DETERMINATION OF THE ANTIOXIDANT POTENTIAL OF TAMBAQUI (*Colossoma macropomum*) RESIDUES FROM THE NORTHERN AMAZON BY THE ABTS METHOD

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ABSTRACT

The increase in fish production over the years has directly contributed to the generation of a large amount of by-products, which consequently raises concerns regarding the treatment, disposal, and environmentally sound management for a sustainable economy. These residues are often not utilized due to the productive sector's lack of knowledge about technological procedures that enable the use of this material. Therefore, as a strategy for recycling and adding value to the product, aiming to make the activity sustainable and economically viable, the objective of this work is to provide protein hydrolysates with antioxidant potential, using residues from the fishing industry in the far north of the Amazon as raw material. Tambaqui residues were hydrolyzed using alcalase, at concentrations of 0.5%, 1.5%, and 3%, substrate concentrations of 1%, 3%, and 5%, a temperature of 50°C, pH 8.0, and evaluated for ABTS radical antioxidant capacity. The results were significant, with inhibition values greater than 80%. Thus, a residual material, which can become a contaminant if discarded uncontrollably, is converted into highly valuable products.

Keywords: Protein hydrolysates 1. Fish waste 2. Proteolytic enzyme 3. Antioxidant 4.

1 INTRODUCTION

There are various economic activities that impact the environment, with fishing being a notable example. This ancient activity is of considerable importance worldwide as a source of food, employment, and income for various economic sectors. Currently, one of the main challenges in the production chain is the low utilization of by-products generated from fish processing. These residues often cause irreversible environmental impacts and pose public health risks due to inadequate management, becoming an environmental issue that needs to be addressed. Additionally, they represent a potential waste of organic matter rich in nutrients.

Fishery industry generates large quantities of fish by-products (heads, viscera, trimmings, roes, frames, cut-offs, skin, and bones) which contain important nutrients, mainly protein and lipids, which can be used to process them into added-value products. Particularly, these wastes contain a high amount of protein-rich material with a crude protein content of 8 to 35%, that are usually converted into low market-value products, such as fish meal, animal feed and fertilizer (CHALAMAIAH et al., 2012; WANG, 2018).

These residues can be hydrolyzed using proteolytic enzymes and possess antioxidant activities. Antioxidants are substances capable of inhibiting or delaying oxidation rates. Thus, antioxidants can neutralize free radicals by donating the electron they need. In this context, antioxidant compounds have been widely used in the treatment and prevention of various diseases and can also be employed in different areas such as the food, cosmetic, and even pharmaceutical industries (DOMÍNGUEZ et al., 2018; FALOWO et al., 2016).

From the perspective of recycling and adding value to residues, this study aims to evaluate the antioxidant potential of tambaqui by-products using the ABTS method, through the enzymatic hydrolysis of these residues, and to provide hydrolysates with antioxidant activity of biotechnological interest for the market.

2 MATERIAL & METHODS

For the production of the hydrolysates, residues obtained from the processing of tambaqui fish (*Colossoma macropomum*), a native species of the Amazon Basin with significant production in captivity in the northern region (Kristinsson & Rasco, 2000), were used. The tambaqui residues (skin, scales, fins, and backbone) were acquired from a fish company located in Boa Vista, RR, Brazil, originating from breeding tanks. For the hydrolysis reaction of the fish industry by-products, the commercial liquid enzyme preparation Alcalase 2.4 L FG, a bacterial endopeptidase produced by submerged fermentation of the microorganism *Bacillus licheniformis*, will be used.

To determine the optimal conditions for obtaining protein hydrolysates, the relationships between the variables incubation time (minutes), substrate concentration (% w/v), enzyme concentration (% w/v), and pH (v/v) in the hydrolysis process were evaluated.

The enzyme concentrations varied between 0.5%, 1.5%, and 3%, the substrate concentrations were 1%, 3%, and 5%, with a fixed temperature of 50°C and pH of 8.0 for all combinations. The hydrolysis time was standardized at 30, 60, 90, 120, and 180 minutes, and during this period, aliquots of each combination were collected for assays, all prepared in triplicates.

For determining antioxidant activity using the ABTS method (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), a 7 mM ABTS stock solution and a 140 mM potassium persulfate solution were initially prepared. To prepare the ABTS radical, 5 mL of the ABTS stock solution was mixed with 88 µL of the potassium persulfate solution (7 mM) and kept in a dark environment at room temperature for 16 hours. Then, 1 mL of this mixture was diluted in PBS buffer (5 mM) until an absorbance of 0.70 ± 0.05 at 734 nm was obtained. In Eppendorf tubes, a 10 µL aliquot of the hydrolysates was mixed with 1 mL of the diluted ABTS solution, and the absorbance (734 nm) was measured after 6 minutes. The results were calculated as a percentage of inhibition (Re et al., 1999).

From the tests carried out, antioxidant activity values were obtained expressed as a percentage of inhibition for the different concentrations of the hydrolysates. Furthermore, all data were obtained from three replications and analyzed in the software R version 4.0.3 (R Core Team 2020), using analysis of variance (ANOVA), and the means compared using the Tukey test with a confidence interval of 95% to determine significant differences in each treatment.

3 RESULTS & DISCUSSION

The commonly used free radical scavenging test for antioxidants is the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) method because ABTS is a stable free radical that provides maximum absorbance at 734 nm. The results obtained for the capture of the ABTS+ radical indicate a high antioxidant power for all tested combinations. There was a significant individual effect ($p < 0.05$) of antioxidant activity on time and the concentrations of enzyme and substrate analyzed. Figure 1 presents the results obtained from the treatments. The results show an increasing percentage of inhibition as the concentrations of enzyme, substrate, and incubation time increase. The antioxidant potential of tambaqui protein hydrolysate at enzymatic concentrations of 0.5, 1.5, and 3% alcalase was 67.84%, 72.96%, and 81.15%, respectively, as shown in Figure 1, letter A.

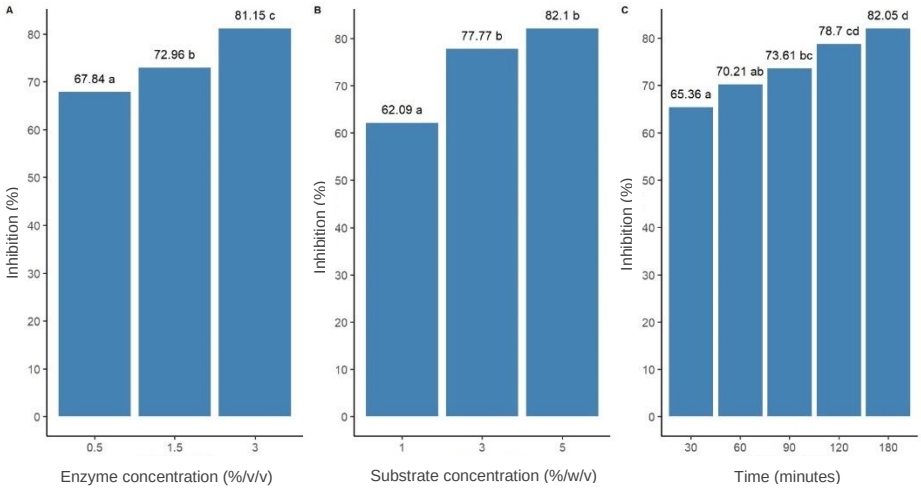

Figure 1 Percentage of antioxidant activity of fish protein hydrolysate on ABTS free radical scavenging. Different lowercase letters in the figure mean significantly different according to Tukey test.

Regarding the incubation time, the minimum activity values were 65.36% at 30 minutes and 82.05% at 180 minutes, indicating that the longer the hydrolysis time, the higher the antioxidant activity. Based on the study conducted by Augustin, Husni, and Putra (2023), the ABTS scavenging activity increases rapidly from 0 to 5 hours of hydrolysis time and then remains constant until 7 hours, similar to our study where activity increased as hydrolysis progressed. According to Le Vo et al. (2016), the antioxidant activity of protein hydrolysate depends on the size and composition of free amino acids; the longer the hydrolysis time, the more free amino acids are obtained. The hydrophobic amino acids that form during hydrolysis, such as proline, leucine, alanine, tryptophan, and phenylalanine, can increase antioxidant activity, as well as tyrosine, methionine, histidine, and lysine, which have antioxidant activity.

For the substrate concentration, the highest inhibition value was 82.1% at a 5% fish concentration. There was no significant variation compared to the 3% concentration (Figure 1, letter B). In the study conducted by Augustin, Husni, and Putra (2021), peptides from the viscera of snakehead fish exhibited free radical scavenging activity. ABTS activity increased significantly ($P<0.05$) as the substrate concentration increased. The percentage of ABTS radical inhibition was 46%, 52%, and 58% at 1%, 2%, and 3% concentrations, respectively. Our results show high antioxidant capacity starting from the initial substrate concentration of 1%, indicating that the higher the substrate concentration, the higher the antioxidant activity. Consequently, more residues will be underutilized, contributing to the reduction of environmental degradation.

4 CONCLUSION

From this perspective, the production of hydrolysates containing bioactive peptides derived from agro-industrial waste presents an interesting recycling strategy linked to the creation of useful products. This approach can generate new value-added ingredients with functional compounds and properties, while also contributing to the reduction of environmental impact. Regarding antioxidant potential, the hydrolysates demonstrated the ability to scavenge the ABTS radical, indicating that tambaqui waste has a high inhibitory effect. However, further research is needed to identify the antioxidant peptides and determine their application in food systems. Thus, the prospecting of new compounds with such activity is a field of growing research, given the high demand.

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