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Utilization of tilapia processing waste for the production of fish protein hydrolysate

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ABSTRACT

Viscera and carcasses represent 60–70% of whole fish body weight, and this processing waste is a known source of bioactive molecules, including proteases. These enzymes can be employed in various biotechnological processes, such as, for preparing fish protein hydrolysate (FPH). Therefore, the objective of this study was to evaluate the use of processing waste from Nile tilapia (*Oreochromis niloticus*) as a source of protein and proteases to produce FPH. Three FPH production conditions were evaluated: two conditions used autolysis with enzymes extracted from the tilapia intestine at different concentrations (FPH_{100}, 100 mg of tissue/mL and FPH_{600}, 600 mg of tissue/mL) and the third used 0.5% (v/v) Alcalase (FPH_{com}), a commercial protease preparation. Protein, amino acids and fatty acids content were calculated as DM basis. After a 4-h reaction, maximum hydrolysis percentages from FPH_{com}, FPH_{100}, and FPH_{600} systems were 34.73 ± 1.44%, 29.21 ± 0.79%, and 41.66 ± 1.33%, respectively. The protein content in the resulting FPS was 584.8 g/kg, 492.3 g/kg, and 508.2 g/kg for FPH_{com}, FPH_{100}, and FPH_{600}, respectively. Methionine and lysine were found at levels of 32.0 and 77.0 g/kg (FPH_{com}), 31.0 and 64.0 g/kg (FPH_{100}), and 33.0 and 69.0 g/kg (FPH_{600}), respectively. Polyunsaturated fatty acid contents of FPH_{com}, FPH_{100}, and FPH_{600} were 101.0 g/kg, 138.0 g/kg, and 70 g/kg, respectively, with a predominance of linoleic acid (C18:2*n*−6). Amino acid composition, lipid profile, and amino acid score suggested that all of the experimental FPHs could be employed as a protein source in diets for aquatic organisms and other farmed animals.

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1. Introduction

Nile tilapia, *Oreochromis niloticus* (L.), is the second most cultivated freshwater fish worldwide, and the filet is the main processed product, yielding 30–40% of whole fish wet weight. Therefore, approximately 60–70% of the fish body is processing waste, which includes meat remains, head, skin, bones, scales, and viscera (Clement and Lovell, 1994). While a fish carcass

Abbreviations: FPH, fish protein hydrolysate; E/S, enzyme:substrate ratio; AA, amino acid; BApNA, benzoyl-DL-arginine-*p*-nitroanilide; SApNA, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide; Leu-*p*-Nan, leucine-*p*-nitroanilide; DH, degree of hydrolysis; TCA, trichloroacetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DMSO, dimethylsulphoxide; BSA, bovine serum albumin; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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has high protein content, fish viscera (mainly stomach, pyloric caeca, and intestine) is an important source of proteases (Bezerra et al., 2001, 2005; Souza et al., 2007; Espósito et al., 2009; Marcuschi et al., 2010; Silva et al., 2011).

Large amounts of protein-rich by-products from the fishery and aquaculture industry are discarded or processed into fish meal. However, the use of these processing wastes to produce fish meal may be restricted, mainly due to its high ash content. In contrast, enzymes are an important tool for the food industry due to their ability to transform raw materials into improved food products. For example, proteases are used to hydrolyze proteins and polypeptides to produce low molecular weight peptides and free amino acids, increasing the digestibility of the product (Shahidi and Kamil, 2001). Therefore, enzymatic hydrolysis is an alternative to recovering protein from fishery and aquaculture processing waste, resulting in a more soluble product known as fish protein hydrolysate (FPH) (Martone et al., 2005; Leal et al., 2009). In this way, production of FPH by proteolytic treatment may be a way to transform cheap pelagic fish, by-catch from trawlers, and fish processing waste into products with improved quality and functional characteristics (Shahidi 1994; Kristinsson and Rasco, 2000).

In this study, we evaluated the use of tilapia processing waste as a source of protein and proteases to produce FPHs. FPHs were also characterized regarding their degree of hydrolysis (DH), molecular weight range of the peptides, proximate chemical composition, amino acid and lipid profiles, and chemical score on the basis of whole-egg protein. Additionally, tilapia proteases extract results were compared with those of a commercial enzyme extract (Alcalase).

2. Materials and methods

2.1. Raw-materials, enzyme extraction and protein determination in intestine crude extracts

By-products (viscera and carcass) from farmed tilapia were kindly donated by Noronha Pescados Ltd., a local fish processing plant in Pernambuco State, Brazil. The materials were brought immediately to the laboratory on ice. By-products comprised tilapia heads, meat remains, skin, bones, and viscera. All other reagents used in enzymatic assays were of analytical grade and purchased from Sigma (St. Louis, MO, USA).

The intestinal were collected and homogenized in distilled water (4 °C) at concentrations of 100 and 600 mg of tissue/mL and these solutions were named ONp_{100} and ONp_{600}, respectively. The resulting preparation was centrifuged (Herolab Unicen MR Centrifuge, Germany) at 10,000 × *g* for 15 min at 4 °C to remove cell debris and nuclei. The supernatant (crude extract) was frozen at −20 °C for subsequent use in enzymatic assays. Soluble protein concentration was determined by the Folin phenol reagent method (Lowry et al., 1951), and absorbance was measured at 750 nm in a microplate reader with bovine serum albumin (BSA) as the standard. This protein measurement method was found to be more suitable for the enzyme extract in this work.

2.2. Enzymatic assay

2.2.1. Total proteolytic activity assay

Total proteolytic activities were determined in all extracts using 1% (m/v) azocasein as the substrate prepared in 0.1 M Tris–HCl, pH 8. The assay was performed by mixing 30 μL of sample with 50 μL of 1% azocasein for 60 min at 25 °C, and the reaction was stopped by adding 240 μL of 10% trichloroacetic acid (TCA) for 15 min. The mixture was centrifuged at 8000 × *g*, for 5 min. The supernatant (70 μL) was mixed with 1 M NaOH (130 μL) and absorbance was measured at 450 nm in a microplate reader (Bio-Rad X-Mark spectrophotometer, Hercules, CA, USA). A blank control was prepared by replacing the sample with 0.1 M Tris–HCl, pH 8.0 (Bezerra et al., 2005). Previous experiments showed that the enzymatic reaction performed under the conditions mentioned above followed first order kinetics during the first 60 min. One unit (U) of enzymatic activity was defined as the amount of enzyme capable of hydrolyzing azocasein to produce a 0.001 absorbance per minute change. Specific enzyme activity was calculated by dividing the enzyme activity (U) per amount of protein in the sample (mg/mL) and expressed as U/mg.

2.2.2. Specific enzyme assays

Trypsin, chymotrypsin, and leucine aminopeptidase activities were determined in 96-well microtiter plates using benzoyl-DL-arginine-*p*-nitroanilide (BApNA), succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (SApNA), and leucine-*p*-nitroanilide (Leu-*p*-Nan) as specific substrates, respectively, prepared in dimethyl sulfoxide. The assay was performed by mixing 30 μL of sample with 140 μL of 0.1 M Tris–HCl, pH 8.0 and 30 μL of 8 mM BApNA, SApNA, or Leu-*p*-Nan for 10 min at 25 °C. Release of p-nitroaniline (product) was measured at 405 nm in a microplate reader (Bio-Rad X-Mark spectrophotometer). A blank control was prepared by replacing the sample with 0.1 M Tris–HCl, pH 8.0 (Souza et al., 2007). One unit (U) of enzyme activity was defined as the amount of enzyme capable of hydrolyzing 1 μmol of BApNA, SApNA, or Leu-*p*-Nan per minute under the established conditions, using a molar coefficient of 9100 mM^{-1} cm^{-1} (Bezerra et al., 2005). Enzymatic specific activity is expressed as mU/mg.

2.3. Fish protein hydrolysates (FPHs)

FPHs ($n = 3$) were produced by fish enzyme hydrolysis adapted from the method described by Cahú et al. (2012). A commercial enzyme (Alcalase, Product Code P4860, supplied by Sigma–Aldrich, St. Louis, MO, USA). at 0.5% (m/v) was adopted

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