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Antioxidant properties of tambatinga (*Colossoma macropomum* x *Piaractus brachypomus*) protein hydrolysates

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Summary: The muscular tissue not recovered of fish filleting process can be utilized as substrate to obtain protein hydrolysates containing bioactive peptides. This research aimed to investigate the effect of enzymatic hydrolysis conditions of muscular tissue from tambatinga's backbone on their antioxidant properties. A Central Composite Rotatable Design (CCRD) was used for evaluate the effects of the following independent variables: hydrolysis time (60 – 180 min) and enzyme concentration of the protease Alcalase® (50 – 200 U/mL), using as responses the antioxidant activities measured by FRAP, ABTS and DPPH methods (μmol of Trolox equivalents per g sample). Degree of hydrolysis was estimated by protein soluble determination in trichloroacetic acid (TCA). Tambatinga backbone was used as substrate for enzymatic hydrolysis and it was prepared in phosphate buffer (0.1 mol/L, pH 8) at a concentration of 10% (m/v). The reaction mixture was incubated at 50 ºC/90 rpm during the time and different concentrations of Alcalase previously defined in the CCRD. After the incubation period, the hydrolysis reaction was stopped in a boiling bath for 20 min. The hydrolyzed material was centrifugated at 10,000 × g/20 min/5 °C, the supernatant was collected and subsequently lyophilized for further analysis. The non-hydrolyzed sample was used as a control assay, prepared under the same process conditions, but without addition of the enzyme. In the evaluated intervals, the independent variables did not present significant effects ($p > 0.05$), not being possible to generate statistically valid mathematical models. However, the individual evaluation of the hydrolysis assays indicated substantial increases in the antioxidant properties of the hydrolysates compared to the control. For the FRAP method, the hydrolysates obtained in assays 6 (200 U/mL; 120 min) and 3 (71.8 U/mL; 162.5 min) showed increases of 94% (24.64 μmol TE g^{-1}) and 71% (21.65 μmol TE g^{-1}), respectively, in relation to control (12.68 μmol TE g^{-1}), indicating the presence of peptides capable of reducing the Fe^{3+}. For the ABTS method, the hydrolysates obtained under the conditions of assays 8 (125 U/mL; 180 min), 5 (50 U/mL; 120 min) and 7 (125 U/mL; 60 min) showed increases of 92% (84 μmol TE g^{-1}), 90% (83.1 TE g^{-1}) and 90% (83.1 μmol TE g^{-1})

compared to the control (43.7 µmol TE g^{-1}), respectively, indicating the presence of peptides capable of donating electrons to stabilize the radical. On the other hand, for the ability to inhibit the DPPH radical, there was no significant ($p > 0.05$) increase in the antioxidant activity of the hydrolysates in relation to control. The determination of the content of TCA-soluble proteins was used as an indirect method of the samples' proteolysis, since this parameter may have a direct relationship with the antioxidant properties of the hydrolysates. The maximum value of TCA-soluble protein was 82% (assay 6), followed by 71% and 57% (assays 3 and 7, respectively), corroborating the highest values of antioxidant activity observed between the assays. The results of the work showed that the enzymatic hydrolysis of tambatinga waste resulted in hydrolysates with antioxidant activity greater than the non-hydrolyzed sample.

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