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Original Article

Yield and centesimal characterization of collagen extracted from the skin of peacock bass *Cichla monoculus*

Rendimento e caracterização centesimal do colágeno extraído da pele de tucunaré *Cichla monoculus*

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Abstract

Fish processing provides waste of around 50.0% to 70.0% of the animal's initial weight, especially the skin. Thus, this residue contains the by-product that allows biopolymers to be obtained, highlighting collagen, which can be widely used in different areas. The present study aimed to evaluate the yield of collagen extracted from peacock bass *Cichla monoculus* skin and to characterize them physicochemically. Twenty-five peacock bass with an average weight of 646 ± 175 g were used. The skin samples were removed by manual filleting and weighed, with an average yield of 3.7%. Subsequently, such models were analyzed for chemical composition, showing 61.8% for moisture, 29.3% for crude protein, 1.5% for ash, 6.3% for total lipids, and 1.2% for non-nitrogenous extract (NNE). Acid-soluble collagen (ASC) presented an average yield of 8.2%, presenting in its analysis of centesimal composition 12.5% of moisture, 82.6% of crude protein, 1.1% of ash, 2.6% of total lipids, and 1.2% NNE. The skin and collagen extracted from the tucunaré skin have technological potential for use in the preparation of products, adding value to these by-products from fish processing.

Keywords: natural polymer, protein, byproduct, fish technology.

Resumo

O processamento do pescado fornece em resíduos cerca de 50,0% a 70,0% do peso inicial do animal, destacando-se a pele. Assim, nesse resíduo encontra-se o subproduto que permite a obtenção de biopolímeros, dando destaque ao colágeno, que pode amplamente ser utilizado em diversas áreas. O presente estudo objetivou fazer uma avaliação do rendimento do colágeno extraído da pele do tucunaré *Cichla monoculus*, e caracterizá-los físico-quimicamente. Foram utilizados 25 tucunarés com peso médio de 646 ± 175 g. As amostras de pele foram retiradas por filetagem manual e pesadas, apresentando rendimento médio de 3,7%. Posteriormente, tais amostras foram analisadas quanto à composição centesimal, apresentando os valores de 61,8% para umidade, 29,3% para proteína bruta, 1,5% para cinzas, 6,3% para lipídios totais e 1,2% para extrato não nitrogenado (ENN). Colágeno solúvel em ácido (ASC) apresentou o rendimento médio de 8,2%, apresentando em sua análise de composição centesimal 12,5% de umidade, 82,6% de proteína bruta, 1,1% de cinzas, 2,6% de lipídios totais e 1,2% ENN. A pele e o colágeno extraído da pele do tucunaré apresentam potencial tecnológico para aproveitamento na elaboração de produtos, agregando valor a esses subprodutos provenientes do processamento do pescado.

Palavras-chave: polímero natural, proteína, subproduto, tecnologia de pescado.

1. Introduction

In the Amazon region, fish is the main commercialized animal protein due to the wide variety of species, the favorable climate, and water availability, making its consumption a source of maintenance and survival for the local population (Alcantara et al., 2015; Silva et al., 2020). Thus, research has been conducted in the production sector to increase supply, improve fish performance, and reduce production costs (Aride et al., 2016, 2017, 2021; Bussons et al., 2021; Liebl et al., 2021, 2022; Lima et al., 2020; Mattos et al., 2023; Nascimento et al., 2021; Polese et al., 2022).

Thus, the growing consumption of fish includes increased production, resulting in a more significant generation of waste such as the tail, head, scales, swim bladder, fins, viscera, cartilage, and skin (Liebl et al., 2021; Oliveira et al., 2017a, b), representing about from 50.0% (Ferraro et al., 2016) to 70.0% of the initial fish weight (Boronat et al., 2023), with Brazil generating around 1,359 tons of by-products in 2022 (Peixe BR, 2023).

Waste improperly disposed of in the environment causes the proliferation of vectors, the production of bad

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smells, the formation of leachate (Decker et al., 2016), and groundwater contamination (Tran et al., 2015). Thus, the use of fish waste in the development of new products is essential, enabling the reduction of impacts caused to the environment (Godoy et al., 2008).

According to Oliveira et al. (2017c), the residues generated in the fish processing chain have aroused interest in the industrial sector because they comprise biomolecules and emerge as alternative sources for obtaining biopolymers. Thus making fish by-products an attractive alternative to valuable products (Nuerjiang et al., 2023). In addition, fish have a low risk of disease transmission, low toxicity, and high yield in the extraction process (Krishnamoorthi et al., 2017; Senaratne et al., 2006) compared to other animals.

Collagen stands out due to its high availability in the animal body (Rosseto et al., 2021), and its principal characteristic is the formation of water-insoluble fibers due to the concentration of hydrophobic amino acids, located internally and externally in the protein (Silva and Penna, 2012). A total of 29 types of collagen families were distinguished, being divided into non-fibrillar and fibrillar collagens (Oliveira et al., 2017c), in which it was observed that their predominant amino acids are hydroxyproline, proline, glycine, glutamic acid, aspartic acid, arginine and alanine (Gómez-Guillén et al., 2011; Cheow et al., 2007).

Type I collagen is the most abundant, being fundamental in the consistency and consistency of tissues; this forms fibrils with an axial periodicity of 67 nm (An et al., 2016), with the collagen protein formed by glycine-X-Y repeats, the X and Y often being proline and hydroxyproline (Nair and Laurencin, 2007). Type I collagen is the most commonly found in marine and freshwater fish, requiring several stages for its isolation, and can be extracted from scales, swim bladder, fins, bones, spine, muscles, and skin (Oliveira et al., 2017c).

Although there are works carried out with the use of collagen extracted from fish, such as the production of regenerative sponges (Yamamoto et al., 2015), biofilm production (Elango et al., 2017), and antioxidant production (Pal and Suresh, 2017), there is still little information on the use of collagen from the peacock bass *Cichla monoculus* (Agassiz, 1831), being found only in *Cichla ocellaris* (Bloch & Schneider, 1801) (Oliveira et al., 2019). In this sense, this work contributes information about the extraction of collagen from the peacock bass skin, thinking of an alternative for the waste generated, determining the yield and the physical-chemical characteristics of the skin, and the total collagen extracted.

2. Materials and Methods

2.1. Sample collection

The fish were collected at the landing port of the Panair Fair (south zone), Educandos neighborhood (3º8'45"S 60º0'38"W), located in the city of Manaus, Amazonas, Brazil. The amount of fish used for the study was 25 individuals with an average weight of 646 ± 125 grams. The fish were transported in isothermal boxes with ice to the Faculty of Agricultural Sciences (FAS) Fish Technology Laboratory of the Federal University of Amazonas (UFAM).

2.2. Skin yield

After being washed in running water, the fish were measured with an ichthyometer and weighed on a digital electronic scale (Toledo®, São Paulo, Brazil), and the data were used to calculate the skin yield. The skin was removed using specific blades (the fillet with skin was removed, then the skin was separated from the fillet with the help of a knife), and weight information was obtained using a digital electronic scale. Then, the yield calculation was performed using the following Equation 1:

$$\left(Weight\ of\ the\ skin\right)/\left(Total\ weight\ of\ the\ fish\right)\ x\ 100 \quad (1)$$

The skin samples used in the yield analysis were kept in a freezer (Electrolux, Paraná, Brazil) at -18 °C for later analyses.

2.3. Raw material pre-treatment and collagen extraction

Through methodological adaptations, collagen extraction from the peacock bass skin was followed by the methodological procedure described by Montero and Gómez-Guillén (2000). All steps took place at a temperature between 18-20 °C. After thawing at room temperature, for each repetition of the analyses (n= 3), 100 g of skin samples previously washed with running water were weighed. After cleaning, the skins were immersed in a NaCl solution (0.8 M) for 10 minutes, using a ratio of 1:6 (skin weight/solution weight), with subsequent rinsing with distilled water and removal of excess water.

Afterward, the skins were immersed in an acetic acid solution (0.05 M), using a ratio of 1:10 (skin weight/solution weight), at room temperature for three hours and then rinsed with distilled water. Subsequently, they remained at room temperature for 24 hours under slow agitation (Certomat®, Germany, Melsungen) in distilled water, using a ratio of 1:20 (skin weight/water weight). After this period, the resultant was filtered in a vacuum pump (Quimis®, Brazil, São Paulo). The filtered material was placed in a non-stick tray in an oven with forced air circulation at 50 °C and then dried for 24 hours. The collagen obtained was placed in a plastic container and saved for yield determination and centesimal analysis.

2.4. Collagen yield

The weight of dry collagen (analytical balance; Gehaka, São Paulo, Brazil) and the weight of the 100-gram skin sample were used to determine the collagen yield. The information obtained was applied in the following Equation 2:

$$\left(Dry\ weight\ of\ collagen\right)/\left(Wet\ weight\ of\ skin\right)\ x\ 100 \quad (2)$$

2.5. Centesimal composition

The analyses carried out in triplicates were carried out on the skin and collagen and are included in the percentage determinations per the standards recommended by the AOAC (1990) and adopted by Instituto Adolfo Lutz, São Paulo (IAL, 2008). These were determined to be moisture-U, ash-C, crude protein-PB, and total lipids-LT by analytical procedures, and non-nitrogenous extract was obtained by difference calculation (ENN= 100 - (U+C+PB+LT)).

2.6. Statistical analysis

The collagen obtained from the peacock bass skin by-product was evaluated for its characteristics, with descriptive statistics being applied to characterize the product (Zar, 2010). The arithmetic means and standard deviation analyses were performed using the computer program "Software R® version 4.0.3".

3. Results and Discussion

After processing by manual filleting, peacock bass *Cichla monoculus* presented an average skin yield of 3.69% (Table 1). This value is lower than Souza and Inhamuns (2011) found when analyzing the same species, with average values of 3.92% and 6.33%, respectively.

Barai et al. (2022), in the flood and drought period, obtained higher yields than those reported in the study when evaluating the skin of the Amazonian species *Astronatus ocellatus* (Agassiz, 1831), *Pellona castelnaeana* (Valenciennes, 1847) and *Leporinus friderici* (Bloch, 1794). Meanwhile, for marine catfish *Sciades herzbergii* (Bloch, 1794), the value was higher (6.2%) than that of the current study (Vasconcelos-Filho et al., 2017). However, Barros et al. (2019) observed a reduction in skin yield for classes of large fish, associating this reduction with the muscle development of the fish.

In different filleting methods, a value similar to that found in the present study was observed for the yield of clean skin of tambaqui *Colossoma macropomum* (Cuvier, 1818), at an average value of 3.56% (Garcia and Maciel, 2021). In different filleting methods, an average However, in the studies carried out by Cirne et al. (2019), lower values were observed for tambaqui (2.7%, 3.0%, and 3.0%) of different weight ranges, and in the studies carried out by Liebl et al. (2021) with tambaqui fed diets containing L-lysine, higher values (10.6%) were observed than the current study.

According to Barai et al. (2022), these differences in yield occur due to the morphometry of the species, which is essential data for the fish industries to avoid waste and add value to the by-product generated. In addition, the skin yield will depend on the operator's skill, so there are no excessive losses during this skin removal process (Oliveira Filho et al., 2020). For the increase of days for cultured fish, the differentiation in the cut yield demonstrates an increase in yields of skin, fillet, head, and fins (Fernandes et al., 2010).

Collagen extracted by the acid-soluble collagen (ASC) method showed an average yield of 8.2% (Table 1). A value higher than that reported by Oliveira et al. (2019) for the *Cicla ocellaris* (Bloch & Schneider, 1801) with a value of 2.9% and Krishnamoorthi et al. (2017) for *Sepia pharaonis* (Ehrenberg, 1831) with values of 1.7% and 3.9% in acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) methods, respectively. However, the result of the present study was considered inferior to those reported by Zhang et al. (2016) for collagen extraction in ASC and PSC methods from the skin of *Oreochromis niloticus* (Linnaeus, 1758) (25.6% and 21.8%) and *Ictalurus punctatus* (Rafinesque, 1818) (25.9% and 28.8%), and similar to the extraction method using acid for *Lates calcarifer* (Bloch, 1790) (8.1%) (Jamilah et al., 2013).

It is worth mentioning that the present study used a temperature that varied between 18-20 °C and different concentrations of the reagents used for collagen extraction compared to the cited authors' studies. According to Oliveira et al. (2017c), the collagen concentration may vary due to existing collagen fibrils in the species, varying the yield between 0.4% and 92.2%. Since collagen yield may vary according to the concentration of acetic acid used (Krishnamoorthi et al., 2017), protein denaturation, environmental temperature (Rigo et al., 2002), time, and pH (Karim and Bhat, 2009).

The centesimal composition of the peacock bass skin showed values of 61.8% for moisture, 29.3% for protein, 1.5% for ash, 6.3% for lipids, and 1.2% for non-nitrogenous extract (Table 2). Vaz et al. (2020) found that for moisture and skin ash of tilapia *Oreochromis niloticus* from tanks, values higher than those of the current study, 93.3% and 2.6%, respectively. For tilapia skin from the river, values of 63.2% and 1.9% are similar to those found for the peacock bass skin investigated in the present study. Bordignon et al. (2012) point out that the moisture value can be associated with the successive washes performed before the polymer extraction process.

The values for moisture, crude protein, and ash found in the present study corroborate the results presented by Franco et al. (2013) working with three species: *O. niloticus*, pacu *Piaractus mesopotamicus* (Holmberg, 1887) and *C. macropomum*, respectively for moisture (67.1%; 64.2%; 61.0%), crude protein (29.1%; 32.7%; 35.7%) and ash (1.8%; 1.3%; 1.4%). However, the lipid value (2.0%, 1.8%, and 1.8%) was considered higher. Junk (1985) mentions that these differences in the chemical composition of the fish are observed due to the great diversity of existing species and are also related to the region's hydrological cycle.

Table 1. Mean yield (%) and standard deviation of the skin and collagen of peacock bass *Cichla monoculus*.

Peacock Bass	Performance (%)
Skin	3.69 ± 1.64[1]
Collagen	8.24 ± 6.10[2]

[1]n= 25 fish; [2]n=3 repeat.

Table 2. Proximate composition (%) and standard deviation of the skin and collagen of peacock bass *Cichla monoculus.*

Fraction	Skin[1]	Colagen[1]
Moisture	61.76 ± 0.82	12.5 ± 0.08
ashes	1.46 ± 0.14	1.05 ± 0.03
lipids	6.29 ± 0.17	2.64 ± 0.20
Protein	29.26 ± 2.62	82.62 ± 0.80
Non-nitrogenous extract (NNE)	1.23	1.19

[1]n=3.

The centesimal composition of collagen extracted from peacock bass skin showed values of 12.5% for moisture, 1.1% for ash, 2.6% for lipids, 82.6% for protein, and 1.2% for non-nitrogenous extract (NNE) (Table 2). Vaz et al. (2020), for collagen moisture extracted in different concentrations of sodium hydroxide, river tilapia skin (5.5%, 5.9%, 5.1%) and tank (3.2%, 5.0%, 7.6%) obtained an inferred value when compared to the current study.

Alfaro et al. (2013) reported that the moisture and ash content of gelatin extracted from the skin of tilapia *Oreochromis urolepis* (Norman, 1922) was higher than that of the present study. Silva et al. (2018) point out that the ash value may be associated with the type of environment and feeding of the species, and the lipid content may influence the ash value in the sample.

The lipid content of the samples analyzed in the present study (Table 2) is considered high when observing the value found by Alfaro et al. (2013) of 0.3%. High lipid values are generally kept in fish that have in their diet high values in the ratio of digestible energy and crude protein (Sampaio et al., 2000). However, in the current study, it was possible to observe a lipid reduction in the skin's lipid content about that of collagen. According to Moia et al. (2021), this reduction occurs due to the pre-treatment used during extraction to obtain the biopolymer.

The protein value obtained was considered low when analyzing the value found by Wang et al. (2013) for tilapia skin collagen on a wet basis (89.0%); on the other hand, the value of the respective work was considered similar to the value observed by Alfaro et al. (2013) for tilapia (81.2%). However, Wang et al. (2013) cite that the protein content is influenced by the extraction method and species used, showing that methods with acidic pH extract protein more than those used at neutral and alkaline pH.

4. Conclusion

The sustainable management of by-products generated in the production chain of peacock bass *Cichla monoculus*, an Amazonian fish species, contributes to the reduction of environmental impacts caused by their inadequate disposal and ensures that fishing activity is socially and environmentally responsible. Where it was possible to extract collagen from peacock bass skin, presenting the technological potential for use in the production of biotechnological products, in addition to adding value to the by-products arising from the processing of the species. The extracted collagen presented favorable characteristics in its proximate composition, an excellent protein source. The results generated in this research provide the scientific community with information that can be used in subsequent studies to improve methods of extraction and characterization of collagen extracted from by-products generated in fish processing.

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