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Study of protein extraction from residues of tambatinga amazon fish

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ABSTRACT

The present work aimed to extract proteins from tambatinga fish residue with physicochemical characterization, a hybrid of the crossing between the female tambaqui (Colossoma macropomum) and the male pirapitinga (Piaractus brachypomus). Protein extraction was performed by the following methods: acid (hydrochloric acid, acetic acid) and basic (sodium hydroxide). The protein fraction was characterized in relation to moisture content, ash, protein and yield. The moisture determination was performed with powder samples, submitted to drying at 40 ° C, in a forced circulation oven, which presented values of 7.29; 11.42 and 3.14% respectively. Regarding the fixed mineral residue (ash), the samples differed from each other, with valuesbetween 81.52; 50.65 and 44.76%. The samples obtained by the different processes presented protein contents of 3.63; 9.36 and 39.55% respectively. And the yield of protein extraction was 8.96; 3.64 and 6.43% for hydrochloric acid, acetic acid and sodium hydroxide, respectively. The obtained results indicate that it is possible to perform protein extraction for fish waste utilization, mainly by the basic extraction method.

Keywords: Protein recovery; Acid extraction; Alkaline extraction

RESUMO

O presente trabalho teve como objetivo extrair proteínas do resíduo de peixe tambatinga com caracterização físico-química, um híbrido do cruzamento entre o tambaqui feminino (Colossoma macropomum) e o pirapitinga masculino (Piaractus brachypomus). A extração de proteínas foi realizada pelos seguintes métodos: ácido (ácido clorídrico e ácido acético) e básico (hidróxido de sódio). A fração proteica foi caracterizada em relação ao teor de umidade, cinza, proteína e rendimento. A determinação da umidade foi realizada com amostras de pó, submetidas à secagem a 40 ° C, em estufa de circulação forçada, que apresentou valores de 7,29; 11,42 e 3,14%, respectivamente. Em relação ao resíduo mineral fixo (cinzas), as amostras diferiram entre si, com valores entre 81,52; 50,65 e 44,76%. As amostras obtidas pelos diferentes processos apresentaram conteúdo proteico de 3,63; 9,36 e 39,55%, respectivamente. E o rendimento da extração proteica foi de 8,96; 3,64 e 6,43% para ácido clorídrico, ácido acético e hidróxido de sódio, respectivamente. Os resultados obtidos indicam que é possível realizar a extração de proteínas de resíduos de peixes, principalmente, pelo método de extração em meio básico.

Palavras-chave: Recuperação de proteínas. Extração ácida. Extração alcalina

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1 INTRODUCTION

Fish production in Brazil is becoming more and more prominent due to the great growth of this activity and may become one of the largest fish producers in the world (BRASIL, 2016). However, the increase of this activity results in an increase of residues, generating concerns to the environmental impacts, since these residues are rich in organic compounds. In addition, wastewater generated in fish industrialization has a high amount of total suspended solids, generating high biochemical and chemical oxygen demand in addition to fish waste, so all wastewater in the process must be taken to the wastewater treatment plant effluents (FELTES et al., 2010).

As the world population grows, so does the demand for protein foods that have better nutritional quality. Fishery resources can contribute significantly to improving this quality (MOREIRA, VARGAS, RIBEIRO, 2001). The production of protein with high added value attributes from fishing activities has been much discussed in recent years, as these activities are capable of generating considerable income in both developed and developing countries. Protein recovery from fish and by-products are high quality sources, providing desirable characteristics such as pseudoplastic, emulsifier and viscoelasticity, as well as sensory (texture, color, softness, taste, aroma), surface (film forming and hydration properties (water sorption, gelatin, solubility, syneresis, viscosity) and others related to texture and rheological characteristics (adhesiveness, elasticity, mass formation, mesh formation, cohesion, gum formation, aggregation) (FREITAS, 2011).

Fish consists mainly of muscle tissue, connective tissue and fat and is considered a food rich in protein, fat, vitamins and minerals. Among the several fish species in Brazil, tambatinga (*Colossoma macroponum x Piaractus brachypomus*) stands out for being one of the most cultivated species, as well as tambaqui (*Colossoma macropomum*). It is originally from South America, but can be found in all states of the Brazilian territory, including Mato Grosso state. In addition, tambaqui (*Colossoma macropomum*), pirapitinga (*Piaractus brachypomus*) and tambatinga hybrid (*Colossoma macropomum x Piaractus brachypomus*) are the most commonly used fish in the fish

processing industry for filet production. Filleting generates a considerable volume of waste that is still little used by the fish industry for food purposes, with the most common destination being discarded in nature, generating environmental problems. Parts of fish discarded as waste include fillet, head, fin, viscera, skin, backbone, trimmings and clippings, which are rich in proteins that can be isolated and used in the enrichment of processed foods, beverages, applications, pharmaceutical companies, among others (SILVA et al., 2018).

Thus, the reuse of fish residues for protein recovery can be an important alternative for reducing environmental impacts, thus contributing to the environment, the processing of fish and also the economic and technological development of the region. In addition, it is important to note that the waste that would be lost during the processing of fish may add value to the waste. In this context, the present work aimed to extract proteins from the tambatinga fish residue with physicochemical characterization, a hybrid produced from the crossing between the female tambaqui (*Colossoma macropomum*) and the male pirapitinga (*Piaractus brachypomus*).

2 MATERIAL AND METHODS

2.1 Raw material preparation

As raw material was used the byproducts (residue) of tambatinga filleting. The fish were filleted and the waste crushed in an industrial blender. Then stored at -18°C until the time of analysis. The residue consisted mainly of head, ridge, fins, leather and scales.

Tambatinga samples were washed twice with 1 L of distilled water to remove excess blood from the raw material and the third wash was performed with 2% (v/v) sodium hypochlorite solution at ratio 2 L water /kg residue. Excess water was removed by twisting mechanism on a clean cotton cloth and the residue thus obtained was used for protein extraction.

2.2 Acid and alkaline extraction of fish proteins

Protein extraction from tambatinga residues was performed through the recovery of the protein fraction via acid and alkaline extraction, according to the methodology suggested by Nolsøe e Undeland (2009).

For alkaline or/and acidic protein extraction a 1L beaker was used. Approximately 300 g of the washed residue was weighed and solubilization reagent NaOH (2 mol L^{-}), 2 mol L^{-} hydrochloric acid solution or 2 mol L^{-} acetic acid solution was added. Subsequently, the pH of the mixture was measured with bench pH to verify the need for pH correction. For alkaline extraction the pH should be between 12.0 and 13.0 and for acid extraction between 1.0 and 2.0.

After pH correction, the samples were allowed to stand for 4 h for solubilization of fish proteins. Then, a double filtration. The filtrate had its pH readjusted to the protein isoelectric point (pH 5.5) for protein precipitation for 48h. The precipitate was oven dried with forced air circulation at 40 ° C. Three repetitions were performed for each extraction (acid or alkaline). The proteins obtained in the different extraction processes were characterized by the amount of yield, moisture, ash and crude protein with acetic acid, hydrochloric acid and NaOH. Analyzes (fixed mineral residue, moisture and yield) were performed in triplicate and the results expressed as mean ± standard deviation, while crude protein analysis was expressed as mean.

2.2 Physicochemical analysis

2.2.1 Fixed mineral residue (ashes)

The determination of the fixed mineral residue was performed according to the technique of the Adolfo Lutz Institute (2008). At the beginning of the process 10 g of sample were weighed in a previously calibrated crucible to discount its dry weight. The sample was then charred at 200 °C and incinerated in a muffle furnace at a temperature of 550 °C until slightly gray in color. Subsequently, the crucible was stored in a desiccator until it reached room temperature and then weighed to obtain the sample weight.

2.2.2 Moisture

The determination of moisture was performed according to the Adolf Lutz Institute (2008). For this analysis, the direct oven drying method at 105 ° C was used. Initially 5 g of the sample were weighed in a Petri dish, previously gauged to discount its weight. The plates were heated for 3 hours and then cooled to room temperature in a desiccator and finally weighed. This heating and cooling operation was performed until constant weight was obtained.

2.2.3 Crude protein

Crude protein content analysis was performed by the Kjeldahl process according to the Association of Official Analytical Chemists (AOAC) techniques (AOAC, 1998). This analysis required 0.3 g of the sample, which was transferred to a digestion tube and added 10.0 mL of concentrated sulfuric acid and 0.5 g of catalytic mixture. The sample tubes were heated (300 °C to 400 °C) for approximately 4 hours. And after reaching room temperature, the contents of the tubes were diluted in distilled water and the solution was transferred to the distillation tube. Then 40.0 mL of 40% NaOH was added of 30.0 mL of a boric acid solution (4%), together with 5 drops of protein indicator, were mixed in a glass vial to perform the titration technique. The solution was then titrated with hydrochloric acid to the turning point. Results were expressed as mean dry basis.

2.2.4 Yield

For the calculation of yield was considered the dry weight of the protein fraction obtained in the extraction process and the wet weight of the waste mass, according to Equation 1

(1)

$$yield\ (\%) = \frac{\text{dry weight of protein fraction}}{\text{wet weight of the residue}} \text{x } 100$$

2.3 Statistical analysis

The results were submitted to analysis of variance and comparison between means (Tukey), with a significance level previously set at 5% ($p < 0.05$) with the aid of *Statistic software* version 7.0.

3 RESULTS AND DISCUSSION

The recovery of the protein fraction of tambatinga residue was performed from acid and alkaline extraction. The product resulting from these extractions had different visual aspects. Extractions with hydrochloric acid and acetic acid were found to have a whitish color and a firm consistency, while the alkaline extraction product had a darker color and a more liquid consistency.

Freitas (2011) in his study of protein recovery through fish residue obtained through the acid solubilization (HCl) process a higher whiteness for surimi, since there was greater removal of hemoglobin during the washing process which was more efficient. The protein recovered from the NaOH-solubilized anchoite residue showed a dark red color, which can be attributed to the precipitation of heme pigments (image not shown). According to Salgado (2015), in his work the proteins recovered after alkaline solubilization, obtained a stronger firmness than the proteins recovered after acid solubilization.

As the major industrial interest in the use of the recovered protein fraction of tambatinga residue is as a protein supplement (SILVA et al., 2018). The product obtained at the end of each extraction was subjected to drying in an oven with forced air circulation at 40 °C. After drying, the samples were ground and subjected to physical chemical characterization in relation to moisture, fixed mineral residue (ashes) and proteins (Table 1).

The determination of moisture is of great importance to define the quality, stability and composition of foods, and may affect the storage, packaging and processing of the product. The water content in food production directly implies the control of the deterioration rate by microorganisms, enzymatic and chemical

reactions that occur during storage. Table 1 shows the moisture content of proteins extracted from tambatinga residue using different extraction methods.

Table 1 – Physicochemical analysis and the yield of the dehydrated protein fraction for the three extraction methods.

Physicochemical parameters	**Extraction Methods**		
	Hydrochloric acid	**Acid acetic**	**Sodium hydroxide**
Moisture (%)	7.29 ± 0.23[a]	11.42 ± 0.24[b]	3.14 ± 0.05[c]
Fixed Mineral Waste (%)	81.52 ± 0.90[a]	50.65 ± 1.16[b]	44.76 ± 0.31[c]
Protein (%)	3.63 ± 0.02[a]	9.36 ± 0.05[b]	39.55 ± 0.07[c]
Yield (%)	8.96 ± 1.59[a]	3.64 ± 0.85[b]	6.43 ± 1.40[c]

by the same letter on the same line do not differ significantly from each other at the 5% probability level by the Tukey test.

Moisture determination was performed with powder samples after the forced-air oven drying process. For the moisture analysis three extraction methods were used: hydrochloric acid, acetic acid and NaOH, which presented values of 7.29%; 11.42%; 3.14% respectively. According to the 95% confidence Tukey test, the three samples differed significantly from each other.

Rebouças et al. (2012) in their study of protein recovery from Nile tilapia filleting residues, obtained 4.85% moisture using extraction with chilled drinking water. This low percentage of water content according to Jay (2005) is an advantage from the preservation point of view of this product, especially considering the microbiological changes that may occur. Drying works by preserving the food due to the removal of water, which microorganisms cannot grow. Thus, from a microbiological point of view, the protein fraction obtained by the alkaline extraction method (NaOH) has advantages over the other methods (extraction with acetic acid and hydrochloric acid), since it presented low humidity.

Ferreira (2015) in his study of extraction of gelatin powder from Nile tilapia by-products by different extraction methods (hydrochloric acid, water, acetic acid) obtained results 9,54; 5.34; 7.54% respectively. Molinari (2014) found identical values (9.54, 5.34 and 7.54%) through the extraction of hydrochloric acid, water and acetic

acid for gelatin extracted from tilapia byproducts. Bueno (2008) found a similar value 9.3% working with the same raw material. These moisture values are within the range obtained in the present study, but different from what was verified by Ferreira (2015), acetic acid presented higher humidity than hydrochloric acid.

According to Bordignon (2010), these differences may occur due to the different methods of washing and preserving the residues before the extraction process begins and especially in relation to the protein drying time after the process. Other studies also report protein fraction moisture values within the range obtained in the present study. Vieira et al. (2011), who evaluated the protein recovery of Nile tilapia filleting residue by extraction with phosphoric acid, observed a humidity between 0.37 and 2.11%, presenting an average of 1.38%, different from that reported by Prentice (2012), which obtained a humidity of 7.0% for all product obtained in protein recovery from hake fillet residues (*Macrodon ancylodon*).

Prestes (2013) verified the moisture content of 12.3% for gelatin produced from bovine collagen (protein) extracted through sulfuric acid and NaOH, while the collagen fiber powder had the lowest water content (6 , 68%). Wolf (2007) reports 10.67% humidity for collagen dust, obtained from bovine skin.

Fixed mineral residue analysis, as also called by some authors as ash, was performed to determine the amount of inorganic matter present in the dried samples. The samples differed with respect to this analysis, ranging from 81.52 to 44.76% as shown in Table 2. The values are higher than those commonly observed in fish protein extraction studies. Nunes (2014) in his study found 21.65% for collagen extracted from tilapia while Ferreira (2015) in protein extraction from Nile tilapia byproducts by different extraction methods (hydrochloric acid, water, acetic acid) obtained inferior results 5.65; 1.8; 3.9% respectively.

Bueno et al. (2011) analyzed the amount of fixed mineral residue present in protein portions from tilapia skins extracted by extraction with acetic acid, hydrochloric acid, water and NaOH and obtained a content of 1.8%. catfish fish performed by Jongjareonrak et al. (2010) presented ash content 0.33%. While Murueta, Toro and Carreño (2007), in a protein recovery study with nine fish species

with different drying methods, obtained fixed mineral residue values between 8.15 to 20.27% by extraction with hydrochloric acid and NaOH. Tavakolipour (2011) found silver carp ash content around 2.2% in samples with acid pretreatment. Meer et al. (1997) evaluated fish meal and soybean meal in tambaqui, observed 3.08% ash, while Jesus (2012) obtained 22.10% ash for tambaqui residue flour.

The fixed mineral residue content for protein recovery precipitate from corvine residue according to Freitas (2011) was 1.61% by extraction with hydrochloric acid and NaOH, similar to that found by Chen, Tou and Jaczynski (2007). which obtained 2.14 and 1.61% (dry basis) for protein recovered from trout processing residues, protein solubilized at pH 2.5 and 3.0 (acid) and precipitated at pH 5.5 (protein isoelectric point), same pH conditions used in the present study. Taskaya et al. (2009) found a content of 3.80% (dry basis) of ash in protein recovery of solubilized carp at pH 11.5 (alkaline) with NaOH. Chen and Jaczynski (2007), when analyzing protein recovered from by-products of solubilization pH 12 trout processing, found 1.61% (dry base) ash, pH conditions close to those employed in this study, which was between 11 and 12.

These differences may be due to the composition of the raw material as they are different species. Also, it is important to note that as it is waste, it is extremely difficult to standardize for comparison purposes. The use of bone-containing wastes for protein extraction may favor the presence of calcium which also contributes to an increase in ash content if not removed.

The samples obtained in this study presented protein contents between 39.55 and 3.63%. It is important to emphasize that the protein fraction extracted with NaOH (alkaline extraction) presented a higher percentage value, differing from the others. According to Salgado (2015), in his study of protein recovery by fish by-products, the values of proteins extracted by alkaline solubilization (NaOH) were higher than the proteins recovered by acid solubilization (hydrochloric acid), 75.9 and 47, 8 respectively.

Protein values obtained in alkaline extraction were similar to those obtained by Chen and Jaczynski (2007), when they analyzed protein recovered from by-products of trout processing with pH 12 of alkaline solubilization (NaOH), obtained 36.48% (dry

basis). extracted protein. Similar result was obtained by Rawdkuen et al. (2009), for protein recovered from tilapia at pH 11.2 with a value of 45.42%, pH conditions also used in this study which was between 11 and 12.

Although alkaline extraction presented values similar to those reported in the literature, in acid extraction the protein levels obtained in the present study are lower than those reported in the literature. According to Martins et al. (2009), the low protein value achieved through acid extraction, is due to the higher risk of lipid oxidation that the acidic process has in relation to alkaline, since heme proteins can be activated as low pH pro-oxidants, too. This low protein content may be associated with the large amount of lipids present in the raw material, which may hinder protein isolation. Batista et al. (2007), report that solubilization at pH close to 2.5 (acid) and 11 (alkaline) may induce denaturation and aggregation of both myofibrillar and sarcoplasmic proteins.

Freitas (2011), by protein extraction with corvina and anchoita residue, obtained 82.87% of protein on dry basis through the acid solubilization process. Also according to the studies by Freitas (2011), protein recovery through corvine and anchoite residues by the alkaline solubilization process obtained approximately 72.96% of protein on a dry basis. Martins, Costa, e Prentice-Hernández (2009) protein recovery in corvina through extraction with acetic acid resulted in 49.7% of crude protein.

According to Rebouças et al. (2012), by performing the protein recovery of Nile tilapia residue obtained a content of 17.48% crude protein in their study through extraction with chilled drinking water. Vidal (2007) found a 16.64% protein content through extraction with hydrochloric acid and NaOH, Vieira et al. (2011) obtained 34.7% protein extracted with phosphoric acid and Kotaki (2005) achieved a result of 9.6% protein through protein recovery performed with Nile tilapia filleting residue extracted with hydrochloric acid. The values quoted are close to those found in this work of protein recovery of tambatinga residue.

Studies from protein recovery of chicken by-products extracted with acetic acid and hydrochloric acid resulted in 78.52% of tarsal proteins and protein extraction

from chicken feet using pre-treatments (ALMEIDA, 2012). Similar to those used in this study with extraction of hydrochloric acid presented from 67.5 to 69.9% of proteins (FERREIRA, 2013).

The yield of protein extraction was between 3.64 and 8.96% similar value of yield for extraction with hydrochloric acid, was found by Ferreira (2015) 10.38% in his study on protein extraction from tilapia by-products. Nile. Trindade (2010) obtained yields between 5.91% and 10.95% through the extraction of hydrochloric acid and NaOH e Silva et al. (2011) performed a work with carp head and obtained lower yields between 1.50 and 2.3% with alkaline (NaOH) and acid (HCl) extraction.

Novato and Viegas (1997) found a yield of 75.5% for Florida red tilapia using hydrochloric acid for extraction. However, Souza (2000) reported lower yields from 57.13% to 61.91% using the same raw material. Vieira et al. (2011) in his study on protein recovery of Nile tilapia filleting residues, obtained approximately 18.34% yield through extraction with phosphoric acid. Already Vidal (2007) working with the same raw material, obtained yield values ranging from 4.64 to 12.83% with acid (HCl) and alkaline (NaOH) extraction. These low yield values found in the present study and also by some authors may be justified by the material losses that occurred during processing, especially in the phase of excess water removal during the washing of waste.

Considering the headless carcass yield, Frascá-Scorvo (2008) working with surubins, found for filet yield 34.70% through extraction with hydrochloric acid, higher values than found in this study. This higher yield may be related to genetic modification, which according to Freato (2007), working with Piracanjuba (*Brycon orbignyanus*), reported that spindle-shaped fishies, longer and plump, have higher carcass and fillet yield.

The method that best suited this work of protein recovery through tambatinga residue was the alkaline extraction performed with NaOH, obtained a lower percentage of moisture which is of great importance for the conservation of the raw material. The fixed mineral residue content also remained low, as compared to the

protein content, it was possible a higher percentage than the other extraction methods adding value to the fish residue.

4 CONCLUSIONS

Given the objectives proposed in this work it is concluded that it is possible to use tambatinga fish residues for protein recovery. And that the most effective method was through alkaline extraction, which provided a significant amount of protein. The physicochemical analyzes obtained from the protein powder presented good centesimal composition, with low moisture content and high ash content.

The insertion of these new products in the market is of great importance for the valorization of the use of these materials, since there is a need to reduce the environmental impacts, increase the benefits to the industry, reduce the costs generated by the waste of raw materials and the search for a development sustainable.

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