FUNCTIONAL EVALUATION OF A PROTEIN CONCENTRATE FROM JUMBO SQUID (*Dosidicus gigas*) VISCERA

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SUMMARY

*Viscera of marine resources represent roughly 10-20% of the total specimen weight and should be studied for utilization in the food industry. In this study, a protein concentrate (PC) was obtained from the viscera of jumbo squids (*Dosidicus gigas*). Protein fractionation of the viscera material yielded 45.08 ±2.09% sarcoplasmic protein, 38.91 ±0.07% myofibrillar protein and 10.27 ±0.18% alkali-soluble proteins, while the PC showed values of 29.53 ±0.17, 51.20 ±0.75% and 15.92 ±0.15%, respectively. The electrophoretic pattern showed the presence of high molecular weight aggregates and sarcoplasmic proteins and/or hydrolyzed proteins of different molecular weights in both systems. It was possible to obtain gels of protein from viscera, but it was not possible to do so from PC. Viscera and PC were not significantly different ($p \geq 0.05$) in emulsification capacity, but the former was more stable; it also showed better foaming capacity than PC, but no difference ($p \geq 0.05$) was detected in the foam stability. The results indicate that the proteins present in the viscera of jumbo squid have low-quality gelling properties, but proteins of this by-product showed good emulsification and foam properties, which could be exploited in the production of food or food ingredients intended for human consumption.*

Introduction

The food industry unavoidably generates by-products as waste, and the fishing industry is not an exception. The processing of fishery products generates bones, skin, fins, heads and viscera as byproducts, which together represent 60-70% of the total body weight (Rustand, 2003; Cavenaghi-Altemio *et al*., 2013). Certain amount of by-products is utilized for animal and aquaculture feed, compost, fertilizer, and other materials, but a high amount is discarded without treatment, causing pollution problems (Bechtel and March, 2012). However, fish byproducts can be important sources of value-added products such as functional proteins, essential fatty acids, enzymes, and other materials for the food industry aimed for human consumption. Hence, the development of technologies for the extraction of functional proteins might provide a means for value-added utilization of by-products (Kil-Yoon *et al*., 2005; Rustad, 2006; Park *et al*., 2008).

Jumbo squid (*Dosidicus gigas*, D'Obigny 1835) represents one of the most important fisheries in Mexico (Cortés-Ruiz *et al*., 2008). At the beginning of the fishery, the mantle was the main portion used for consumption; however, recently, tentacles have been used as octopus imitation, and they are considered a product with major value in the market. This results in a more efficient use of this species. The rest of the body (viscera, fins, and head)

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EVALUACIÓN FUNCIONAL DE UN CONCENTRADO DE PROTEÍNAS DE VÍSCERAS DE CALAMAR GIGANTE (*Dosidicus gigas*)

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RESUMEN

*Las vísceras de los recursos pesqueros representan del orden del 10-20% del peso total del animal, y pudieran ser estudiadas para su posible utilización en la industria alimentaria. En este estudio se obtuvo un concentrado proteico (CP) a partir de vísceras de calamar gigante (*Dosidicus gigas*). El fraccionamiento proteico de las vísceras dio como resultado un 45,08 ±2,09% de proteína sarcoplásmica, 38,91 ±0,07% de proteína miofibrilar y 10,27 ±0,18% de proteína soluble en alcali; mientras que las proteínas del CP mostraron valores de 29,53 ±0,17; 51,20 ±0,75 y 15,92 ±0,15%, respectivamente. En ambos sistemas, el patrón electroforético mostró la presencia de agregados de alto peso molecular y proteínas sarcoplásmicas y/o proteína hidrolizada de distintos pesos moleculares. Fue posible obtener geles a partir de proteínas de víscera, pero imposible de las proteínas del CP. Respecto a la capacidad emulsificante, las proteínas de vísceras y CP no mostraron diferencias significativas (p≥0,05), pero las primeras fueron más estables y también presentaron mayor capacidad espumante que las proteínas del CP, pero sin detectarse diferencias significativas en la estabilidad (p≥0,05). Los resultados indican que las proteínas presentes en las vísceras tienen baja capacidad gelificante, pero poseen buena capacidad emulsificante y espumante, lo cual podría ser explotado en la industria alimentaria para la producción de ingredientes de alimentos.*

AVALIAÇÃO FUNCIONAL DE UM CONCENTRADO DE PROTEÍNAS DE VÍSCERAS DE LULA GIGANTE (*Dosidicus gigas*)

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RESUMO

*As vísceras dos recursos pesqueiros representam entre 10 e 20% do peso total do animal, e poderiam ser estudadas para sua possível utilização na indústria alimentícia. Neste estudo se obteve um concentrado protéico (CP) a partir de vísceras de lula gigante (*Dosidicus gigas*). O fracionamento protéico das vísceras deu como resultado um 45,08 ±2,09% de proteína sarcoplásmica, 38,91 ±0,07% de proteína miofibrilar e 10,27 ±0,18% de proteína solúvel em alcali; enquanto que as proteínas do CP mostraram valores de 29,53 ±0,17; 51,20 ±0,75 e 15,92 ±0,15%, respectivamente. Em ambos os sistemas, o padrão electroforético mostrou a presença de agregados de alto peso molecular e proteínas sarcoplásmicas e/ou proteína hidrolizada de distintos pesos moleculares. Foi possível obter géis a partir de proteínas de víscera, mas impossível das proteínas do CP. Em relação à capacidade emulsificante, as proteínas de vísceras e CP não mostraram diferenças significativas (p≥0,05), mas as primeiras foram mais estáveis e também apresentaram maior capacidade espumante que as proteínas do CP, mas sem detectar-se diferenças significativas na estabilidade (p≥0,05). Os resultados indicam que as proteínas presentes nas vísceras têm baixa capacidade de gelificação, mas possuem boa capacidade emulsificante e espumante, o qual poderia ser explorado na indústria alimentícia para a produção de ingredientes de alimentos.*

is discarded (Torres-Arreola *et al*., 2008). Additionally, the mantle has been used for production of functional protein concentrates that are the basis of sirimi manufacture (Sanchez-Alonso *et al*., 2007; Cortés-Ruiz *et al*., 2008; Dihort-García *et al*., 2011; Galvez-Rongel *et al*., 2014). However, there are no studies related to obtaining protein concentrates from jumbo squid by-products.

Viscera represent ~10% of the total weight of the jumbo squid, and could be a good raw material for the obtaining of protein concentrates (Martínez-Vega *et al*., 2000). The latter might be an alternative to exploitation of functional properties of proteins present in viscera, providing an integral use and avoiding to be discarded. The conventional method of obtaining fish protein concentrates involves muscle washing with water in proportions ranging from 1:4 to 1:8 (muscle/water). During the washing cycles lipids, pigments and sarcoplasmic proteins are removed, while the myofibrillar proteins are concentrated (Gómez-Guillén *et al*., 1997). The proteins obtained might have good functional properties applicable in the food industry, such as gel, foam or emulsion forming ability (Borderías and Montero, 1988). In order to contribute to a better use of squid by-products and reduce pollution problems due to their disposal, the aim of this work was to obtain a protein concentrate from jumbo squid viscera using conventional methods and to study their chemical and functional properties.

Materials and Methods

Raw material

Jumbo squid (*Dosidicus gigas*) was harvested off the coast of Kino Bay, Mexico (28ºN, 112ºW) in May 2012. Twenty specimens exhibited mantle length of 31.6 ±2.5cm and total weight of 757 ±7.50g. The viscera were manually removed from the mantle, discarding the hepatopancreas (digestive gland). The remaining fraction of the viscera was packed in bags and transported on ice to the laboratory. The elapsed time between capture and arrival at the laboratory did not exceed 12h. The viscera samples were stored at -20ºC until their utilization. The total fraction of viscera had an average weight of 138.3 ±8.5g, while for the fraction without the hepatopancreas it was 75.66 ±5.68g. The latter fraction was used for the obtaining of the protein concentrate.

Preparation of protein concentrate (PC)

The squid viscera were mixed with chilled distilled water (~4ºC) in a ratio of 1:5 (viscera:water) and homogenized at 1000rpm for 1min by employing a tissue homoge-

nizer (WiseTis HG-15D; Witeg, Germany). The homogenate was sifted to eliminate stromal protein, and then centrifuged at 15000*g* for 15min at 4ºC using a refrigerated centrifuge (Stratos Biofugue, Sorvall, USA). The precipitate was considered as the protein concentrate (PC) and was stored at 4-5ºC during its evaluation.

Proximate composition

Moisture, fat and ash of viscera and PC were determined according to the AOAC Official Methods (2005), while the protein content was quantified by the micro-Kjeldahl method proposed by Woyewoda *et al*. (1986). Protein yield was expressed as a percentage of net protein present in PC with respect to the total net protein present in viscera.

Protein fractionation

Sarcoplasmic, myofibrillar, alkali-soluble, and stroma proteins were fractionated based on their solubility following the separation methodology proposed by Hashimoto *et al*. (1979) with minimal modifications. An aliquot of 20g of sample was mixed with 200ml of a phosphate buffer of ionic strength I= 0.05 (19.1mM Na_2HPO_4, pH 7.5) and homogenized at 1000rpm for 1min (WiseTis HG-15D; Witeg, Germany). The homogenized sample was centrifuged and the precipitate mixed with 10 volumes of the same buffer, and centrifuged again. The two supernatants were mixed and trichloroacetic acid (TCA) was added until a final concentration of 5% (w/v). The precipitate obtained was considered as the sarcoplasmic protein fraction, while the supernatant was considered as non-protein nitrogen compounds fraction. Initial precipitate (insoluble at I= 0.05) was homogenized with 10 volumes of phosphate KCl buffer of I= 0.5 (0.45M KCl, 19.1mM Na_2HPO_4, pH 7.5) and centrifuged. The precipitate obtained was mixed with the same buffer and centrifuged again. Supernatants were mixed and considered as the myofibrillar protein fraction. The resulting precipitate was submitted overnight to exhaustive extraction with 10 volumes of 0.1N NaOH with continuous shaking and then it was centrifuged. The supernatant obtained was considered as the alkali soluble proteins fraction and the final residue was considered as the stromal protein fraction. All the operations were performed at 4ºC, and centrifugation at 5000*g* for 15min using a refrigerated centrifuge (Stratos Biofugue, Sorvall, USA). Protein concentration in each fraction was determined by the micro-Kjeldahl method according to the procedure of Woyewoda *et al.* (1986).

Electrophoretic profile (SDS-PAGE)

The protein samples were mixed with a solution made of 8M urea, 0.1mM phenylmethylsulphonyl fluoride (PMSF), 10mM ethylenediaminetetraacetic acid (EDTA), 0.01% NaN_3 and 0.6M KCl. The electrophoretic profile of the two protein systems (viscera: V; protein concentrate: PC) was analyzed by polyacrylamide gel electrophoresis (PAGE) using sodium dodecyl sulfate (SDS), with and without β-mercaptoethanol, in a discontinuous gel (4% stacking gel and 10% separating gel) according to Laemmli (1970). A Mini PROTEAN 3 Cell Multi-Casting Chamber (Bio-Rad Laboratories, USA) was used. Twenty µg of protein were loaded into each lane of the gels, and a broad range molecular weight protein standard solution (Sigma-Aldrich, MO, USA) containing myosin (200k Da), β-galactosidase (116kDa), phosphorylase b (97kDa), bovine serum albumin (66kDa), glutamate dehydrogenase (55kDa), ovalbumin (45kDa), glyceraldehyde 3-phosphate dehydrogenase (36kDa), carbonic anhydrase (29kDa), trypsinogen (24kDa), trypsin inhibitor (20kDa), α-lactalbumin (14.2kDa) and aprotinin (6.5kDa) was used. Electrophoretic runs were performed at room temperature (25ºC) at 120V. After electrophoresis, the gel was stained with 0.5% (w/v) Coomassie Brilliant Blue R-250 in 40% (v/v) methanol and 7 % (v/v) acetic acid, and the gel was de-stained with 40% (v/v) methanol and 7 % (v/v) acetic acid.

Gel forming ability

Heat-set gels were prepared for each protein system (V and PC) and their gel forming ability analyzed in terms of the folding test (FT) and the texture profile analysis (TPA) (Cortés-Ruiz *et al.,* 2008). For the heat-set gel preparation, protein systems were adjusted at 80% of moisture with addition of sucrose and 2.5% NaCl, and the mix was homogenized for 1min. Each sol was placed in glass petri dishes (1cm height) that were packed in sealed plastic bags, and were heat-set in a water bath at 90ºC for 30min. Heat-set gels were immediately chilled to 2-4ºC, and stored refrigerated for 24h before proceeding to functional evaluation.

The gels were tempered for 60min at room temperature prior to analysis. The FT was carried out as described by Tanikawa *et al*. (1985). The test was conducted by folding a 3.0mm by 30.0mm 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 (Lanier, 1992): 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 but 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 TPA was conducted on cylinder-shaped samples of uniform dimensions (1cm diameter and 1cm height) obtained from each gel using a sharp-edged plastic tube. Texture was measured using a texturometer (TA-XT2 Plus; Texture Technologies, USA). Compression forces at 50% of the original gel sample height were used to compute gel strength (g_f), fracturability (g_f), cohesiveness (%) and elasticity (%).

Water holding capacity (WHC)

WHC was measured using the methodology reported by Dihort-García *et al.* (2011). A 5g portion of each gel was centrifuged at 3000*g* for 20min at 4ºC in a refrigerated centrifuge (Stratos Biofugue, Sorvall, USA). The resulting WHC was expressed as a percentage of water retained with respect to the total amount of water present in the sample before centrifugation.

Color

Before the gel preparation of each protein system (V and PC), the color of sols was measured by tri-stimulus colorimetry using a colorimeter (model CR-300, Konica Minolta Sensing, USA). Samples were packed into petri dishes (1cm height) and the readings were taken at four different points. Color coordinates for degree of luminosity (L), redness/greenness (+a/-a), and yellowness/blueness (+b/-b) were obtained. From the color coordinates, the whiteness index (WI) was calculated as $WI= (100 - ((100 - L)^2 + a^2 + b^2)^{1/2})$ (Lanier, 1992).

Emulsifying properties

Emulsifying capacity (EC) and emulsion stability (ES) for V and PC were determined according to the method described by De la Fuente-Betancourt *et al.* (2008). A total of 9ml of edible oil were added to 50ml of protein solution at 0.3% (w/v). The mixture was homogenized at high speed at room temperature (WiseTis HG-15D; Witeg, Germany). Then, the homogenate was centrifuged at 2000*g* for 5min at 20ºC. The volume of the emulsion was measured for each protein concentrate. The EC was calculated as the

ratio between the volume of the emulsion formed and the initial volume of the mixture. The ES for each concentrate was determined by heating the emulsion at 80ºC for 30min. The emulsion volume was measured after heating. ES was reported as the EC percentage of the remaining emulsion after heating.

Foaming properties

Foaming capacity (FC) and foam stability (FS) of V and PC were evaluated according to the method proposed by Sathe and Salunkhe (1981). A total of 50ml of a 1% (w/v) protein solution was used. The solution was mixed at high speed for 2min using a homogenizer (WiseTis HG-15D; Witeg, Germany). Next, it was immediately poured into a graduated cylinder. The FC was calculated by measuring the foam volume at minute zero. The FS was evaluated by monitoring the foam volume after 60min.

Statistical analysis

The experiment was conducted three times (n=3) and each determination was performed in triplicate. Descriptive statistics (mean and standard deviation), one-way analysis of variance (ANOVA) and multiple comparison by the Tukey test were applied. Data analysis was performed using the JMP 5.0.1 statistical package.

Results and Discussion

Samples

Jumbo squid (*Dosidicus gigas*) viscera (V) had a mean weight of 138.3 ±8.5g, and that of the hepatopancreas was 62.6 ±4.16g. V represented 18.23% of the total weight of whole squid, while viscera without hepatopancreas amounted to 10.01%. This yield is higher compared to the one obtained by Martínez-Vega *et al.* (2000), who reported 10.86% of yield for total fraction of viscera from jumbo squid captured on May 1992 in Santa Rosalía, Baja California, México. This difference in yield may be due to factors of species variability as size, sex, physiological maturity, food, and other factors (Rustad, 2006).

Proximate composition and protein yield

The chemical composition of jumbo squid has been associated to the reproductive stage and time of capture, and also to factors of variability of the species (Rustad, 2006; Taheri *et al.*, 2013). Proximal composition on dry and wet basis of jumbo squid viscera (V) and its protein concentrate (PC) are listed on Table I. Proximal composition on dry basis of V (without hepatopancreas) was different than that reported by Martínez-Vega *et al.* (2000) for the total fraction of viscera from the same species (71.86% protein, 9.04% lipids, 6.09% ash and 13.01% others). Protein and lipid content of V was higher and lower, respectively, than that reported by Martínez-Vega *et al.* (2000). These differences could be related, mainly, to capture season and specimens size (Rustad, 2006). Also, the low content of lipids of V could be attributable to hepatopancreas removal, because in lean marine organism like squid, lipid content is concentrated generally in liver or hepatopancreas (Phillips *et al.*, 2002). Muscle with a low lipid content is desirable for obtaining protein concentrates used for surimi production, due to less susceptibility to oxidation reactions and/or protein-lipid aggregation, which can cause a decrease in functionality of muscle proteins (Saeed and Howell, 2002). V on wet basis show similar crude protein content as the mantle (14.3 ±0.6%) of the same species (Cortés-Ruiz *et al.*, 2008). Also, the protein content of V can be compared to that obtained for complete fraction of viscera of Alaska pollock (*Theragra chalcogramma*; 15.2%), Pacific cod (*Gadus macrocephalus*; 13 %), and pink salmon (*Oncorhynchus gorbuscha*; 15.3%) (Bechtel, 2003).

TABLE I
PROXIMATE COMPOSITION ON DRY AND HUMIDITY BASE OF JUMBO SQUID VISCERA (V) AND ITS PROTEIN CONCENTRATE (PC)

Component	Dry basis		Wet basis	
	V	PC	V	PC
Moisture	-	-	82.57 ±0.09 b	91.53 ±0.22 a
Crude protein	79.04 ±0.64 a	80.87 ±2.98 a	13.46 ±0.44 a	6.58 ±0.38 b
Lipids	4.55 ±0.41 b	5.43 ±0.68 a	0.79 ±0.07 a	0.46 ±0.06 b
Ash	11.67 ±0.08 a	5.85 ±0.19 b	2.03 ±0.01 a	0.49 ±0.02 b
Others*	4.74	7.85	1.15	0.94
NPN**	-	-	4.42 ±0.14 a	0.96 ±0.05 b

Values are the mean ±standard deviation of n= 3. Different superscripts within the same rows indicate significant differences ($p<0.05$).
*Calculated by difference.
**Non protein nitrogen.

As expected, protein, lipid, ash and non protein nitrogen content of V decreased considerably by the washing and centrifugation stages for obtaining PC. The moisture content of PC was higher than V, which can be attributed to fixation of water in the protein structure of V during the washing process. This could have had repercussions on the functional behavior of PC, mainly in the gels preparation. Protein concentrate of viscera from marine species has not been obtained before, so the results obtained for this experiment were compared with protein concentrates obtained from jumbo squid mantle, fillet and/or viscera from fish species. PC showed a protein yield of 49.04 ±1.70% with respect to initial net protein content of V. This protein yield is lower than those obtained by Galvez-Rongel *et al.* (2014) and Encinas-Arzate *et al.* (2014) for protein concentrates from mantle of the same species (55.9 and 69.49%, respectively). The protein yield from PC means that ~50.96% of the total protein of the raw material (V) was solubilized and discarded in the supernatant after centrifugation. The high content of water soluble proteins has already been reported for viscera from fish species. Bechtel (2003) reported 61.6, 36 and 47.7% of water soluble protein for viscera of Alaska pollock, Pacific cod and pink salmon, respectively.

Protein fractionation

Protein fractions obtained for jumbo squid viscera (V) and its protein concentrate (PC) are shown in Table II. V showed high content of soluble protein at the I= 0.05 (sarcoplasmic protein) with respect to that reported by Cortés-Ruiz *et al.* (2008) of 34.7% and Dihort-García *et al.* (2011) of 29.14% for mantle in the same species. This was expected, because components of viscera have naturally a high content of sarcoplasmic proteins, mainly enzymes, due to their physiological function, the digestion of food (Bechtel, 2003). Also, peptides could be present in sarcoplasmic fraction as products of enzymatic hydrolysis. This agrees with the high content of water-soluble proteins reported by Bechtel (2003) for viscera from fish species, as described

TABLE II
PROTEIN FRACTIONS OF JUMBO SQUID VISCERA (V) AND THEIR PROTEIN CONCENTRATE (PC)

Protein fraction (%)	V	PC
Soluble at I= 0.05	45.08 ±2.09 a	29.53 ±0.17 b
Soluble at I=0 .5	38.91 ±0.07 b	51.20 ±0.75 a
Alkali-soluble	10.27 ±0.18 b	15.92 ±0.15 a
Insoluble	5.72 ±0.05 a	3.34 ±0.24 b

Values are the mean ±standard deviation of n= 3. Different superscripts within the same rows indicate significant differences ($p<0.05$).

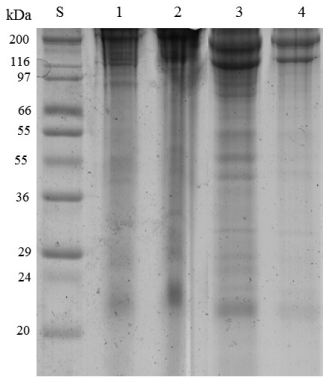

Figure 1. Electrophoretic profile of jumbo squid (*Dosidicus gigas*) viscera (V) and protein concentrate (PC). Lane S: wide range standard; lanes 1 and 2: V and PC; lanes 3 and 4: V and PC with addition of β-mercaptoethanol.

previously. The content of soluble protein at I= 0.5 (myofibrillar protein) of V increased after the centrifugation stage for the production of the protein concentrate (PC). The latter is because of discarding water soluble protein in the wash water (supernatant) and subsequent concentration of myofibrillar proteins.

The content of the alkali-soluble fraction of V was in the range reported by Cortés-Ruiz *et al.* (2008) at 2.4% and by Dihort-García *et al.* (2011) at 14.11% for jumbo squid mantle. Dihort-García *et al.* (2011) attributed the high content of alkali-soluble fraction to differences in capture season and size of the studied specimens. But the presence of the alkali-soluble fraction in viscera could be attributed to the formation of protein aggregates during frozen storage before PC production. The alkali-soluble fraction and insoluble fraction (stromal protein) of V were concentrated after the production of PC.

Electrophoretic profile (SDS-PAGE)

The polyacrylamide gel electrophoresis (SDS-PAGE) is an analytical method in which proteins are separated according to their size. The electrophoretic profile of V and PC proteins is shown in Figure 1. Under denaturing conditions (without β-mercaptoethanol), both protein systems showed intense bands at the top of the gel, which could be attributed to the presence of high molecular weight aggregates. These aggregates might be those which were solubilized in the alkaline solution (0.1M NaOH). Moreover, under reducing conditions (with β-mercaptoethanol) the aggregates disappeared and intense bands of 163.23, 115.26 and 100.7kDa in V, and 185.7 and 132kDa in PC, were noticed. The band at 100.7kDa could be paramyosin (PM), a protein usually present in mollusks (Kijowski, 2001; Hooper and Thuma *et al.*, 2005). A 97kDa molecular weight for paramyosin from mantle jumbo squid has been reported (Cortés-Ruiz *et al.*, 2008), which is similar to that obtained in the present study for viscera of the same species. In both V and PC, thin and soft bands were noticed along the lane, which may indicate the presence of a sarcoplasmic protein fraction, where the majority could correspond to enzymes and/or peptides produced by hydrolysis of digestive proteases.

TABLE III
PARAMETERS OF GEL-FORMING ABILITY OF JUMBO SQUID VISCERA (V) AND THEIR PROTEIN CONCENTRATE (PC)

Parameter	Gel of V	Gel of PC
Water holding capacity (%)	98.86 ±1.02 a	95.80 ±0.18 a
Folding test	5.0 ±0.00 a	1.66 ±0.58 b
Hardness (g_f)	116.01 ±8.7	-
Fracturability (g_f)	Not present	-
Elasticity (%)	88.9 ±0.03	-
Cohesiveness (%)	65.9 ±0.02	-

Values are the mean ±standard deviation of n= 4. Different superscripts within the same rows indicate significant differences ($p<0.05$).

Gel-forming ability

Gel-forming ability has been used as an indicator of myofibrillar protein functionality, because it determines the feasibility of using a fishery resource as surimi or restructured products (Kim, 2003). The gel-forming ability of V and PC was evaluated in terms of texture profile analysis (TPA) and folding test (FT), and the gels obtained were used to determine water holding capacity (WHC). The parameters of gel-forming ability of V and PC are shown in Table III. Gels made of PC were not suitable for TPA evaluation, while gels obtained from viscera (V) could be evaluated. This is interesting because in a study by Cavenaghi-Altemio *et al.* (2013) it was found that it was not possible to obtain sausages from tilapia viscera due to technological problems observed during cooking. However, the gels obtained from V showed different characteristics compared to gels elaborated from squid mantle. All studies performed with mantle or fin from jumbo squid have shown higher hardness than those obtained in the present study; however, the gels made with viscera showed higher cohesiveness and elasticity (Cortés-Ruiz *et al.*, 2008; Dihort-García *et al.*, 2011; Galvez-Rongel *et al.*, 2014).

Hardness, elasticity and cohesiveness parameters of the gels are related to the concentration and structural integrity of the proteins, as well as to protein type (Pacheco-Aguilar *et al.*, 2000); therefore, the lack of gel-forming ability of PC could be due to low protein concentration. Regarding structural integrity, V showed two disadvantages: a high concentration of sarcoplasmic proteins (45.08 ±2.09%), which might be mainly hydrolytic enzymes from the digestive tract. It is known that the sarcoplasmic proteins coagulate at 62- 68°C and exhibit a low gelling ability (Kijowski, 2001). Therefore, the gelling capacity of the myofibrillar proteins of both protein systems undoubtedly was affected by the coagulation of the sarcoplasmic proteins during heat treatment. Likely, this effect might have been lower in the PC gel, due to a lower proportion of sarcoplasmic proteins in comparison to V. Nevertheless, although it is considered that the sarcoplasmic proteins interfere with gelation of the myofibrillar fraction, several studies have reported that addition of low concentration of sarcoplasmic proteins increased the strength of myofibrillar protein gels (Karthikeyan *et al.*, 2004; Pérez-Mateos *et al.*, 2004; Yongsawatdigul and Piyadhammaviboon, 2007; Jafarpour and Gorczyca, 2009). On the other hand, protein degradation during processing by endogenous or exogenous enzymes present in the viscera probably affected the formation of the tridimensional protein network during sol-gel transition (Rustand, 2003). No fracturability was noticed in V gels, which exhibited a rubbery consistency, as corroborated sensorially by pressing the gel with the fingers. This behavior could be also due to the high content of sarcoplasmic fraction.

The folding test (FT) is a subjective test that complements the values obtained in the texture profile analysis (TPA). In this study, V gel showed better results than PC gel. This evaluation confirmed the absence of fracturability found in V gels, obtaining a

TABLE IV
COLOR PARAMETERS OF JUMBO SQUID VISCERA (V) AND THEIR PROTEIN CONCENTRATE (PC)

Parameter	V	PC
Luminosity (*L**)	52.56 ±0.24 a	54.35 ±0.51 a
Redness (*a**)	1.99 ±0.06 a	1.64 ±0.03 b
Yellowness (*b**)	9.54 ±0.10 a	6.84 ±0.20 b
Hue angle	86.87 ±0.23 a	85.03 ±0.11 b
Whiteness	51.57 ±0.25 b	53.81 ±0.53 a

Values are the mean ± standard deviation of n= 3. Different superscripts within the same rows indicate significant differences ($p<0.05$).

AA quality, because none of them broke down. Furthermore, the PC gels were of C-D quality (1.66), as most of them were broken immediately when folded in half. The PC gel behavior can be attributed to the low concentration of protein.

Water holding capacity (WHC)

The WHC is defined as the ability and capacity of muscle proteins to retain and immobilize water when any external force such as centrifugation or pressure is applied. The WHC of the V and PC gels showed no significant difference ($p \geq 0.05$). The WHC presented by both protein systems (V and PC) was higher than those reported for jumbo squid mantle and its respective neutral protein concentrate, reported by Cortés-Ruiz *et al.* (2008). This difference might be due to the differences in protein concentration and type of proteins present. However, although PC had a low protein proportion, it showed a similar value than that of V. This could be because PC had a higher fraction of myofibrillar proteins, which are responsible in a 97% for the WHC (Kijowski, 2001).

Color

Color is one of the most important factors that influence the acceptance of foods by consumers. Proper color along with texture are two critical quality attributes of restructured fish food products as surimi (Taskaya *et al*., 2010). The color parameters obtained for PC and V are shown in Table IV. An apparent pink-brown color was obtained for both systems, which may be due to the presence of melanin (the main component of squid ink) and the inherent color of the different organs that are part of the viscera, since usually the internal organs of certain marine organisms have pigments such as carotenes and hemoproteins (Russo *et al.,* 2003).

The luminosity (L*), redness (a*) and yellowness (b*) values placed both protein systems (V and PC) in the red-yellow quadrant of the color solid. Differences ($p<0.05$) in color between V and PC might be due to the washing processes performed for obtaining the PC, which could have eliminated color producing compounds, obtaining lower values of a* and b* in PC in relation to V. L* values found in V and PC are considered acceptable for surimi; however, the whiteness values of the two systems were not. In fish, the color of protein concentrates can determine its application in a particular product (Taskaya *et al*., 2010). It has been mentioned that surimi must present L* values >50 and low but positive values of a* and b* parameters (Lanier, 1992). Likewise, the product must have whiteness values >75 (Pérez-Mateo *et al*., 2004). Nonetheless, the discoloration of protein concentrates does not limit their use as food or food ingredient, since they can be utilized in various products. For example, white protein concentrates are useful for obtaining kamaboko, pink proteins concentrates can be used for sausages preparation, while red proteins paste serve for shrimp, lobster, crab and salmon imitation and, thus, different colors can be exploited in other areas of the food industry (Simpson, 2007).

Emulsifying properties

The emulsifying capacity (EC) and emulsion stability (ES) of proteins from viscera (V) and its protein concentrate (PC) are shown on Table V. The EC of V and PC showed no significant difference ($p \geq 0.05$), and was similar to that reported for protein concentrates obtained from *D. gigas* via acidic, alkaline and neutral dissolution, but less than a protein concentrate obtained by direct isoelectric precipitation (Galvez-Rongel *et al*., 2014). Similar results have been reported by De la Fuente-Betancourt *et al*. (2008), who studied the EC of squid mantle proteins and its effect during storage in ice, reporting an emulsion percentage at days 0 and 15, of 23 and 33%. The ES obtained indicated that PC had a significantly lower value in comparison with V. The ES of both systems was low copared to proteins obtained from squid mantle; in this sense, Galvez-Rongel *et al*. (2014) assessed the ES of protein concentrates obtained via acidic, alkaline, neutral dissolutions, and by mean of direct isoelectric precipitation, finding a stability of nearly 100%, with the exception of the neutral protein concentrated, which showed a stability of 79%, similar to that obtained for V in this study. The results of emulsifying properties indicate that the viscera have an emulsifying capacity similar to that reported for neutral protein concentrates obtained from squid mantle, but slightly less stable.

TABLE V
EMULSIFYING AND FOAMING PROPERTIES OF JUMBO SQUID VISCERA (V) AND THEIR CONCENTRATE (PC)

	V	PC
Emulsifying capacity (%)	20.90 ±0.97 a	19.21 ±0.98 a
Emulsifying stability (%)	74.25 ±3.11 a	59.16 ±2.79 b
Foaming capacity (%)	43.93 ±2.48 a	37.49 ±1.18 b
Foaming stability (%)	32.39 ±2.10 a	28.85 ±2.23 a

Values are the mean ± standard deviation of n= 4. Different superscripts within the same rows indicate significant differences ($p<0.05$).

The differences in ES between V and PC were attributed to the higher protein content of the sarcoplasmic fraction (albumins, enzymes and/or peptides of hydrolyzed myofibrillar proteins) in V. Additionally, the autolysis carried out by proteases from the digestive tract, generating hydrolyzed proteins or peptides might confer emulsion stability. It has been reported (García-Barrientos *et al*., 2006) that moderate proteolysis of myofibrillar proteins can stabilize emulsions because partially denatured proteins in oil-water can improve the ES for the unfolding and coverage of a larger area of dispersed phase (lipid globules).

Foaming properties

The foaming capacity (FC) and foaming stability (FS) proteins from viscera and its protein concentrate are shown on Table V. FC was higher for the V system ($p<0.05$), which was expected. As was mentioned above for the emulsifying properties, a limited enzymatic hydrolysis in V could have improved the FC. The FC obtained in this study was higher than that reported by Galvez-Rongel *et al.* (2014) for a neutral protein concentrate obtained from squid mantle (19%); however, it was lower than that reported by the same authors for protein concentrates obtained via acid and alkaline dissolution, 78 and 80% respectively. Regarding FS, no difference was found between the two systems ($p \geq 0.05$). However, FS was low in comparison to that obtained for proteins from squid mantle (73%; De la Fuente *et al*., 2008) and protein concentrates obtained from the same organism (83%; Galvez-Rongel *et al*., 2014). Results of foaming properties indicate that the V and their respective PC have better foaming ability, but are less stable, with respect to neutral protein concentrates obtained from squid mantle, and a similar behavior was found for the emulsifying property.

As well as emulsifying properties, a possible enzymatic hydrolysis in V and in the PC may have improved foaming properties. This might be due to an increase of molecular flexibility and exposure of hydrophobic groups. However, a severe hydrolysis could increase the concentration of low molecular weight peptides, which cannot form cohesive films at the interface and thus impair ES (Damodaran, 2008).

There is little information about surface properties of

proteins of marine origin, as most of the studies on functionality focus on gelling property; however, surface properties are protagonists in many products of the food industry (Galvez-Rongel *et al*., 2014).

Conclusions

A protein concentrate from jumbo squid viscera with poor yield was obtained, due to the elimination of a high proportion of the soluble protein in the wash water. Proteins from viscera were characterized by having a high content of sarcoplasmic protein and low proportion of myofibrillar protein, and possibly a high proteolytic activity. All these characteristics have a direct impact on their functional properties, especially in gelation, obtaining gels with low-quality for proteins from viscera and no gelling capacity for its protein concentrate. However, the proteins of this byproduct can be used for the manufacture of foams or emulsions, because they were favorable and even better than those of neutral protein concentrates obtained from mantle where the viscera was slightly better in comparison with the obtained protein concentrate. The proteins from jumbo squid viscera have good capacity for the formation of emulsions and foams, and can be considered as an ingredient for food manufacture or food ingredients in which these properties are desirable. Nevertheless, additional studies must be conducted in order to determine the possible accumulation of heavy metals in jumbo squid viscera, as well as the microbial load or pathogenic microorganisms in the viscera, although viscera from other organisms have been used for human consumption. Hence, it is possible to use viscera from nonconventional sources for human or animal consumption.

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