EVALUATION OF THE ANTIOXIDANT CAPACITY (DPPH) OF TAMBAQUI (Colossoma macropomum) WASTE FROM THE AMAZON

Thaylanna C. C. Fagundes[1]*, Kezia M. S. Assunção[1], Ana P. F. Correa[1], José T. de Paiva[2] & Marcos J. S. Vital[1]

[1] *Programa de Pós-graduação em Recursos Naturais/PRONAT/UFRR, Universidade Federal de Roraima, Boa Vista, Brasil.*
** Corresponding author's email address: thaylannacorreia@gmail.com*
[2] *Departamento de Zootecnia/UFRR, Boa Vista, Brasil.*

ABSTRACT

Among the various economic activities that cause impacts on the environment, the fishing sector stands out due to the large generation of waste at all stages of its production process, from capture to the sale of fish. From this, it becomes necessary to use alternatives for the reuse of waste generated by this activity, which can provide added value products for the industry, due to the enormous protein content of fish. An alternative that contributes to reducing environmental degradation is the use of enzymatic technology to recycle these by-products. The objective of this work was to produce protein hydrolysates and evaluate the antioxidant capacity from enzymatic hydrolysis, using as raw material by-products generated from the fishing industry in the extreme north of the Amazon. Tambaqui residues were hydrolyzed using alcalase, at concentrations of 0.5, 1.5 and 3%, substrate concentrations of 1, 3 and 5%, temperature of 50°C, pH 8.0 and evaluated for antioxidant potential (DPPH). The results obtained suggest the application of these protein hydrolysates in various areas of biotechnology, aiming to insert/maintain Brazil at the forefront of an emerging area of research, with great potential for generating new products.

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

1 INTRODUCTION

The fishing industry has great relevance in the world market, constituting an activity with high economic potential in several countries such as Brazil, being considered one of the main productive activities in the Amazon region in continuous growth, considering the favorable environmental conditions such as a favorable climate in temperatures, light and humidity, favoring the large production of farmed fish. The Brazilian North Region has been standing out in the global production of native fish, increasing the scale of the market, through automated production in captivity, with high efficiency (PEREIRA, 2020; PEIXE BR, 2022).

The problem related to waste from the fishing industry is the underuse of this material and environmental degradation due to its improper disposal, being a contaminant when the waste remains in the industrial plant itself, due to rapid deterioration, or the waste can be an environmental contaminant causing high environmental impact. This waste is often not used due to the lack of knowledge in the production sector about technological procedures that enable the use of this material. From these byproducts it is possible to obtain protein hydrolysates through an enzymatic hydrolysis process, which may have functional properties such as antioxidant activity. (CORRÊA et al., 2014).

From this perspective, the production of hydrolysates with bioactive peptides from fishing waste presents itself as an interesting strategy for recycling and adding value to these wastes, so that the activity becomes sustainable and economically viable. Therefore, the purpose of this work is to make hydrolysates with antioxidant potential available, using as raw material by-products from the fishing industry in the extreme north of the Amazon.

2 MATERIAL & METHODS

The raw material used to produce hydrolysates with biological activities were obtained from the processing of tambaqui fish (*Colossoma macropomum*), a species native to the Amazon Basin with significant captive production in the North region. Skin, scales, fins and spinal column were used, crushed in 100 mL of phosphate buffer (0.2M) to produce protein hydrolysates. For the hydrolysis reaction, the commercial liquid preparation enzyme Alcalase 2.4 L FG, supplied by LNF Latino-Americana, was used. Hydrolysis was carried out following the method described by Kristinsson and Rasco (2000), with adaptations.

In order to determine the ideal conditions for obtaining protein hydrolysates, the ratio between the variables incubation time (minutes), substrate concentration (% w/v) and enzyme concentration (% w/v) in the hydrolysis process were evaluated. The enzyme concentrations varied from 0.5, 1.5 and 3%, the substrate concentration in amounts of 1, 3 and 5% and a fixed temperature of 50°C and pH 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 to perform the antioxidant assay using the DPPH method, according to the methodology proposed by Brand-Williams et al (1995), with modifications.

The DPPH method was used to determine the antioxidant activity of protein hydrolysates from tambaqui residues. DPPH is a stable radical with an intense purple color. The reaction of this with other radicals, electrons or hydrogen atoms present in fish protein hydrolysates leads to a loss of color at a wavelength of 515 nm. Marked decreases in absorbance at this wavelength when DPPH is reacted with the sample indicate the presence of compounds with free radical scavenging activity. For the DPPH free

radical capture method, 50 µL of lyophilized hydrolysates were mixed with 1.95 mL of 0.06 µM DPPH solution in test tubes and kept in a dark environment for 45 minutes. The samples were read using a UV-Vis spectrophotometer, with a wavelength of 515 nm.

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 by antioxidants is the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method because DPPH is a stable free radical that provides maximum absorbance at 515 nm. The antioxidant activity in this assay was shown by its ability to scavenge DPPH free radicals, thus forming colorless non-DPPH-H free radicals. The hydrolysate ability of Tambaqui fish protein using 0.5, 1.5, and 3% alcalase enzymes to reduce DPPH free radicals was 60.22, 71.65, and 71.72%, respectively, as shown in Figure 1, letra A.

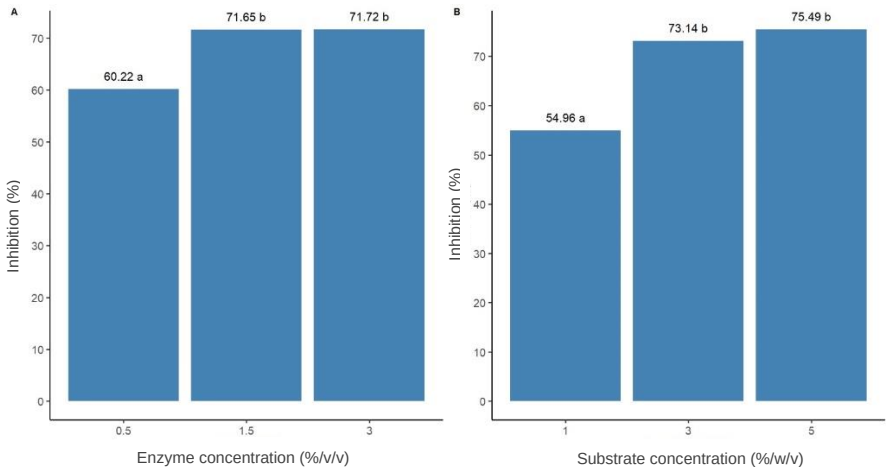

Figure 1 Percentage of antioxidant activity of fish protein hydrolysate on DPPH free radical scavenging. Different lowercase letters in the figure mean significantly different according to Tukey test.

Based on Figure 1, it can be statistically stated that there is a difference in the use of alcalase enzyme concentrations of 0.5, 1.5, and 3% in their ability to scavenge DPPH free radicals ($p < 0.05$). In contrast, the use of alcalase enzyme concentrations of 1.5 and 3% did not show any difference. From these data, it can be seen that the use of 3% alcalase enzyme provides a relatively greater DPPH free radical scavenging value compared to the others. This shows that the peptides produced from protein breakdown are functional. According to Elias et al. (2008) and Ngo and Kim (2013), protein hydrolysate serves as the main source of antioxidant peptides and becomes more active after hydrolysis.

The results for substrate concentration show that the inhibition capacity of 1%, 3%, and 5% DPPH was 54.96%, 73.14%, and 75.49%, respectively, as shown in Figure 1, letter B. Statistically, there is no significant difference between using 5% and 3% fish, but there is a difference compared to 1%. These results are similar to the previous study by Puspawati et al. (2020), which reported that the DPPH radical inhibitory activity increased with higher enzymatic and substrate concentrations of hydrolysates from Snakehead murrel (Channa striata) fish waste.

4 CONCLUSION

This work aims to provide protein hydrolysates with bioactive peptides that have antioxidant activity greater than 70%, obtained through the enzymatic hydrolysis of by-products generated by the fishing industry. The goal is to position Brazil at the forefront of an emerging research area with great potential for generating new products. Additionally, it is important to develop research based on biotechnological innovations to expand research projects in the Amazon region, facilitating the dissemination of new products.

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