM with MW around 75 kDa according to Kijowski (2001). The electrophoretic protein profile for APC suggested a stronger metalloprotease activity in this material as shown by a wider HMM band and a more noticeable LMM band which are not clearly observed for SM and NPC. This fact could be due to pH induced conformational changes in proteins structure making them more liable to enzyme hydrolysis. Moreover, results suggest that the involved enzyme is stable over the pH changes included in the APC process (acid, pH 3.2; isoelectric precipitation, pH 5.5; neutral, pH 7.3). The myofibrillar proteins constitute the muscle protein fraction soluble at $I = 0.5$ (Hultin et al., 1995). From those, myosin is the responsible of the functional and technological attributes of fish muscle proteins and surimi, thus its autolysis could leads to deterioration of the gel-forming ability of surimi and surimi-like materials such as NPC and APC.

Moisture and protein contents of SM, NPC, APC, and those of their correspondent sols and gels are shown in Table 3. As mentioned elsewhere, moisture content of sols was adjusted during their preparation to 80% with the addition of NaCl and sucrose. This adjustment generated a dilution effect in the protein content of sols with differences ($p < 0.05$) among them. Regardless of the known impact that protein content/integrity had on the functionality of proteinaceous materials, evaluation of functionality was carried out with no further adjustment in their protein contents. The interest at this point was to evaluate the concentrates as they came out of process having its moisture content as criteria of uniformity.

The gel-forming ability (GFA) of SM and both protein concentrates (NPC, APC), was evaluated in terms of their texture profile analysis (TPA), along with the folding test (FT) and water holding capacity (WHC). The pH of sols was adjusted to 7.3 during the sol preparation. This adjustment above the initial muscle pH of 6.1 was done to counteract at some extent the protein aggregation detected for the APC (Table 2) and facilitate the formation of sol.

Theoretically, protein need to be soluble to interact with water, fat and themselves, thus its solubility is determined to estimate its availability to exert technological roles such as water holding, emulsifying or gelling (Smith, 1988). Consequently, protein extracted at $I = 0.5$ is used as an index of the myofibrillar protein solubility and its availability to gel (Suzuki, 1981). Changes in WHC of gels indicate denaturation/degradation of their constituent myofibrillar proteins by changes in charge and/or structure. The higher ($p < 0.05$) WHC-1 and WHC-2 (Table 4) of NPC gels as compared with those of SM is due to the increment in concentration of $I = 0.5$ soluble protein as result of washing (Table 2). Likewise, the highest ($p < 0.05$) WHC-1 of APC is the result of an over-all higher protein content in that system, however, its lower ($p < 0.05$) WHC-2 indicated

Table 3
Moisture and protein in concentrates, sols and gels

System	Moisture (%)			Crude protein (% wet basis)		
	Initial	Sol	Gel	Initial	Sol	Gel
SM	83.7 ± 0.9^{b}	80.0	$78.6 \pm 1.0^{a,b}$	14.3 ± 0.6^{b}	13.8 ± 0.6^{b}	15.2 ± 0.4^{b}
NPC	89.2 ± 1.9^{a}	80.0	77.5 ± 1.3^{b}	10.1 ± 1.7^{c}	9.2 ± 1.6^{c}	11.0 ± 0.9^{c}
APC	81.8 ± 1.3^{b}	80.0	79.1 ± 0.5^{a}	16.6 ± 1.7^{a}	15.2 ± 1.5^{a}	16.4 ± 0.5^{a}

Figures are means and standard deviation of $n = 3$. Figures in the same column with the same superscript are equal ($p > 0.05$). SM: squid muscle. APC: acidic protein concentrate. NPC: neutral protein concentrate.

Table 4
Functionality of sample gels

Parameter		SM gel	NPC gel	APC gel
WHC-1		64.6 ± 6.4^{a}	81.5 ± 10.6^{b}	95.3 ± 2.0^{c}
WHC-2		3.3 ± 0.3^{a}	5.7 ± 0.9^{b}	4.6 ± 0.1^{c}
Quality (FT)		2.8 ± 0.7^{a}(C–A)	3.3 ± 1.2^{a}(C–AA)	5.0 ± 0.0^{b}(AA)
Hardness (g_f)	75%-C	464.2 ± 102.8^{a}	618.4 ± 295.0^{a}	1849.5 ± 459.2^{b}
	90%-C	1181.7 ± 434.1^{a}	1580.3 ± 994.8^{a}	6109.3 ± 1354.1^{b}
Fracture (g_f)	75%-C	366.4 ± 84.1^{a}	605.0 ± 294.0^{a}	No registered
	90%-C	345.3 ± 109.2^{a}	640.0 ± 333.1^{a}	No registered
Gel Strength(g_f cm)	75%-C	114.8 ± 33.0^{a}	174.7 ± 31.6^{a}	455.4 ± 74.2^{b}
	90%-C	241.5 ± 77.4^{a}	427.5 ± 71.8^{a}	1160.0 ± 274.0^{b}
Elasticity (%)	75%-C	56.6 ± 7.9^{a}	73.6 ± 18.9^{b}	86.7 ± 6.8^{c}
Cohesiveness (%)	75%-C	37.1 ± 10.6^{a}	42.0 ± 9.8^{a}	62.7 ± 6.0^{b}

WHC-1: (retained H_2O/total H_2O) × 100. WHC-2: (g H_2O/g protein). 75%-C and 90%-C: percentage of the compression applied. Figures are the means and standard deviation of $n = 3$. Figures in the same row with the same superscript are equal ($p > 0.05$). SM: squid muscle. APC: acidic protein concentrate. NPC: neutral protein concentrate.

that pH changes affected its capacity to bind water, probably as the result of the combined effect of protein aggregation (Table 2) and autolysis (Fig. 1). Results indicated that the effect of treatments on the WHC of gel is better reported in terms of WHC-2, since this functional property should not be attributed to the total protein content but rather to the integrity.

Gels obtained from SM and NPC were smoother and softer than those from APC due to their lower protein content. The APC had a 7.9% more protein than SM and 49% more than NPC (Table 3). Remarkably, FT and TPA data (Table 4), indicated that gels from APC displayed better gel-forming ability due to its higher protein content, regardless of its lower content of $I = 0.5$ soluble protein and WHC-2 ($p < 0.05$).

The TPA parameter of hardness and brittleness indicated a more rigid gel ($p < 0.05$) for APC when compared with those from NPC and SM. This difference might reflect differences in protein concentration as previously mentioned. Pacheco-Aguilar, Crawford, and Lampila (1989), reported that hardness is an indicator of protein per unit weight available to form a gel. Additionally, gel strength, elasticity and cohesiveness, parameters related with structural integrity of proteins were also higher ($p < 0.05$). The APC displayed an outstanding ability to gel highly superior to that of SM and the NPC, giving gel samples with a gel strength of 455 and 1160 g cm at 75% and 90% compression respectively, and an AA folding test grade. Sánchez-Alonso et al. (2007) reported gels made of a protein concentrate of the same species with gel strength in the range 340–440 g cm. Remarkably, gel strength for APC was 2.6–2.7-fold higher than that for NPC at both compression levels (75% & 90%), while 4.0–4.8-fold higher than that for SM. Smaller but significant differences ($p < 0.05$) were observed for cohesiveness and elasticity between APC and NPC or SM (Table 4).

Results suggest that protein aggregates (alkali soluble protein) present in the APC were solubilized by a synergistic effect of ionic strength and heating during sol formation and sol–gel transition, and thus regaining functionality. This fact indicates that acid aggregation was weak and that during heating protein recovered their hydration properties. Elasticity and cohesiveness data correlated well with results from the FT, since gels with lower FT values showed also lower elasticity and cohesiveness. Accordingly with Cortés-Ruiz et al. (2001) and Hultin and Kelleher (1999), results indicate that even after acid precipitation functionality of protein was preserved. However, the results also suggested that protein concentration in APC could mask the impact of the detected degree of hydrolysis in its GFA (Fig. 1).

The colour parameters L and WI for sols and gels are shown in Table 5. Both, the process and the sol-gel transition, had a significant effect ($p < 0.05$) on colour. Unexpectedly sols from NPC had lower L and WI values than those from SM and APC. This apparent discrepancy could be related to the light refraction phenomena (Palombo & Wijngaards, 1990), due to a higher hydration and solubilization capacities of NPC proteins ($I = 0.5$) during sol formation. Data suggests that the protein–protein aggregates in the APC that remain as such even after neutralization adversely affect light refraction and hence the overall colour of sols and gels.

Table 5
Colour parameters of sols and gels

System		L	Whiteness index (WI)
SM	Sol	65.2 ± 4.8^{c}	65.1 ± 4.7^{c}
	Gel	81.0 ± 1.4^{a}	79.8 ± 0.9^{a}
NPC	Sol	53.3 ± 1.0^{e}	53.2 ± 1.0^{e}
	Gel	75.2 ± 3.6^{b}	74.5 ± 3.3^{b}
APC	Sol	60.3 ± 1.8^{d}	59.8 ± 1.7^{d}
	Gel	66.3 ± 2.9^{c}	66.0 ± 2.9^{c}

Values are means and standard deviation of $n = 3$. Means in the same column with the same superscript are equal ($p > 0.05$). SM: squid muscle. APC: acidic protein concentrate. NPC: neutral protein concentrate.

Results indicate that whitening improvements as result of the heat-set gelation were less pronounced for APC. These results highly differs from those of Sánchez-Alonso et al. (2007), who reported a *WI* of 79.5 for a giant squid concentrate (*D. gigas*) obtained by washing in salted solution and isoelectric precipitation, however the authors did not elaborate about *WI* of gels. Whiteness is regarded as a useful index for the overall colour evaluation of surimi and surimi gels (Lanier, 1992). Surimi with a *WI* of 75 or more is considered of excellent quality (Hultin and Kelleher, 1999). Overall, the colour properties of gels obtained in this study were considered acceptable with *WI* between 66 and 79.8.

4. Conclusions

Giant squid (*D. gigas*) muscle proved to be a potential raw material for elaboration of protein concentrates of excellent functional quality when submitted to the acidic dissolution and isoelectric precipitation process. The result of the present study constitutes a significant advance with relation to overcoming the unsuitability of applying the traditional surimi process to squid.

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