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PROPERTIES OF THE ARGENTINE ANCHOVY AND WHITEMOUTH CROAKER MUSCLE PROTEINS OBTAINED BY ALKALI SOLUBILIZATION PROCESS

PROPRIEDADES DAS PROTEÍNAS DE MÚSCULO DE ANCHOITA E CORVINA OBTIDAS PELO PROCESSO DE SOLUBILIZAÇÃO ALCALINA.

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ABSTRACT

The aim of this study was to evaluate functional properties and microbiological characteristics of recovered proteins of anchovy (*Engraulis anchoita*) and whitemouth croaker (*Micropogonias furnieri*) through the process of alkaline solubilization and isoelectric precipitation, using different solubilization at pH 11 (NaOH and KOH) and precipitation at pH 5.5 (HCl and H_3PO_4) reagents. Analyses of water holding capacity were carried out (at pH 3,5,7,9 and 11), oil holding capacity and *Salmonella* sp, *Escherichia coli* and *Staphylococcus aureus*. The water holding capacity was lowest at pH 5. The low value of the proteins recovered by alkaline solubilization process also indicates changes in the protein. The highest oil holding capacity was observed in whitemouth croaker concentrates (NaOH/H_3PO4) at 5.6 ml oil.g protein^{-1}. As for microbiological analyses, results showed no *Salmonella* in 25 g for all treatments and maximum count of 2.75 x 10^2 CFU.g^{-1} for coagulase positive *Staphylococcus* for the muscle of anchovy concentrated. The protein concentrate obtained by combining NaOH/H_3PO4 showed better functional quality. This process is an alternative to recover fish protein of low commercial value that may be used as an ingredient in foods.

Keywords: Fish, solublization, funcionaty, quality

RESUMO - O objetivo deste estudo foi avaliar as propriedades funcionais e as características microbiológicas da proteina recuperada de músculo de anchoita (*Engraulis anchoita*) e corvine (*Micropogonias furnieri*) obtida pelo processo de solubilização alcalina e precipitação isoelétrica usando diferentes solventes de solubilização (NaOH e KOH) a pH 11 e precipitação (HCl e H_3PO_4) a pH 5.5. Foram realizadas analises da capacidade de retenção de água na faixa de pH 3,5,7,9 e 11; capacidade de retenção de óleos; *Salmonella* sp, *Escherichia coli* e *Staphylococcus aureus*. Baixa capacidade de retenção de água foi encontrado em pH 5 para todos as amostras, e esse baixo valor obtido indica mudanças na proteína ocorrido pelo processo de solubilização alcalina. Alta capacidade de retenção de óleo foi observado no concentrado de corvina (NaOH/H_3PO_4) 5,6 ml oil/g protein. Quanto as análises microbiológicas os resultados apresentaram ausência de Salmonella sp em 25 g para todos os tratamentos e contagem máxima de 2,75 x 10^2 UFC/g para *Staphylococcus coagulase* positiva para o concentrado de músculo de anchoita. A proteína concentrada obtida pela combinação NaOH/H_3PO_4 mostrou melhor qualidade funcional. Este processo é uma alternativa para recuperar proteínas de pescados de baixo valor comercial e pode ser usado como ingredientes em alimentos.

Palavras-chave: pescado, solubilização, funcionalidade, qualidade.

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INTRODUÇÃO

Proteins play a very important role in stability, functionality and texture of food systems. This functionality is governed by physicochemical and structural properties of proteins like intrinsic amphiphilic character, surface activity, molecular weight, net charge, solubility, conformational flexibility and extrinsic conditions of the aqueous medium such as pH, ionic strength and temperature (BRYANT & McCLEMENTS, 2000).

The fish muscle proteins have the advantage of possessing a high biological value, due to the sensitivity to hydrolysis and balanced amino acid composition, particularly those which tend to be limiting in the vegetable proteins such as methionine and cysteine.

Another relates to the use of fish advantage is the fact that certain species are not suitable for marketing, usually because of lack of satisfactory performance respecting to filleting, due to body structure and present a low commercial value (BÁRZANA & GARIBAYGARCÍA, 1994; FONTANA et al., 2009).

The isolated protein generally has good functional and nutritional properties that can be used in a higher value product (GEHRING et al., 2011; TAHERGORABI et al., 2012). This contribution is very important because of the world population increasing, so there is need to get products that have good properties (ALU'DATT et al., 2012).

Knowledge of the specific functional properties of isolate and concentrate tends to facilitate direct your application, contributing to a better use, resulting in products with higher nutritional and technological quality (CENTENARO et al., 2009). The water retention capacity is very useful in the manufacture of meat products, preventing water loss in the cooking process, and which usually improves the texture of foods.

The oil holding capacity is of great importance in the formulation of food, being able to influence the order of addition of dry ingredients into the mixture, besides being used to determine the mixing times using a uniform distribution of oil or fat in the dry mixtures (CHAUD & SGARBIERI, 2006).

The works aims to evaluate functional properties and microbiological characteristics of recovered proteins of anchovy (*Engraulis anchoita*) and whitemouth croaker (*Micropogonias furnieri*) through the process of alkaline solubilization and isoelectric precipitation, using different solubilization and precipitation reagents.

MATERIAL AND METHODS

Raw material

The raw material used was muscle from the anchovy (Engraulis anchoita) captured off 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 croaker muscle (Micropogonias furnieri) provided by Pescal S.A. fish industry located in the city of Rio Grande - Rio Grande do Sul state. The anchovy and croaker 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 2.0 $g.L^{-1}$, filleted and stored frozen at (- 18° C) until use.

Obtaining the recovered protein

The process of alkaline solubilization was performed as described by Kristinsson et al., (2005) and Nolsoe & Undeland (2009) with slight modifications. The filets were minced and then homogenized (IKA Model RW 20DZM.n) in a ratio of 1:9 (w/v) with distilled water at 4 °C for 60 s. Protein solubilization was performed at 3-4 °C in controlled temperature by an ultrathermostatic bath (Quimis, model 214 D2) for 20 minutes under constant stirring with propeller stirrer shaft (IKA, model RW 20DZM.n). Two alkaline solutions, 1N KOH and 1N NaOH were used for protein extraction at pH 11.0 for 20 min then centrifuged (SIGMA, Model 6-15) at 9000 × g for 20 min. Soluble proteins were subjected to isoelectric precipitation (pH 5.5) with addition of 1N HCl solution and 1N H_3PO_4. A second centrifugation was performed at 9000 x g for 20 min, where the precipitate referred to as recovered protein was mixed with cryoprotectants (0.3% polyphosphate, 4% sorbitol and 4% sucrose).

Microbiological analysis

Microbiological analysis was performed using the method recommended by APHA (2001) for Coagulase-Positive Staphylococcus, Salmonella and Escherichia coli.

Determination of the water holding capacity (WHC)

WRC was determined according to the method by Regenstein et al., (1984), adapted to laboratory conditions. Protein dispersion at 1% with

varying pH (3, 5, 7, 9 and 11) was prepared. 2 ml of 0.1M NaCl was added to the dispersion to obtain a homogeneous paste, and then added to the appropriate buffer according to the corresponding pH up to the volume of 40 ml, the dispersion was stirred for 15 minutes and centrifuged at 8667 x g for 20 minutes. Soluble proteins in the supernatant were quantified by the method defined by Bradford (1976), and deducted from the total protein of the original sample. The WHC was determined as follows.

$$WHC = \frac{Amount\ water\ retained\ oil\ (\mathrm{g})}{Original\ Protein\ mass\ (g)}\ \mathrm{x}100 \quad \text{Eq.(1)}$$

Determination of the oil holding capacity (OHC)

ORC was determined according to the method described by Fonkwe & Singh (1996). 0.5 g of protein was weighed and mixed with 10 ml of soybean oil in centrifuge tubes and stirred for 10 min. in a tube stirrer at speed 4 (56 PROENIX AP).

Later, the mixture was centrifuged at 8667 x for 20 minutes, and the difference between the added oil and the oil that is not retained was considered as the amount of oil retained by the protein. The data was obtained using the equation 2.

$$OHC = \frac{Retained\ oil\ (mL)}{Protein\ mass\ (g)}\ x\ 100 \quad \text{Eq.(2)}$$

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 tests, with the significance level of 5%, using Statistica 7.0 software.

RESULTS

Microbiological analysis Microbiological evaluation is of great importance during the production and storage of food products. Freshly caught seafood often has a large numbers of microorganisms on its surface (NOLSØE & UNDELAND, 2009). The results of microbiological analysis for recovered fish proteins are shown in Table 1. These results are in agreement with the RDC No. 12, January 2, 2001 (Brazil, 2001) which regulates the quality standards for products derived from fish, i.e surimi and similars, which require the absence of Salmonella in 25g and allows maximum count of 5 x 10^2 CFU.g^{-1} for coagulase-positive Staphylococcus.

Table 1: Microbiological analysis of recovered proteins by the process of alkaline solubilization

Recovered Protein	*Salmonella* sp/25g	*Escherichia coli* (NMP/g)	*Staphylococcus aureus* (UFC/g)
Anchovy Muscle (KOH/HCl)	Absent	< 3.0	$1.52x10^2$
Anchovy Muscle ($NaOH/H_3PO_4$)	Absent	< 3.0	$2.52x10^2$
Croaker Muscle (KOH/HCl)	Absent	< 3.0	$2.75x10^2$
Croaker Muscle ($NaOH/ H_3PO_4$)	Absent	< 3.0	Absent

Functional properties

Table 2 shows the water holding capacity (WHC) as a function of pH. It is observed that for all samples, WHC increased at pH 3.0, showed a decrease in pH 5.0 and increased again at pH's 7.0, 9.0 and 11.0. It was verified by Tukey test that no significant difference (P> 0.05) was presented between the muscle proteins of croakers using different solubilization reagents at pH 5. The same was observed for the solubilized anchovy muscle protein. Among the pH's studied, maximum values were observed at pH 11 for the croaker muscle proteins with 15.34% and 22.50%, and at pH 3, 23.64% was obtained.

Table 2: Values pH of water holding capacity (WHC) presented by fish proteins recovered by the process of alkaline solubilization.

Recovered protein	pH				
	3	5	7	9	11
Anchovy Muscle (KOH/HCl)	9.46±0.52 ^{c}	2.34±0.18 ^{b}	3.35±0.58 ^{b}	6.08±0.71 ^{b}	11.31±0.59 ^{c}
Anchovy Muscle ($NaOH/H_3PO_4$)	15.38±0.65 ^{b}	3.12±0.02 ^{b}	5.89±0.01 ^{a}	5.59±0.98 ^{b}	2.12±0.04 ^{d}
Croaker Muscle (KOH/HCl)	nd	4.34±0.05 ^{ab}	5.10±0.70 ^{ab}	9.36±5.73 ^{ab}	15.34±0.55 ^{b}
Croaker Muscle ($NaOH/H_3PO_4$)	23.64±0.17 ^{a}	6.05±2.19 ^{a}	6.35±2.05 ^{a}	12.13±2.32 ^{a}	22.50±0.42 ^{a}

Averages of three determinations (n = 3) ± standard deviation. The same letters in the same column do not differ by Tukey test p>0.05. nd- not detected.

For anchovy muscle protein, low pHs were obtained for the studied WHC. The low WHC of proteins recovered by the process of acid or alkaline solubilization also indicates changes in the protein. However, the treated proteins had their structure changed as a result of intermolecular charge repulsion induced by extremes of pH (Hultin & Kelleher, 1999).

Batista et al. (2007) found a reduced capacity for water holding in protein extracted from sardine muscle for both alkaline solubilization processes (approximately 3%).

The oil holding capacity (OHC) is one of the most important functional properties in the elaboration of products. The oil retention mechanism is mainly due to physical capture of the oil by the protein and is an important functional feature, required mainly by the meat and emulsified products industries (Sathivel & Bechtel, 2006).

The Figure 1 shows the values of the OHC of proteins recovered by alkaline solubilization process. In this study, the OHC values obtained were 3.73 and 5.63 mL of oil/g of protein for the croaker muscle protein isolates, and 4.70 and 4.40 mL of oil/g of protein for the anchovy muscle protein isolates, which indicates that there is a high amount of hydrophobic regions in proteins which favor them with the oil. Similar results were found by Sathivel & Bechtel (2008), who obtained 4.8 mL of oil/g of sole muscle protein isolate solubilized at pH (11), while Fontana et al., (2009) found 4.7 ml of oil/g of protein for croaker muscle concentrate obtained by the alkaline processes.

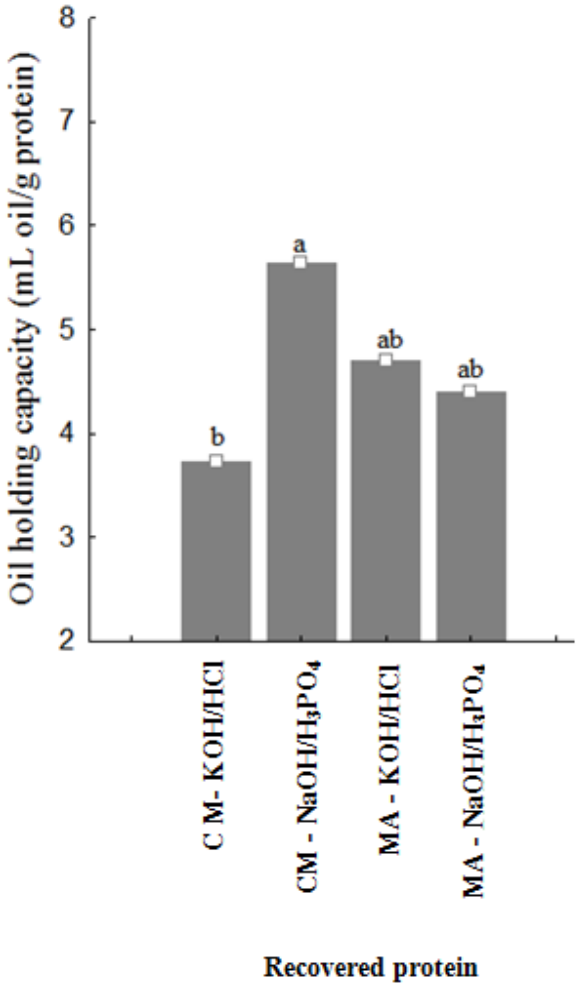

Figure 1: Oil holding capacity of proteins recovered by alkaline solubilization process. AM- Anchovy Muscle; CM- Croaker Muscle.

CONCLUSIONS

It was found that the functional properties of the protein isolates of fish can be modified according to the type of solvent used and the type of raw material used.This difference to the functional properties can be made available for various technological purposes in food formulations according to the characteristics of the desired product.

The microbiological results presented in accordance with the standards of the Brazilian law, this shows that the isolated protein can be used for direct application.

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