Journal of Aquatic Food Product Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/wafp20

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Version of record first published: 11 Oct 2008.

To cite this article: J. A. Cortes-Ruiz MSc, R. Pacheco-Aguilar PhD, G. Garciasanchez MSc & M. E. Lugo-Sanchez MSc (2001): Functional Characterization of a Protein Concentrate from Bristly Sardine Made Under Acidic Conditions, Journal of Aquatic Food Product Technology, 10:4, 5-23

To link to this article: http://dx.doi.org/10.1300/J030v10n04_02

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ABSTRACT. A protein concentrate from bristly sardine (*Opisthonema libertate*) was produced under acidic conditions and its gel-forming ability evaluated by texture profile analysis and folding test index. Process protein recovery and lipid removal were greater than 60% and 85%, respectively. A protein-protein aggregate, insoluble in 0.56 M NaCl, was

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This work is part of the graduate thesis research of J. A. Cortés-Ruiz. Appreciation is extended to CIAD, A.C. for its support to this work. Authors want to thank Dr. Michael Morrissey for his review and comments on the manuscript.

detected during sol preparation. The protein concentrate showed a good-to-excellent gel-forming ability with an averaged folding test of 4.5. Storage of whole fish for five days at 0°C affected the gel-forming ability of the concentrate.

KEYWORDS. Sardine, protein concentrate, protein functionality, surimi

INTRODUCTION

Given the high nutritional value of aquatic foods and its importance in the world nutrition, most fisheries resources are destined for human consumption. In 1997, world fisheries production was 122.1 million tons, of which 76.2% was destined for human consumption, mainly as fresh, refrigerated, frozen, dried-salted and canned products. The remaining 23.8% was used for feed production (meal and fish oil) (FAO, 1997) and was comprised of small pelagic fish species like anchovy and sardine (FAO, 1991).

Surimi is a successful fish protein extract of highly functional characteristics and is found in many international markets. Traditionally, surimi technology has been applied to lean species, since they present few problems related to the removal of fat. Several fisheries that have potential for large-volume surimi production are small pelagic fish stocks. However, the muscle of small pelagic species, such as sardine, can have fat contents as high as 20%, which can be a problem in surimi processing (Pacheco-Aguilar et al., 2000; Méndes et al., 1997). Fish mince in high fat species require a more elaborate washing process causing low recoveries and a reduced stability and functionality of the protein (Méndes et al., 1997).

Over the past few years, the use of underutilized small pelagic species have been considered with great interest for the production of surimi and surimi based products. Recently, several research efforts have been designed to solve the technological problems present in the use of small pelagic species for surimi. Kelleher and Hultin (1999) and Pacheco-Aguilar et al. (2001), have developed alternative methodologies to conventional surimi processing to produce a functional protein concentrate from small pelagics. The main objective of the present work was to produce a protein concentrate from bristly sardine (*Opisthonema libertate*), a small pelagic fish from the Gulf of California, applying a procedure based on the isoelectric precipitation of muscle protein and to compare its functionality with that produced by the traditional procedure for making surimi from fatty fish. The effect of fish freshness in process recovery and protein functionality of the concentrate was also evaluated.

MATERIALS AND METHODS

Raw Material

Bristly sardine (*Opisthonema libertate*) was harvested off the Gulf of California coast by a commercial fishing vessel from the Port of Guaymas, Mexico. From November of 1999 to March of 2000, five different sampling lots (20-30 kg/each) no more than 6 h post-catch, were obtained at the moment of unloading at the plant dock. Broken and bruised specimens were discarded. Undamaged sardines (15.5-18.5 cm long) were packed in alternate layers of ice and fish for transportation to the laboratory for processing. Elapsed time from capture to start of processing never exceeded 24 h. All experimental fish were held in crushed ice (0°C) and transported to the Laboratories of Centro de Investigación en Alimentacion y Desarrollo, Hermosillo, Mexico. Upon arrival to the laboratory a random sample of 10 sardines were separated and measured to register average weight and length. Six to eight kg of round fish were processed into skinless fillets by hand and thoroughly washed. Visceral and blood contamination was carefully avoided. Each sample (approximately 2 kg) was divided into three working lots and kept in ice until used. The first and second lots (A and B) (Freshness-1) were processed immediately to make the protein concentrates while the third lot (C) (Freshness-2) was kept at 0°C for 5 days before processed.

Proximate Composition

Proximate analyses were carried out for sardine muscle and protein concentrates. Moisture, protein, non-protein nitrogen (NPN) and ash content was determined according to AOAC (1990); lipid content was determined by the chloroform–methanol method described in Woyewoda et al. (1986).

Color

Color was measured in muscle, protein concentrates and their correspondent heat-set gels. Color was measured using a HunterLab D25 Reflectance Tristimulus Colorimeter (Hunter Assoc. Laboratory, Reston, VA). Color coordinates were used to measure the degree of lightness (L), redness (+a) or greenness (−a), and yellowness (+b) or blueness (−b). Additional color traits such as whiteness, hue angle ( ) and total color difference (E) were calculated from Hunter "L," "a" and "b" values (Park et al., 1996; McLellan et al., 1995). Whiteness was calculated by the equation: $100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$ (Lanier, 1992).

pH

Samples (muscle and concentrates) were prepared according to Martin (1992) by blending 2 g of sample with 18 mL of distilled water for 1 min at 22°C in a Tissumizer tissue homogenizer (Model TR-10, Tekmar Co., W. Germany). The pH was measured using a Corning digital pH meter (Model 240, Corning Inc., NY).

Manufacturing of Protein Concentrate by the Ishikawa Method (PC-I)

Lot A (1 kg) was used for the protein concentrate following the Ishikawa process (PC-I) as described in Rousell and Cheftel (1988) with some modifications. The PC-I thus obtained was used as control. The process included 4 washing exchanges with a mince to water ratio of 1:5 (w/w). For the first washing exchange a 0.5% sodium bicarbonate solution was used instead of water. Water was used for the second, third and fourth washing exchanges. For each exchange, minced flesh and water mixtures were gently stirred 5 min (0-4°C), then allowed to settle for 30 min in a refrigerated chamber (0°C) prior to manual dewatering using gauze cloth with a 20 × 12 weave. Final moisture content was approximately 80%. The PC-I was packed in plastic bags, sealed and kept under ice in a refrigerated chamber until used. Elapsed time before using PC-I for analyses and evaluation of its gel-forming ability did not exceed 12 h. A mass balance was applied to calculate solid and protein recovery and removal of lipid and minerals.

Manufacturing of Protein Concentrate by the Isoelectric Precipitation Method (PC-IP)

The process includes the procedure outlined by Kelleher and Hultin (1999) with some modifications. Lots B and C of 500 g each were used in this part of the experiment. The minced flesh was mixed with 4.5 L of water (0°C) and homogenized for 10 min in a Tissumizer Model TR-10 homogenizer (Tekmar Co., W. Germany). The homogenate was acidified by adding 1M HCl until a pH 3.2 was reached. The homogenate was then divided into 10 fractions of 500 mL each and centrifuged under vacuum for 15 min at 16,000 × g in a Beckman Model J2-21 refrigerated centrifuge (Beckman Instrument Inc., Palo Alto, CA). After centrifugation 4 fractions were distinguished in the centrifuge flask (a fatty supernatant, a protein solution phase, a gelatinous protein intermediate phase and a precipitate). The upper lipid phase was decanted and discarded. The protein solution phase (PSP) was decanted and kept at 0°C. The last two phases at the bottom of the flasks were collected and reprocessed as described above by adding 4.5 L of water and adjusting the pH at 3.2. The new PSP was collected and mixed along with the previous one. The protein content of the

composite PSP was precipitated by adjusting the pH of the solution to 5.5 using 1 M NaOH and separated by centrifuging at 16,000 × g for 10 minutes. Supernatant was decanted and the PC was further centrifuged at 1,000 × g for 1 min to eliminate more water. Final PC moisture contents was approximately 80%. The PC-IP thus produced was packed in plastic bags, sealed and kept under ice in a refrigerated chamber until used. Elapsed time before using the PC-IP for analyses and evaluation of its gel-forming ability did not exceed 12 h. A mass balance was applied to the process to calculate solid and protein recovery and removal of lipid and minerals.

Gel Preparation

Test samples of 120 g of PC-I were mixed after the addition of 2.5 g NaCl/100 g of PC 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%. Mixing was continued until the sol temperature reached 5°C. Each sol was packed into a Petri dish (1 cm height) and vacuum sealed in moisture/vapor-proof film bags (Nylon 0.75 MIL-Adhesive-Polyethylene 2.25 MIL, 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 10°C in a ice-water mixture and held overnight at 2-4°C prior to texture evaluation.

Texture Profile Analysis (TPA) and Folding Test (FT)

Fish gels were tempered for 30-40 min to ambient temperature (23°C) prior to analysis. Six cylinder-shaped samples of uniform dimensions (1.5 cm diam × 1 cm long, 0.75-0.85 g) were cut from each gel using a sharp-edged copper tube. Texture was measured in a Stable Micro Systems texturometer (Texture Technologies Corp., NY) with the 3.9 cm diam compression plunge attached to a 25 kg load cell. Compression forces at 90% and 75% (double bite analysis) of the original gel sample length were used to compute hardness, brittleness, cohesiveness and elasticity. Results were reported as described by Abbott (1972). For each textural parameter 7 samples/gel/replicate were evaluated.

The gel-forming ability was also evaluated using the folding test described by Tanikawa et al. (1985). The test was conducted by folding a 3.0 mm thick by 2.5 cm diam 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 SA = extremely elastic gel (no cracks on folding into quarters); grade A = moderately elastic gel (no cracks on folding in half; cracks on folding into quarters); grade B = slightly elastic gel (some cracks on folding in half); grade C = non-elastic gel (breaks into pieces on folding in half); and

grade D = poor gel (breaks into pieces with finger pressure). Ten samples/gel/replicate were evaluated.

Water Holding Capacity (WHC)

WHC was evaluated by the technique outlined by Jiang et al. (1985). A portion of 5 g of each gel was centrifuged at 3,000 × g for 20 min (4°C). WHC was expressed as g of water held per g of protein in the gel (Chin et al., 1993).

Salt-Soluble Protein (SS-P)

SS-P was carried out in both mince and PC following the methodology described by Camou and Sebranek (1991) with small modifications. An aliquot of 3.5 g of sample was mixed with 35 mL of saline solution (pH 8.3) containing 0.56 M NaCl, 17.8 mM $Na_5P_3O_{10}$ and 5 mM NaN_3. Sample container was placed in an ice-bath. The mixture was homogenized for 1 min, avoiding foaming by fitting the mouth of the sample container with parafilm. The homogenate was centrifuged at 12,800 × g for 1 h at 2°C. Protein concentration in the supernatant was determined by the Biuret method (Gornall et al., 1949). Solubility was expressed as g of solubilized protein/g of protein in the sample.

SDS-PAGE Electrophoresis

Polyacrylamide gel electrophoresis (PAGE), using both a dissociating sodium dodecyl sulfate (SDS) and mercaptoethanol-SDS buffer system in a discontinuous gel (4% stacking gel and 10% running gel) were run according with Laemmli (1970). A Mighty Small II SE 250 electrophoretic chamber (Hoefer Scientific Instrument, San Francisco, CA) was used. Minced muscle, saline extract from concentrates and the whole concentrates were analyzed for their electrophoretic protein patterns. The extraction solutions were a 0.56 M NaCl and a dissociating system (8 M urea, 0.1 mM PMSF, 10 mM EDTA, 0.01% NaN_3 and 0.6 M KCl). Electrophoretic runs were performed at 18-22°C for 2.5 h at 80 volts. A high-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. Resulting gels were analyzed by comparing densitometric tracings of the protein bands in a GS-710 densitometer (BioRad Laboratories, Richmond, CA).

Statistical Analysis

Data was analyzed using one-way ANOVA and Tukey-Kramer multiple comparison test with a significance level of 5% (Montgomery, 1991). Three

replicates were carried out for each treatment. All analyses were carried out in duplicate.

RESULTS AND DISCUSSION

Muscle Composition

Table 1 shows the relation of sampling period to weight, size and yield of the edible fraction. Sampled fish complied with the minimal commercial size stipulated by the Mexican Ministry of Fisheries for sardine (NOM-003-PESC-1993). Data for total size was similar to the reported by Ramirez-Suarez (2000) for the same species. Average yield recovery was 34.8 2.6%, which agreed with the expected yields fish as reported by Suzuki (1981).

Muscle composition of fish was not affected ($p \geq 0.05$) by the degree of freshness nor sampling period (Table 2). Composite averages (dry weight basis) were 6.4 0.7, 13.2 0.5 and 76.5 0.3, for ash, lipid and protein, respectively (data not shown). The lipid content in muscle of sardine species has been reported to fluctuate widely. Matzumori et al. (1979) reported a fat content varying from 2.6 to 18.4%, Pacheco-Aguilar et al. (2000) from 1.1% to 8.4%, Ramirez-Suarez et al. (2000) from 10.2% to 36.8% (dry-weight), while Jacquot (1961) from 5 to 15% for mature sardine. The lipid content of sardine muscle in this study was considered low (3.3% wet-weight). This low fat content could favor the application of the proposed methodology for the species.

NPN which includes free amino acids, peptides, guanidine compounds, nucleotides, urea and quaternary ammonium compounds was determined as part of the chemical composition of muscle. According with Simidu (1961), the

TABLE 1. Effect of sampling period in the weight, size and edible yield of bristly sardine.

Sampling period	Weight (g)[1]		Standard size (cm)*[1]		Total size (cm)**[1]		Yield (%)[2]	
09 Nov. 1999	132.0	14.5 [a]	19.0	0.7 [a]	23.0	1.0 [a]	33.4	1.4 [a,b]
18 Jan. 2000	98.8	8.5 [b]	17.6	0.5 [b]	21.8	0.6 [b]	31.4	1.0 [a]
13 Feb. 2000	142.3	20.9 [a]	19.4	1.0 [a]	23.9	1.1 [a]	34.4	0.3 [a,b]
04 Mar. 2000	126.6	7.3 [a,b]	18.8	0.5 [a]	23.4	0.5 [a]	37.5	0.6 [b]
29 Mar. 2000	109.3	20.5 [a,b]	17.6	0.9 [b]	21.6	1.1 [b]	37.5	1.8 [b]

*Without including caudal fin. **Including caudal fin.
[1] Values are mean and standard deviation of 10 specimens (n = 10).
[2] Values are mean and standard deviation of two sardine batches (n = 2). Means in the same column with the same superscript are equals ($p \geq 0.05$).

TABLE 2. Chemical composition of protein concentrates (% dry weight basis).

	Ash		Lipid		Protein		NPN	
Freshness-1								
PC-I	3.9	0.8 [a]	11.1	5.4 [b]	74.6	7.2 [b]	0.23	0.05 [a]
PC-IP	2.2	1.5 [a]	4.1	3.0 [a]	86.0	6.4 [a]	0.14	0.05 [a]
Freshness-2								
PC-IP	3.5	1.3 [a]	2.8	2.3 [a]	85.8	3.7 [a]	0.16	0.05 [a]

Figures are the mean and standard deviation of n = 5.
Means in the same column with the same superscript are equals ($p \geq 0.05$).

normal content of NPN in sardine muscle varies from 500 to 600 mg/100 g muscle. In this study, the averaged NPN content for fresh sardine (< 24 h post-catch at 0°C) was 410 20 mg/100 g muscle, while 320 40 mg/100 g muscle was found for 5-day old samples at 0°C. Similar results in NPN reduction were reported by Pacheco-Aguilar et al. (2000) for Monterey sardine stored at 0°C for 15 days. NPN data along with the organoleptic characteristics of the sample (data not shown) suggest that the experimental fish was still near its optimal condition after day storage at 5°C.

Composition of Protein Concentrates

The chemical composition of the PC-I and PC-IP are shown in Table 2. Results suggest that the degree of fish freshness had no effect ($p \geq 0.05$) on the composition of PC-IP. Since concentrates had final moisture content of approximately 80%, their lipid content in a wet-weight was lower than 1% for the PC-IP and 2.2% for the PC-I. The lipid content for the PC-IP was similar to those reported for surimi from lean species (Chang Lee et al., 1989; Pacheco-Aguilar et al., 1989). The operations for the PC-IP process such as muscle homogenization, dissolution of protein at acidic pH (3.2) followed by its isoelectric precipitation at pH 5.2 and centrifugation facilitated the removal of lipid compared with the traditional PC-I process. Similarly, the PC-IP process recovered both sarcoplasmic and myofibrilar protein, while much of the sarcoplasmic and some myofibrilar protein were lost on the PC-I process during the consecutive water exchanges.

Process Yields

Recoveries of solids and protein and removal of lipid (Table 3) were dependent on the process with higher solids and protein recovery ($p < 0.05$) achieved by PC-IP. Values for solids and protein recoveries and lipid removal were 57%, 72%, and 34%, respectively. No statistical difference ($p \geq 0.05$)

TABLE 3. Process yield.

Process	Solids Recovery (%)	Proteins Recovery (%)	Lipids Removal (%)
PC-I	36.8 4.1 [a]	38.2 5.8 [a]	67.4 6.9 [a]
PC-IP Freshness-1	55.6 2.4 [b]	64.2 3.2 [b]	88.3 4.8 [b]
PC-IP Freshness-2	59.8 2.2 [b]	67.4 1.2 [b]	92.6 2.6 [b]

Figures are the mean and standard deviation of n = 5.
Means in the same column with a same superscript are equals ($p \geq 0.05$).

was detected for yields between both PC-IP's (fresh and 5-day old samples). Protein recovery for the PC-IP's was on average 65.8%, which exceeded the recovery of 63% reported by Kelleher and Hultin (1999) for capelin (*Trisopterus minutus*) using the same procedure. Pacheco-Aguilar et al. (2001) recovered 57.5% and 55.8% of total protein in making surimi from Monterey sardine (*Sardinops sagax caerulea*), by applying procedures that involve acidic and alkaline washing operations.

In addition to the stroma protein that was precipitated out in the process, some protein was lost during the centrifugation following protein solubilization at pH 3.2. A gelatinous soft gel was formed in the interphase "stroma-dissolved protein." As noted by Kelleher and Hultin (1999), most of this gel is protein and could be potentially recovered by redissolving. In order to clarify this and to increase the over-all protein recovery, the precipitate obtained after this centrifugation was re-processed and further increased the protein recovery to 76%.

Removal of lipid constitutes the foremost problem in using oily fish species as raw material to produce functional protein concentrates including surimi. Lipid promotes protein denaturation and loss of functionality during the storage of the concentrate. In this study, about 90% of the total lipid content in sardine muscle was removed by the PC-IP. Complete disruption of muscle and cell membranes during the homogenization stage is essential for a high lipid removal. Results suggested that removal of lipid could be improved by increasing both homogenization and centrifugation forces during the production of concentrate. Pacheco-Aguilar et al. (2001) reported a removal of lipid no higher than 67% by modifying water pH during the washing exchanges. Neither muscle homogenization nor centrifugation were involved in their process.

Color

The color parameters "L," "a," "b," hue angle and whiteness index for mince, PC's and heat-set gels are shown in Table 4. The unwashed mince (control)

TABLE 4. Color parameters for the sardine mince, concentrates and gels.

	L		a		B		1		B2	
Sardine mince	38.0	1.7^{d}	5.8	0.3^{c}	7.7	1.0^{c}	52.6	4.0^{c}	37.2	1.6^{e}
PC-I	52.9	1.1^{b}	1.8	0.2^{b}	10.0	0.4^{a}	80.0	1.4^{a}	51.8	1.07^{c}
PC-I (Gel)	62.1	1.3^{c}	−0.3	0.2^{a}	11.7	0.7^{a,b}	91.3	0.9^{b}	60.3	1.15^{d}
PC-IP (Neutralized pH 7.2)	46.3	1.6^{a}	0.3	0.5^{a}	10.5	0.8^{a}	90.4	6.4^{a,b}	45.2	1.5^{a}
PC-IP (Gel)	50.0	2.4^{b}	0.7	0.4^{a}	12.9	0.5^{b}	86.8	1.6^{a,b}	48.3	2.2^{b,c}

Values are mean and standard deviation of n = 5. Means in the same column with a same superscript are not significantly different ($p > 0.05$). [1] : Hue Angle. [2]B: Whiteness index

showed a dark red/yellow hue due to the presence of myoglobin in muscle and possibly by the contamination of mince with hemoglobin and skin pigments (Park, 1995). It had an average whiteness value of 37.2. Similar data for other sardine species has been reported in the literature (Ramirez-Suarez et al., 2000; Terán-Peralta, 1994; Hernández-Cervantes, 1993). In their study, Ramirez-Suarez al. (2000) indicated that the increase in "L" and the decrease in "a" in the washed sardine mince was mainly due to the removal of myoglobin and suggested that the change in "a" value as result of washing was the major contributing factor to improve whiteness. Similarly, our results indicated that the process had a significant effect in the color of concentrates and that the parameter "a" was the most affected ($p < 0.05$) by the process. Additionally, the acidic condition of the PC-IP process more efficiently removed ($p < 0.05$) the red pigments from the mince resulting in a more yellowish hue for this concentrate. However, PC-I was lighter and whiter in color. Data suggests that some of the protein-protein aggregates generated at the acidic pH during the PC-IP process might remain after the neutralization step, adversely affecting light refraction and hence the over-all color of the concentrate. This protein aggregation effect may mask the removal of dark/red pigments observed in the PC-IP process. Further studies are required to clarify this.

Whiteness is regarded as an useful index for the overall color evaluation of surimi (Lanier, 1992). Surimi with a whiteness index of 75 or more is considered of excellent quality (Kelleher and Hultin, 1999). In either process, whiteness data in the present study was far from optimum. Results indicated that changes in color (hue angle and whiteness) as result of the heat-set gelation were more positively pronounced for PC-I. As mentioned previously, disadvantages of PC-IP color could be related to aggregated protein structures present prior to gelation. The degree of fish freshness did not show any significant effect on color attributes neither in mince nor concentrates nor heat-set gels (data not shown).

Protein Functionality

In order to evaluate the functionality of the PC-IP, its final pH was adjusted from 5.5 to 7.2 prior to analysis (Table 5). The pH adjustment of the PC-IP above the original muscle pH of 6.1-6.2 was done to contrast protein aggregation and facilitate its solubility during the preparation of sols. It is known that changes in pH alter protein structure resulting in conformational modifications and changes in surface hydrophobicity affecting functionality (Watabe et al., 1991; Haard, 1992). Kelleher and Hultin (1999) reported that in spite of changes in pH to which the muscle proteins are subjected to in their acid-wash method, proteins preserve their functionality.

In the present study, a dramatic ($p < 0.05$) loss of protein solubility from concentrates was observed for PC-IP samples. These samples decreased 5 and 6 fold in their ability to be extracted as salt-soluble protein as compared with mince and PC-I samples. The data indicated that protein aggregates remained in the PC-IP even after adjustment of pH to 7.2 and that the ionic strength of the extraction solution was not enough to bring the protein aggregates into solution. The PC-IP concentrate showed an adequate gel-forming ability, however, and the process affected the water holding capacity (WHC) of heat-set gels (Table 5). The WHC of protein gels depends on the physicochemical properties of their proteins such as hydrophobicity, solubility and dispersion capacity (Smith, 1988). Changes in WHC of protein gels indicate protein denaturation by changes in the charge and structure of their constituent proteins

TABLE 5. pH and functional properties of PC's and their heat-set gels.

Freshness-1	**pH**	**Proteins Solubility[1]**	**Water Holding Capacity[2]**
Sardine fillet	6.1 0.1 [a]	0.64 0.11 [b]	*
PC-I	7.0 0.2 [b]	0.75 0.07 [b]	*
PC-I (Gel)	*	*	5.0 0.5 [b]
PC-IP (Neutralized at pH 7.2)	7.2 0.1 [b]	0.13 0.06 [a]	*
PC-IP (Gel)	*	*	4.0 0.3 [a]
Freshness-2			
Sardine fillet	6.2 0.1 [a]	0.72 0.08 [b]	*
PC-IP (Neutralized at pH 7.2)	7.3 0.2 [b]	0.18 0.04 [a]	*
PC-IP (Gel)	*	*	4.1 0.3 [a]

[1] g soluble protein/g total protein, [2] g water held/g total protein, * not applicable.
Values are mean and standard deviation of n = 5. Means in the same column with the same superscript are equals ($p \geq 0.05$).

(Gómez-Guillén et al., 1997a,b; Hasting et al., 1990). WHC data confirmed the fact that protein aggregates prevail to some extent after the adjustment of PC-IP at pH 7.2 and subsequent sol formation. Mendes et al. (1977) showed that WHC of a protein gel should not be attributed to its total protein content but rather to its quality. Protein solubility and WHC were not affected ($p \geq 0.05$) by the degree of fish freshness.

Moisture content of heat-set gels were adjusted to 80%, however their protein content were not equal since chemical composition of concentrates were different (Table 2). The PC-IP concentrates had an average 15% more protein than the PC-I sample. The gel-forming ability is a functional property that reflects the extent of protein-protein and protein-water interaction in a protein-water system. An excess in protein-protein interactions may result in a hard and inelastic gel, while an excess of protein-water interactions produces a soft and fragile gel. Remarkably, both PC-IP samples showed ability to gel regardless of the low solubility of the protein present in the system (Table 5). Results shown in Table 6 also indicated that PC-I and PC-IP made from fresh sardine had a good-to-excellent gel forming ability as measured by the folding test (FT) procedure. The TPA parameters of hardness and brittleness indicated a more rigid gel ($p < 0.05$) for the PC-IP when compared with the gels from the PC-I. This difference might reflect differences in protein concentration as mentioned previously. Pacheco-Aguilar et al. (1989) reported that hardness is an indicator of protein per unit weight available to form a gel. On the other hand, TPA parameters of elasticity and cohesiveness, properties related to the structural integrity of proteins, were equal ($p \geq 0.05$) for both gel types. Results suggest that protein aggregates present in the PC-IP were disrupted by a synergistic effect of ionic strength and heating during sol formation and sol-to-gel transition. This indicates that such aggregation was weak and that during heating, proteins recovered their hydration properties. Elasticity and

TABLE 6. Texture parameters of heat-set gels.

Parameter	% Compression	Gel from PC-IP (F1)		Gel from PC-IP (F2)		Gel from PC-I	
Cohesiveness (%)	75	31.7	5.0 [a]	26.9	5.0 [b]	32.1	5.0 [a]
Elasticity (%)	75	66.3	8.0 [a]	58.1	7.0 [b]	68.5	6.0 [a]
Folding test		4.5	0.35 [a,1]	3.1	0.82 [b,2]	4.75	0.25 [a,1]
Fracturability (kg_f/g)	90	2.880	0.64 [a]	2.292	1.03 [a]	2.211	0.76 [a]
Hardness (kg_f/g)	90	9.280	1.51 [a]	9.382	1.55 [a]	6.771	1.76 [b]

[1]Quality A to AA, [2]Quality B , according to the FT scale.
Values are mean and standard deviation of n = 3.
Means in the same row with a same superscript are equals ($p \geq 0.05$).

cohesiveness data correlated well with results from the FT. Our results agreed with those reported by Kelleher and Hultin (1999) for lean fish species, as even under acidic conditions, functionality of proteins was highly preserved. The TPA parameters of elasticity and cohesiveness for the PC-IP gels were affected ($p < 0.05$) by the degree of freshness. These results agreed with those of the FT. Results showed that the post-mortem events in sardine muscle during the 5-day storage prior to its utilization significantly affected ($p < 0.05$) the functionality of the concentrate.

Our results agreed with those in the literature (Pacheco-Aguilar et al., 2001; Ramirez-Suarez et al., 2000; Alvarez et al., 1999; Alvarez and Tejada, 1997) that different sardine species could be used to elaborate protein concentrates and surimi with good-to-excellent gel forming ability resulting in heat-set gels with FT grades of AA to A.

Electrophoretic Analysis of PC

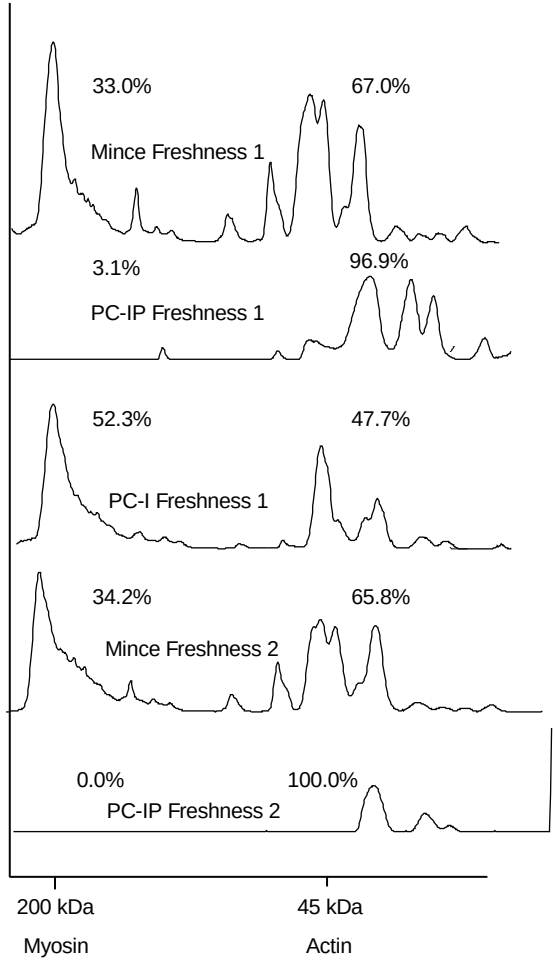
Densitograms of the electrophoretic protein profiles of protein solutions extracted from mince and PCs used for the analysis of protein solubility are shown in Figure 1. Densitograms for mince from both fresh and 5-day-old sardines and PC-I showed proteins of high molecular weight (MW) (200 kDa) indicating the presence of non-aggregate myofibrilar (myosin) protein in the system. However, the absence of high MW protein in the extract is highly noticeable for the PC-IP samples. This result correlates well with the low protein solubility detected for those PCs (Table 5), suggesting a mechanism that involved protein-protein aggregation as the result of processing under acidic conditions.

Densitograms in Figures 2 and 3 showed the presence of myosin and high MW protein aggregates in mince and all the protein concentrates. Electrophoretic data was similar for all materials suggesting that both hydrophobic and covalent protein-protein aggregation were involved. A noticeable peak at the right-hand side of 200 kDa was observed for 5-day-old mince and both PC-IPs indicating proteolytic activity in the samples.

CONCLUSIONS

The PC-IP samples showed good gel-forming ability when compared with the conventional procedure employed to make surimi from oily species. Compared to the Ishikawa process, water usage was reduced by 55% and yield increased by 41%. Yield could be further improved through better homogenization and centrifugation. The acidic condition used during the PC-IP process promoted a protein-protein aggregation that requires further study to establish its nature. Additional work is needed to determine how this protein aggrega-

FIGURE 1. Densitogram of the electrophoretic profiles (ME-SDS-PAGE) of protein extracted from mince and protein concentrates using a solution 0.56 M NaCl.



tion can be more efficiently disrupted during the sol-to-gel transition with adjustment of ionic strength, pH and the use of polyphosphates. The effect of frozen storage in the stability and functionality of the protein system of the PC-IP needs to be evaluated in detail. Results showed that freshness affected functionality of the PC-IP. Overall, this process proved to be a good alternative to elaborate a functional protein concentrate from a small pelagic fish species such as bristly sardine.

FIGURE 2. Densitogram of the electrophoretic profiles (SDS-PAGE) of protein extracted from mince and protein concentrates using 8 M urea.

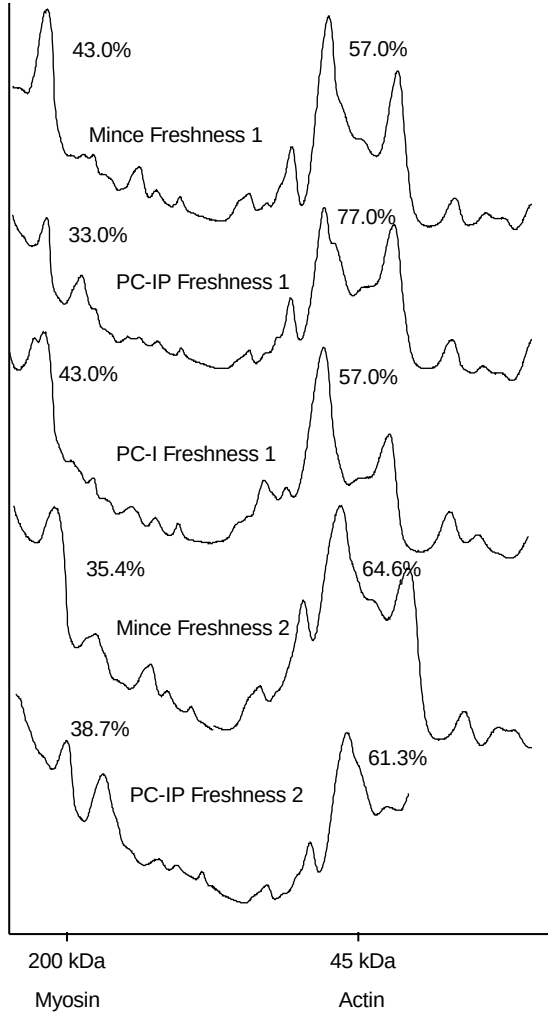
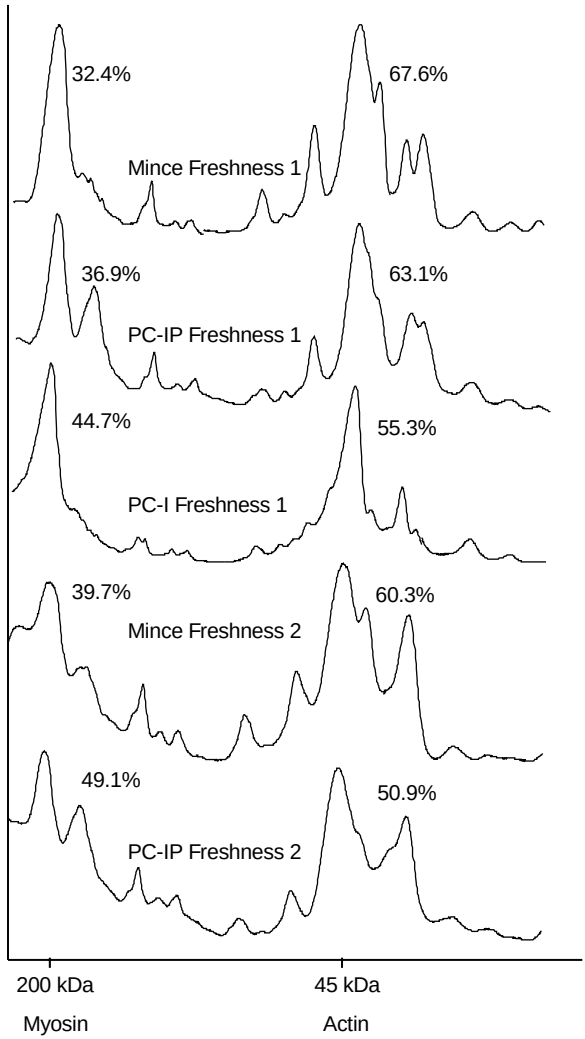


FIGURE 3. Densitogram of electrophoretic profiles (ME-SDS-PAGE) of protein extracted from mince and protein concentrates using 8 M urea.



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