

## RECOVERY OF ANCHOVY (*ENGRAULIS ANCHOITA*) AND WHITEMOUTH CROAKER (*MICROPOGONIAS FURNIERI*) PROTEINS BY ALKALINE SOLUBILISATION PROCESS

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The aim of this study was to evaluate the physical, chemical, and functional properties of recovered proteins of anchovy (*Engraulis anchoita*) and whitemouth croaker (*Micropogonias furnieri*) through the process of alkaline solubilisation and isoelectric precipitation, using different solubilisation (NaOH and KOH) and precipitation (HCl and H<sub>3</sub>PO<sub>4</sub>) reagents. The tests showed high protein level, and the lowest lipid reduction (94.5%) was found in the recovered protein of anchovy, the lowest yield of the process was 76.1%. The highest whiteness (78.8 and 74.2) was found in whitemouth croaker proteins. The solubilisation of the recovered protein was studied in the pH range (3, 5, 7, 9, and 11). The maximum solubility was at pHs 3 and 11 and minimum solubility was at pH 5 in the species under study.

**Keywords:** fish, muscle protein, pH shift process, solubility

With the growing awareness that marine resources are finite, numerous efforts are underway to improve the utilization of species of low commercial value (NOLSOE & UNDELAND, 2009). Obtaining fish protein isolates is an alternative method to diversify and improve the use of these species. It will contribute a protein source of high value for various food products and products of industrial purposes, including milk substitutes, protein supplements, flavour enhancers, drink stabilizers. It can also be used as fat substitute, yielding a product with flavour and texture similar to traditional beef and/or pork (MARTINS et al., 2009).

The alkaline solubilisation process was developed on the principle that the solubility of a protein material, homogenized in water, is affected by the pH of the mixture, with separation of lipids and removal of unconsumed material, such as bones, scales, skin, etc. (UNDELAND et al., 2002; GEHRING et al., 2011). Under extreme or alkaline conditions, the charging of the myofibrillar proteins and the cytoskeleton changes, leading to repulsion of like-charged molecules and to interaction with water, thereby resulting solubility (NOLSOE & UNDELAND, 2009). This fact has shown significant potential as a method for maximum recovery of proteins and the proteins that are recovered by this process exhibit good functionality (RAWDKUEN et al., 2009).

Many factors influence the protein solubility: the quality of raw material, the solid/liquid relation, pH, temperature, centrifugation speed, and ionic strength. However, good recovery

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during the extraction process is vitally important as it determines the amount of protein in the concentrate or isolate (KAIN et al., 2009).

The aim of this study was to evaluate the physical, chemical, and functional properties of anchovy and whitemouth croaker proteins recovered by the alkaline solubilisation process.

## 1. Materials and methods

### 1.1. Material

*1.1.1. Raw material.* The raw material used was anchovy (*Engraulis anchoita*) and whitemouth croaker (*Micropogonias furnieri*) muscles. The anchovies were captured in the coast of Rio Grande do Sul, on cruises conducted by the “South Atlantic Oceanographic Vessel”, owned by the Federal University of Rio Grande – FURG, and the whitemouth croakers were provided by Pescal S.A., a fish industry located in the city of Rio Grande - Rio Grande do Sul. The fish were transported in coolers with ice to the Laboratory of Food Technology, Federal University of Rio Grande – FURG, where they were cleaned with chlorinated water 2 g l<sup>-1</sup>, filleted, and stored frozen at –18° C until use.

### 1.2. Methods

*1.2.1. Obtaining the recovered protein.* The process of alkaline solubilisation was performed as described by FREITAS and co-workers (2011) (Fig. 1). Combinations of KOH/HCl and NaOH/H<sub>3</sub>PO<sub>4</sub> were used.

*1.2.2. Proximate composition.* All analyses were performed according to the A.O.A.C. (2000). Moisture: according to the methodology no. 950.46, until constant weight. Protein: according to the method of Kjeldahl no. 928.08. Lipids: according to the methodology no. 960.39. Ashes: according to the gravimetric method no. 920.153, with calcination at 550 °C.

*1.2.3. Lipid reduction.* Lipid reduction was calculated from the difference between total lipids in the raw material (dry basis) and total lipids present in the recovered protein (dry basis) (KRISTINSSON & LIANG, 2006).

*1.2.4. Process yield.* Process yield was calculated from the ratio between the amount of recovered protein obtained at the end and the amount of raw material (muscle) used in each process.

$$(\%) \text{ Yield} = \frac{\text{Recovered protein mass}}{\text{Muscle mass}} \times 100 \quad (1)$$

*1.2.5. Colour.* Colour was determined by using a Minolta colorimeter model CR-400 (Minolta Camera Co. Ltd., Osaka, Japan) and CIELAB system to measure the degree of Luminosity (L\*), redness (a\*), and yellowness (b\*). Whiteness was calculated according to RAWDKUEN and co-workers (2009):

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

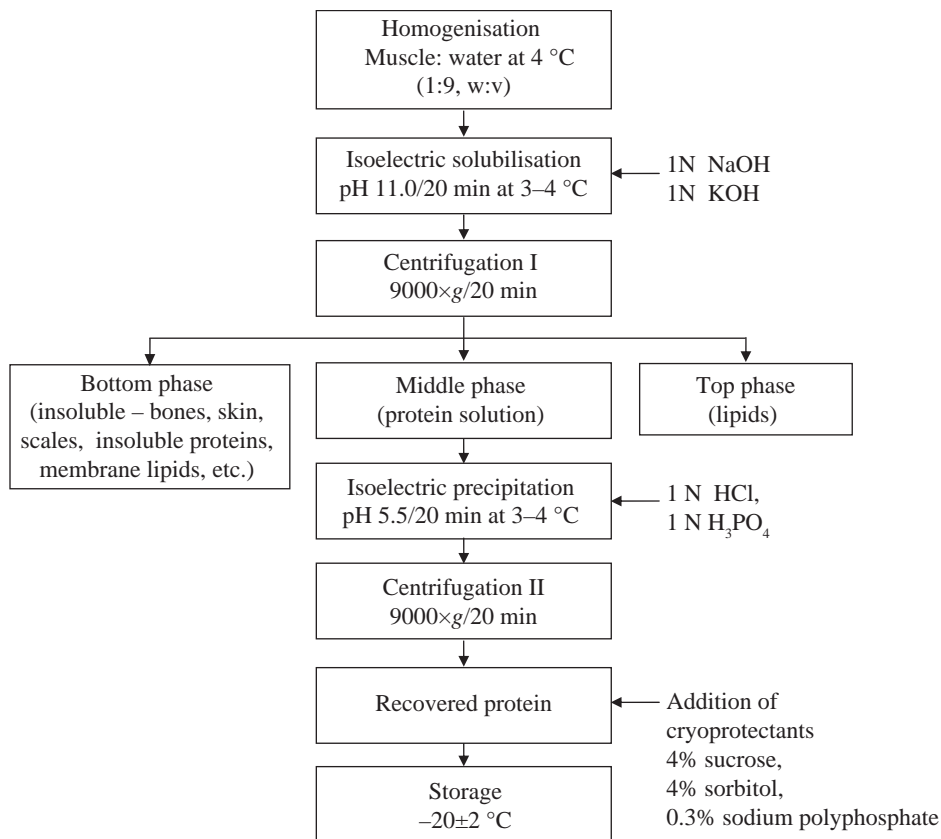


Fig. 1. Flowchart of the recovery process of fish protein using alkaline solubilisation

### 1.2.6. Functional properties

1.2.6.1. *Solubility*. Solubility was determined according to the method proposed by CHALAMAIAH and co-workers (2010) and TADPITCHAYANGKON and co-workers (2010), adapted to laboratory conditions. The amount of soluble protein in the supernatant was determined by the Folin-Ciocalteu method according to LOWRY and co-workers (1951). The solubility of the protein was calculated according to the following equation:

$$(\%) S = \frac{\text{Amount of protein in the supernatant}}{\text{Amount of protein in the sample}} \times 100 \quad (3)$$

For the calculation of protein in the supernatant a standard curve of albumin was used.

1.2.7. *Statistical analysis*. The experiments were conducted in triplicate. Results were expressed as mean and standard deviation values. The results were evaluated using analysis of variance (ANOVA) and Tukey's multiple comparison test, with the significance level of 5%, using Statistica 7.0 software.

## 2. Results and discussion

### 2.1. Proximate composition

The proximate compositions of the recovered proteins are demonstrated in Table 1. As there was no significant difference between the protein contents of the two species, the reagent used for solubilisation and precipitation of the protein had no significant influence, i.e., both were sufficient for a high protein solubilisation. The percentage of moisture in the recovered protein of the different species used ranged between 74 and 89%. Such results are similar to the ones found by CHEN and co-workers (2009), MARMON and co-workers (2009), and TASKAYA and co-workers (2009).

Table 1. Proximate composition on dry basis, lipid reduction, and process yield of the proteins recovered by alkaline solubilisation

Treatments	Protein (%)	Moisture (%)*	Lipids (%)	Ashes (%)	Lipid reduction (%)	Process yield (%)
Anchovy protein (KOH/HCl)	94.2±0.1 <sup>a</sup>	89.1±0.5 <sup>a</sup>	0.09±0.02 <sup>c</sup>	4.8±0.4 <sup>a</sup>	98.1±0.4 <sup>a</sup>	90.2±7.6 <sup>a</sup>
Anchovy protein (NaOH/H <sub>3</sub> PO <sub>4</sub> )	94.0±0.2 <sup>a</sup>	74.7±0.3 <sup>d</sup>	0.65±0.04 <sup>a</sup>	2.3±0.3 <sup>b</sup>	94.5±0.7 <sup>b</sup>	76.1±2.4 <sup>c</sup>
Whitemouth croaker protein (KOH/HCl)	96.7±0.1 <sup>a</sup>	78.9±0.2 <sup>c</sup>	0.17±0.03 <sup>b</sup>	0.68±0.01 <sup>d</sup>	97.8±0.4 <sup>a</sup>	80.9±5.2 <sup>ab</sup>
Whitemouth croaker protein (NaOH/H <sub>3</sub> PO <sub>4</sub> )	95.9±0.8 <sup>a</sup>	80.9±0.9 <sup>b</sup>	0.14±0.02 <sup>bc</sup>	1.6±0.1 <sup>c</sup>	98.2±0.2 <sup>a</sup>	85.4±2.5 <sup>ab</sup>
Raw material						
Anchovy muscle	76.1±1.8	76.8±0.1	11.8±0.1	4.8±0.5		
Whitemouth croaker muscle	88.1±4.9	79.5±1.2	7.6±0.7	4.9±0.4		

Averages of three determinations (n=3) ±standard deviation.

The same letters in the same column do not differ by Tukey's test P>0.05.

\* Wet basis

The separation system resulted in good removal of lipids: the highest content was 0.65% for anchovy extracted with NaOH and precipitated with H<sub>3</sub>PO<sub>4</sub>. The lipid content in fresh whitemouth croaker and anchovy was 7.6 and 4.7%, respectively. The process of alkaline solubilisation caused a reduction of lipid content in the fish samples, 94.5% to 98.2% for the studied samples. These results were higher than those found by RAWDKUEN and co-workers (2009), who obtained 88.6% of lipid reduction for the protein extracted from tilapia muscle by the alkaline process using sodium carbonate and HCl.

The reduction in ash content is attributed to the removal of water-soluble minerals present in fresh fish and the absence of spines. The highest amount of ash was found in the recovered protein of anchovy (KOH/HCl). This probably occurred during the separation of soluble and insoluble proteins, when some fraction of insoluble proteins may have blended with the soluble ones, resulting in a higher amount of ash. The highest process yield was obtained in the sample of anchovy (KOH/HCl): 90.2%.

## 2.2. Colour

The values of  $L^*$ ,  $a^*$ ,  $b^*$ , and whiteness are reported in Table 2. It can be noticed that the brightness values differ statistically among themselves ( $P < 0.05$ ). The recovered proteins of the whitemouth croaker were whiter, since the whitemouth croaker muscle “in natura” has a characteristic of milky white colour (BORGES et al., 2007), while the anchovy muscle “in natura” presented dark colour and pigments, such as melanin. However, the protein solubilised with KOH showed the highest luminosity, which can be verified by the whiteness calculations, with values of 78.8 and 68.1 for the whitemouth croaker and anchovy proteins, respectively. KOH was more efficient in the removal of heme proteins than NaOH. CHAIJIAN and co-workers (2006) found whiteness values of 65.67 and 63.55 in alkaline concentrates of sardines and mackerel, respectively. These results were similar to the values obtained for whiteness of recovered protein of anchovy muscle.

Table 2. Values of  $L^*$ ,  $a^*$ ,  $b^*$ , and whiteness of proteins recovered by the process of alkaline solubilisation

Treatments	$L^*$	$a^*$	$b^*$	Whiteness
Anchovy protein (KOH/HCl)	70.4±0.7 <sup>c</sup>	-0.17±0.62 <sup>b</sup>	11.8±1.0 <sup>a</sup>	68.1±0.9 <sup>c</sup>
Anchovy protein (NaOH/H <sub>3</sub> PO <sub>4</sub> )	62.4±0.2 <sup>d</sup>	0.49±0.11 <sup>a</sup>	10.9±0.2 <sup>a</sup>	60.8±0.2 <sup>d</sup>
Whitemouth croaker protein (KOH/HCl)	79.7±0.6 <sup>a</sup>	-2.2±0.2 <sup>c</sup>	5.5±0.9 <sup>b</sup>	78.8±0.5 <sup>a</sup>
Whitemouth croaker protein (NaOH/H <sub>3</sub> PO <sub>4</sub> )	74.9±0.7 <sup>b</sup>	-1.9±0.2 <sup>c</sup>	6.2±0.06 <sup>b</sup>	74.2±0.2 <sup>b</sup>
Raw material				
Anchovy muscle	44.3±1.1	3.3±0.2	8.6±0.4	43.5±0.6
Whitemouth croaker muscle	58.6±0.6	8.8±0.7	14.5±0.5	55.3±0.8

Averages of three determinations (n=3) ±standard deviation.

The same letters in the same column do not differ by Tukey's test  $P > 0.05$ .

The values of  $a^*$  indicate a tendency to colour green, probably due to lower retention of native heme proteins in the final material, except in the recovered protein of anchovy muscle solubilised with NaOH, which presented a tendency to colour red, due to the presence of myoglobin in the muscle and possible contamination with haemoglobin. All the analysed samples presented a tendency to colour yellow (value of  $b^*$ ). The reagent used for the recovery of the anchovy muscle protein did not influence significantly the value of  $b^*$ . However, the results for anchovy protein presented higher tendency to yellow, probably due to the fact that skin pigments, which were possibly dragged during protein solubilisation, remained with the proteins during the isoelectric precipitation. The presence of such pigments contributes to a yellow colour tendency ( $b^*$ ) and to whiteness reduction (GEHRING et al., 2011).

KRISTINSSON and LIANG (2006) found values of  $L^*$  72.7,  $a^*$  -2.0, and  $b^*$  7.5 in whitemouth croaker concentrates by using NaOH and HCl. The aforementioned tendency is similar to the ones presented by this study of recovered protein of whitemouth croaker.

### 2.3. Functional properties

Figure 2 shows the solubility values for the recovered proteins. In this study, it can be observed that the lowest solubility values were obtained at pH 5, due to the fact that proteins commonly exhibit their minimum solubility at their isoelectric point (pI), i.e., the protein–protein interaction increases, because the electrostatic forces of the molecules are minimal and less water interacts with protein molecules. The recovered protein of whitemouth croaker showed the lowest solubility at this pH.

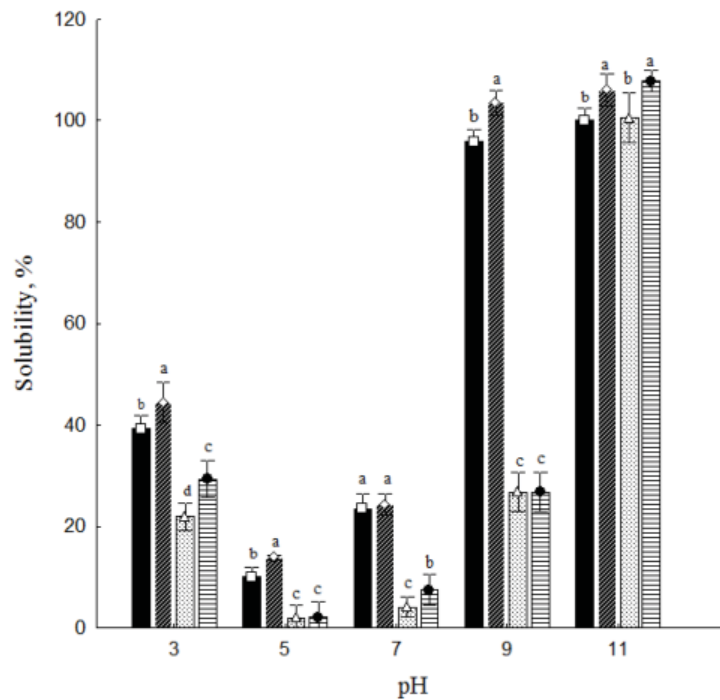


Fig. 2. Solubility values of fish proteins recovered by the process of alkaline solubilisation. AM-Anchovy protein; CM-Whitemouth croaker protein. ■: AM - KOH/HCL; ▨: AM - NaOH/H<sub>3</sub>PO<sub>4</sub>; ▩: CM - KOH/HCl; ▪: CM - NaOH/ H<sub>3</sub>PO<sub>4</sub>

For proteins of different raw materials and solubilisation/precipitation reagent, the highest solubilisation occurred at pH 3 and 9, and at pH 11 they were fully solubilised. The proteins are all charged positively or negatively at pH below or above their isoelectric point, respectively, which, therefore, increases the electrostatic repulsion between like-charged protein molecules, thereby increasing their solubility (YONGSAWATDIGUL & PARK, 2004). Results obtained in this study were similar to those found by FONTANA and co-workers (2009).

At neutral pH, low solubility of the recovered proteins of whitemouth croaker was observed. Generally, fish proteins are less soluble at neutral pH. The denaturation of the proteins during processing may have contributed to the decreased solubility of the protein through unfolding and exposure of the hydrophobic groups. The results obtained for this species were lower than those found by SATHIVEL and BECHTEL (2008), who obtained solubility value of 14% at pH 7.0 for sole fillet isolates extracted with NaOH (pH 11) and precipitated with H<sub>2</sub>SO<sub>4</sub> (pH 5.5).

### 3. Conclusions

It was possible to obtain solubilised protein of different fish species using solubilisation and precipitation with different reagents. Proteins recovered by alkali solubilisation process resulted in a product with high protein and lipid reduction. The protein isolates obtained by combining NaOH/H<sub>3</sub>PO<sub>4</sub> showed better functional quality. It was observed that the solubilised protein from the anchovy muscle showed better results than from whitemouth croaker. However, the whitemouth croaker proteins were whiter. This process is an alternative to recover fish protein from fish of low commercial value, to be used as food ingredients.

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