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# ENZYMATIC HYDROLYSIS FOR VALUE ADDITION TO MECHANICALLY SEPARATED MEAT AND ITS INCLUSION IN BREADED FISH PRODUCTS

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## ABSTRACT

Fish production and demand have been increasing worldwide. Along with this increase, fish processing generates waste, such as mechanically separated meat, which can be used for human consumption and in the production of protein hydrolysates. The objective of this work was to produce protein hydrolysate from Tambatinga hybrid fish (a cross between *Colossoma macropomum* (Tambaqui) and *Piaractus brachypomus* (Pirapitinga)) using mechanically separated meat (MSM) and papain enzyme, and to add these hydrolysates to breaded products. The hydrolysates were produced by combining enzyme concentration (2,0, 1,0, 0,66, 0,33, and 0,22%) and hydrolysis time (40 and 60 minutes). The obtained hydrolysates were characterized according to the degree of hydrolysis and percentage of free radical scavenging. Subsequently, breaded products were produced with three concentrations of the hydrolysate (7%, 10%, and 13%) and characterized according to their nutritional composition. Tambatinga mechanically separated meat (MSM) proved to be a promising raw material for obtaining protein hydrolysate. since approximately 20% of its composition consists of proteins. The percentage of free radical scavenging of the obtained hydrolysate was influenced by the reaction time and the enzyme concentration used. In this context, the incorporation of tambatinga protein hydrolysate into breaded products proved to be a viable alternative for enriching the product's protein content, contributing to the development of foods with higher nutritional value.

**Keywords:** protein hydrolysate. aquaculture; food security; Tocantins.

# HIDRÓLISE ENZIMÁTICA PARA AGREGAÇÃO DE VALOR A CARNE MECANICAMENTE SEPARADA E SUA INCLUSÃO EM EMPANADOS DE PEIXE

## RESUMO

A produção e demanda de pescado tem aumentado no mundo inteiro. Aliado a isso, ocorre a geração de resíduos oriundos do seu processamento, como a carne mecanicamente separada que pode ser utilizada na alimentação humana e na produção de hidrolisados proteicos. O objetivo desse trabalho foi de produzir hidrolisado proteico de peixe híbrido Tambatinga (cruzamento entre o *Colossoma macropomum* (Tambaqui) e *Piaractus brachypomus* (Pirapitinga), a partir da carne mecanicamente separada (CMS) utilizando a enzima papaína e adicionar esses hidrolisados em empanados. A produção dos hidrolisados foi realizada mediante combinação dos fatores de concentração enzimática (2.0; 1.0; 0.66; 0.33; e 0.22%) e tempo de hidrólise (40 e 60 minutos). Os hidrolisados obtidos foram caracterizados quanto ao grau de hidrólise e percentual de sequestro de radicais livres. Em seguida foram produzidos empanados com três concentrações do hidrolisado (7%. 10% e 13%) que foram caracterizados quanto à sua composição nutricional. A carne mecanicamente separada (CMS) de tambatinga mostrou ser uma matéria-prima promissora para a obtenção de hidrolisado proteico, uma vez que aproximadamente 20% de sua composição é constituída por proteínas. O percentual de

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sequestro de radicais livres do hidrolisado obtido foi influenciado pelo tempo de reação e pela concentração enzimática empregada. Nesse contexto, a incorporação do hidrolisado proteico de tambatinga em produtos empanados mostrou-se uma alternativa viável para o enriquecimento proteico do produto, contribuindo para o desenvolvimento de alimentos com maior valor nutricional.

**Palavras-chave**: hidrolisado proteico; piscicultura; segurança alimentar; Tocantins.

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## INTRODUCTION

Global fish production has been increasing since 1994. As consumption increases, so do the demand for fish and global aquaculture production (Faveret and Siqueira, 1997; Vidal *et al*., 2019). According to data from the Food and Agriculture Organization of the United Nations (FAO), total global aquaculture production reached a record of 214 million tonnes in 2020, and fish consumption is expected to increase in all regions of the world by 2030, with the largest increase projected for Latin America (33%), followed by Oceania (28%), Asia (20%), and Africa (7%) (Naylor *et al*., 2021; FAO. 2022).

Brazil has great potential for aquaculture due to its favorable conditions and abundant water resources, which support the production of several species (Souza *et al*., 2021). This potential is further enhanced by a combination of factors. including the tropical climate. vast land and ocean areas. self-sufficiency in grain production. essential for feed production. and species diversity.

According to data from the Brazilian Fish Farming Association (Peixe BR). fish farming has been the fastest-growing animal production activity in recent years. In 2022, Brazil produced 860,355 tonnes of farmed fish (tilapia, native fish, and other species), representing a 2,3% increase compared to the 841,005 tonnes produced in 2021 (Peixe BR. 2023).

The state of Tocantins. characterized by high temperatures and abundant water resources. saw an increase in fish production. from 18,100 tonnes in 2024 to 20,400 tonnes in 2025, with tucunaré, tambaqui, and their hybrid, tambatinga, among the main species produced. Tambatinga results from the cross between female tambaqui (*Colossoma macropomum*) and male pirapitinga (*Piaractus brachypomus*) (Castilho and Pedroza, 2019; PeixeBR, 2026; Oliveira *et al*., 2020).

Tambatinga is widely used in the fish processing industry for fillet production. This process, known as filleting, leaves a large amount of protein-rich waste on the fish carcass, which is often used for the production of animal feed, oils or even discarded into the environment (Silva *et al*., 2020; Silva *et al*., 2018).

Thus, one way to use filleting waste is through the production of mechanically separated meat (MSM), which consists of the edible portion of the fish mechanically separated from the carcass (Bernadino Filho *et al*., 2019).

An alternative for utilizing these trimmings is the use of MSM in the production of flours, breaded products, sausages, nuggets, frankfurters, and canned products, with good results in terms of nutritional quality and consumer acceptability (Guimarães and Calixto, 2017).

Another promising approach for processing MSM is its use in the production of fish protein hydrolysate (FPH) obtained through enzymatic hydrolysis. an effective method for transforming underutilized protein sources into products of high biological value with excellent nutritional and medicinal properties. This process concentrates this macronutrient and allows its incorporation into a variety of preparations for human consumption (Chalamaiah *et al.*, 2015; Neves *et al.*, 2016; Barbosa, 2021).

Several enzymes, such as alcalase, papain, pepsin, flavourzyme, neutrase, protamex, trypsin, α-chymotrypsin, pancreatin, pronase, bromelain, and thermolysin, have been used to hydrolyze fish proteins for the production of protein hydrolysates (Noman *et al*., 2018).

Despite its significant advantages for the utilization of fish processing trimmings, enzymatic hydrolysis involves several complexities that remain poorly understood. Its efficiency is directly affected by processing conditions, particularly the ideal substrate pH, temperature, and contact time with the food matrix. These factors influence the overall yield, the required enzyme concentration, and the final degree of hydrolysis (Egerton *et al*., 2018).

Therefore, the utilization and transformation of fish filleting waste into products that meet market demands and benefit consumers represent a new source of income for processing companies. In addition to increasing revenue, these products can provide nutritional benefits and offer a new option for human consumption while reducing environmental impacts through waste reduction (Neves *et al*., 2016). In this context, this study aimed to produce protein hydrolysate from the hybrid fish tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) using mechanically separated meat and the enzyme papain. and to develop breaded products containing this hydrolysate for human consumption.

## MATERIALS AND METHODS

### Obtaining Mechanically Separated Meat

The raw material used in this study consisted of processing waste from the hybrid fish (*Colossoma macropomum* × *Piaractusaa brachypomus*). obtained from the Piracema Conservation Reserve. in the municipality of Almas. Tocantins. Brazil.

The carcasses resulting from filleting were processed using fish deboning machines to obtain mechanically separated tambatinga meat (MSTM). After processing. the MSTM was stored in food-grade plastic bags and frozen at −18 °C until use.

### Physicochemical Analyses of Mechanically Separated Meat and Breaded Products

In order to determine moisture content. approximately 3 g of the sample was weighed in a previously dried and tared porcelain crucible and then placed in an oven at 105 °C until constant weight was reached. After this process. the crucible was transferred to a desiccator and then weighed with the dried sample. The difference between the initial and final sample weights was attributed to the amount of water lost during the drying process. corresponding to the moisture content of the sample. according to the methodology described by the Association of Official Analytical Chemists (AOAC, 2000).

For the determination of total lipid content in MSTM, 3 g of the dried sample was weighed into a cellulose thimble and placed in a previously dried and tared reboiler. Hexane was then added to the reboiler until the sample contained in the thimble was submerged. Subsequently, the reboiler was coupled to the heating block of the Soxhlet apparatus at 70 °C and maintained under reflux for approximately 3 hours. After this period, the thimble was suspended above the hexane level for 2–3 hours to remove the solvent. Following hexane evaporation, the reboiler was placed in an oven at 105 °C until constant weight was reached (AOAC. 2000).

For protein assessment, 100 mg of dry matter was weighed and transferred to a digestion tube, followed by the addition of 600 mg of $K_2SO_4$, 300 mg of $CuSO_4$, and 5 mL of 98% $H_2SO_4$. The tube was placed in a digestion block at 400 °C until the sample became colorless. Subsequently, the tube containing the digested sample was coupled to the Kjeldahl apparatus. and an Erlenmeyer flask containing 10 mL of boric acid was placed at the condenser outlet. Then, 15 mL of 50% NaOH was added to the digested sample. A total of 100 mL of condensate was collected in the Erlenmeyer flask and titrated with HCl (0.02 N) until the color changed from green to red. Equation 1 was used to calculate the nitrogen content.

$$\%\, Nitrogen\ (\%\, N) = \frac{(Va - Vb)\ x\ 14.007\ x\ Na\ x\ CFa)}{Ms\ (mg)}\ x\ 100 \quad \text{Equation 1}$$

Where $V_a$ = volume of hydrochloric acid used for sample titration (mL); $V_\beta$ = volume of hydrochloric acid used for blank titration (mL); 14,007 = constant equivalent to the atomic mass of N; $N_a$ = normality of the hydrochloric acid used; $CF_a$ = correction factor for hydrochloric acid; and $M_s$ = mass of the sample used for distillation (mg). Subsequently, the protein content of the sample was calculated by multiplying % N by a conversion factor of 6,25 (AOAC, 2000).

To quantify ash. 5 to 10 g of the dried sample was weighed. After weighing. the samples were placed in a muffle furnace at 550 °C for 4 hours until white ash was obtained. The samples were then cooled in a desiccator and weighed (AOAC, 2000).

### Hydrolysate Production

The hydrolysate was produced according to the methodology proposed by Paiva *et al.* (2015), with some adaptations. MSTM was weighed, transferred to a Falcon tube, and homogenized with distilled water at a ratio of 1:10 (MSTM solids/mL of water). Papain was added after adjusting the temperature to 60 °C, considered the optimal temperature for papain activity (Venetikidou *et al*., 2025), at an enzyme protein/substrate protein ratio. Enzymatic hydrolysis was performed in a water bath under constant agitation, and the enzymatic reaction was stopped by heating at 90 °C for 15 minutes.

To evaluate the effect of enzyme concentration (g of enzyme protein/g of substrate protein). the following concentrations were used: 2,0%, 1,0%, 0,66%, 0,33%. and 0,22% at 60 °C, with hydrolysis times of 40 and 60 minutes. The pH values of the assays remained within the range established for optimal papain activity (4,5–6,6).

### Chemical Analyses of the Hydrolysate

For the determination of the degree of hydrolysis (%DH), the OPA (o-phthaldialdehyde)

reagent was prepared according to Church *et al.* (1983), and the degree of hydrolysis (%DH) was calculated according to Spellman *et al.* (2003), with adaptations, using Equation 2. Where:

$$\%\, DH = \frac{\alpha\text{-amino nitrogen (AN)}}{Total\ Nitrogen}\ x\ 100 \quad \text{Equation 2}$$

For the determination of total antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, the soluble fraction of the hydrolysate was used. A 0,1 mL aliquot of the sample was added to 3,9 mL of DPPH solution, according to the methodology proposed by Rufino *et al.* (2007), and the percentage of DPPH radical scavenging was calculated based on the standard.

The readings were performed after 120 minutes using a spectrophotometer at 515 nm. and the results were obtained according to Equation 3:

$$\%\, FRS = \frac{CA-SA}{CA}\ x\ 100 \quad \text{Equation 3}$$

Where: FRS = Free Radical Scavenging; CA = Control Absorbance; SA = Sample Absorbance.

**Preparation of MSTM Breaded Products with Added Protein Hydrolysate**

The hydrolysate used in the preparation of the breaded products was selected based on the highest degree of hydrolysis, considering enzyme concentration and hydrolysis time. After selection, the whole hydrolysate (soluble and insoluble fractions) was incorporated into the formulation of MSTM breaded fish products.

The ingredients used in the breaded product formulations were purchased from local markets in Palmas, Tocantins, Brazil, except for the hydrolysates, which were produced at the Food Technology Laboratory of the Federal University of Tocantins, Palmas campus.

After obtaining the hydrolysates from mechanically separated tambatinga meat, four breaded product formulations were developed: a traditional formulation without hydrolysate addition (CF) and three formulations containing MSTM protein hydrolysate at concentrations of 7% (F7). 10% (F10), and 13% (F13), partially replacing the meat in the formulation.

ITAll ingredients used in the formulations were individually weighed using a digital analytical balance (Table 1). The ingredients were added to the MSTM and mixed until a homogeneous mixture was obtained. Subsequently, portions of the mixture were shaped into breaded products. The portions were then coated with predust (corn-based), followed by batter (consisting of milk, wheat flour, starch, and salt), and finally with breading (corn-based). After this process, all breaded products were arranged in horizontal layers and stacked on trays, properly labeled, and frozen at −18 °C until analysis.

**Table 1**. Proportion of ingredients used in the formulation of breaded products with different concentrations of tambatinga protein hydrolysate.

| Ingredients (grams) | Breaded product formulations (grams) | | | |
|---|---|---|---|---|
| | **CF** | **F7** | **F10** | **F13** |
| | **%** | **%** | **%** | **%** |
| Mechanically separated tambatinga meat | 82 | 75 | 72 | 69 |
| Hydrolysate | 0 | 7 | 10 | 13 |
| Textured soy protein | 5 | 5 | 5 | 5 |
| Corn starch | 4 | 4 | 4 | 4 |
| Dehydrated onion | 2.5 | 2.5 | 2.5 | 2.5 |
| Dehydrated chives | 0.5 | 0.5 | 0.5 | 0.5 |
| Dehydrated garlic | 0.4 | 0.4 | 0.4 | 0.4 |
| Paprika | 0.2 | 0.2 | 0.2 | 0.2 |
| Black pepper | 0.2 | 0.2 | 0.2 | 0.2 |
| Water | 5 | 5 | 5 | 5 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 |

Notes: CF - Breaded fish product containing 0% tambatinga hydrolysate; F7 - Breaded fish product containing 7% tambatinga hydrolysate; F10 - Breaded fish product containing 10% tambatinga hydrolysate; F13 - Breaded fish product containing 13% tambatinga hydrolysate.

**Statistical Analysis**

For the production of tambatinga protein hydrolysates, a completely randomized design was adopted, with five enzyme/substrate ratios (2,0, 1,0, 0,66, 0,33, and 0,22%) and two hydrolysis times (40 and 60 minutes), with three replicates. For the characterization of breaded products containing tambatinga protein hydrolysate, a completely randomized design was used, with four formulations (0% – without hydrolysate addition; 7%, 10%, and 13% hydrolysate addition) and three replicates. Statistical analysis was performed using SISVAR software, adopting a 95% confidence interval. The results obtained from the physicochemical analyses were subjected to analysis of variance, and Tukey's test was used to compare the means (Ferreira, 2019)

## RESULTS AND DISCUSSION

**Chemical Composition of Mechanically Separated Tambatinga Meat (MSTM)**

According to the results, the moisture content of mechanically separated meat from the tambatinga hybrid showed a mean value of 78,7% (Table 2). This value was close to the 74,55% reported by Macena (2017) for MSTM, while Veit (2012) found a mean moisture content of 78,36% for mechanically separated tilapia meat.

The moisture content of mechanically separated fish meat can be influenced by intrinsic factors such as species, age, body composition, and the anatomical region used, as well as technological factors related to the mechanical separation process, applied pressure, sieve diameter, washing steps, and storage conditions (Kirschink *et al*., 2013).

It should be noted that there is no specific Technical Regulation on Identity and Quality (RTIQ) establishing a reference moisture content for mechanically separated fish meat. However, moisture content is one of the most important parameters evaluated in fish, as it is related to its stability, quality, and composition (Veit, 2012).

Regarding lipid content. a mean value of 2,8% was found in MSTM, as shown in Table 2. Amaral *et al*. (2013) found mean lipid contents of 5,1% and 14,9% in mechanically separated meat from Aracu and Mapará fish, respectively.

Factors such as physiological characteristics. Diet, and season are considered determinants of lipid levels in fish (Bordignon *et al*., 2010). In addition, the filleting process may leave adipose tissue attached to the carcass, consequently increasing lipid percentages (Dallabona *et al.*, 2013).

Regarding the production of protein hydrolysates from mechanically separated meat. Nilsang *et al*. (2005) reported that high lipid contents may adversely affect the enzymatic hydrolysis process.

Regarding protein content. a mean value of 20,2% was found (Table 2). This value was

considered high compared to those reported in the literature by Terres-Ribeiro *et al*. (2020). Signor *et al*. (2018), and Freitas *et al*. (2012), who observed mean protein contents of 12,26%, 11,89%, and 9,75%. Respectively, in mechanically separated Nile tilapia meat. However, it is important to note that fish composition may vary according to several factors, such as sex, size, age, and species (Macedo-Viegas *et al.*, 2008).

**Table 2**. Chemical composition of mechanically separated tambatinga meat (MSTM)

| **Component** | **Mean Values** |
|---|---|
| Moisture (%) | 78.7 ± 0.5 |
| Total Lipids (%) | 2.8 ± 0.4 |
| Protein (%) | 20.2 ± 0.8 |
| Ash (%) | 1.1 ± 0.1 |

Values are expressed as mean ± standard deviation.

Signor *et al.* (2020) evaluated mechanically separated tilapia meat and found that the pressing process altered its proximate composition. The reduction in moisture content resulted in a proportional increase in lipid and protein contents.

The protein content of the raw material is one of the main factors determining the efficiency of protein hydrolysate production. as it is directly related to enzymatic hydrolysis yield and the amount of peptides generated. In addition, protein composition influences the amino acid profile, degree of hydrolysis, and functional and bioactive properties of the hydrolysates, such as solubility, emulsifying capacity, water-holding capacity, and antioxidant activity (Chalamaiah *et al.*, 2012).

Regarding ash content, a value of 1,1% was obtained, which was similar to the values reported by Oliveira Filho *et al.* (2010) and Kirschnik *et al.* (2013), who found 1,14% and 1,11%, respectively, in mechanically separated Nile tilapia meat.

The determination of ash content is an important parameter of the nutritional composition of mechanically separated fish meat, as it represents the total mineral content of the raw material. It can also provide information on the efficiency of the mechanical separation process, since high ash contents may indicate greater incorporation of bone fragments (Guimarães *et al.*, 2017)

**Production of Hydrolysates**

Table 3 presents the results for the degree of hydrolysis (%DH). showing an increase in this variable with increasing enzyme concentration. It was also observed that the mean degree of hydrolysis values obtained for samples hydrolyzed for 60 minutes were higher than those obtained for samples hydrolyzed for 40 minutes (Table 3).

Furthermore. hydrolysis time had a significant influence. as the sample hydrolyzed at the same enzyme concentration for 40 minutes showed a lower value of 31.45%. No statistically significant difference was observed between samples hydrolyzed for 40 and 60 minutes at a mean enzyme concentration of 0.83% (Table 3).

The samples with the lowest degree of hydrolysis were those hydrolyzed with the lowest papain concentration (0.22%). with samples hydrolyzed for 40 minutes showing significantly lower mean values (11.78%) (Table 3).

According to Marçal *et al.* (2021). the degree of hydrolysis represents the number of peptide bonds that are hydrolyzed. Hydrolysates with a degree of hydrolysis greater than 10% can be used as protein supplements and may improve the nutritional properties of food products.

**Table 3.** Mean degree of hydrolysis (%) of mechanically separated tambatinga meat (MSTM) hydrolyzed with papain at different enzyme concentrations and hydrolysis times

| Tambatinga Mechanically Separated Meat Hydrolysates | | Degree of Hydrolysis (%) |
|---|---|---|
| Hydrolysis time (min) | Enzyme concentration (%) | |
| 40 | 0.22 | 11.78[h] |
| 60 | 0.22 | 17.02[g] |
| 40 | 0.33 | 18.60[f] |
| 60 | 0.33 | 20.86[e] |
| 40 | 0.66 | 23.66[d] |
| 60 | 0.66 | 26.00[c] |
| 40 | 1.0 | 26.41[c] |
| 60 | 1.0 | 26.49[c] |
| 40 | 2.0 | 31.45[b] |
| 60 | 2.0 | 32.97[a] |

Lunelli (2015) also used papain for the enzymatic hydrolysis of tilapia processing waste. varying the reaction time. According to the author. papain increased the degree of hydrolysis when a reaction time of 120 minutes was used. reaching a value of 25.28%. This result indicates that hydrolysis time influences the hydrolytic process.

The effect of increasing enzyme concentration was also reported by Centenaro *et al.* (2009) in the production of protein hydrolysate from croaker using alcalase. demonstrating the influence of this variable on the production of fish protein hydrolysates.

The mean percentages of free radical scavenging at different papain concentrations are presented in Table 4. According to the results. the highest free radical scavenging values were observed in samples hydrolyzed for 60 minutes at enzyme concentrations of 0.66%. 1.0%. and 2.0%. and in samples hydrolyzed for 40 minutes at an enzyme concentration of 2.0%. These samples did not differ significantly. with mean values ranging from 46.03% to 51.34%. No statistically significant difference was observed among samples hydrolyzed for 60 minutes at enzyme concentrations of 0..33% and 0.22% and samples hydrolyzed for 40 minutes at concentrations of 0.22%. 0.33%. 0.66%. and 1.0% (Table 4).

Higher percentages of free radical scavenging were reported by Fonseca (2014) when evaluating the antioxidant activity of peptides derived from protein hydrolysates of cobia muscle and processing waste (60.77% and 60.25%. respectively). The hydrolysates were produced using flavourzyme. a fungal protease complex consisting of exopeptidases and endopeptidases and produced by submerged fermentation of a selected strain of Aspergillus oryzae. with a hydrolysis time of 30 minutes.

Analyzing the hydrolysate obtained from mechanically separated anchovy meat. Piotrowicz (2012) observed 43.4% free radical scavenging using alcalase for 1 hour of hydrolysis at pH 8.0 and 50 °C. with a hydrolysate concentration of 7 mg mL−1. In addition. the author observed a similar effect using flavourzyme for 5 hours at pH 7.0 and 50 °C. with 45.4% free radical scavenging at a hydrolysate concentration of 5 mg $mL^{-1}$.

It should be noted that the antioxidant activity of protein hydrolysates is related to the size of the bioactive peptides produced during the hydrolytic process. with peptides containing 2 to 20 amino acids and a molecular weight of 6 kDa being considered more active (Nepomuceno. 2018). In addition. some studies have shown that protein hydrolysates with a high degree of hydrolysis and low-molecular-weight peptides interact more effectively with free radicals. thereby interfering with the oxidation process (Rajabzadeh *et al.*. 2018; Sukkhown *et al.*. 2018).

**Table 4**. Free radical scavenging (FRS) of mechanically separated tambatinga meat (MSTM) hydrolyzed with papain at different enzyme concentrations and hydrolysis times

| Tambatinga Mechanically Separated Meat Hydrolysates | | %FRS |
|---|---|---|
| Hydrolysis Time (min) | Enzyme Concentration (%) | |
| 40 | 0.22 | 39.53$^{d}$ |
| 60 | 0.33 | 40.71$^{cd}$ |
| 60 | 0.22 | 40.84$^{cd}$ |
| 40 | 1.0 | 41.02$^{cd}$ |
| 40 | 0.66 | 41.09$^{cd}$ |
| 40 | 0.33 | 42.78$^{bcd}$ |
| 60 | 1.0 | 46.03$^{abc}$ |
| 60 | 0.66 | 47.22$^{ab}$ |
| 40 | 2.0 | 48.59$^{a}$ |
| 60 | 2.0 | 51.34$^{a}$ |

Several studies indicate that protein hydrolysates and bioactive peptides may exert a range of beneficial health effects. including antioxidant. anti-inflammatory. antihypertensive. and antidiabetic activities. However. despite the promising findings reported in experimental studies. current knowledge regarding the mechanisms of action. bioavailability. safety. and efficacy of these compounds in humans remains limited. herefore. further research. particularly well-designed clinical trials. is warranted to confirm and expand the existing evidence regarding their potential health benefits (Santos-Sánchez et al.. 2026; Zaky et al.. 2022).

**Composition of Breaded Products**

According to the results presented in Table 5. the control formulation differed significantly from all formulations containing protein hydrolysate in terms of moisture content. The highest mean moisture content was observed in the control formulation. at 58.50%. In contrast. the formulations containing 10% and 13% hydrolysate showed the lowest mean values. at 50.17% and 51.79%. respectively. with no significant difference between them.

Moisture is an important factor that can promote microbial growth. including fungal growth. Similar differences were reported by Veit (2012) with the incorporation of Nile tilapia protein hydrolysates into breaded fish products. Compared to the control sample. the moisture content of breaded products containing hydrolysate was also lower.

However. bioactive peptides can improve water retention in meat systems and alter water distribution and migration patterns due to their hygroscopic effect. A very high proportion (>75%) of specific amino acids. such as asparagine. serine. glutamine. histidine. arginine. tyrosine. lysine. and glycine. has been reported to contribute to water-holding capacity (WHC) (Nuñez *et al.*. 2021).

Table 5 presents the ether extract values. showing no statistically significant difference between the control formulation and the formulations containing protein hydrolysate. Mean values ranged from 2.44% in samples containing 13% protein hydrolysate to 2.48% in samples without hydrolysate addition (control formulation).

Signor *et al.* (2020). when incorporating mechanically separated tilapia meat into breaded products. observed a lipid content of 2.86%. However. according to the researchers. this value may be associated with lipids derived from the frying process. It should be noted that. in the present study. the breaded products were baked in a gas oven. and no additional lipids were incorporated into the formulations. other than those naturally present in the raw materials used in their preparation.

Regarding the protein content of the breaded products. Table 5 shows significant differences among all formulations. with higher hydrolysate concentrations resulting in significantly higher protein contents. The lowest mean value (22.83%) was observed in the control sample. while samples containing 13% protein hydrolysate showed a mean value of 31.72%.

Bourscheid (2015). when evaluating the optimization of the enzymatic hydrolysis process of chicken by-products and their application in hamburgers. observed that the addition of hydrolysate obtained using Protamex (a protease complex obtained from two *Bacillus* species. *Bacillus*

*licheniformis* and *Bacillus amyloliquefaciens*) resulted in a protein content of 17.97%. which was 6% higher than that of the control sample.

Regarding the crude fiber content of the formulations presented in Table 5. no statistically significant difference was observed. with mean values ranging from 0.44% to 0.47%. This may be attributed to the fact that the formulations differed only in the amount of mechanically separated meat and the percentage of protein hydrolysate. which did not affect the mean crude fiber values.

The ash contents presented in Table 5 showed no significant differences among the formulations. Veit (2012). when evaluating the inclusion of tilapia protein hydrolysate in breaded fish products. found an ash content of 1.28% in the breaded product containing hydrolysate obtained using the Protamex enzyme.

The carbohydrate content of the breaded products showed no significant differences among the different hydrolysate concentrations. as presented in Table 5. ranging from 11.80% in the formulation containing 10% hydrolysate to 13.16% in the control formulation. However. this variation may be associated with the breading process. as it was performed manually. resulting in a lack of standardization during the process (Veit. 2012).

The caloric values of the 10% and 13% formulations were significantly higher than those of the other formulations. This may be associated with their higher protein contents. since no significant differences were observed in lipid and carbohydrate contents. which are also included in the calculation of caloric value.

**Table 5**. Nutritional composition of breaded fish products containing different concentrations of MSTM protein hydrolysate

| **Components (g/100g)** | **Formulations** | | | |
|---|---|---|---|---|
| | **CF** | **F7%** | **F10%** | **F13%** |
| Moisture | 58.50±0.84$^{a}$ | 54.72±0.71$^{b}$ | 50.17±0.78$^{c}$ | 51.79±1.51$^{c}$ |
| Ether extract | 2.48±0.05$^{a}$ | 2.46±0.09$^{a}$ | 2.47±0.12$^{a}$ | 2.44±0.07$^{a}$ |
| Protein | 22.83±0.48$^{d}$ | 27.44±0.0.0$^{c}$ | 29.66±0.48$^{b}$ | 31.72±0.58$^{a}$ |
| Crude fiber | 0.46±0.07$^{a}$ | 0.44±0.04$^{a}$ | 0.47±0.01$^{a}$ | 0.44±0.05$^{a}$ |
| Ash | 2.63±0.05$^{a}$ | 2.62± 0.06$^{a}$ | 2.71± 0.04$^{a}$ | 2.72± 0.06$^{a}$ |
| Carbohydrates | 13.16± 0.32$^{a}$ | 12.13± 0.52$^{a}$ | 11.80± 0.49$^{a}$ | 12.44± 0.94$^{a}$ |
| Caloric value (kcal) | 165.41±3.56$^{c}$ | 181.34±3.14$^{b}$ | 202.29±3.73$^{a}$ | 194.93±4.21$^{a}$ |

Notes: CF: breaded fish product without MSTM hydrolysate; F7%: breaded fish product containing 7% MSTM hydrolysate; F10%: breaded fish product containing 10% MSTM hydrolysate; F13%: breaded fish product containing 13% MSTM hydrolysate. Means followed by the same lowercase letter within a row do not differ significantly according to Tukey's test at the 5% probability level.

## CONCLUSION

Mechanically separated tambatinga meat proved to be an excellent substrate for the production of protein hydrolysate. as protein accounted for approximately 20% of its nutritional composition. Among the conditions evaluated. hydrolysis of mechanically separated tambatinga meat with 2% papain for 60 minutes resulted in the highest degree of hydrolysis. The results also indicated that the percentage of free radical scavenging of the protein hydrolysate was influenced by hydrolysis time and enzyme concentration. Overall. the addition of protein hydrolysate to breaded products made from mechanically separated tambatinga meat proved to be a viable alternative for increasing the protein content of the product.

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