ELECTROPHORETIC PROFILE OF PROTEINS PRESENT IN THE RINSE WATER OF SURIMI PRODUCED WITH BULLFROG (*Lithobates catesbeianus*) MEAT

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Abstract - The aim of this study was to characterize the approximate molecular weight of soluble proteins present in the rinse water of surimi produced with bullfrog meat. The electrophoretic profile was used to characterize the soluble proteins from four washes (1stRW, 2ndRW, 3rdRW and 4thRW) of surimi produced from mechanically separated back (MSB) of bullfrog. The proteins were characterized by gel electrophoresis (SDS- PAGE). As a result, it was found that the content of soluble proteins in the rinse water of surimi showed higher level in the 1stRW (2.420 mg / mL), followed by proportional reductions in the 2nd RW (1.590 mg / mL), 3rd RW (0.620 mg / mL) and 4thRW (0.305 mg / mL). The electrophoretic profile revealed a greater abundance of proteins with relative molecular weight in the range from 31.0 to 52.0 kDa and little bands of weight less than 24.0 kDa. The presence of protein bands weighing between 76 and 225 kDa for the 1stRW was also verified, and in the other washes, bands were repeated in the same range, but in lower amounts. The proteins found can be recovered and have great potential for application in food products, as in the production of biofilms.

I. INTRODUCTION

Bullfrog meat is nutritionally distinguished due to its high content of proteins of high biological value and its low fat content [1]. Brazil has the best technology for the production of *Lithobates catesbeianus* in captivity and is one of the largest world's producers [2]. The legs (including the thigh) are the main edible and marketable part, which increases the cost of the meat due to the great waste of other cuts. The back, for example, consists of chest and arms and due to its low commercial value is used to obtain mechanically separated back (MSB) [3], and as raw material in the preparation of restructured products [4] [5].

There is a wide range of new products that can be used in the better utilization of frog MSB, among which includes surimi. The technology used to produce surimi allows adding functional, nutritional and commercial value for the production of restructured products [6]. In the industrial manufacturing process of surimi, meat is minced and washed repeatedly with cold water to remove sarcoplasmic proteins and impurities such as lipids to produce a tasteless and odorless product. As a result of washing, about 40-50 g/100 g of pulp solids from fish (mainly containing water soluble proteins) are lost throughout the process [7]. Thus, 40-50 % of the product is considered improper for consumption and is discarded into the environment, which has caused a negative environmental impact. The recovery of proteins present in the rinse water of surimi would not only reduce the environmental impact and costs of waste disposal but also generate potential profits [7]. Some studies have been conducted aiming to use these proteins in obtaining proteases [8], in the production of edible biofilm [9] and re-use in surimi production [7].

Thus, the objective of this study was to quantify and analyze the electrophoretic profile of soluble proteins present in the rinse water of surimi produced with bullfrog (*Lithobates catesbeianus*) meat.

I. MATERIALS AND METHODS

The mechanically separated backs (MSB) of bullfrogs (*Lithobates catesbeianus*) were provided by the Laboratory of Frog Breeding and Aquaculture Products (LRPA) - CCHSA / UFPB. The animals were slaughtered at an average live weight of 230 ± 20 g, had their legs (thighs) separated and back submitted to mechanical deboning using mechanical deboner prototype (Patent MU 8200639-3).

Surimi with MSB was produced according to methodology described by Kuhn et al. [10] with modifications (Fig. 1).

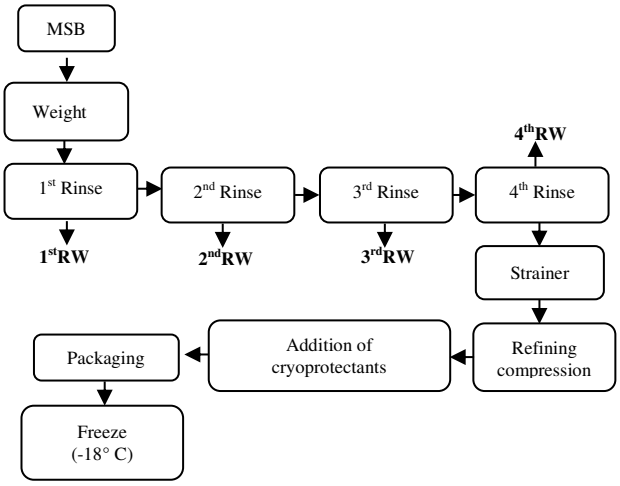

Figure 1. Surimi manufacturing process

MSB was submitted to four successive washes in polyethylene tanks with cold water (T = 5°C), and the volume of water used was three times the sample weight (3:1, water / pulp). In each washing cycle, the pulp was wrapped in cotton cloth and drained through compression in stainless steel cylinder with pore size of 32 mesh. After each wash, rinse waters were collected (1stRW, 2ndRW, 3rdRW and 4thRW). Analyses of moisture, lipid, protein (N × 6.25) and ash in the MSB of bullfrogs were performed according to procedure described by Association of Official Analytical Chemists [11].

For the quantification of soluble proteins in the rinse water of surimi, the aqueous residue was centrifuged in refrigerated centrifuge (model 2K15, Sigma, Germany) for 3 min at 3000 rpm to pellet insoluble proteins. The supernatant was used to quantify soluble proteins by the method of Bradford [12] using bovine serum albumin (BSA) as analytical standard. The protein quantification reading was performed using spectrophotometer at wavelength of 595 nm.

The molecular profile of proteins present in the rinsing waters was determined by polyacrylamide gel electrophoresis (SDS-PAGE) according to method of Lammeli [13]. For this, protein samples were solubilized in 0.0625 M Tris-HCl buffer containing 2 % SDS, 2% 2-mercaptoethanol, 10% glycerol and 0.010 % bromophenol blue, followed by the application of an aliquot in the stacking gel of 4 g/100 g and 12.5 g/100 g in running polyacrylamide gel (10 x 10.5 cm with 0.30 mm spacers) submitted to a constant current of 25 mA for approximately 2 hours. Commercial standard Full-Range Rainbow™ Molecular Weight Marker from GE Healthcare was used as molecular weight marker. After electrophoresis, the gel was stained according to procedure described by Weber and Osborne [14]. The dye solution was prepared using 1% Coomassie Blue R-250 (Sigma Chemical Co.), 40% methanol, 10% acetic acid in distilled water. Bleaching was performed with solution containing 10% acetic acid and 20% methanol in distilled water.

For better visualization of protein bands, the gel was submitted to development with silver nitrate, which was dehydrated with a 50% ethanol solution, being submitted to three washes of 20 minutes each. Then, a sodium thiosulfate solution was added (20mg/100 ml H_2O) and held for 1 minute under gentle agitation. After this time, three quick washes were made in distilled water, then adding the silver nitrate solution (200 mg + 74 µL of formaldehyde in 100 mL H_2O), maintained for 20 minutes under gentle agitation. After this time, the gel was submitted to three rapid washes in distilled water and the developing solution was added (6 g of calcium carbonate + 50 µL formaldehyde + 2 ml of sodium thiosulfate in 100 mL H_2O). To stop the development, a 13% acetic acid solution was added [15].

III. RESULTS AND DISCUSSION

The proximate composition analyses of the MSB of bullfrogs (*Lithobates catesbeianus*) are presented in Table 1. This table shows the large amount of proteins present in the raw material of surimi produced with MSB.

Table 1. Proximate composition of MSB samples

Sample	MSB
Moisture (%)	76,75
Ash (%)	2,30
Protein (%)	18,19
Lipid (%)	2,74

Regarding the contents of soluble protein, 1stRW contained the highest level (2.420 mg / mL), followed by 2ndRW (1.590 mg / mL), 3rdRW (0.620 mg / mL) and 4thRW (0.305 mg / mL) (Fig. 2). The same behavior was observed in a study with surimi made with catfish (*Nemipterushexodon*), with levels of 1.23 mg / mL, 0.64 mg / mL, 0.54 mg / mL, respectively, in three rinse waters [7].

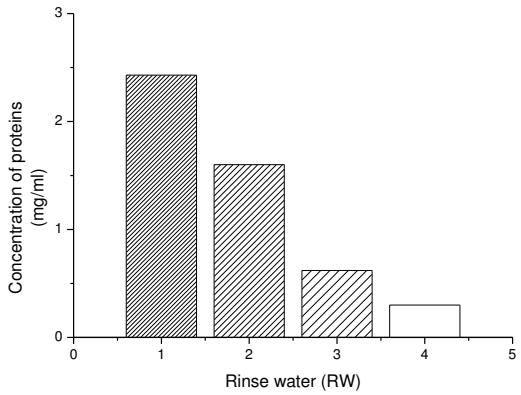

Figure 2. Concentration of water-soluble proteins in surimi rinse water at different wash stages.

The electrophoretic profile obtained by PAGE-SDS of proteins present in the four washings is illustrated in Figures 3 and 4.

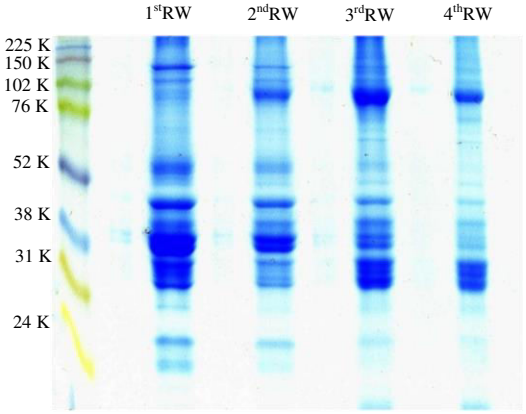

Figure 3. SDS-PAGE patterns of water-soluble proteins in surimi rinsewater at different wash stages stained with Coomassie

After examining the bands present on the electrophoresis gel, it was found that most of the soluble proteins of the 1stWS had relative molecular weight (MW) between 31.0 and 52.0 kDa, with some bands of molecular weight below 24.0 kDa. Some proteins weighing between 76 and 225 kDa were also observed. Proteins of the 2ndWS, 3rdWS and 4thWS had similar molecular weights, although present in lower amounts. Similar results were found by Bourtoom et al. [7], where the range of higher protein concentration was between 23.2 and 71.6 kDa. Iwata et al. [16] also found soluble proteins in the range between 30.0 and 66.6 kDa, and a well-defined band of approximately 98.0 kDa in the rinse water of surimi prepared with blue marlin (*Makaira mazara*).

Silver stained gels have better sensitivity in the detection of proteins present in low concentrations. Thus, after staining the gel with silver (Figure 4), bands of lower molecular weight were observed, which were not previously observed when the gel was stained with Coomassie. It was also observed that for proteins with molecular weight close to 52 kDa and less than 24 kDa, there was a decrease in their concentrations in solution with succesive washing cycles.

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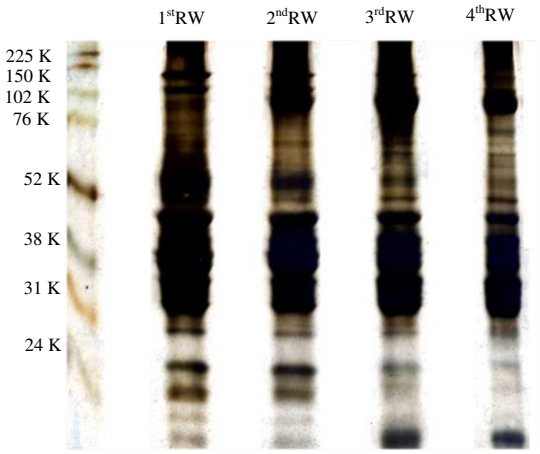

Figure 4. SDS-PAGE patterns of water-soluble proteins in surimi rinse water at different wash stages stained with silver nitrate

As previously mentioned, the largest amount of soluble proteins present in the washing cycles are in the range from 31.0 to 52.0 kDa. Studies have used these proteins in the production of edible biofilms [16], and claim that substitution of up to 10% of recovered proteins keeps the gel features such as hardness, elasticity, color and water retention [17].

These proteins can also be recovered by lyophilization, which in turn, when added to food products, enrich these products with high biological value proteins and avoid the disposal of these waters into the environment.

IV. CONCLUSIONS

Soluble proteins recovered from the rinse water of surimi produced with mechanically separated back (MSB) of bullfrog have great potential for application in food products and in the production of biofilms. Future studies are needed to define new ways of using these proteins.

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