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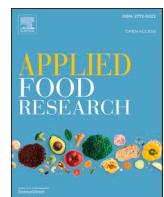
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Extraction of collagen from by-products of Amazonian fish tambaqui, *Colossoma macropomum* and pirarucu *Arapaima gigas*

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ABSTRACT

Several high-value commercial products, such as collagen, have been developed from by-products of the fish processing industry due to their wide range of applications and importance for human health. This study aimed to extract, determine the yield, and characterize the collagen obtained from the scales of *Colossoma macropomum* and *Arapaima gigas*. The samples underwent two treatments: uncrushed scales (T1) and crushed scales (T2). The moisture content in tambaqui scales showed average values of 47.71 % (T1) and 12.63 % (T2), while lipid content was 1.39 % (T1) and 1.33 % (T2). Protein content was 41.04 % (T1) and 72.95 % (T2). For pirarucu, higher values were observed for ash (28.80 % in T1), lipids (1.06 % in T1), and protein (48.40 % in T2), while higher moisture content was recorded for T1 (28.54 %). The collagen extraction yields for *C. macropomum* and *A. gigas* were 2.97 % and 1.48 % for T1, and 1.51 % and 0.85 % for T2, respectively. FTIR and XRD analyses confirmed that the collagens extracted from both species are primarily composed of type I collagen. These findings contribute to biotechnological research by adding value to the by-products of these two commercially important species in the Amazon Region of Brazil.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO, 2022) has reported that the expansion of fishing and aquaculture (Novaes et al., 2024) processing has contributed to a significant increase in processed fish by-products, reaching up to 70 % of the total production. Studies by Nóbrega et al. (2024); Batista (2011) and Miranda & Lens (2021) have emphasized the importance of utilizing these materials, given their valuable biological composition. These by-products serve as a sustainable source of bioactive compounds such as enzymes, peptides, polyunsaturated fatty acids (PUFAs), minerals, and collagen (Coppola et al., 2021). Therefore, extracting collagen from fish processing by-products represents an innovative and sustainable strategy to add value, reduce economic losses, and mitigate environmental impacts (Bhuimbar et al., 2019).

In Brazil, the production of fish by-products is particularly relevant. In 2021, the country produced approximately 841,005 tons of fish, with

the state of Amazonas contributing 21,000 tons, including 6888 tons of tambaqui (*Colossoma macropomum*) from fish farming and 194,350 tons of pirarucu (*Arapaima gigas*) from managed fishing areas (Brazilian Institute of Geography & Statistics - IBGE, 2021). Tambaqui farming generates up to 45 % of by-products, with a skinless fillet yield ranging from 26 % to 29 %, and scales representing approximately 2.6 % (Souza & Inhamuns, 2011). Similarly, juvenile tambaqui yields reported by Liebl et al. (2021) ranged from 22.91 % to 28.81 % for skinless fillets and 8.27 % to 13.63 % for skin and scales, depending on diet. For pirarucu, Barbosa et al. (2022) reported a yield of 57.88 % for skinless fillets, 9.86 % for waste, 20.37 % for head and carcass by-products, and 8.07 % for skin and scales.

Despite their low economic value, fish scales are rich in biologically valuable compounds and can serve as raw material for various products (Kubitza, 2016; Bussons et al., 2021; Liebl et al., 2022; Polese et al., 2022; Chen et al., 2022a). Scales contain keratin, biopolymers, and collagen—a fibrous, insoluble protein found in connective tissues (Shi

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et al., 2019). Collagen plays a critical physiological role, with types I (tropocollagen) and III being the most prevalent in the human body, each characterized by unique amino acid compositions, molecular domains, and structural arrangements (Squire & Parry, 2017).

The acid-soluble collagen (ASC) extraction method applied in this study offers notable advantages compared to enzymatic and thermal extraction methods, which are often associated with higher collagen degradation and lower yields. For instance, studies by Liu et al. (2012) and Pal & Suresh (2017) highlighted the loss of structural properties in collagen when using pepsin or high-temperature methods. The ASC method, in contrast, better preserves type I collagen, as demonstrated by Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) analyses, and generates lower chemical waste, contributing to environmental sustainability.

Collagen from aquatic animals also presents unique advantages compared to land-based sources, which are associated with disease transmissibility risks and religious restrictions (Heidari & Rezaei, 2022). Aquatic collagen has no risk of transmitting diseases, is non-toxic, has high prosthetic compatibility, and is particularly rich in type I collagen, making it suitable for diverse applications (Krishnamoorthi et al., 2017; Shi et al., 2019; Felician et al., 2018; Wu et al., 2022). Given this context,

this study aimed to utilize the by-products of tambaqui (*C. macropomum*) and pirarucu (*A. gigas*) scaling to extract collagen using the ASC method. The study also evaluated the yield and physicochemical characteristics of the extracted collagen, contributing to the sustainable valorization of fish by-products and advancing research on collagen's biotechnological applications.

2. Material and methods

2.1. Raw materials

Approximately 500 g of pirarucu (*Arapaima gigas*) scales were obtained from juvenile fish aged between three and four years, managed in the Inambé and Sapateiro lakes, located in the city of Fonte Boa, Amazonas, Brazil. Similarly, approximately 500 g of tambaqui (*Colossoma macropomum*) scales were obtained from the formal fish market at Feira da Panair, located in the city of Manaus, Amazonas, Brazil (Fig. 1). Pirarucu samples were obtained from fisheries using nets. The tambaqui samples were obtained from fish farms in which the fish were removed with a trawl net. Then the fish were filleted, and the by-production were collected. It is important to emphasize that these quantities were

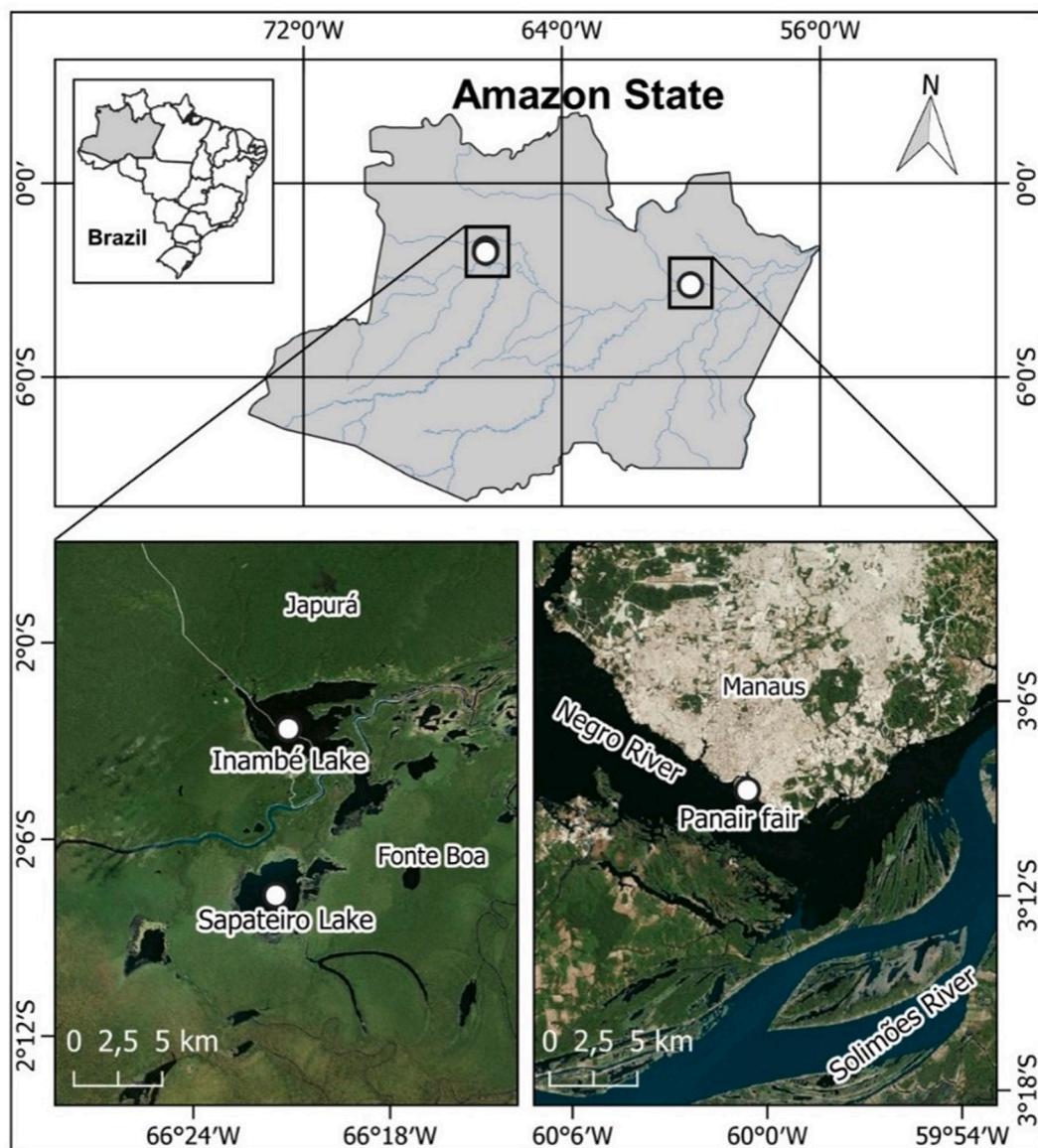


Fig. 1. Location of collection points for by-products of *Arapaima gigas* and *Colossoma macropomum* scaling.

sufficient to perform all the analyses and extractions described in this study.

2.2. Sample preparation

Pirarucu scales were processed without the skin, while tambaqui scales were kept attached to the fish skin. All scales were washed under running water, dried at 50 °C in an air circulation oven for 8 h, and cut with specialized scissors into pieces with a thickness of 1 to 3 cm. The experiment included two treatments: uncrushed scales and crushed scales. The crushed fraction was processed using a hammer mill (model IMBRMAQ/FM/01/007) at the National Institute for Amazonian Research (INPA). All analyses performed on these samples, including proximal composition and collagen yields, were conducted in triplicate to ensure the reproducibility and reliability of the data. Samples were used based on dry weight.

2.3. Physicochemical analyzes

The analyses of the proximal composition of the raw materials from the uncrushed and crushed scales of each species were conducted in triplicate, following the Analytical Standards of the Adolfo Lutz Institute (São Paulo, 2008) as recommended by the AOAC (2019). Moisture content was determined using the oven-drying method (105 °C) with air circulation until a constant weight was achieved. For ash determination, the material was carbonized in a Compact industrial stove (Metal Four®, São Paulo, Brazil) using a Bunsen burner and subsequently incinerated in a muffle furnace at 550 °C until it achieved a light gray or white color. Total lipid content was measured using the Soxhlet method with a Soxhlet extractor (Tecnal®, São Paulo, Brazil) and petroleum ether as the solvent. Total nitrogen content was determined using the Kjeldahl method, which was performed with a Kjeldahl distillation apparatus (Tecnal®, São Paulo, Brazil) and a digestion system (Tecnal®, São Paulo, Brazil), applying a conversion factor of 6.25 to calculate the protein content.

2.4. Collagen extraction

Samples with an average weight of 100 g of scales were immersed, in triplicate, in a solution of 0.05 M sodium hydroxide (1:20 w/v) for 12 h, with solution changes every 6 h, following the protocols of Zhang et al. (2011) and Carpio et al. (2023). The scales were then demineralized by placing them in a 10 % sodium chloride solution (1:10 m/v) for 24 h, washed with distilled water, immersed in hydrochloric acid (0.4 mol/L; 1:10 m/v) for 90 min, neutralized, and dried in an oven (40 °C) for 24 h. Degreasing was performed by immersing the scales in a 10 % butyl alcohol solution (1:20 v/v) for 36 h, followed by washing and drying the material in an oven (70 °C) for 8 h.

To extract the collagen from the scales, a 0.5 M acetic acid solution (1:40 w/v) was used with constant homogenization in an ultra-thermostatic bath (QUIMIS®; model Q214M2, São Paulo, Brazil) with water circulation, coupled to a mechanical stirrer, for 72 h at 20 °C. Buffering was performed using sodium carbonate buffer and 0.9 M sodium bicarbonate until obtaining a pH of 8.87 was achieved. Centrifugation was conducted at 11,000 rpm for 45 min at 4 °C. The processed material was subjected to three dialysis cycles: the first in a 0.1 M acetic acid solution and the last two in distilled water for 36 h. Finally, the dialyzed materials were frozen and lyophilized, obtaining ASC.

2.5. Collagen yield

To determine the yield of collagen extractions, the following equation was used (Alves et al., 2018):

$$\text{Performance (\%)} = \frac{M}{Mo} \times 100$$

Where: M dry weight (g) of lyophilized collagen and Mo = weight of the by-product sample used (g).

2.6. Spectroscopy in the fourier transform infrared region (FTIR)

For the structural characterizations of collagens, 16 scanning spectra (waves: 4000 to 500 cm⁻¹; resolution: 2 cm⁻¹) were collected at room temperature, using a FTIR (Shimadzu®, model IRTracer-100, Tokyo, Japan). The analysis was carried out using the Attenuated Diffuse Reflectance (ATR) method with zinc selenide (ZnSe) crystal, with subsequent comparison between the collagens obtained from tambaqui and pirarucu and the standard commercial bovine type I collagen.

2.7. X-ray diffraction (XRD)

X-ray diffraction analysis was performed to determine the structural characteristics of collagen samples. A Shimadzu® XRD-700 equipment (Tokyo, Japan) was used under the following conditions: CuK α radiation (wavelength 1.5406 Å), a generator voltage of 40 kV, and a current of 30 mA. The diffraction data were collected over a 2θ range of 5° to 60° with a step size of 0.02° and a scanning speed of 1° per minute. The crystallographic parameters, including d-spacing values, were calculated using Bragg's equation. The resulting diffraction patterns were analyzed to identify peaks characteristic of collagen's ordered triple-helical structure and mineralized hydroxyapatite, as referenced in standard databases (ICDD card no 9-432). The samples were analyzed at room temperature, and diffraction peaks were compared with commercial bovine type I collagen for validation. The findings were used to confirm the structural integrity and mineral composition of the collagen extracted from tambaqui and pirarucu scales.

2.8. Statistical analysis

The data regarding the parametric assumptions of normality and homogeneity of variance were analyzed using the Shapiro-Wilk and Levene tests, respectively. To verify the difference in determining the proximal composition and collagen yield of uncrushed scales and crushed scales of each species analyzed, a Student's t-test was performed. All statistical analyses were conducted in the R Statistical Software (R Core Team, 2021), considering $\alpha = 0.05$ statistically significant.

3. Results and discussion

3.1. Proximal composition

Table 1 presents the average results for the proximal composition of the uncrushed and crushed scales of the Amazonian fish tambaqui and pirarucu. Vidal et al. (2011) reported an average moisture percentage of 1.38 % in the protein concentrate of Nile tilapia by-products, which is

Table 1
Proximal composition of uncrushed and crushed tambaqui *Colossoma macropomum* and pirarucu *Arapaima gigas* scales.

	Sample	Proximal Composition (%) *			
		Moisture	Ash	Fat	Crude Protein
Tambaqui	Uncrushed scale	47.71 ± 0.80	9.77 ± 0.45	1.39 ± 0.28	41.04 ± 1.22
	Crushed Scale	12.63 ± 0.25	11.80 ± 0.11	1.33 ± 0.01	72.95 ± 0.06
	Uncrushed scale	28.54 ± 0.94	28.80 ± 0.80	1.06 ± 0.06	31.63 ± 2.10
	Crushed Scale	10.85 ± 0.24	17.18 ± 0.73	0.20 ± 0.04	48.40 ± 0.81

* Number of repetitions -3.

lower than the values observed in the current study (Table 1). The pirarucu scales showed total lipid content ranging from $1.39 \pm 0.28\%$ in uncrushed samples to $1.33 \pm 0.01\%$ in crushed samples, with a significant difference ($p < 0.001$). However, according to the literature, pirarucu scales are rich in calcium and phosphorus, and the inverse relationship between calcium and phosphorus and lipid content may explain the lower lipid values observed (Torres et al., 2008, 2012).

According to Contreras-Guzmán (1994), the determination of ash indicates the richness of the sample in mineral elements. The ash values in pirarucu scales varied between $28.54 \pm 0.94\%$ and $17.18 \pm 0.73\%$ for uncrushed and crushed scales, respectively. The high mineral content can be attributed to the reduction of water content, which correlates with the high concentrations of calcium and phosphorus. The average ash value in crushed samples is below the range of 39 % to 45 % reported by Torres et al. (2008) in their study of a laminated nanocomposite structure in scales of the Amazonian fish (*Arapaima gigas*).

The protein content in pirarucu scales was $31.63 \pm 2.10\%$ for uncrushed samples and $48.40 \pm 0.8\%$ for crushed samples. A study by Muyonga et al. (2004) reported protein levels ranging from 20.3 % to 21.3 % in skins with scales of young and adult Nile Perch (*Lates niloticus*), which is lower than the values observed in the present study. However, most studies indicate that these variations are influenced by factors such as the age and growth of scale structures (Silva & Stewart, 2006; Chen et al., 2022b; Kroboth et al., 2024).

Variations observed in the proximal composition of tambaqui and pirarucu scales, particularly in moisture, lipid, and ash contents, can be attributed to differences in species-specific physiology, age, and environmental conditions, as highlighted by Torres et al. (2008) and Vidal et al. (2011). The higher ash content in pirarucu scales may be related to the mineralized structure of the scales, which are rich in calcium and phosphorus. In contrast, the lower lipid content aligns with the inverse relationship between lipids and mineral composition reported in the literature (Torres et al., 2012).

3.2. Collagen yield

Table 2 represents the average yield results (%) of acid-soluble collagen (CAS) from uncrushed and crushed scales of tambaqui and pirarucu. The average yield of collagen extraction from tambaqui scales ranged from $2.97 \pm 0.21\%$ to $1.48 \pm 0.04\%$ between crushed samples and non-crushed samples, presenting a significant difference ($p < 0.01$), the by-product used in the extraction of tambaqui scales is found. Adhering to a thin superficial layer of the fish skin influences the higher collagen content obtained.

The collagen yield ($p < 0.03$) obtained from pirarucu scales ranged from 0.85 % to 1.51 % for crushed and uncrushed scales, respectively, with the highest yield observed in uncrushed samples (Table 2). Pirarucu scales have a mineralized coating (a fraction with greater pigmentation) that contains a significant amount of calcium hydroxyapatite - Hap (Torres et al., 2008). The presence of Hap, when mixed with the more malleable parts containing collagen fibers, may lead to a reduction in collagen yield during the extraction process (Torres et al., 2012; Amorim et al., 2020; Mouss et al., 2022).

According to Thuy et al. (2014) and Heidari & Rezaei (2022), fish collagen yields can vary widely, from 0.43 % to 92 %, depending on factors such as species, collagen fiber cross-links, age of the animal, raw

material used, temperature, time, pH, habitat, and extraction method. Notably, younger animals tend to have a higher collagen content. Duan et al. (2009) evaluated the properties of collagen from Carp (*Cyprinus carpio*) scales and reported a yield of 1.35 %, which is lower than those observed in this study for tambaqui in both treatments. Similarly, Liu et al. (2012) and Pal & Suresh (2017) reported yields of 2.7 % using enzymatic (pepsin) and acid methods on the scales of Bighead Carp (*Hypophthalmichthys nobilis*) and Rohu Carp (*Labeo rohita*).

Other studies have reported consistent collagen yields from fish scales: Carp (*Cyprinus carpio*), 1.35 % and 1.06 % (Duan et al., 2009); Nile tilapia (*Oreochromis niloticus*), 3.2 % (Chen et al., 2016); Red snapper (*Pagrus major*), and Nile tilapia (*Oreochromis niloticus*), 2 % (Ikoma et al., 2003); Seabass (*Lates calcarifer*), 0.38 % and 1.06 % (Chuaychan et al., 2015); Silver carp (*Hypophthalmichthys molitrix*), 1.45 % (Zhang et al., 2010); Brama australis (*Southern ray bream*), 1.5 % (Sionkowska et al., 2015).

The higher collagen yield observed in uncrushed pirarucu scales (1.51 %) compared to crushed scales (0.85 %) may be attributed to the larger fragment size in uncrushed samples. In contrast, the lower yield in crushed samples could be influenced by high temperatures generated during the grinding process, which may lead to early denaturation and loss of collagen molecules (Ahmad & Benjakul, 2010; Heidari & Rezaei, 2022). High temperatures can cause the destruction of hydrogen bonds, resulting in the denaturation of collagen chains and the loss of the triple helix conformation (Pati et al., 2010).

According to Wang and Feng (2005); Jeevithan et al. (2014), and Veeruraj et al. (2013), partial or complete separation of collagen occurs when hydrogen bonds are broken due to increased temperatures, which disrupt adjacent polypeptide chains. Furthermore, Balian & Bowes (1977) stated that collagen in scales is not entirely solubilized in 0.5 M acetic acid or other acidic solutions. This is likely due to the presence of covalent cross-links in the collagen molecules, particularly in the telopeptide regions, where aldehyde groups condense, reducing the solubility of collagen protein in acidic environments (Li et al., 2018; Zhang et al., 2007).

Additionally, these results reinforce the advantages of the ASC extraction method compared to other methods reported in the literature, such as enzymatic and thermal extraction. For instance, the collagen yield extracted from tambaqui and pirarucu scales was higher than that obtained from similar species using alternative methods, as described by Duan et al. (2009) and Liu et al. (2012). Furthermore, the FTIR and XRD analyses confirmed the preservation of the structural properties of type I collagen, an aspect that can be compromised in methods employing high temperatures or more aggressive chemical agents (Ahmad & Benjakul, 2010; Veeruraj et al., 2013). These results demonstrate not only the efficiency but also the sustainability of the ASC method, which generates a lower amount of chemical waste and promotes the valorization of fish by-products, contributing to the reduction of the environmental impact associated with fish processing.

Moreover, the feasibility and cost-effectiveness of the ASC method are highlighted by its operational simplicity and reduced reliance on aggressive chemical reagents, lowering waste disposal costs and environmental impacts. Furthermore, the use of fish by-products, often discarded as low-value waste, adds economic value by transforming these materials into high-demand products in the biomedical and biotechnological markets. This approach aligns with global trends in sustainable processing, promoting a circular economy and reducing waste in the production chain.

3.3. Fourier transform infrared spectroscopy (FTIR)

The absorption peaks were located in the characteristic amide regions, corresponding to Amide A, Amide B, and Amides I, II, and III, respectively. According to Heidari & Rezaei (2022), the stretching vibration of N—H bonds generally occurs within the wavenumber range of $3400\text{--}3440\text{ cm}^{-1}$ in the amide A bands. However, lower frequencies due

Table 2
Yield of acid-soluble collagen extracted from uncrushed and crushed scales of tambaqui *Colossoma macropomum* and pirarucu *Arapaima gigas*.

Collagen yield (%) *			
	Uncrushed scale	Crushed Scale	p-value
Tambaqui	2.97 ± 0.21	1.48 ± 0.04	$p < 0.01$
Pirarucu	1.51 ± 0.34	0.85 ± 0.14	$p < 0.03$

* Number of repetitions: 3.

to the formation of hydrogen bonds can alter the wavenumber (Duan et al., 2009; Bhuimbar et al., 2019).

In the present study, for tambaqui, the band for Amide A was observed at 3285 cm^{-1} (Fig. 2), likely resulting from the stretching of the N—H group in peptides involved in hydrogen bonding or thin stretching bands (Pati et al., 2010; Veeruraj et al., 2013). This result is consistent with values reported for other fish species, *Lates calcarifer* (3292 cm^{-1}) (Sinthusamran et al., 2013), *Hypophthalmichthys nobilis* (3330 cm^{-1}) (Noohi et al., 2023) and *Oreochromis niloticus* (3340 cm^{-1}) (Li et al., 2018).

The Amide B band, related to CH_2 asymmetric and CH_3 symmetric stretching, was identified at 2917 cm^{-1} . This band exhibited an intense vibration, possibly due to the presence of H_2O trapped within the porous structure of the scale (Abe & Krimm, 1972; Muyonga et al., 2004; Yu et al., 2014). Nevertheless, the result is within the range reported for *Lates niloticus* (2924 cm^{-1}) (Muyonga et al., 2004), *Hypophthalmichthys nobilis* (2930 cm^{-1}) (Noohi et al., 2023), and *Hypophthalmichthys molitrix* (2931 cm^{-1}) (Faralizadeh et al., 2021).

In the literature, Amide I bands, typically located in the range of $1750\text{--}1600\text{ cm}^{-1}$, are primarily associated with the stretching vibrations of the carbonyl group ($\text{C} = \text{O}$) along the polypeptide backbone, indicating the presence of secondary structures or hydrogen bonding coupled with COO groups (Payne & Veis, 1988). In this study, Amide I showed maximum absorption at 1630 cm^{-1} , which is within the range reported for *Oncorhynchus mykiss* (1635 cm^{-1}) (Heidari & Rezaei, 2022) and *Cyprinus carpio* (1596 cm^{-1}) (Bhagwat & Dandge, 2016).

The Amide II band exhibited maximum absorption at 1540 cm^{-1} , attributed to in-plane bending and deformation of N—H groups coupled with C—N stretching vibrations (Payne & Veis, 1988; Zanaboni et al., 2000). Amides III, located in the ranges of 1023 cm^{-1} and 658 cm^{-1} , describe N—H bending vibrations coupled with C—N stretching and C—H vibration, exhibiting the triple-helical structure of collagen. Jackson & Mantsch (1995) highlighted that Amide III is a complex region with stretching vibrations and C—H and N—H bonds in flat bending configurations. Collagen in this region is influenced by intermolecular interactions and absorptions resulting from CH_2 group movements along the glycine backbone and proline side chains. At 658 cm^{-1} , the region is associated with $\text{P} = \text{O}$ group stretching. The spectral bands for tambaqui and pirarucu collagen (Fig. 2 and 3) align with those found in standard commercial bovine type I collagen, indicating the preservation of structural properties.

These results of FTIR analysis highlighted the preservation of the molecular structure characteristic of type I collagen extracted from tambaqui and pirarucu scales. This structural integrity, confirmed by the characteristic bands of Amide A, B, I, II, and III, is a distinguishing

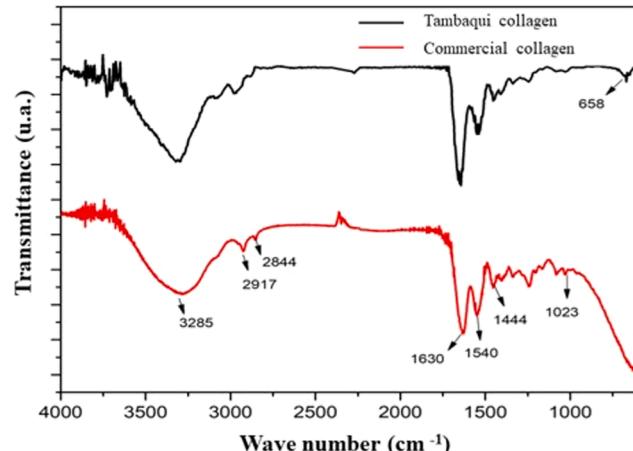


Fig. 2. FTIR spectra were observed for collagen from tambaqui scales and type I collagen, a commercial standard.

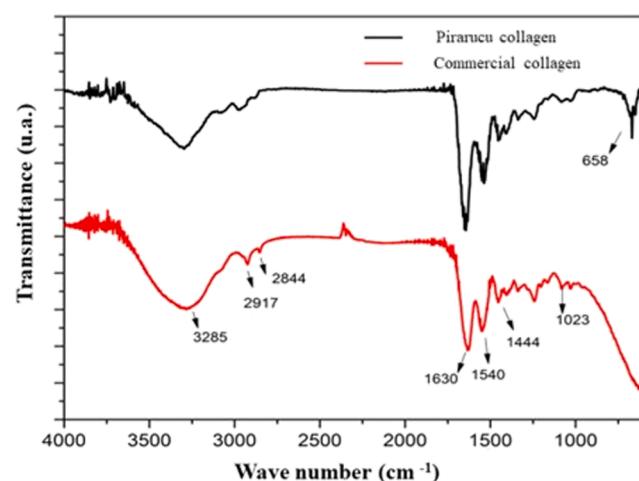


Fig. 3. FTIR spectra observed for collagen from pirarucu scales and type I collagen, commercial standard.

feature of the ASC method compared to techniques that employ high temperatures or aggressive chemical agents, which are often associated with collagen fiber degradation (Veeruraj et al., 2013; Ahmad & Benjakul, 2010). These findings reinforce the applicability of the ASC method not only for obtaining high-purity collagen but also for its potential application in biomedical and biotechnological products, standing out as an efficient and sustainable alternative compared to conventional methods.

3.4. X-ray diffraction (XRD)

Collagen proteins possess a 3D structure with voids capable of facilitating the growth of hydroxyapatite (HA) nanoparticles (Taton, 2001), a phenomenon referred to as collagen mineralization. This HA-collagen composite material was confirmed in the present study through XRD and FTIR analyses. The collagen sample obtained from pirarucu scales (Fig. 4) exhibited diffraction peaks characteristic of the hydroxyapatite structure (ICDD card no 9-432). These peaks, observed at $2\theta = 25.9^\circ, 32.2^\circ, 39.8^\circ, 46.7^\circ, 49.5^\circ$, and 53.2° , correspond to the crystallographic planes (002), (211), (310), (222), (213), and (321), respectively (Mohammad et al., 2013).

These results confirm the presence of hydroxyapatite and collagen,

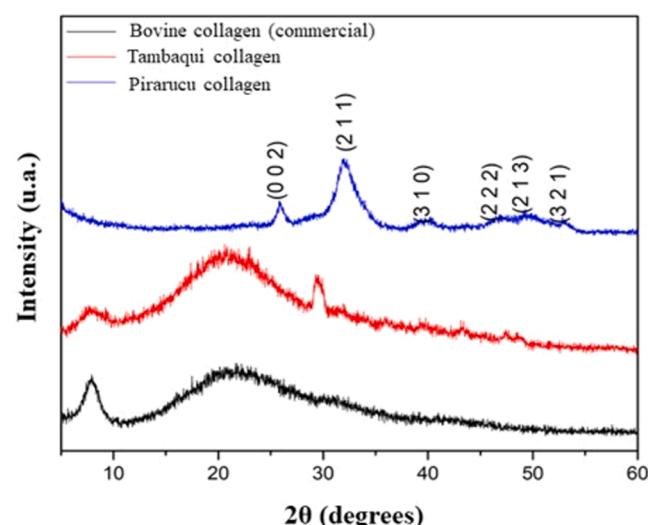


Fig. 4. X-ray diffraction (XRD) for collagen from tambaqui, pirarucu and commercial scales.

as also indicated by FTIR analysis. The diffractograms for bovine collagen (commercial) and collagen obtained from tambaqui scales were similar (Fig. 4), validating that the extracted collagen shares characteristics identical to commercial collagen. The diffraction peaks demonstrate that this protein exhibits an ordered structure (Cameron, 2007). To confirm these results, two diffractograms were analyzed using Bragg's equation: $d(\text{\AA}) = \lambda/2\sin\theta$ (where λ is the wavelength of the X-rays (1.54 Å), and θ is the Bragg diffraction angle to calculate the minimum value of the repeat spacings (Vinila et al., 2022).

The low-angle peaks of CB and CT were found at $2\theta = 7.89^\circ$ and 7.93° , respectively, corresponding to the distances between collagen molecular chains. The d-spacing values for these peaks were calculated as 11.07 Å and 11.34 Å, respectively. Secondary diffraction peaks were observed at 20.98° and 20.03° , indicative of diffuse scattering caused by the structural layers of collagen fibrils. The ddd-spacing values for these peaks were 4.34 Å and 4.31 Å, respectively.

A third diffraction peak, corresponding to the unit height typical of the triple-helical structure (0.29 nm), was also observed (Giraud-Guille, 2000). This peak represents the triple-helical collagen molecule and the single-helical chain. These findings confirm that the collagen sponges maintain their tri-helical and crystalline structures (Muthukumar et al., 2014).

The X-ray diffraction analysis corroborated the FTIR results by confirming the preservation of the ordered three-dimensional structure of type I collagen, with characteristic peaks associated with the triple helix and mineralized collagen. These results indicate that the ASC method preserves the structural characteristics of collagen, which is essential for its industrial and biomedical applications. Furthermore, unlike methods that employ high temperatures, which can compromise the molecular organization of collagen (Wang & Feng, 2005; Veeruraj et al., 2013), the ASC method demonstrated efficiency in extraction without significant impacts on protein structure. This reinforces the importance of the ASC method as a viable, sustainable approach with lower environmental impact for the valorization of fish by-products.

Additionally, the XRD patterns observed in the collagen extracted from tambaqui and pirarucu scales demonstrated characteristics consistent with type I collagen, as validated by commercial bovine collagen standards and reported by Giraud-Guille (2000). However, minor differences in peak intensities and spacing compared to previous studies, such as Torres et al. (2012), may result from species-specific structural properties and the presence of hydroxyapatite in pirarucu scales, which affects the overall crystalline arrangement.

4. Conclusion

This study successfully demonstrated the extraction and characterization of acid-soluble collagen (ASC) from the scales of tambaqui (*Colossoma macropomum*) and pirarucu (*Arapaima gigas*), two fish species of significant commercial importance in the Amazon region. The results highlighted that the form of scale processing influenced both the proximal composition and collagen yield, with uncrushed scales yielding higher amounts of collagen compared to crushed scales. This finding underscores the importance of scale structure preservation during processing to optimize collagen extraction. The collagen extracted was identified as type I collagen, confirmed through FTIR and XRD analyses, with properties comparable to commercial bovine type I collagen. These results not only validate the quality of the collagen obtained but also demonstrate its potential for industrial and biomedical applications due to its structural integrity and high purity.

CRediT authorship contribution statement

Alexandre Augusto Barai: Methodology, Investigation, Formal analysis, Conceptualization. **Antônio José Inhamuns:** Writing – original draft, Methodology, Investigation. **Tiago Cabral Nóbrega:** Methodology, Investigation, Formal analysis. **Cristiane Cunha Guimarães:**

Methodology, Investigation, Formal analysis, Conceptualization. **Lígia dos Santos Mourão:** Methodology, Investigation. **Antonio Fabio Lopes de Souza:** Methodology, Investigation. **Fagnaldo Braga Pontes:** Methodology, Investigation. **Flávia Dayane Félix Farias:** Writing – original draft, Methodology, Investigation. **Joana Maia Mendes:** Methodology, Investigation. **João Paulo Ferreira Rufino:** Methodology, Formal analysis. **Adriano Teixeira de Oliveira:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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