ACTA
AMAZONICA | http://dx.doi.org/10.1590/1809-4392202402841

ANIMAL AND FISHERIES SCIENCES | ORIGINAL ARTICLE

Biomechanics evaluation of Amazonian tambaqui (*Colossoma macropomum*) skin puncture after preservation with glycerol 98%

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ABSTRACT

Tambaqui (*Colossoma macropomum*) skin represents a promising alternative for wound treatment due to its histological characteristics and mechanical properties. This study aimed to evaluate tambaqui skin preserved with glycerol 98% using mechanical puncture tests. A total of 85 tambaqui skins were randomly divided into two groups: Group 1 (G1, n = 40) - skins not subjected to glycerol preservation; Group 2 (G2, n = 45) - skins preserved in 98% glycerol. Group 2 samples were immersed in glycerol 98% and refrigerated at 5° C for 32 days. All skins underwent mechanical puncture testing to assess maximum puncture force, resistance, and deformity. Group 2 (G2) exhibited significantly higher puncture force and resistance values, and lower deformity values compared to G1. A strong correlation was found between maximum puncture force and resistance. Tambaqui (*C. macropomum*) skins showed greater maximum puncture strength and higher tissue resistance, with less deformity, after being preserved in glycerol 98% at a temperature of 5°C for 32 days.

KEYWORDS: Amazônia, biological membranes, fish, puncture test, wound.

Avaliação biomecânica de punctura da pele de tambaqui (*Colossoma macropomum*) conservada em glicerina

RESUMO

A pele de tambaqui (*Colossoma macropomum*) representa uma alternativa promissora como biomembrana devido às suas características histológicas e propriedades mecânicas. O estudo teve por objetivo avaliar as características mecânicas da pele do tambaqui após conservação com glicerina (98%). Foram utilizadas 85 peles divididas aleatoriamente em dois grupos: Grupo 1 (G1, n = 40) – peles não submetidas à conservação em glicerina (98%), e Grupo 2 (G2, n = 45) – peles submetidas à conservação em glicerina (98%) e refrigerada à 5° C durante 32 dias. Todas as peles foram submetidas ao ensaio mecânico de punctura para determinar a força máxima de punctura, a resistência e a deformidade. As peles do grupo G2 apresentaram valores da força de punctura e da resistência significativamente maiores, e valores de deformidade menores em relação às peles do grupo G1. Foi identificada uma correlação forte entre a força máxima de punctura e a resistência. As peles de tambaqui (*C. macropomum*) apresentaram maior força máxima de punctura e resistência tecidual maior, e deformidade menor, após conservadas em glicerina (98%) à temperatura de 5º C durante 32 dias, e posteriormente reidratadas com solução salina (0.9%) durante 20 minutos.

PALAVRAS CHAVE: Amazônia, membrana biológica, peixe, teste punctura, ferida.

CITE AS: Silva, B.R.A; Santos, I.F.C; Mascarenhas, A.R.P; Prado, E.J.R; Bergamin, A.C; Pereira, V.L; Moraes, A.C; Pizzigatti, D; Andrade, E.R. 2025. Biomechanics evaluation of Amazonian tambaqui (*Colossoma macropomum*) skin puncture after preservation with glycerol 98%. *Acta Amazonica* 55: e55af24284.

INTRODUTION

Tambaqui (*C. macropomum*) is a native fish of the Amazon region in Brazil and is considered one of the species with the greatest potential for national fish farming (Lima et al., 2020; Liebl et al., 2022; Polese et al., 2022). It is the leading species in national aquaculture production, offering producers a potential profit margin of up to 108%, attributed to its excellent meat quality and adaptability to captivity (MPA, 2011; Silva et al. 2022). This success is due to its rapid body mass gain and high market acceptance within the region (Bussons et al. 2021; Silva et al. 2022).

In 2022, tambaqui (*C. macropomum*) production in Brazil increased by 1.8% compared to the previous year, reaching a total of about 109 million kg. Of this, 72% originated from the North region, which leads tambaqui production within the country (IBGE, 2022; Procopio et al. 2023). Rondônia state ranked fifth nationally in tambaqui production, contributing 50.7 million kg, followed by Maranhão with 10.9 million kg, and Pará with 8 million kg (IBGE, 2022; Procopio et al. 2023). This high production is attributed to favorable climatic conditions and strong consumer demand for various processed products, such as fresh and frozen fillets and specialty cuts (Aride et al. 2020; Nascimento et al. 2020).

The skin of tambaqui is considered a by-product and is often discarded by the fish industry (Nóbrega et al. 2024). However, it can be repurposed as a biomembrane, similar to the skins of tilapia (*Oreochromis niloticus*), which are used in wound healing applications (Lima Júnior et al. 2017; Araújo et al. 2018; Miranda and Brandt, 2019; Gimenez et al. 2019; Rotondano Filho et al. 2021). Tilapia skins are effective biological dressings or tissue substitutes, aiding wound treatment by preventing contamination and infection, promoting local neovascularization, and providing mechanical protection to wounds (Wietecha and Dipietro 2013; Araújo et al.,2018). Tambaqui skin, due to its thicker collagen fibers and greater tensile strength compared to tilapia skin (Franco et al. 2013), represents a promising alternative for wound treatment. These characteristics highlight its potential value for medical applications, particularly in developing biomaterials for wound healing.

For fish skins to be used as biomembranes, efficient sterilization and preservation methods are crucial, along with mechanical tests to evaluate their suitability as biomaterials for medical purposes, such as wound treatment (Wietecha and Dipietro 2013). Various conservation processes, such as glycerination, can have both positive and negative effects, emphasizing the importance of conducting comprehensive mechanical testing.

Glycerol is widely used for preservation due to its cost-effectiveness and ease of application. Its primary mechanism of action involves cell dehydration, which contributes to its bacteriostatic effect against gram-negative and gram-positive microorganisms, although it may not be effective against sporulating forms (Bastos et al. 2005; Oliveira et al. 2009).

This study aims to evaluate the mechanical properties of tambaqui skin after preservation in 98% glycerol using mechanical puncture tests. Currently, there is a lack of research in this area, making this study essential for providing initial insights into the safety and efficacy of tambaqui skin for potential veterinary and human applications. We test the hypothesis that glycerol preservation will enhance tissue resistance and reduce deformability in tambaqui skin. The findings of this study will provide valuable insights into the effects of glycerol preservation, establishing a foundation for utilizing this cost-effective method to preserve tambaqui skin for future use as a biomembrane.

MATERIALS AND METHODS

The study was approved by the Ethics Committee on the Use of Animals under protocol number 033/2023. The sample size was determined using the "power.t.test" function from the "stats" package in R software within the RStudio integrated development environment (Version 1.0.143 – © 2009-2016, RStudio, Inc.). This test set the power at 0.80 and the significance level at 0.05 for a two-sample, two-tailed hypothesis test.

Experimental environment and samples

The tambaqui (*C. macropomum*) skins were sourced from a fish industry located in Vale do Paraíso city, Rondônia, Brazil (Latitude: 10º26'52" S, Longitude: 62º08'03" W, Altitude: 204 meters). The skins were manually extracted from the bilateral dorsal region using knives and immediately placed in a refrigerated thermal box at 10º C for 60 minutes before processing. The skins underwent the removal of fat and muscle residues from their inner surfaces using a metal spatula, followed by washing with distilled water. Skin samples were standardized to dimensions of 10 cm in length, 5 cm in width, and 1 mm in thickness for mechanical tests.

Experimental protocol

Eighty-five (85) tambaqui skins were randomly allocated to two groups: Group 1 (G1, n= 40) - skins not preserved in glycerol 98%; and Group 2 (G2, n= 45) - skins preserved in glycerol 98%. Skins of G1 underwent puncture testing immediately after the removal of fat and muscle, and skins of G2 were immersed in glycerol 98% (MaxxLine Laboratory, São Paulo, Brazil) and refrigerated at 5° C for 32 days. After the preservation period, the skins were rehydrated with saline solution 0.9% for 20 minutes (Oliveira et al. 2009), and then subjected to the mechanical puncture test.

Mechanical puncture test

The mechanical puncture test was performed to evaluate the puncture resistance and deformation characteristics of

the skins. A penetrometer (Marconi MA933, São Paulo, Brazil) was utilized for this purpose. The device operated at a frequency of 60 Hz, with a current of 0.5 A and a power rating of 100 W. The penetrometer tip used in the test had a conical shape, an angle of 30º and a base area of 12.37 mm² (with specifications of height= 80 mm, upper diameter= 3 mm, and lower diameter= 4 mm) (Figure 1).

The skins were stretched and securely fastened onto a pine wood base for the mechanical puncture tests. The pine wood base measured 5 cm in height, 10 cm in external length, and 5 cm in external width, with internal dimensions of 7 cm in length and 2 cm in width (Figure 2a). Aluminum fasteners were used to secure the skins to the base (Figure 2b). These fasteners ensured stable fixation, keeping the skins taut and properly positioned throughout the mechanical testing process.

The distance between the penetrometer tip and the skin was manually adjusted (Lago et al. 2020) and the cell displacement speed was 20 mm/minute. The maximum puncture force (in Newtons, N) and deformation (in millimeters, mm) values were obtained from the penetrometer's software (Engco, Marconi MA933, São Paulo, Brazil). The skin resistance (MPa) was calculated using the formula (Sobral *et al.* 2001):

R - puncture resistance, *Fmax* - maximum puncture force exerted on the skin (N), *ACs* (mm²) - area where the puncture force was exerted on the skin, *A* (mm) - internal area of the fixation base of the skin, and *E* (mm) - skin thickness. *A* was equal to 1 mm and the thickness of the skins was 1 mm, the constant *ACs* used was 2 mm².

Statistical analysis

The data were processed using R software, and univariate analysis of variance employed the "lme4" package for fitting linear mixed-effects models, following a Completely Randomized Experimental Design (CED) as a mixed model. Fixed effect factors were the treatment groups, while each sampling from the sample population was assumed to be a random effect factor. Each sample repetition was accepted as completely independent and randomly assigned within each fixed effect factor.

Figure 1. Photographic image of the penetrometer connected to the laptop (A) and the conical tip penetration probe with a 30º angle and 12.37 mm² base area.

Differences between groups were assessed using t-tests ($p < 0.05$). Normality and homoscedasticity assumptions were verified through standardized residual analysis, applying the Cramer-Von Mises test ($p > 0.05$) and Bartlett test ($p > 0.05$), respectively.

RESULTS

The average weight of the untreated skins was 9.74 g, and for those preserved in glycerol 1.90 g. Skins preserved in glycerol (G2) showed significantly higher puncture force and resistance values ($p < 0.05$) compared to untreated skins (G1), (Figure 3a,b). The maximum puncture force for G1 was 108.62 ± 25.62 N, whereas for G2 it was 128.60 ± 24.76 N.

The average resistance for G1 was 54.31 ± 12.81 MPa/mm², whereas for G2 it was 64.30 ± 12.38 MPa/mm². Skins preserved in glycerol (G2) showed significantly lower deformation values ($p < 0.05$) compared to untreated skins (G1), measuring 7.23 ± 1.09 mm for G2 and 8.49 ± 1.70 mm for G1 (Figure 3c).

The maximum puncture force and deformation were visualized using Engco® software (Figure 4). A strong correlation between maximum puncture force and deformity was identified in both groups (Figure 5).

DISCUSSION

This study aimed to evaluate the maximum puncture strength, resistance, and deformability of tambaqui (*C. macropomum*) skin after preservation in 98% glycerol. The use of tambaqui skin as a biological membrane shows potential for advancing

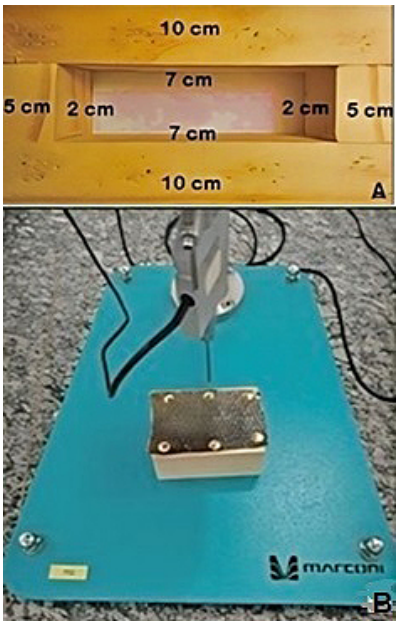

Figure 2. Pinewood base (**A**) and the skin fixed using aluminum fasteners (**B**), before being subjected to the puncture test.

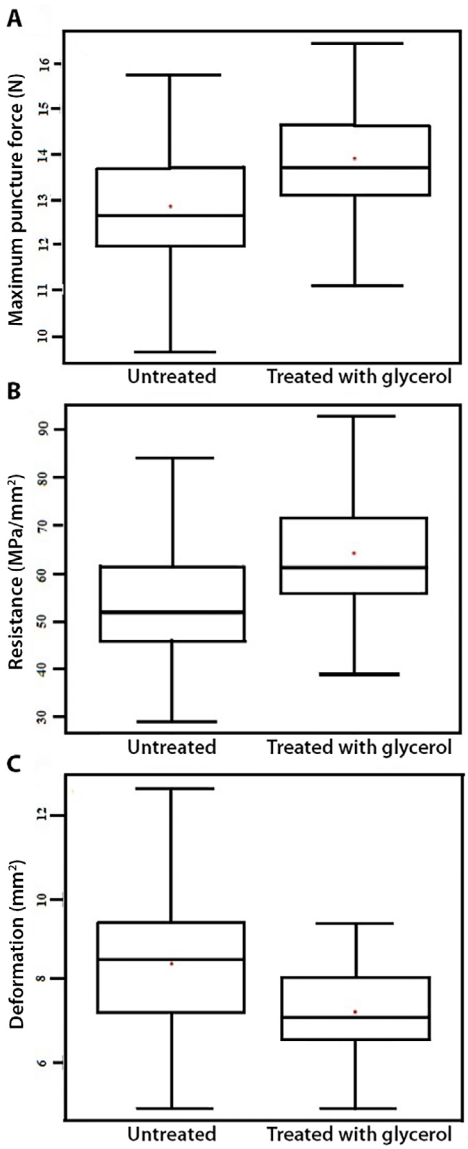

Figure 3. Mechanical properties of tambaqui skin (averages and standard deviations), compared for untreated and glycerin 98% treated (for 32 days) samples. **A**) Maximum puncture force (N), **B**) resistance (MPa/mm^2), and **C**) deformation (mm^2).The red dot in the figure corresponds to the average.

scientific research and promoting environmental conservation, as it repurposes a by-product of the fishing industry. The findings confirmed our hypothesis that preservation in glycerol significantly enhances both maximum puncture force and tissue resistance while reducing tissue deformability.

Tambaqui (*C. macropomum*) skin presents a promising alternative for wound treatment due to its thick collagen fibers and its native Amazonian origin, contrasting with tilapia, which is an exotic species (Etene 2021). Moreover, the tambaqui skin has both a thick longitudinal deep layer and a transversal superficial layer of collagen fibers, whereas tilapia skin has only thinner fibers (Franco et al. (2013). Collagen fibers are crucial components of biological membranes, guiding tissue reconstruction and facilitating biodegradability and biocompatibility (Alves et al. 2015).

Biomembranes derived from fish are less likely to transmit diseases compared to those from warm-blooded animals (Franco et al. 2013). Another factor supporting the viability of tambaqui skin as a biological membrane is its epidermis, which contains a significant number of mucin-producing cells which provide lubrication and protection (Franco et al. 2013). Moreover, tambaqui skin exhibits higher values of tensile strength, elasticity, and maximum breaking strength compared to tilapia skin (Franco et al. 2013; Alves et al. 2015).

The choice of 98% glycerol as a preserving medium was based on its widespread use in Veterinary Medicine for preserving biological membranes, its ability to inhibit membrane decomposition and microbial growth (Araújo et al. 2018), and its mild immunosuppressive properties, which help prevent rejection. Glycerol is also both cost-effective and readily available (Oliveira et al. 2009; Araújo et al. 2018).

The methodology used for preserving tambaqui skins in glycerol and subsequent rehydration with a saline solution 0.9% for 20 minutes aligns with findings in the literature (Araújo et al. 2018). The use of glycerol for conserving various biomaterials is well-documented. Brum et al. (2002) successfully preserved equine pericardium for 11 years before its use in tracheoplasty in dogs. Similarly, a xenogeneic

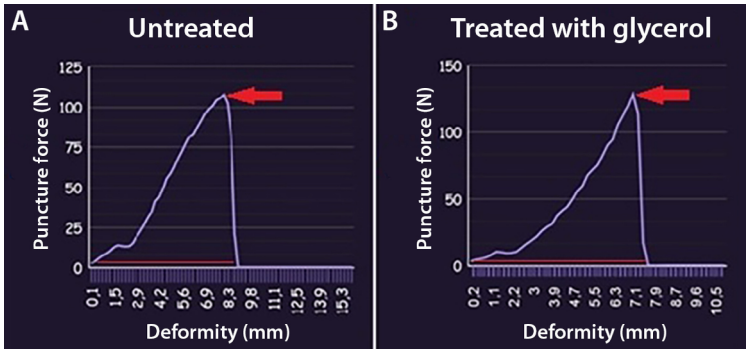

Figure 4. Progression of the deformation dynamics for the tambaqui (*C. macropomum*) skin, showing the maximum puncture force (N) (red arrow) and the beginning and end of the deformation (mm). Graphs generated by the Engco® software of the penetrometer, from untreated and glycerin 98% treated (for 32 days) samples.

auricular cartilage was preserved in glycerol for less than 30 days without identifying clinical signs of rejection or infection (Rappeti et al. 2003).

In the present study, we opted to cool the skins in a refrigerator at 5º C, instead of room temperature which contrasts with literature (Brun et al. 2002; Araújo et al. 2018), given that high temperatures in the region of the study which could have induced their degradation. A study conducted by Gioso et al. (2002) showed that tissues preserved in glycerol at room temperature prevented the formation of intra - and extracellular crystals, as well as harmful electrolyte changes to cells and the extracellular matrix. The formation of intra- and extracellular crystals was not observed in the present study due to the absence of freezing of the skins.

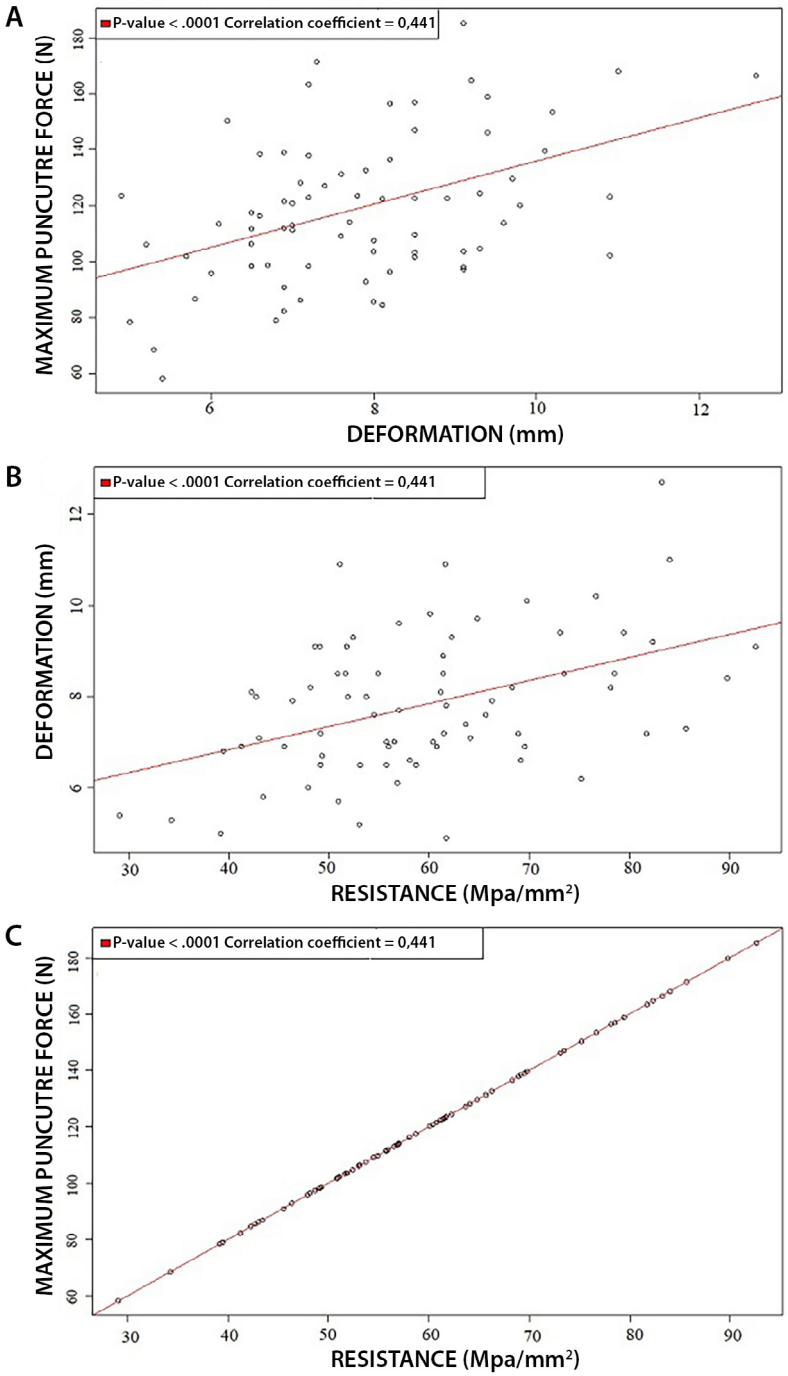

Figure 5. Correlations among the biomechanical properties of tambaqui skin. (**A**) Correlation between the maximum puncture force (N) and deformation (mm); (**B**) deformation (mm) and resistance (MPa/mm^2); and (C) maximum puncture force (N) and resistance (Mpa/mm^2).

The increase in the weight of the skins treated with glycerol (G2) can be attributed to the substance's hydrophilic action. Glycerol replaces a significant portion of intracellular water without altering the ionic concentration of the cells (Oliveira et al. 2009; Araújo et al. 2018). This temporary replacement of intracellular water with glycerol induces greater preservation of the skins, particularly concerning the action of microorganisms. However, rehydration with a saline solution (0.9%) is required before use, as outlined in the present methodology.

Mechanical tests are crucial for the evaluation of biological membranes and can vary depending on the preservation method (Andarawis-Puri et al. 2012; Gut et al. 2016). Glycerol reduces intermolecular forces between protein molecules, thereby increasing flexibility and extensibility (Hanani et al. 2013). However, not always this effect has been found; Nile tilapia skins, for example, exhibited lower tensile strength, progressive tearing, elasticity, and maximum strength values when compared to tambaqui skins (Franco et al. 2013).

In the present study, significant differences in maximum puncture force, resistance, and tissue deformation were identified between skins preserved in glycerol (G2) and those not preserved (G1). The significant increase in maximum puncture force and resistance values, along with the significant decrease in skin deformity in G2 compared to G1, was associated with glycerol's ability to enhance resistance (Hanani et al. 2013; Araújo et al. 2018; Lago et al. 2020). These findings suggested a uniform dispersion of glycerol in the extracellular matrix of tambaqui skin.

CONCLUSION

The preservation of tambaqui (*C. macropomum*) skins in 98% glycerol at a temperature of 5°C for 32 days has shown beneficial and promising effects, as it significantly increases tissue resistance and reduces deformity. Translational and clinical studies should be conducted to advance an appropriate protocol for the use of tambaqui skin in medical applications.

ACKNOWLEDGMENTS

The authors thank the Laboratório de Solos, Universidade Federal de Rondônia, campus Rolim de Moura – RO - Brasil; Pescado Rodrigues (Vale do Paraíso, Rondônia) – RO - Brasil; Pró-Reitoria de Pós-Graduação Universidade Federal de Rondônia – RO – Brasil - (PROPESQ – UNIR) (PVC1282-2023, Edital 01/2023/DPesq/PROPESQ/UNIR).

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RECEIVED: 13/08/2024
ACCEPTED: 30/12/2024
ASSOCIATE EDITOR: Andre Willerding
DATA AVAILABILITY: The data supporting the findings of this study have been published in this article.