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Coupling of ultrafiltration and enzymatic hydrolysis aiming at valorizing shrimp wastewater

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ABSTRACT

The objective of this work was to obtain a protein hydrolysate from the wastewater generated during shrimp cooking, by coupling ultrafiltration and enzymatic hydrolysis processes. Initially, the effluent was concentrated by ultrafiltration, reaching a protein concentration factor of 3.2. The concentrated effluent was then enzymatically hydrolyzed, aiming at obtaining peptides with antioxidant capacity. The effects of some process variables – temperature (55–75 °C), pH (7–9) and enzyme/substrate (E/S) ratio (0.1–2.5%) – on the degree of hydrolysis and the antioxidant capacity were evaluated. The increase in temperature and pH resulted in lower degree of hydrolysis and higher antioxidant capacity. The conditions selected as the most suitable were: temperature of 75 °C, pH of 9.0 and E/S ratio of 0.1%. The hydrolysates produced at these conditions were also evaluated for total amino acid content and electrophoretic profile, showing a suitable amount of essential amino acids that covers the recommended daily needs.

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1. Introduction

Food industries are responsible for around half of the organic industrial pollution found in the world (Papargyropoulou, Lozano, Steinberger, Wright, & Ujang, 2014). Among them, the fish industry generates a great amount of wastewater from washing, thawing, filleting and cooking, which have different characteristics. The water consumption and its organic charge vary according to the type of process being considered: filleting, for example, generates big water volumes with moderate organic charge; on the other size, operations such as baking and salting result in lower volume and more charged effluents, particularly in the canning industry (Cros, Lignot, Jaouen, & Bourseau, 2006). Furthermore, the fish industry effluents have high turbidity, strong yellow-green color and unpleasant odor. Therefore, they must undergo a suitable treatment before being disposed. In this sense, the recovery of molecules present in the fish wastewaters, such as proteins, is an alternative that can generate high added value products, besides representing an opportunity for reduction of water treatment costs.

The capture production of fish, crustaceans and molluscs in Brazil in 2013 was about 765 thousand tons. In a world level, this value was close to 92.6 million tons, of which 3.4 million tons corresponded to shrimp production (FAO, 2015). According to Cros et al. (2006), processes such as thawing and rinsing can generate approximately 5 and 3.5 L of effluent per kg of fish in seafood industries, while cooking generates around 1 L/kg, which can largely vary according to the amount of cooked material. Therefore, these effluents represent an alternative source for protein recovery, which is generated annually in a large amount.

Ultrafiltration is a process that has been used by some dairy industries with the aim of concentrating milk and whey proteins. It uses porous membranes able to retain molecules with molar mass within the range of 1–100 kDa (Mulder, 1991), thus promoting the concentration of molecules such as proteins and allowing the flow of water and some minerals. In the case of other protein-rich effluents, such as those from the fish industry, ultrafiltration can also represent an alternative treatment, aiming at both the protein recovery/concentration and the reduction of environmental pollution.

Among the processes that have been used to add value to products and wastes with high protein content, hydrolysis can also be considered a promising one. It consists of the cleavage of protein molecules into peptides of various sizes and diverse amino acid

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compositions and can be performed by acids, alkalis or enzymes. However, the use of enzymes allows for a better and more efficient control over the protein hydrolysis process (Kristinsson & Rasco, 2000). Another advantage of the enzymatic hydrolysis over the chemical hydrolysis is the improvement of protein functional properties such as solubility, emulsifying capacity and texture, thus showing great applicability in food products. Enzymatic hydrolysis has been studied for several types of protein sources, such as seaweed by-products (Laohakunjit, Selamassakul, & Kerdchoechuen, 2014), lupin protein (Boschin, Scigliuolo, Resta, & Arnoldi, 2014), grape juice protein (Younes, Cilindre, Jeandet, & Vasserot, 2013) and whey protein (Salami et al., 2010).

In human nutrition, protein hydrolysates can be used as supplements, with the possibility of being adopted in diets for people with digestion problems or protein malabsorption, due to its high digestibility and the essential amino acids availability. According to Bhaskar, Modi, Govindaraju, Radha, and Lalitha (2007), preparations rich in small peptides, especially di- and tripeptides obtained by partially hydrolyzing proteins, have greater nutritional value and more efficient gastrointestinal absorption as compared to the intact protein and free amino acids.

In addition to these advantages, protein hydrolysates are also considered as sources of biologically active peptides. The so-called "bioactive peptides" can affect many physiological body functions, showing antioxidant capacity, antihypertensive activity, immunomodulatory and antimicrobial properties (Clare & Swaisgood, 2000). Several studies have associated the peptides bioactivity to their antioxidant potential, showing that the antioxidant property of protein hydrolysates depends on several factors such as the type of protein, the enzyme used and the degree of hydrolysis reached (Dong et al., 2008; Theodore, Raghavan, & Kristinsson, 2008; You, Zhao, Cui, Zhao, & Yang, 2009). According to Mahmoud (1994), the hydrolysates functionality also depends on its amino acid composition and on the length of their polypeptide chains.

In this sense, the objective of this work was to evaluate ultrafiltration and enzymatic hydrolysis as alternative methods for recovering and adding value to the wastewater from the shrimp processing industry.

2. Materials and methods

2.1. Material

The wastewater generated during shrimp cooking was provided by a local company (Rio de Janeiro, Brazil).

Alcalase® 2.4 L (Novozymes, Bagsvaerd, Denmark), a commercial endopeptidase obtained from *Bacillus licheniformis*, was used to promote enzymatic hydrolysis.

2.2. Concentration by ultrafiltration

Before concentration, the effluent was passed through a 100 μm mesh, on order to remove solids in suspension, facilitating the ultrafiltration process.

Ultrafiltration was carried out in a system consisting of four tubular modules (TIA, Techniques Industrielles Appliquées, Bollene, France), with the membranes configured in series, resulting in an effective permeation area of 0.022 m^2. Ceramic membranes (Pall Corporation, New York, USA) with an average pore size of 10–20 kDa were used. The process was performed at 35 °C, with a recirculation rate of 900 L/h and transmembrane pressure of 5 bar.

The shrimp wastewater was analyzed, before and after concentration, with respect to moisture content, total protein, ash and lipids, as well as for mineral profile (A.O.A.C., 2005).

2.3. Enzymatic hydrolysis

As previously mentioned, the commercial proteolytic enzyme preparation Alcalase® 2.4 L), with activity of 11,360 U/mL, measured with azocasein as the substrate spectrophotometrically at 428 nm against the blank (Charney & Tomarelli, 1947), and density of 1.25 g/mL, was used as biocatalyst.

Enzymatic hydrolysis was carried out in a thermostatically controlled stirred-batch reactor (800 rpm), following the pH-static procedure proposed by Adler-Nissen (1985). The enzyme was added to the effluent and the reaction pH was kept constant by the continuous addition of 1 N NaOH. The volume of consumed alkali was recorded at regular intervals, up to 6 h. The reaction was stopped by heating the effluent up to 90 °C for 10 min, in order to inactivate the enzyme.

A central composite experimental design was used to evaluate the influence of three process variables – temperature (55–75 °C), pH (7–9) and enzyme/substrate (E/S) ratio (0.1–2.5%) – on the degree of hydrolysis and the antioxidant capacity of the resulting hydrolysates. Three levels of each variable were chosen for the trials, including the factorial points and three central points, giving a total of 11 combinations (Table 1). The following polynomial equation was fitted to data:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

where β_n are constant regression coefficients, y is the response (degree of hydrolysis or antioxidant activity), and x_1, x_2 and x_3 are the coded independent variables (temperature, pH and E/S, respectively).

The analysis of variance (ANOVA), test for the lack of fit, determination of the regression coefficients and the generation of three-dimensional graphs were carried out using the Statistica 7.0 software (StatSoft, Tulsa, USA).

2.3.1. Degree of hydrolysis (DH)

The degree of hydrolysis was determined by the pH-stat method and defined as the percent ratio of the number of peptide bonds cleaved (h) to the total number of bonds available for proteolytic hydrolysis (h_{total}) (Adler-Nissen, 1985), being calculated according to Eq. (2):

$$\text{DH } (\%) = \frac{h}{h_{total}} \times 100 = \frac{B \times N_b}{MP \times \alpha \times h_{total}} \times 100 \quad (2)$$

where B is the NaOH consumption (ml) to keep the pH constant during the reaction; N_b is the normality of the base; MP is the mass of protein (g); h_{total} is the total number of peptide bonds in the protein substrate, calculated from amino acid analysis by summing the micromols of each individual amino acid per gram of protein; and α is the degree of dissociation of the α-NH_2 groups, expressed as:

$$\alpha = \frac{1}{1 + 10^{\text{pK}-\text{pH}}} \quad (3)$$

The pK value varies with temperature and can be estimated by Eq. (4) (Kristinsson & Rasco, 2000):

$$\text{pK} = 7.8 + \frac{298 - T}{298 \times T} \times 2400 \quad (4)$$

Considering mean molecular weight of amino acids around 125 g/mol (Rodríguez-Díaz, Kurozawa, Netto, & Hubinger, 2011; Silva, Park, & Hubinger, 2010) and total amino acid content of 75.74 g/100 g (Table 5), h_{total} of shrimp wastewater, used to calculate DH, was 6.0 meq/g of protein.

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