Tambaqui (*Colossoma Macropomum*) Leather Tanning: A Study on the Influence of Skin Morphology on the Physical-Mechanical Properties of Leather

by

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Abstract

The fish production chain presents losses of up to 35%, generating a large amount of waste. Using these residues to produce value-added materials boost fish production towards a sustainable path. The use of fish skin to obtain leather is increasingly attracting the market for fashion and luxury products. Besides considered an exotic material with a unique design, fish leather also has good mechanical properties. Due to the morphology of fish skin, different mechanical responses can be observed, depending on the leather cutting direction. However, there are no specific technical standards for sampling this material. In this scenario, 45 Tambaqui skins were tanned with chromium associated with oxazolidine, and the mechanical properties of the leather were evaluated in four cutting directions: parallel, perpendicular, dorsal-ventral and ventral-dorsal, all concerning the cephalocaudal line of the fish. Skin and leather micrographs revealed layers of parallel collagen fiber bundles distributed in superimposed layers oriented obliquely to the preceding one. Insertions of collagen fibers perpendicular to the leather surface were also observed, joining the deeper layers to the superficial layers. Tensile strength (TS) and elongation (E) results indicated statistical differences, with higher TS results for the perpendicular cut and greater elongation for the parallel cut. The tear strength was higher for the ventral-dorsal cut, statistically differing from the parallel cut. The data indicate that the cutting direction of the specimen can influence the physical-mechanical behavior of the leather, reinforcing the importance of standardization for the qualitative evaluation of fish leather.

Introduction

Fish farming is one of the main branches of aquaculture and has been intensified worldwide. In Brazil, production surpassed the mark of 860 thousand tons in 2022, with Tilapia (*Oreochromis niloticus*) as the main cultivated species.[1] Among the native fish, Tambaqui (*Colossoma macropomum*) stands out. Native to the Amazon and Orinoco river basins, the Tambaqui is considered a "round" fish with small scales and protective lamellae, featuring a typical design.[2,3] According to the Food and Agriculture Organization of the United Nations (FAO), the amount of loss in the fish production chain reaches 35%, double the losses in the processing of meat products[4]. The use of these residues in value-added products contributes to boosting the fish chain towards a sustainable path. Among fish waste, the skin is of great interest for reuse in the form of leather or as a source of collagen and its peptides. Each skin has a specific natural pattern, giving the leather a unique character that appeals to the fashion and luxury goods industries.[5] FAO considers the use of fish skins in fashion design articles one of the actions that contribute to the Blue Growth Initiative[4], fulfilling the agenda of the Sustainable Development Goals (SDGs) elaborated by the UN. The initiative brings together strategies and actions to improve the use of water resources, seeking economic, environmental, and social benefits.

In addition to the exclusive and differentiated appearance, fish leather has good resistance due to the skin morphology and the organization of collagen fibers.[6,7] The dermis is made up of layers of overlapping collagen fibers bundles. These layers are composed of continuous and parallel fibers superimposed at different angles along the body, varying from 50 to 70° concerning the preceding layer.[8] Such an arrangement gives the skin/leather of fish a highly organized structure that influences its qualitative properties.

The growing interest in fish leather promotes the need for sampling standardization methods to evaluate intrinsic quality. So far, there are no specific standards for sampling fish leather. However, due to the morphology and organization of collagen fiber bundles in the skin, the specimens' cutting direction can influence the leather's physical-mechanical behavior. In this sense, the present work aims to investigate the structure of the Tambaqui skin and the influence of the cutting direction on the mechanical properties of the leather.

Material and Methods

Material

Tambaqui (*Colossoma macropomum*, n=45), a species registered in the National System for the Management of Genetic Heritage

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and Associated Traditional Knowledge under N° A3B1887, were purchased from the Mar & Rio Pescados (São José do Rio Preto, SP). Busan® 7600 bactericide (Buckman, USA) was used for skin preservation. Other reagents used in the tanning process were of commercial analytical grade.

Methods

Fish skin composition

Skin samples (200 g) were analyzed for their centesimal composition. Crude protein contents were determined by method 45 (Protein - Dumas Method), and ash contents by method 5 (Ashes or Mineral Matter)[9]. Lipids were determined following standard methodology.[10]

Histological analysis of Tambaqui skin

Skin fragments were fixed in 10% neutral buffered formalin for 24 hours. The fixed samples were cut to a thickness of 6 μm, stained using Hematoxylin/Eosin and Masson's Trichrome techniques, and mounted on glass slides with Entellan®[11]. The slides of histological sections were observed and photographed under a Leica DM 2500P Optical Microscope with Polarized Light, positioning the rotating circular stage of the microscope at 45°.

Tanning process

The fish were stunned by thermal shock in an ice bath. The cut lines were made in the fish's gill, dorsal, ventral and caudal regions. Subsequently, with the aid of pliers, the skins were removed, fleshed, and preserved in salt and bactericide (NaCl and Buzan® 7600) until processing.

In the tanning process, the steps of soaking, liming (Table I), deliming, bating, pickling, tanning (Table II), retanning I, basification, retanning II, and fat liquoring (Table III) were carried out. Factors such as pH, water temperature, drum rotation speed, and time were monitored throughout the process and are shown in Tables (I-III).

Table I
Pre-tanning process conditions of Tambaqui skin.

Process	Water (%) $m.m^{-1}$	Reagents	Reagent (%) $m.m^{-1}$ *	°C	Run time (min)	pH	Remarks
Soaking	200	Water		25			
		Bactericide	0.1		1440		Drain
	200	Water		25	10		Drain
	100	Water		25	10		Drain
	100	Water		25			
		surfactant**	0.3		10		Drain
	100	Water		25			
		surfactant**	0.3		10		Drain
	200	Water		25			
		Sodium carbonate	0.2				
		surfactant**	0.3		240		Run 10'/h Drain
Liming	100	Water		25	10		pH = 8.5 - 9.5 < 2.0° Be*** Drain
	100	Water		25			
		Sodium sulfide	1.5				
		surfactant**	0.4				
		Lime powder ($Ca(OH)_2$)	2.5		240		
	100	Water		25	840		Run 10'/h (14 h) Drain
	200	Water		25	10		Drain

*The percentage of reagent corresponds to the initial mass of the skins.
**anionic + non-anionic surfactants, free of nonylphenol ethoxylated
***Baume degrees.

Table II

Tanning process conditions of Tambaqui skin.

Process	Water(%) m.m-1	Reagents	Reagent (%) m.m-1*	°C	Run time	pH	Remarks
Deliming	200	Water		25			
		Ammonium chloride	0.5		10		Drain
	200	Water		25			
		Ammonium chloride	2.0				
		Deliming agent**	1.0				
		surfactant	0.6		20	8.2	Phenolphthalein; pH < 8.2
Bating	100	Water		37	5		Drain
	200	Water		37	5		Drain
	200	Water		30-34			
		Pancreatic enzyme	0.05				
		surfactant	0.6		60		Fingerprint test
	100	Water		25			
		Formic acid	0.1		60	6.8	Drain
	100	Water		25			
		Ammonium chloride	1.0				
		surfactant	1.0		60	7.3	Drain
Pickling	100	Water		25			
		Sodium chloride	10.0		10		
		Formic acid	2.0		10	3.2	
					840		Run 10'/h (14 h)
Tanning		Water		25			Pickling water
		Chromium powder (33% basic)	10.0		60	2.8	
		Oxazolidine	3.0				
		Acrylic retanning agent	5.0				
		Sulphited fish oil	0.5				
		Fatty alcohol-based	0.5		40		
		Glycol esters based	0.2		90		
		Sodium formate	1.0		20	3.2	
		Sodium bicarbonate	0.5		30	3.3	
		Sodium formate	1.0		20	3.5	
		Sodium bicarbonate	1.5		20	4.1	
					840		Drain and horse up overnight

*The percentage of reagent corresponds to the initial mass of the skins.

**Combination of organic acids and inorganic salts.

Table III

Retanning and finishing process conditions of Tambaqui leather.

Process	Water (%) m.m^{-1}	Reagents	Reagent % m.m^{-1} *	°C	Run time	pH	Remarks
Retanning I/ Pre lubrification	200	Water		35			
		Formic acid	0.2				
		Oxalic acid	0.2				
		surfactant	1.0		20	3.4	Drain
	100	Water		35			
		Chromium powder (33% basic)	3.0				
		Sulphited fish oil	1.0		30		
		Polymer retanning agent	3.0		30		
		Acrylic retanning	6.0		30		
Basification		Sodium formate	2.0		20	4.5	
		Sodium bicarbonate	1.5	40	90	5.9	Drain
	200	Water		25	10		Drain
Retanning II/ Dyeing	60	Water		25			
		Mimosa powder	4.0				
		Phenolic retanning agent	3.0				
		Oxazolidine	0.5				
		Neutralizing agent**	3.0				
		Blue aniline	3.0				
		Turquoise aniline	1.0		45		
		acrylic retanning	5.0		25		Cutting Check
	150	Water		50			
		Formic acid	2.5		30	4.3	Drain
	200	Water		55			
		Formic acid	0.2		10		Drain
Fat liquoring/ Fixation	200	Water		55			
		Synthetic oil-based	5.0				
		Fatty alcohol-based	3.0				
		Sulphited fish oil	2.0				
		Glycol esters based	0.5		45		
		Formic acid	2.9	55	50	3.2	4 × 10' + 20'
		Cationic oil***	1.0		15	3.0	

*The percentage of reagent corresponds to the initial mass of the skins.

**Based on aromatic sulfonic acids and aliphatic dicarboxylic acids.

***Aliphatic Esteramide /Polyglycol Ether

Tambaqui leather scanning electron microscopy (SEM) analysis
For scanning electron microscopy (SEM) evaluation, the leather was cut with a razor blade and fixed in a semicircular stub using carbon tape. Images were obtained using a scanning electron microscope (FEI Quanta 250), operated in low vacuum (LV) mode and with an accelerating voltage of 15 kV.

Tensile and tear strength tests
In this study, the side of the fish was not considered an influencing factor, and random distribution was performed to remove the specimens. One of the sides was used to obtain the samplings for the tensile strength test (3 samples), and the other for the tests referring to the tear strength (3 samples).

The physical-mechanical behavior of the Tambaqui leathers was evaluated according to the technical standards for tensile strength (ISO 3376:2020) and tearing strength (ISO 3377-2:2016). The determination of thickness and conditioning of the samples followed the guidelines described in the technical standards ISO 2589:2016 and ISO 2419:2012, respectively.

The Tambaqui leathers were cut in four different directions: parallel (Figure 1A), perpendicular (Figure 1B), dorsal-ventral (Figure 1C), and ventral-dorsal (Figure 1D), all concerning the cephalocaudal line of the fish.

Statistical analysis
The experiments were carried out following a completely randomized experimental design, in which 45 fish were randomly selected for each test specimen direction. The variance analysis of the tensile and tear strength results was performed using the least squares method with a statistical model that included the fixed effect of the cutting direction (parallel, perpendicular, dorsal-ventral, and ventral-dorsal).

Results and Discusion

Tambaqui skin composition
Tambaqui skin showed 69.7% moisture, 27.1% crude protein, 1.2% lipids, and 0.2% ash (wet basis). Factors such as type of feed, sex, and age at slaughter can influence skin composition, explaining the differences in the literature.[3,12]. Regarding the protein content, this difference may also be related to the nitrogen conversion factor used.

Considering the lipid content, ideally, they should be below 4%.[13] At higher lipid levels, the tanning process becomes more difficult. Therefore, paying attention to the first stages of the process is critical for the success of the procedure.

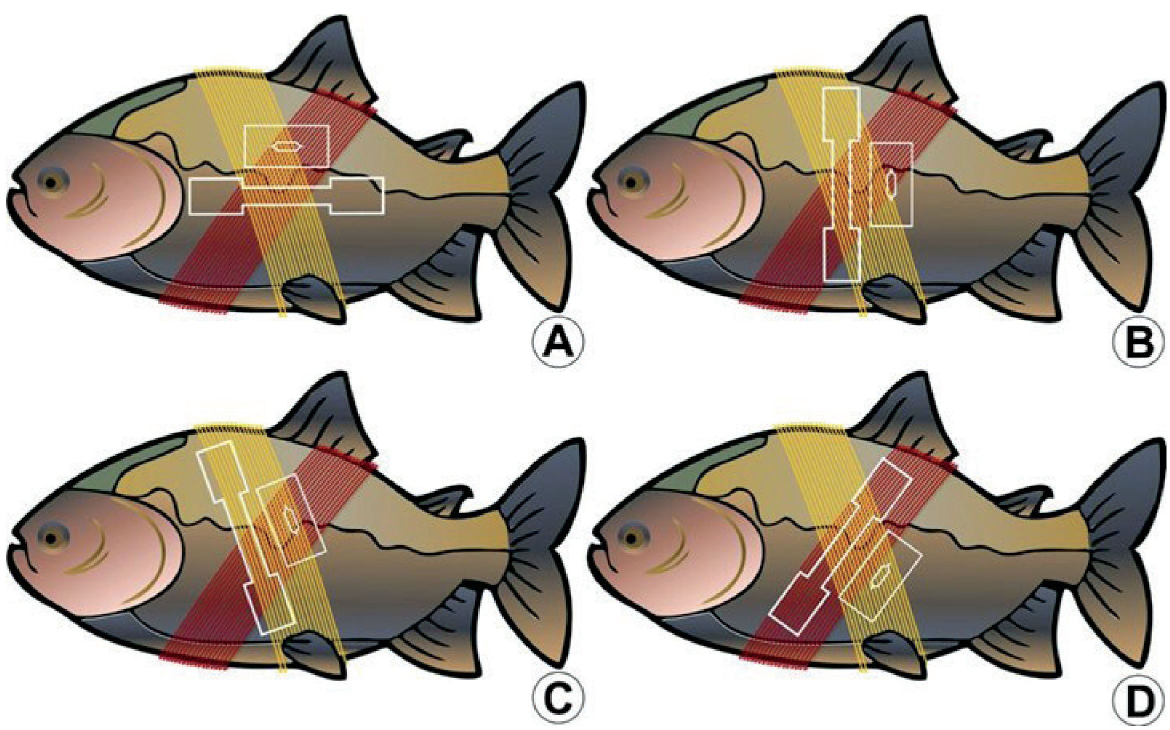

Figure 1. Graphical representation of the Tambaqui leather cuts obtained in the parallel **(A)**, perpendicular **(B)**, dorsal-ventral **(C)**, and ventral-dorsal **(D)** directions concerning the cephalocaudal line.

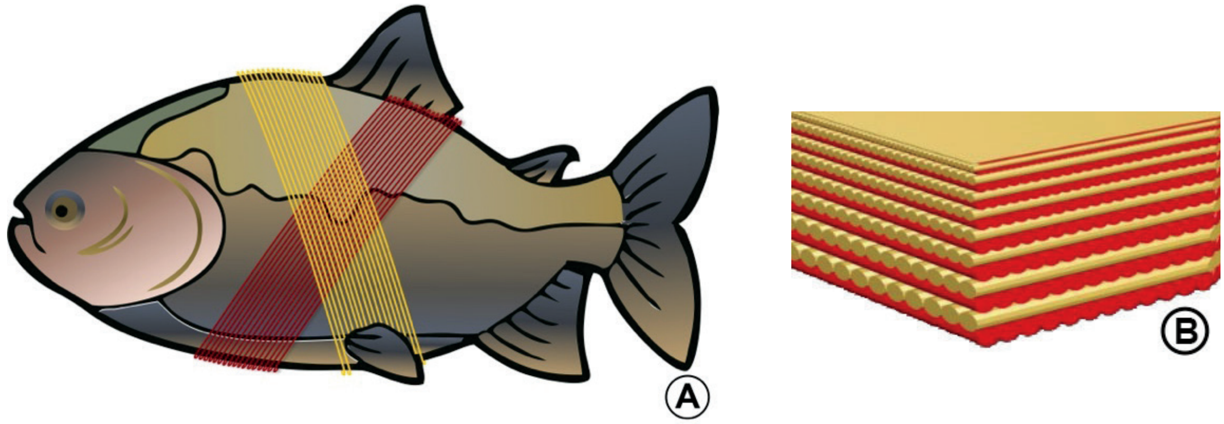

Figure 2. Parallel collagen fibers bundles distributed in a helical fashion around the Tambaqui body **(A)**, arranged in superimposed layers, oriented in an oblique direction to the preceding one **(B).** (Adapted from Nadol et al [15]).

Histological analysis of Tambaqui skin

The dermis of fish has overlapping layers of parallel collagen fibers bundles. These bundles are helically distributed along the body (Figure 2A). Each layer is oriented obliquely to the previous one, also differing in thickness (Figure 2B). The overlap between the layers can present different angulations depending on the body region (head, center, tail).[8,14]

Through the histological analysis of Tambaqui skins, evidenced by optical microscopy of polarized light, it is possible to verify the overlapping layers, with deeper regions presenting thicker layers of collagen fiber bundles (Figure 3A). Furthermore, collagen fibers arranged vertically can be noted, joining the superficial layers to the deeper ones (Figure 3 A and B, arrow 4). They are distributed in intervals and have the function of restricting the sliding movement of the layers.[8] These structures were also identified in the skin of other aquatic species such as eels[16] and frogs[17]. In the study by Allen et al.[18] the structure of *Monacanthus tuckeri* skin, a small fish that inhabits Atlantic corals, is presented in the form of micrographs and schematic drawings, showing the presence of collagen fibers inserted perpendicularly to the layers. Eastman & Hikida[19] describe the stratum compactum of the Ploughfish dermis (*Gymnodraco acuticeps*) as a thick matrix formed by collagen fibers regularly arranged in parallel layers, united by vertical columns of collagen fibers.

Scanning electron microscopy (SEM) analysis of Tambaqui leather

In this study, the organization of collagen fiber bundles in Tambaqui leather is shown in Figure 4A. Like in skin, collagen fiber bundles are organized in layers (Figure 4 A and B, arrow 1) interspersed

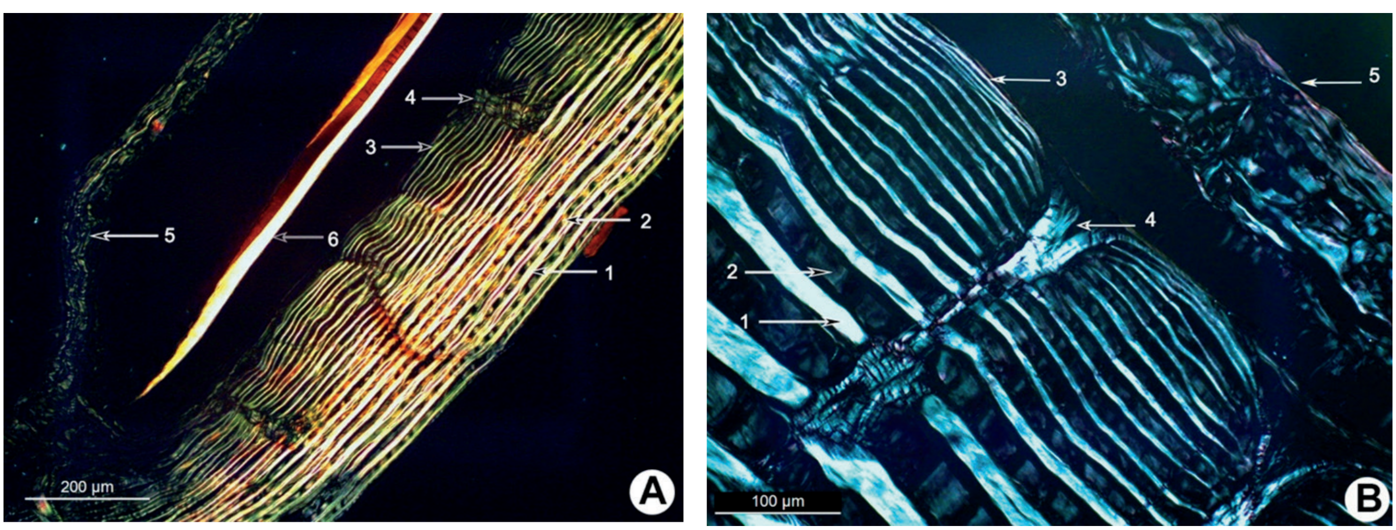

Figure 3. Images of the histological section of Tambaqui skin in the central region (between head and tail, and back and belly), evidenced by optical microscopy of polarized light. Layers with shiny collagen fibers bundles with the same vibration direction **(1)**, alternating with layers of collagen fibers bundles with different vibration directions **(2)**, collagen fibers bundles of the superficial dermis **(3)**, vertically inserted collagen fibers **(4)**, lamellae of the scale **(5)** and scale **(6)**. Staining: Masson's Trichrome (MT) (Figure **3A**) and hematoxylin and eosin (Figure **3B**).

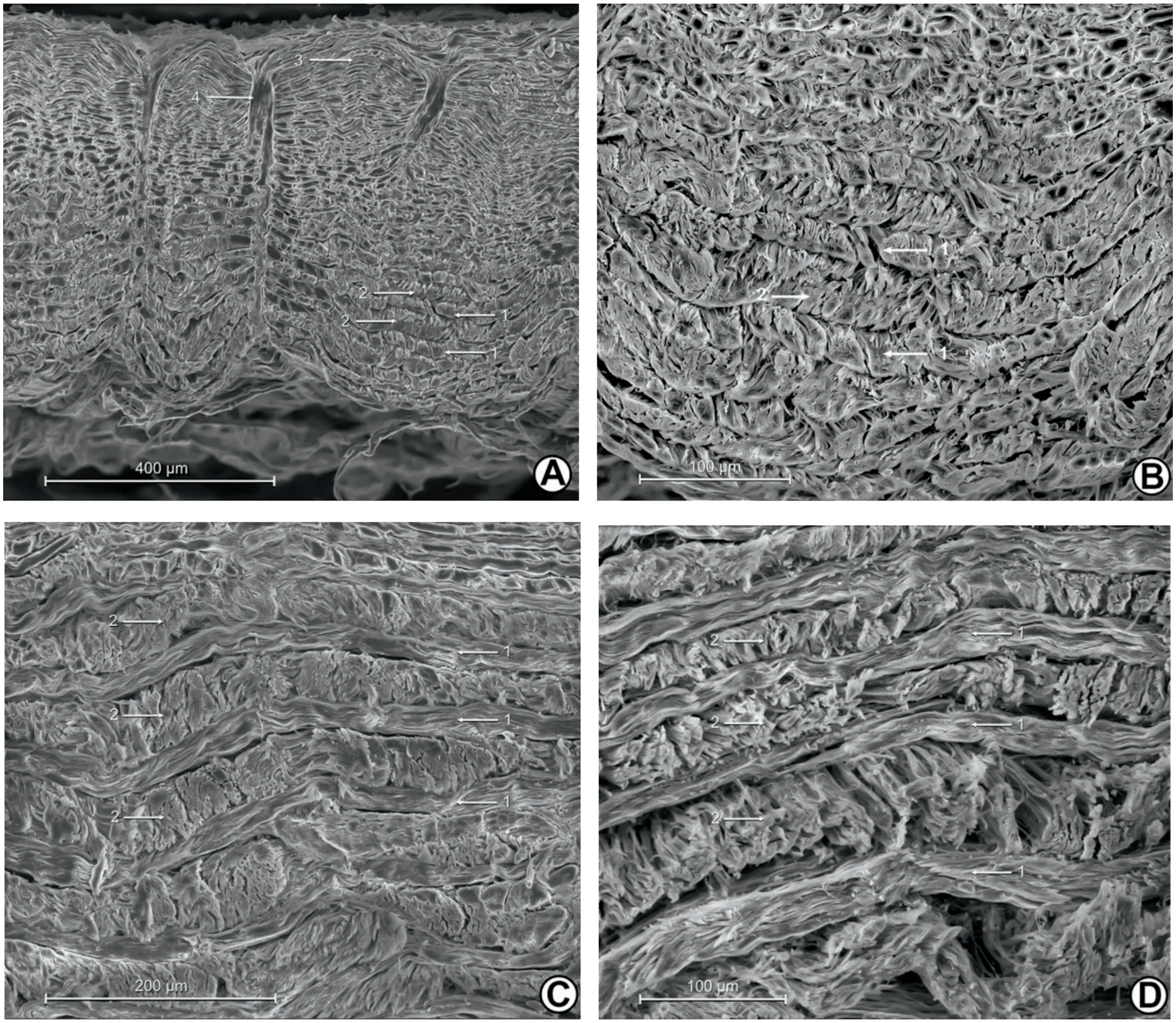

Figure 4. SEM image of the perpendicular section to the Tambaqui leather surface. Parallel cutting direction **(A** and **B)**, dorsal-ventral **(C)**, and ventral-dorsal **(D)** concerning the cephalo-caudal line of the fish. Layers with collagen fibers bundles organized in the same direction (arrow 1), alternating with layers of collagen fibers bundles with an oblique direction (arrow 2), collagen fibers from the superficial dermis (arrow 3), and perpendicular collagen fibers projections to the leather surface (arrow 4). Author: Luciana dos Reis Fernandes.

by collagen fiber bundle arrangements with different orientations (Figure 4 A and B, arrow 2). These layers are present throughout the leather structure, thinner when close to the surface (Figure 4A, arrow 3). Collagen fibers arranged vertically are also presented (Figure 4 A, arrow 4). Other works have also identified perpendicular projections for different species of fish.[8,14,20]. In the case of Tambaqui (Figure 4A, arrow 4), these joints appear throughout the entire structure of the leather, which may favor the material's resistance as they prevent slipping between layers.

When cut in a parallel direction (Figure A and B), the layers are composed of collagen fiber tips, oriented in oblique directions to each other. As for the dorsal-ventral and ventral-dorsal directions, the collagen fibers appear as tops, alternated by layers of more elongated collagen fibers. Collagen fiber bundles aligned and oriented towards the traction force axis can provide better resistance to the leather.[21,22] Observing the dorsal-ventral cut (Figure 4C), fiber tops (arrow 2) appear oriented to the left, while in the ventral-dorsal (Figure 4D), they appear slanted towards to the right (arrow 2). Considering the difference in the collagen fiber arrangements, different mechanical responses can be observed depending on the cutting direction.

Tensile and tear strength tests

In this study, four cutting directions were chosen to evaluate the physical-mechanical properties of Tambaqui leather: parallel (PL), perpendicular (PP), dorsal-ventral (DV), and ventral-dorsal (DV) directions, considering the cephalocaudal line.

The graphs in Figure 5A-C show a significant difference ($p \leq 0.05$) between treatments. As reported in other studies the perpendicular cut direction showed greater tensile strength (Figure 5A).[20,23,24] Cuts in the ventral-dorsal direction come in second with 15.13 N/mm^2. Such results are attributed to the greater alignment of the fibers (Figure 4D), oriented to the tension force axis. The specimens cut in the parallel direction showed the lowest tensile strength (8.38 N/mm^2) but the highest elongation percentage (Figure 5B).

Other authors also observed the effect of collagen fiber arrangements on the mechanical properties of fish skins and leather.[25,26] Kirt & Khora[27] observed differences between the concentration, distribution, and orientation of collagen fibers in the ventral and dorsal regions on the mechanical behavior of puffer skin (*Lagocephalus gloveri*). The results indicate greater tensile strength for the skin in the ventral area, attributed to the higher density and alignment of collagen fibers.

A minor effect of collagen fiber orientation was observed for tear strength (Figure 5C). A statistically significant difference was observed only between the ventral-dorsal and parallel cuts ($p \leq 0.05$). In this case, the test evaluates the material's resistance by applying a force from the high-stress concentration (tear) point. According to Maina et al.[6] the leather resistance to tearing is more related to the softness and plasticity, characteristics acquired during the tanning process. However, the authors also suggest a positive correlation between tensile strength and tear strength, which was not observed in the present experiment.

Our results corroborate previous studies that also identified differences in the mechanical properties of fish skins and leather cuts in different directions.[25,26] This reinforces the need to develop adequate methodologies for sampling and qualitative evaluation of fish leather.

Conclusion

Chrome-tanned Tambaqui leathers were evaluated concerning morphology and mechanical properties. Collagen fibers distributed in layers, observed in scanning electron microscopy (SEM) images, showed the organization of the fish dermis, as reported in the literature. Physical-mechanical analyzes of Tambaqui leathers indicate that different cutting directions of the specimens influence the qualitative properties. Samples cut perpendicular to the cephalocaudal line of the fish showed greater tensile strength. The parallel cuts presented the highest values in assessing the elongation capacity until failure. Regarding tearing strength, cuts in the ventral-dorsal direction showed greater resistance. Thus, the anisotropy of collagen and its influence on the mechanical properties of leather was demonstrated, indicating the need for specific technical standards for the sampling and qualitative evaluation of fish leather.

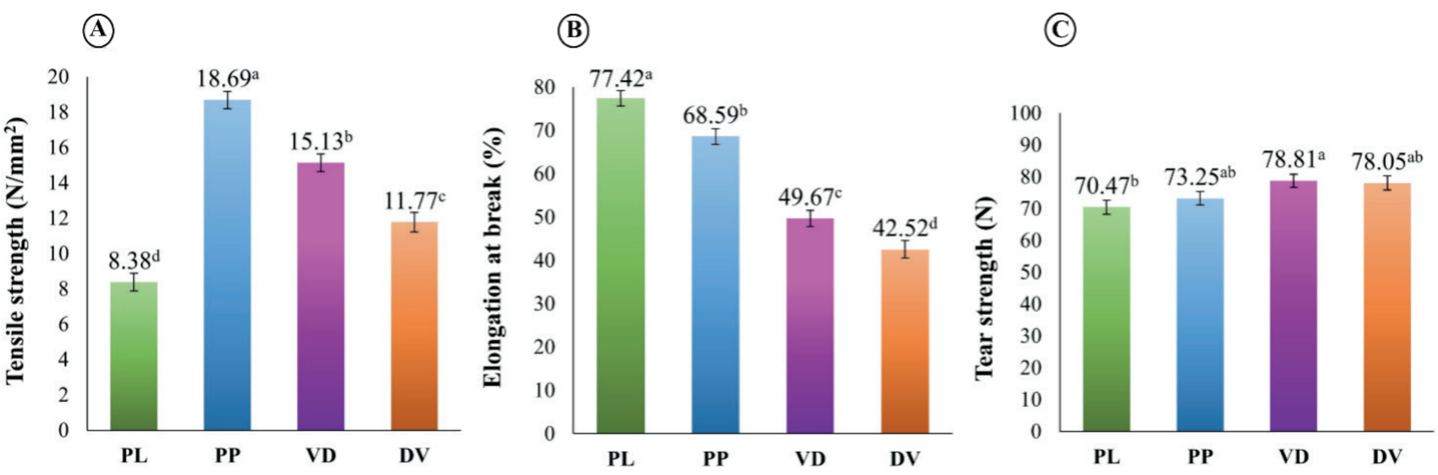

Figure 5. Tensile strength, elongation at break and tear strength of Tambaqui leather cut in the parallel (PL, green), perpendicular (PP, blue), ventral-dorsal (VD, purple), and dorsal-ventral directions (DV, orange). Different lowercase letters indicate statistically significant differences between samples (Tukey, $p<0.05$).

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Statements and Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Credit authorship contribution statement

Jessica Valéria de Campos: Conceptualization, Investigation, Methodology, Data curation; Fernanda Ramalho Procopio: Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing – original draft; Waldomiro Barioni Júnior: Formal analysis; Ana Rita de Araujo Nogueira: Supervision, Validation, Writing – review & editing; Manuel Antonio Chagas Jacintho: Conceptualization, Supervision, Methodology, Investigation, Validation, Writing – review & editing.

References

1. Peixe BR AB da P: Anuário Brasileiro da Piscicultura. 1–65, 2023
2. Gomes LC, Simões LN, Araujo-Lima CARM: Tambaqui (Colossoma macropomum). In: *Espécies nativas para piscicultura no Brasil*, edited by Baldisserotto B, Gomes LC, pp 175–204, 2010
3. Franco MLRS, Franco NP, Gasparino E, Dorado DM, Prado M, Vesco APD: Comparação das peles de tilápia do Nilo, pacu e tambaqui: Histologia, composição e resistência. *Archivos de Zootecnia* 62: 21–32, 2013
4. FAO: Achieving Blue Growth. 2018
5. Jacinto MAC, Ferrari WA: Pele de peixe: Uma matériaprima abundante e inesgotável. *Revista do couro* 18: 30–31, 1992
6. Maina P, Ollengo MA, Nthiga EW: Trends in leather processing: A Review. *International Journal of Scientific and Research Publications (IJSRP)* 9: p9626, 2019
7. Cavali J, de Souza MLR, Silva de Oliveira Kanarski P, Coradini MF, Vieira Dantas Filho J: Tanned leather of the paiche Arapaima gigas Schinz, 1822 (Arapaimidae) with extracts of vegetable origin to replace chromium salts. *PLoS One* 17: e0261781, 2022
8. Motta PJ: Anatomy and Functional Morphology of Dermal Collagen Fibers in Sharks. *Copeia* 1977: 454, 1977
9. Sindirações: Compêndio Brasileiro de Alimentação Animal. São Paulo:
10. AOCS: Rapid Determination of Oil/ Fat Utilizing High Temperature Solvent Extraction. Oficial Procedure. American Oil Chemists´ Society. 1–4, 2017
11. Prophet E, Mills B, Arrington J, Sobin LH: Manual de Métodos Histotecnológicos del Instituto de Patología de las Fuerzas Armadas de los Estados Unidos de América. 5th edition. Washington, 1995
12. Moia PJS, Lourenço L de FH, Sousa CLL, Batista JTS, Joele MRSP, Araujo EAF: Efeito dos plastificantes sorbitol e glicerol em filmes de gelatina da pele de tambaqui (Colossoma macropomum). *Revista Brasileira de Tecnologia Agroindustrial* 15: 2021
13. Hoinacki E: Peles e couros: origens, defeitos, industrialização. 2nd edition. Porto Alegre, RS: SENAI/RS;
14. Junqueira LCU, Joazeiro PP, Montes GS, Menezes N, Pereira-Filho M: The collagen fiber architecture of brazilian Naked catfish skin. *Brazilian J. Med. Biol. Res* 16: 313–316, 1983
15. Nadol JB, Gibbins JR, Porter KR: A reinterpretation of the structure and development of the basement lamella: An ordered array of collagen in fish skin. *Dev Biol* 20: 304–331, 1969
16. Fishelson L: Skin morphology and cytology in marine eels adapted to different lifestyles. *Anat Rec* 246: 15–29, 1996
17. Greven H, Zanger K, Schwinger G: Mechanical properties of the skin of Xenopus laevis (Anura, Amphibia). *J Morphol* 224: 15–22, 1995
18. Allen JJ, Akkaynak D, Sugden AU, Hanlon RT: Adaptive body patterning, three-dimensional skin morphology and camouflage measures of the slender filefish *Monacanthus tuckeri* on a Caribbean coral reef. *Biological Journal of the Linnean Society* 116: 377–396, 2015
19. Eastman JT, Hikida RS: Skin structure and vascularization in the Antarctic notothenioid fish Gymnodraco acuticeps. *J Morphol* 208: 347–365, 1991
20. Souza MLR de, Viegas EMM, Nakaghi LSO, Dourado DM, Kronka S do N, Goes ES dos R: Morfologia, composição centesimal e alterações ocorridas no processo de curtimento da

pele da tilápia do Nilo. *Research, Society and Development* 10: e35810817240, 2021

21. Whelan A, Duffy J, Gaul RT, O'Reilly D, Nolan DR, Gunning P, et al.: Collagen fibre orientation and dispersion govern ultimate tensile strength, stiffness and the fatigue performance of bovine pericardium. *J Mech Behav Biomed Mater* 90: 54–60, 2019
22. Ward AG: The mechanical properties of leather. *Rheol Acta* 13: 103–112, 1974
23. Hilbig CC, Fockink DH, Maluf MLF, Boscolo WR, Feiden A: Resistência do Couro de Tilápia e Composição Centesimal da Pele nas Operações de Ribeira e Curtimento. *Scientia Agraria Paranaensis* 12: 258–266, 2013
24. Souza MLR, Gasparino E, Penha BG, Coradini MF, Goes ESR, Gonçalves AA: Physicochemical and mechanical characteristics of cobia (Rachycentron canadum, Linnaeus, 1766) leather submitted to different tanning agents in the retanning step. *International Journal of Latest Research in Science and Technology* 6: 8–13, 2017
25. Wairimu PM, Ollengo MA, Nthiga EW: Physical Properties of Chrome-Tanned Nile Perch (Lates niloticus) Fish Leather. *Jounal - Society of Leather Technologists and Chemists* 103: 314–317, 2019
26. Zheng Y, Guo C, Li L, Ma Y: Unique morphology and mechanical property of Chinese sturgeon (Acipenser sinensis) fish skin. *IET Nanobiotechnol* 14: 281–288, 2020
27. Kirti, Khora SS: Mechanical properties of pufferfish (*Lagocephalus gloveri*) skin and its collagen arrangement. *Mar Freshw Behav Physiol* 49: 327–336, 2016