Functional, thermal, and physicochemical properties of proteins from Argentine croaker (*Umbrina canosai*) recovered by solubilization/precipitation or a washing process


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**Abstract**

Proteins were recovered from Argentine croaker by two methods: alkaline extraction-isoelectric precipitation and a washing process with cold distilled water and sodium chloride. The objective of this study was to evaluate the effect of the recovery method on the physicochemical, structural, thermal and functional properties of proteins recovered from the Argentine croaker muscle. The alkaline extraction-isoelectric precipitation and washing process yielded significantly different (p < 0.05) protein contents of 92.13% (as isolated protein) and 88.70% (as myofibrillar protein), respectively. Polar amino acids comprise approximately 61.50% and 59.80% of the myofibrillar and isolated protein, respectively. The digestibility of the myofibrillar and isolated protein did not differ significantly (p > 0.05). The isolated protein had a lower lightness (81.04) than the myofibrillar protein (83.87) but high redness (2.55) and yellowness (15.47) values. The total enthalpy of denaturation of the myofibrillar protein (1.47 J/g) was higher than that of the isolated protein (0.33 J/g). The myofibrillar protein showed more compact ultrastructures and higher protein degradation than the isolated protein. The solubility and water-holding capacity of the isolated protein were higher than those of the myofibrillar protein at the same pH. Functional studies show that pH-shift processing may improve the functional properties of Argentine croaker proteins relative to myofibrillar protein. However, the results also show that some of the characteristics, as color and thermal properties, of the isolated protein were lower than those of the myofibrillar protein, suggesting that the two materials can be used to obtain different products with high added value.

**Keywords**

Fish  
Protein  
Alkaline extraction  
Washing  
Properties

**Article history**

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**Introduction**

The Argentine croaker (*Umbrina canosai*) is a fish species caught in large amounts in the Southern Brazil coast. However, this species is underutilized and has low commercial value (Lempek *et al.*, 2007). Meanwhile, exploitation of natural resources and increased environmental pollution have created a need for a more value-extracting use of fish processing plant residues and fish species of low commercial value (Martins *et al.*, 2011). Muscle proteins from underutilized fish species are nutritive and easily digested and exhibit good functionality, making them desirable for various food applications. However, the use of fish proteins as a food or food ingredient has been limited by their low stability compared to mammalian and vegetable proteins. Efforts are currently ongoing to better utilize these species. Many authors are using these species to obtain protein isolates and washed minced fish, which can be considered products of high added value (Nolsøe and Undeland, 2009; Rawdkuen *et al.*, 2009; Rocha *et al.*, 2013; Rocha *et al.*, 2014; Yarnpakdee *et al.*, 2014).

Protein isolates have been shown to have good functionality for application in different food formulas (Nolsøe and Undeland, 2009; Gehring *et al.*, 2011) and contain sufficient amounts of all essential amino acids for adult humans (Foh *et al.*, 2012; Marmon and Undeland, 2013). According to Tahergorabi *et al.* (2012), the protein isolate obtained by acid- and alkaline-aided solubilization has shown significant potential for protein recovery from muscle. Solubilization or precipitation processing allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids and the removal of materials not intended for human consumption, such as bones, scales, skin, connective tissue, cellular membranes, and neutral storage lipids through centrifugation (Nolsøe and Undeland, 2009; Gehring *et al.*, 2011). In their studies, Foh *et al.* (2012) report that protein isolates are more stable and
have elevated functionality than unprocessed protein. Isolated proteins have been largely utilized as a protein basis for biodegradable films, as functional ingredients, and as protein hydrolysate, among materials (Gehring et al., 2011; Tahergorabi et al., 2012; Rocha et al., 2013; Tahergorabi et al., 2014; Rocha et al., 2014). According to Kristinsson and Liang (2006), washing is one of the most important steps in surimi manufacturing, as it improves the gel-forming ability due to the leaching of a considerable amount of fat and sarcoplasmic proteins. According to Nolsøe and Undeland (2009), the washing process involves a sequence of washes with cold water or a slightly alkaline solution. Fish sarcoplasmic proteins are a large family of proteins that include myogens and enzymes and are soluble in water or low ionic strength solution (Nolsøe and Undeland, 2009; Gehring et al., 2011). Many water-soluble compounds are diluted, and some of the neutral fat is removed. However, the loss of sarcoplasmic proteins and some of the myofibrillar proteins into the washing water reduces the total protein yield. Limpan et al. (2010) obtained a fish myofibrillar protein with a protein content of 88.3%, which was utilized in film forming solutions. The objective of this study was to evaluate the effect of the recovery method on the physicochemical, structural, thermal and functional properties of proteins recovered from Argentine croaker muscle. This fish species is underutilized and has low commercial value, which due to its protein content awake interest for improved use thereof.

Material and Methods

Material

The fish species used in this study was the Argentine croaker (Umbrina canosai), provided by a fish processing company from the city of Rio Grande, Southern Brazil. The fish was transported in ice-filled containers to the Laboratory of Food Technology at the Federal University of Rio Grande (FURG). The fish were washed in chlorinated water at a concentration of 5 ppm at 4 °C and then beheaded, eviscerated and filleted. The samples were packaged in plastic bags and stored in a freezer at -18 °C. The sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium chloride (NaCl) and chemicals used in the protein functional analysis were purchased from Synth (Diadema, São Paulo, Brazil).

Preparation of Argentine croaker protein isolates (CPI)

The alkaline extraction and acid precipitation method described by Nolsøe and Undeland (2009) was used to prepare CPI, with some modifications. The muscle of Argentine croaker was homogenized with distilled water (proportion 1:9, w/v). The pH was adjusted to 11.2 with 1 mol.L-1 NaOH, and the mixture was stirred in a stainless steel reactor for 20 min at 4°C. After solubilization, the sample was centrifuged at 9000 x g (Hanil, Supra 22K, Korea) for 20 min at 4°C. During centrifugation, the sample separated into three phases. The upper phase (neutral lipids) was discarded, the middle phase (soluble proteins) was subjected to isoelectric protein precipitation, and the bottom phase (insoluble proteins) was discarded. The pH of the soluble proteins was adjusted to pH 5.0 with 1 mol.L-1 HCl and stirred in a stainless steel reactor for 20 min at 4°C. The sample was centrifuged at 9000 x g for 20 min at 4°C. The CPI was freeze dried (Liotop, São Carlos, Brazil), ground in a knife-mill (Tecnal, TE-633, Piracicaba, Brazil), sieved through N° 42 mesh (0.35 mm) and stored at -18°C.

Preparation of fish myofibrillar protein (FMP)

FMP was obtained according to Limpan et al. (2010) with some modifications. The Argentine croaker muscle (CM) was homogenized in cold distilled water (ratio 1:3, w/v) at 13,000 rpm (Fisatom, 712, São Paulo, Brazil) for 2 min, followed by filtering through a layer of nylon cloth. The CM was homogenized with cold NaCl (50 mmol.L-1) for 5 min at 13,000 rpm and filtered through a layer of nylon cloth. The washing process was repeated twice. The FMP obtained were then freeze dried (Liotop, L108, São Carlos, Brazil), ground in a knife-mill (Tecnal, TE-633, Piracicaba, Brazil), sieved through a N° 42 mesh (0.35 mm) and stored at -18°C.

Chemical composition of myofibrillar protein and protein isolates

The chemical composition of CPI and FMP were conducted in triplicate according to the AOAC (2000) method considering the analytical N° 992.15, 923.03, 960.39 and 925.30 protein (N x 6.25), ash, fat and moisture analyses, respectively.

Digestibility

The in vitro digestibility of the protein samples was determined in triplicate according to Akeson and Stahman (1964), with some modifications, as follows. First, 1 g of protein was added to 10 mL of pepsin (3.0 mg/mL) and dispersed in 0.1 mol.L-1 HCl. The mixture was shaken at 90 rpm using a shaker (Cientec CT-712RNT, Piracicaba, Brazil) at 37 °C for 3 h and then neutralized with 0.3 mol.L-1
NaOH and treated with 10 mL pancreatin (4.0 mg/mL) in phosphate buffer at pH 8.0. The mixture was shaken at 130 rpm in a shaker at 37°C for 24 h. The reactions were stopped by adding an equal volume of 10 mL trichloroacetic acid (30%, w/v) (TCA), and the volume was adjusted to 50 mL with TCA (5%, w/v). The protein precipitates were removed by centrifugation at 5000 x g for 20 min, and the supernatant was filtered. The TCA-soluble nitrogen in the supernatants was determined by the micro-Kjeldahl method (N x 6.25).

Determination of amino acid composition

The amino acid composition was determined in triplicate according to a method proposed by Bidlingmeyer et al. (1984). The samples were previously hydrolyzed with distilled 6 mol.L⁻¹ HCl for 22 h at 110 ± 1°C.

Color characteristics

CPI and FMP colors were measured in triplicate using a colorimeter (Minolta, CR-400, Osaka, Japan) with the CIE $L^*$, $a^*$ and $b^*$ color scale according to the HunterLab method (1997). Illuminant D65 was used, and the color values were $L^*$= darkness (0) to brightness (+); $a^*$= greenness (−) to redness (+); and $b^*$=blueness (−) to yellowness (+). The CPI and FMP were added to a sample cup to a thickness of 5 mm. Readings were acquired by rotating the sample cup clockwise in five different positions.

Thermal properties

The thermal properties of CPI and FMP were determined using differential scanning calorimetry (TA-60WS, Shimadzu, Kyoto, Japan) according to Meng and Ma (2001) with some modifications. The CPI and FMP samples (2.0 mg) were weighed into aluminum pans, and 10 µL of phosphate-buffered saline (PBS; 0.1 M, pH 7.0) was added. The pans were hermetically sealed in aluminum pans and scanned at a heating rate of 10 °C/min over the range 30 – 200 °C. A sealed empty pan was used as a reference. The temperature at which denaturation started, known as the onset denaturation temperature (Tonset), was calculated as the intercept of the baseline and the extrapolated maximum slope of the peak. The peak denaturation temperature (Tpeak) was considered to be the temperature of maximum heat flow. The enthalpy of thermal denaturation was calculated from the area of the endothermic peak.

Thermogravimetric analysis (TGA)

The thermal gravimetric analysis (TGA) of CPI and FMP was conducted using a Shimadzu DTG 60 instrument (Osaka, Japan) according to Kummar et al. (2014) with modifications. Samples of 8 - 10 mg were heated from 30 to 600°C at a rate of 10°C/min. Nitrogen was used as a purge gas at a flow rate of 50 mL/min. The weight losses of the samples were measured as a function of temperature.

Scanning electron microscopy (SEM)

Microstructure studies of myofibrillar and isolated proteins were performed in duplicate using a scanning electron microscope (JEOL, JSM 6610, Tokyo, Japan). The samples were deposited on aluminum stubs using double-sided adhesive carbon conductive tape and coated with a thin gold layer. The morphology of the surface of the samples was imaged at an acceleration voltage of 20 kV.

Protein solubility

Protein solubility was determined in triplicate according to the method of Tadpitchayangkoon et al. (2010), with minor modifications. The solubilities of CPI and FMP were determined at pH values between 3 and 11. The protein (0.5%, w/v) was dispersed in 50 mL distilled water under magnetic stirring (Warmnest, HJ-3, Curitiba, Brazil), and then the pH was adjusted to the desired value using HCl or NaOH at 0.1 or 1 mol.L⁻¹. After 30 min of stirring, the dispersions were centrifuged (BioSystems, MPW-350R, Curitiba, Brazil) at 8667 x g for 20 min, and the supernatant was filtered. The protein contents of the CPI and FMP were determined according to Lowry et al. (1951) by measuring the absorbance at 660 nm of the supernatants using bovine serum albumin (BSA) as standard curve (0.04 - 0.42 mg/mL). Protein solubility was expressed as the percentage of protein in the supernatant relative to the total protein in the initial dispersion.

Water absorption capacity (WAC)

The WAC of the CPI and FMP was measured at different pH values between 3 and 11 in triplicate according to Regenstein et al. (1979). A 1% (w/v) protein dispersion was prepared in 2 mL of NaCl (0.1 mol.L⁻¹), and the buffer solution corresponding to the desired by pH was added up to a volume of 40 mL. The dispersion was homogenized for 15 min in a magnetic stirrer (Warmnest, HJ-3, Curitiba, Brazil) at room temperature, and the samples were then centrifuged (BioSystems, MPW-350R, Curitiba, Brazil) for 20 min at 8667 x g. Finally, the supernatant was filtered. The soluble proteins in the supernatant were quantified by the method of Lowry et al. (1951) relative to the total protein of the original sample. The WAC was determined as follows:
reported that the alkaline solubilization of Channel catfish (*Ictalurus punctatus*) yielded a lower lipid content compared with the surimi process. According to Gehring et al. (2011), using basic pH resulted in greater fat reduction in the recovered proteins.

The ash content in CPI (1.51%) was significantly lower (p < 0.05) than that in FMP (3.32%). This content can be verified by the presence of residues of the sodium chloride utilized in washing the minced fish. Tokur et al. (2006) reported an ash content of 1.80% in washed minced fillets from mirror carp (*Cyprinus carpio L*.). Rocha et al. (2013) verified that Argentine anchovy protein isolate presented an ash content of approximately 1.0%. In studies of the effects of washing treatments and alkali extraction on the gelation characteristics of striped catfish (*Pangasius hypophthalmus*) muscle protein, Tadpitchayangkoon and Yongsawatdigul (2009) observed a reduction in ash content (0.86%) of the protein isolate when compared with water-washed mince (3.39%) or alkali-washed mince (3.67%). The high concentration of ash in myofibrillar protein is explained by the accumulation of NaCl during the washing process.

**Digestibility of proteins**

An important parameter for protein nutritional quality is digestibility. According to Tahergorabi et al. (2015), muscle proteins are of high nutritional quality due to their high biological value and bioavailability. The digestibility of the nutrients must be known to fully evaluate the significance of nutrient concentration. The digestibility of the CPI and FMP samples is shown in Table 1. The CPI and FMP did not show significantly different digestibilities (p > 0.05). Food processing can both increase and decrease the protein digestibility. Induced structural changes can make the protein more or less accessible for digestive enzymes, and protein oxidation may decrease the digestibility by, e.g., creating S–S bonds (Marmon and Undeland, 2013). However, in this study, changes in protein digestibility were not observed when comparing the protein digestibilities obtained by the pH-shift and minced washing methods. Marmon and Undeland (2013) verified that pH-shift-isolated proteins from herring (*Clupea harengus*) retained good digestibility.

**Color characteristics**

The color characteristics differed between the CPI and FMP, as shown in Table 1. CPI had a significantly (p < 0.05) lower value of L* (81.04) compared with FMP (83.87). However, CPI had significantly (p < 0.05) higher a* (2.55) and b* (15.47) values. The

\[
WAC = \frac{W}{wP}
\]

Where W is the mass of water retained by protein (g) and wP is the initial mass of protein that absorbed water (g).

**Statistical analysis**

The samples analytic determinations were performed in triplicate and standard deviations were reported. Means were compared by Tukey’s test at 5% level of significance by analysis of variance (ANOVA) using the software Statistica (Version 5.0, by StatSoft, Inc., Tulsa, U.S.A).

**Results and Discussion**

**Chemical composition of myofibrillar and isolated proteins**

The chemical composition of CPI and FMP is presented in Table 1. The moisture content of CPI was significantly (p < 0.05) higher than that of FMP. In this study, the CPI had a higher protein content and lower lipid content than did FMP (p < 0.05). The CPI also had a higher protein content than that reported by Rocha et al. (2013), who obtained a protein content of 88.8% for the Argentine anchovy (*Engraulis anchoita*) protein isolate. Marmon and Undeland (2013) obtained an isolated protein from herring (*Clupea harengus*) fillets with a protein content of 86%, lower than that found in this study. According to Limpian et al. (2010), the myofibrillar protein from Bigeye snapper (*Priacanthus tayenus*) presented 88.3% total protein, similar to the value found in the present study. García and Sobral (2005) obtained myofibrillar proteins from freeze-dried Nile tilapia with a protein content of 80%, a lower value than that reported in this study. According to Nolsøe and Undeland (2009), water-soluble compounds are diluted and removed during the washing, and the loss of sarcoplasmic proteins and some myofibrillar proteins into the wash water reduces the total protein yield. Tahergorabi et al. (2012) suggested that fish lipids are also removed from the recovered muscle proteins to a greater degree when solubilization is conducted at basic pH. According to Yampakdee et al. (2014), when the alkaline solubilization process is applied, proteins are more likely to dissociate under alkaline conditions, mainly due to the enhanced repulsion. As a result, lipids could be released to a greater extent. After solubilization, these components were separated on the basis of density and solubility differences. This was verified in this study when comparing CPI and FMP. Kristinsson et al. (2005)
Table 1. Physicochemical and thermal characterization of Argentine croaker protein isolate (CPI) and myofibrillar protein (FMP)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CPI</th>
<th>FMP</th>
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<tbody>
<tr>
<td>Protein (%)</td>
<td>92.13 ± 0.02</td>
<td>88.70 ± 0.80</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>2.25 ± 0.08</td>
<td>2.03 ± 0.13</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>1.18 ± 0.07</td>
<td>3.90 ± 0.11</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.51 ± 0.07</td>
<td>3.32 ± 0.02</td>
</tr>
<tr>
<td>Digestibility (%)</td>
<td>98.04 ± 0.06</td>
<td>97.97 ± 0.06</td>
</tr>
</tbody>
</table>

Mean values ± standard deviations of three replicates; Different letters in the same line indicate significant differences (p < 0.05).

lower L' value was probably due to higher levels of denatured and oxidized heme proteins recovered using the alkaline-aided process than using other methods (Rawdkuen et al., 2009). The brightness is likely due to the greater retention of native heme proteins in the final material, as the redness (a') was higher for CPI than FMP (p < 0.05). Foh et al. (2012) obtained a fish protein isolate from tilapia 

![DSC thermogram of protein isolated from tilapia (Oreochromis niloticus)](image)

The differential scanning calorimetry (DSC) thermogram of protein isolated from tilapia (Oreochromis niloticus) showed that proteins recovered from silver carp (Hypophthalmichthys molitrix) by isoelectric solubilization/precipitation may also have undergone acid-induced and base-induced denaturation, contributing to the lack of an

**Thermal properties**

Many studies have evaluated the thermal denaturation of fish muscle proteins and fish protein isolate (Rocha et al., 2013; Yu et al., 2014). Table 1 shows the onset (Tonset) and maximum (Tmax) temperatures for endothermic transitions as well as the net heat energy (enthalpy, ΔH) required for the reaction to occur for the CPI and FMP samples. These curves are typical of the thermal behavior of fish muscle proteins, as reported by several authors (Yongswatdigul and Park, 2004; Taskaya et al., 2009; Foh et al., 2012). The CPI exhibited an endotherm for Tmax1 (53.42 °C), similar to that of the FMP (53.51 °C). Medina-Vivanco et al. (2007) reported the denaturation temperatures of myosin and actin of myofibrillar proteins from tilapia muscle as being 54 and 76 °C, respectively. According to a study by Bertram et al. (2006) on meat, three denaturation steps occur, which can be primarily ascribed to myosin denaturation (40-60 °C), sarcoplasmic protein and collagen denaturation (60-70 °C), and actin denaturation (80 °C). The lower transition temperature indicated destabilization of the protein structure; thus, less energy was required to denature the proteins. It was verified in this study that the CPI and FMP showed lower enthalpies of denaturation, namely, ΔH1: 0.32 J/g and ΔH1: 0.40 J/g, respectively. Thermal denaturation of food proteins requires heat input (i.e., is an endothermic reaction) to disrupt the hydrogen bonds. This reaction is detected as an endothermic peak, and the heat energy (enthalpy, ΔH) required for the reaction to occur is represented by the area under the peak (Taskaya et al., 2009). Foh et al. (2012) presented a differential scanning calorimetry (DSC) thermogram of protein isolated from tilapia (Oreochromis niloticus) muscle solubilized at pH 11, which exhibited a single endothermic transition with a total enthalpy, ΔH or 0.0206 J/g.

Taskaya et al. (2009) showed that proteins recovered from silver carp (Hypophthalmichthys molitrix) by isoelectric solubilization/precipitation may also have undergone acid-induced and base-induced denaturation, contributing to the lack of an
endothermic transition for the proteins. This was observed by Yongswatdigul and Park (2004) in their studies with rockfish (Sebastes flavidus) protein isolate obtained from alkaline or acidic solubilization treatment, showing that the energy required for thermal denaturation in DSC was minimal. While the minced rockfish exhibited typical endothermic transitions, the acid- and base-solubilized rockfish proteins did not undergo these transitions. However, Tahergorabi, Sivanandan and Jaczynski (2012) verified that the denaturation of chicken breast protein isolate extracted from alkaline solubilization at pH 11.5 presented a denaturation enthalpy of 2.0 J/g. The differences in the endothermic transitions found in this work and in theirs might be partially due to the different sample conditions used. The CPI exhibited an endotherm with a $T_{\text{max}}$ (72.19°C) similar to that of the FMP (72.71°C). However, the FMP showed a higher $\Delta H_2$ (1.47 J/g) and $\Delta H_{\text{total}}$ (1.87 J/g) than did the CPI (0.01 J/g and 0.33 J/g, respectively). This suggests that FMP requires more energy for thermal denaturation, as the process used to obtain it does not include a severe acid-alkali treatment. According to Rocha et al. (2013) the temperatures for endothermic peaks tend to vary depending on the muscle type, pH and heating conditions. Lee et al. (2007) suggest that the enthalpy decreases with increasing solution pH because the structure of the protein unfolds at greater distances from the isoelectric point during the solubilization process. However, according to Kristinsson and Hultin (2003), the fish myosins are typically characterized by a less stable structure that their mammalian counterparts, as indicated by their lower thermal resistance and aggregation.

### Amino acid composition

The nutritional value of a food depends on the type and amount of amino acids available for body functions (Tahergorabi et al., 2015). The amino acid compositions of CPI and FMP are presented in Table 2. In both proteins, the amino acids present in the greatest concentrations were glutamic acid, aspartic acid, lysine and leucine. Similar results were reported by Paschoalick et al. (2003) in myofibrillar proteins obtained from muscle proteins of Nile tilapia. However, the content of these amino acids is significantly higher in CPI than in FMP (p < 0.05). The polar amino acids correspond to approximately 61.50% and 59.80% of the total amino acids in FMP and CPI, respectively. According to Cuq et al. (1995), the amino acid composition of proteins determines the type and quantity of interactions between the side chains (for example, their interactions with water). These interactions are produced by amino acids with ionizable (Arg, His, Lys, Pro, Trp) and non-ionizable (Asp, Asn, Cys, Gln, Glu, Ser, Thr, Tyr) polar groups, which are connected to water via hydrogen bridges. Hydrophobic interactions

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>CPI</th>
<th>FMP</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>5.87 ± 0.06</td>
<td>6.18 ± 0.14</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.02 ± 0.01</td>
<td>6.78 ± 0.13</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.9 ± 0.02</td>
<td>11.36 ± 0.17</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.27 ± 0.01</td>
<td>0.24 ± 0.00</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>16.47 ± 0.23</td>
<td>17.94 ± 0.48</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.55 ± 0.06</td>
<td>3.87 ± 0.08</td>
</tr>
<tr>
<td>Proline</td>
<td>3.17 ± 0.00</td>
<td>3.17 ± 0.08</td>
</tr>
<tr>
<td>Serine</td>
<td>3.97 ± 0.22</td>
<td>4.62 ± 0.08</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.41 ± 0.03</td>
<td>4.34 ± 0.18</td>
</tr>
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</table>

Mean values ± standard deviations of three replicates; Different letters in the same line indicate significant differences (p < 0.05).
can be formed by joining the side chains of amino acids with hydrophobic groups (Ala, Val, Leu, Ile, Met, Pro). According to Zhao et al. (2012), protein solubility is usually associated with hydrophilicity/hydrophobicity balance, which depends on the amino acid composition. In this study, the FMP exhibited a hydrophilic character because of the quantity of polar amino acids present in the sample. According to Tahergorabi et al. (2015), the nutritional quality of a protein source is determined by the presence of all nine essential amino acids (EAAs). The protein isolates contain sufficient amounts of all essential amino acids to cover adult human needs (Marmon and Undeland, 2013). It can be noted that the CPI contained slightly higher amounts of all essential amino acids than did the FMP. There were larger differences between the CPI and FMP in terms of the non-essential amino acids than the essential amino acids. The collagen is also rich in un-modified proline and glycine (Gómez-Guillén et al., 2011). The decrease in glycine in CPI (p < 0.05) is a strong indicator that the collagen present in large amounts in the skin has been more effectively removed during the pH-shift process than that in the fish myofibrillar protein obtained from the washing process.

**Scanning electron microscopy (SEM)**

The SEM micrographs of CPI and FMP are shown in Figure 1. The surface of the CPI, shown in Figure 1a, exhibits a large fragmentary structure with the particles forming a multilayered sheet-like structure. According to Liu et al. (2010), this morphology may result form the samples being near the isoelectric point (pI), which would result in almost no net charge on the molecules. The lack of charge may cause the molecules to be randomly aggregated. However, the surface of the FMP, shown in Figure 1b, appeared to be folded or wrinkled and more uniform, and a compact and homogeneous structure was observed.

**Thermo-gravimetric analysis (TGA)**

The TGA thermograms presented in Figure 2 show the thermal degradation of CPI and FMP. Two main weight loss stages were observed for all samples. The first weight loss stage for CPI (5.2%) and FMP (4.1%) was observed from 53.4 to 68.4 °C and from 59.9 to 75 °C, respectively, which may have been associated with the loss of free water adsorbed in the samples. The second stage of weight loss (52.4% and 61.9%) appeared at temperatures from 286.5 to 357 °C and from 286.5 °C to 366.3 °C for CPI and FMP, respectively. This transition shows that FMP experienced greater protein degradation than CPI. A reduction in thermal stability at higher temperatures can be promoted by changes in the protein structure and the rupture of the low energy intermolecular bonds that maintain the protein conformation (Oujifard et al., 2013). According to Oujifard et al. (2013), the weight loss at this stage was mostly associated with the degradation of the larger or associated protein fraction. According to the DSC results in this study, the FMP requires more energy for thermal denaturation because the process used to obtain it does not involve a severe acid-alkali treatment. However, the CPI showed a lower weight loss than FMP. The chemical composition of the FMP and CPI verified that FMP has higher lipid and ash contents, which may explain the thermo-gravimetric analysis results.

**Protein solubility and water-holding capacity (WHC)**

The protein solubility is believed to be a prerequisite for many functional properties, including gelation and emulsification (Kristinsson et al., 2005). Figure 3a shows the solubility of CPI and FMP. The minimum solubilities were observed at pH 5 and pH 7 for CPI and FMP, respectively, and the maximum solubilities were observed at pH 3 and pH 11.
However, at pH 11, CPI showed a higher solubility (94%) than did FMP (70.1%) (p < 0.05). Foh et al. (2012) verified that protein isolated from the muscle of tilapia (*Oreochromis niloticus*) had a solubility above 60% at pH 3, with the highest solubility being achieved using alkaline solubilization (81.34%). These authors verified that the solubility was lowest in the pH range of 4.5 to 5.5 and highest at pH 2 to 3 and pH 11 to 12. According to Kristinsson and Hultin (2003), acid and alkali treatments are known to solubilize the muscle proteins, which might explain why the protein obtained from acid and alkali treatments had consistently higher solubility than the untreated protein. According to Kristinsson et al. (2005), the greater solubility at extreme pH values was attributed to the increase in the positive or negative charge of the protein, leading to electrostatic repulsion among the proteins. According to Foh et al. (2012), the protein solubility at various pH values may provide a useful indication of how well protein isolates will perform when incorporated into food systems.

According to Kristinsson et al. (2005), WHC is related to such properties as texture, body, viscosity, and adhesion. Figure 3b shows the WHC of CPI and FMP at pH 5. The CPI and FMP have minimum WHCs of 4.07 g water/g protein and 4.17 g water/g protein, respectively. The WHC increased significantly (p < 0.05) at pH 11. At pH 11, the WHC of CPI (15.80 g water/g protein) was higher than that of FMP (9.80 g water/g protein) (p < 0.05). According to Liu et al. (2010), in denatured proteins, –CO– and –NH become positive and negative polarization centers at polypeptide chains, respectively. These centers could form a multilayer water system, and the hydrogen bonds between proteins could contribute to the structure entrapping free water. This study verified that the energy required for the thermal denaturation of CPI during DSC was lowest, as shown in Table 1. The FMP showed a higher ΔHtotal (1.87 J/g) than CPI (0.33 J/g). This finding suggests that FMP requires more energy for thermal denaturation because the process used to obtain it does not include a severe acid-alkali treatment. Thus, the partially denatured structure of the CPC contributes to its ability to entrap free water. According to some authors (Liu et al., 2010; Tahergorabi et al., 2015), above the isoelectric point, myosin swelled and bound a large volume of water because of the presence of many charged groups and repulsive forces. At pH values closer to isoelectric point, the proteins tended to coagulate due to increased protein–protein interactions. Meanwhile, the protein hydration gradually became weak due to the changes in the hydration states of the charged amino acids, decreasing their interaction with water and thus decreasing the WHC. In this study, the same behavior was verified for the WHC at more extreme pH values, such as 3 and 11. However, the CPI has a higher WHC than the FMP at the same pH value. According to Nolsoe and Undeland (2009), the proteins from aquatic species recovered by solubilization/precipitation (SP) exhibit increased surface hydrophobicity and reactive –SH groups. Therefore, the pH-induced folding of fish proteins during SP processing is believed to contribute to the technologically useful functionalities of the recovered proteins, such as increased WHC, gel-forming ability and emulsification.

**Conclusion**

The functional, thermal, and physicochemical properties of myofibrillar and isolated proteins from the Argentine croaker were evaluated. The results reveal that CPI showed a higher protein content, redness, yellowness, solubility, and water-holding
capacity and a lower lipid content, lightness, polar amino acids, total enthalpy of denaturation and weight loss than FMP. The CPI and FMP did not show significant differences in digestibility. Scanning electron microscopy indicated that the FMP had more compact ultrastructures than did the CPI. These results suggest that these changes are largely influenced by the alkaline treatment. Isoelectric solubilization/precipitation more efficiently recovers functional and nutritious protein isolates than the washing process.

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