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ARTIGO *REVISTA BRASILEIRA DE ENGENHARIA DE PESCA*

NILE TILAPIA (*Oreochromis niloticus* Linnaeus 1758) IN MESOHALINE WATER OVERPRODUCES SKIN DERMATAN SULFATE

TILÁPIA DO NILO (*Oreochromis niloticus* Linnaeus 1758) EM ÁGUA MESOALINA SUPERPRODUZ DERMATAM SULFATO DE PELE

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Abstract Tilapia culture in saline waters has been a reality, but the effects on the skin-glycosaminoglycans-(GAGs) composition are little known. This study evaluated the (fresh/dehydrated) skin of marked freshwater/10‰-mesohaline tilapia on its morphometry/biomass, water content (WC) and GAGs yield. Skin (n=10/5 group^{-1}) was determined on the percentage (w w^{-1}) from the total fish or fresh tissue, with WC by difference in dehydrated mass. GAGs from fresh/dehydrated skin digestion with papain were analyzed for metachromasy using cationic dye and structurally by infrared spectroscopy. Results showed no difference between morphometry *vs*. fresh skin yield (2.60±0.12/2.68±0.11%, p>0.05) from total fish, but there were (p<0.05) in total residual (8.13±0.64/8.26±0.76g *vs*. 3.33±0.25/4.97±0.35g), relative mass (41.00±0.35 *vs*. 60.56±1.46%), WC (58.95±0.34% *vs*. 39.40±1.45%) and level of GAGs (0.06±0.00% *vs*. 0.15±0.00/0.19±0.01%) from tilapia (fresh *vs*. dehydrated) skin between both origins. Samples had dermatan, except for dermatan/chondroitin chain in freshwater tilapia skin. The study suggested that the skin GAGs composition changes as a compensatory response to the saline effect, but does not affect tilapia performance.

Key words: teleost, mucous matrix, osmotic stress, adaptation, salinity.

Resumo Tilapicultura vem sendo uma realidade em águas salinas, porém pouco são conhecidos os efeitos sobre a composição de glicosaminoglicanos (GAGs) de pele. Avaliaram-se, de tilápia comercial (dulcícola/mesoalina 10‰), a morfometria/biomassa, conteúdo de água (CA) e rendimento de GAGs da pele (fresca/desidratada). Foi determinada a porcentagem (m m^{-1}) de pele (n=10/5 grupo^{-1}) do peixe inteiro ou tecido fresco, com diferença na massa desidratada pelo CA. Os GAGs, da digestão com papaína da pele fresca/desidratada, foram analisados para metacromasia usando corante catiônico e, por espectroscopia de infravermelho, estruturalmente. Os resultados não mostraram diferença entre morfometria *vs*. rendimento de pele fresca (2,60±0,12/2,68±0,11%; p>0,05) do peixe inteiro, porém no resídual total (8,13±0,64/8,26±0,76g *vs*. 3,33±0,25/4,97±0,35g), massa relativa (41,00±0,35 *vs*. 60,56±1,46%), CA (58,95±0,34% *vs*. 39,40±1,45%) e nível de GAGs (0,06±0,00% *vs*. 0,15±0,00/0,19±0,01%) houveram (p<0,05) da pele de tilápia (fresca *vs*. desidratada) entre ambas origens. As amostras apresentaram dermatam, exceto na pele das tilápias dulcícolas para cadeia dermatam/condroitim. Estudo sugeriu que muda a composição de GAGs na pele em resposta compensatória ao efeito salino, mas não efeta o desempenho da tilápia.

Palavras-chave: teleósteo, matriz mucosa, estresse osmótico, adaptação, salinidade.

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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Introduction

Aquaculture is an agribusiness that arises as an alternative means of aquatic organisms' world production due to increasing declines in fisheries, the causes of which range from overfishing to climate change affecting natural stocks. This productive system of food security aim to produces animal protein because the escalating demand for food according to Fao (2024)' recent data. Traditionally, commercial fish farming has been carried out in freshwater to still take advantage of natural water bodies or reservoirs built by human (Moreira et al., 2001); however, the use of saline waters has made fish farming viable in certain regions as an promissing strategy to the production of fish intended for human consumption with desirable attributes set (Suresh & Lin, 1992).

Among the most cultivated freshwater species has Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), which is a well-known fish worldwide for its hardiness, resilience in different cultivation systems, and high zootechnical performance (Moreira et al., 2001), as well as its tolerance a wider range of salinity as a stressor parameter on the growth, survival rate, reproduction, chemical composition and other physiological changes related to fish metabolism under the effect of saline stress (Suresh & Lin, 1992; Mohamed et al., 2021). The use of Nile tilapia fish at moderate salinity would allow a viable strategy for some regions with salinized water and reduced rainfull, but with negative impacts in its osmoregulation capacity (Boeuf & Payan, 2001) suggesting a more limited salinity between 5 and 10 g L^{-1} (Suresh & Lin, 1992). As a consequence of this production, there would be the generation of solid wastes (*e.g.*, skin, gill, viscera and bone) from commercial or artisanal filleting (Moreira et al., 2001), which would also offer opportunities for obtaining natural biopolymers with biotechnological/bioeconomic potential, such as glycosaminoglycans (GAGs) (Liu et al., 2025).

The fish filleting-derived wastes are sources in GAGs (known as mucopolysaccharides) occupying the extracellular matrix and cell membranes; covalently cross-linked to proteins making known polymeric structures as proteoglycans; and their chemical features of the backbone of linear long-chain exhibiting carboxylate or sulfate groups with differences in position and molecular configuration (Liu et al., 2025). The GAGs family comprises chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate, heparin, heparan sulfate, and hyaluronic acid (the only uncharged), where they have each disaccharide unit hexosamine (glucosamine, *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine), uronic acid (L-iduronic acid or D-glucuronic acid), or galactose, connected by glycosidic bonds (Badri et al., 2018). This generic class of sulfated polysaccharides within the animal body that determines usefulness has been well-emphasized on their functionalities for industrial applications in various fields, including medicine, biotechnology and food (Badri et al., 2018), as also bioeconomic perspective for circular economy (Liu et al., 2025). In terms of total biomass, sulfated polysaccharides from animals are less abundant than in other aquatic organisms, including seaweed and seagrass which their composition would reflect to the environmental responses (Pomin & Mourão, 2008). However, to the best our knowledge, the effect of water salinity lack of investigation to understand the natural mucosal (GAGs) barrier sensitivity of Nile tilapia fish.

Skin of bony fish has a morphological architecture that varies with the species, as well as in composition and resistence of this external organ (Franco et al., 2013). It is composed by epidermis presenting glands that secrete mucus, and dermis with predominantly fibrous structure, and due to its extracellular-matrix primarily constituted by collagens and GAGs (Moreira et al., 2001), skin of fish has also been a relevant theme for studies related to manufactures (*e.g.*, clothes, gloves and tanning) (Franco et al., 2013), besides biomedical applications (Alves et al., 2015). Skin tissue is rich in DS GAG chains and their compositional percentage varies with the species of fish that habit the aquatic systems (Dellias et al., 2004; Souza et al., 2007) or when animals are cultured (Rodrigues et al., 2009; Rodrigues et al., 2025).

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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Nile tilapia skin structure has been extensively investigated (Franco et al., 2013; Alves et al., 2015) and a DS was found and partially characterized (Pereira et al., 2021; Fernandes et al., 2025) with anticoagulant (Rodrigues et al., 2011; Salles et al., 2017) and antioxidant (Nascimento et al., 2021) properties, being it still an alternative tool in the seminal cryopreservation of fish (Nascimento et al., 2021; Pereira et al., 2021). As the salinity level can be a stressful condition for Nile tilapia (Suresh & Lin, 1992; Souza et al., 2019; Mohamed et al., 2021; Palmer et al., 2024), the current study investigated the effects of an isosmotic mesohaline condition (0 *vs*. 10‰) on the biochemical changes in available biomass from marked Nile tilapia skin by-product, as well as the impact of this low salinity on the extraction yield concerning matrix DS composition from *in natura* or dehydrated fish skin, and on a comparative structural basis by Fourier Transform Infrared (FT-IR) spectroscopy.

Material and Methods

Nile tilapia skin samples

The sex-reversed Nile tilapia (*O. niloticus*) skin was freshly provided from a fish processing unit located at the municipatility of Fortaleza, Brazil. A total of twenty specimens was acquired and, then, transported on ice (1:1 kg ratio) at sialed isothermal boxes at the Marine Biotechnology (MarBio) Laboratory following the Fernandes et al. (2025)' protocol for bony fish and stored at -20°C until experimental use. In plastic bags, the fish were separated into two groups of ten individuals each, based on water source (fresh or mesohaline water, 0 *vs*. 10‰) in which they were cultured and, consequently, popularly marketed (Table 1). The specimens of Nile tilapia were registered in SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) platform under code AA4816B.

Table 1. Biometry and total skin mass of marked Nile tilapia according to water source.

fish group* (n = 10)	salinity (‰)**	lenght (cm)***	height (cm)****	weight (g)*****	total biomass (kg)*******	total fresh skin (g)*******
freshwater	0	28.87 ± 0.30	9.81 ± 0.30	430.80 ± 27.26	4.380	112.74
mesohaline	10	31.88 ± 1.66	10.64 ± 0.66	580.30 ± 75.83	5.803	154.04

* origin of fish farming; ** local information; *** lenght from the tip of the snout (Palmer et al., 2024); **** length from the dorsal region to abdominal cavity; ***** whole mass of the fish; ****** total weight of the fish sample; ******* total weight of *in natura* waste removed from the fish' muscle.

In MarBio laboratory, the biometry of the tilapia individuals (Table 1) was performed using a rudimentary ichthyometer and a commercial balance on a 1 g precision scale, respectively, before fish filleting procedure. From treated (washed, scalined and eviscerated) fish, the fresh skin of Nile tilapia was carefully removed with knife and, when necessary, clam by hand; being it then cleaning to eliminate adherent muscle (Fernandes et al., 2025; Rodrigues et al., 2025). The total fish biomass and total fresh skin were also weighed, respectively (Table 1). After the total skin sampling, this source material from fish body was separated in sialed plastic bags into two groups of five units of skin samples, per water origin, as represented in figure 1. The fresh skin waste was kept in freezer at -20°C (Moreira et al., 2001) and those dehydrated in an oven with air circulation (45°C, 24 h - Fernandes et al., 2025) mantained in closed recipient until both GAGs extraction (Rodrigues et al., 2011; Salles et al., 2017). The yield of fresh or dehydrated skin samples (n = 10 or 5) from the Nile tilapia were separately estimated as percentage (%) from the total fish biomass as following (*Eq. 1*):

$$\text{Waste yield (n = 10 or 5): fish skin total biomass}^{-1} \times 100 \qquad (1)$$

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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For constitution water analysis, the skin samples (n = 5) separately dehydrated for 24 h in an oven under air circulation (at 45°C) were weighted and their yields based on respective fresh waste as following (*Eq. 2*):

Water content (n = 5): fresh fish skin dehydrated fish skin^{-1} × 100, or water loss -1 (*2*)

Based on available (fresh or dehydrated) Nile tilapia skin masses recovered from *Eq. 1* determination, they were further used into two groups of five fish waste each for GAGs extraction, following the experimental design illustrated in figure 1.

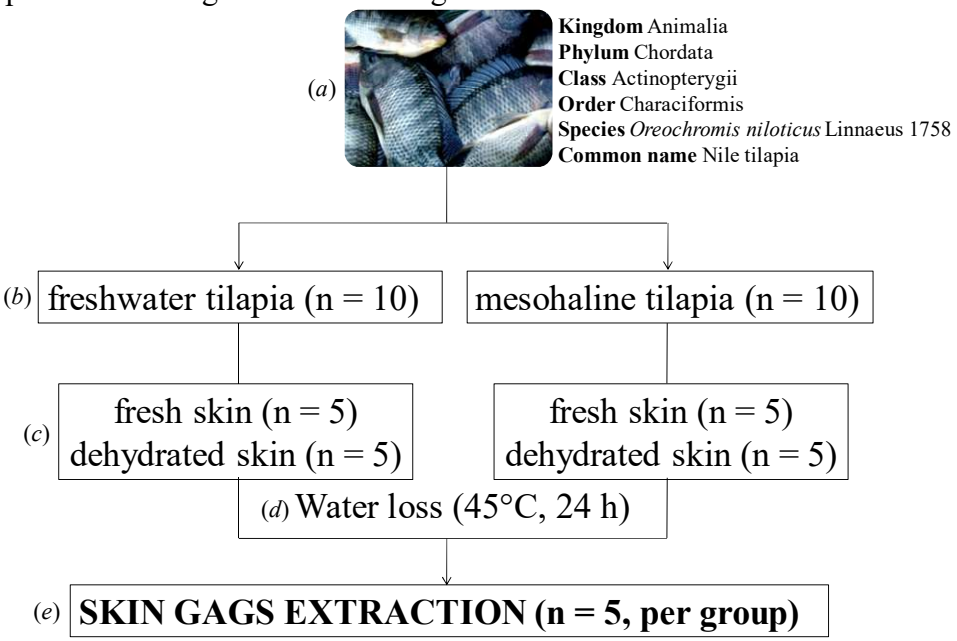

Figure 1. Experimental design for the use of Nile tilapia biomass (*a*) followed by group separation, per water source, of individuals (*b*) with their respective skins (*c*), skin water loss determination (*d*), and GAGs extraction procedure (*e*).

For GAGs extraction, it was applied the enzymatic method as previosuly described for Nile tilapia (*O. niloticus*) skin (On.skinGAGs) (Rodrigues et al., 2011; Salles et al., 2017; Nascimento et al., 2021) based on scheme shown in figure 2, in which crude extracts rich in GAGs were partially purified from the fresh or dehydrated skin samples. For this, both forms of preparated skin were weighed on an initial basis of 8 g and, then, the raw samples putted in glass flasks for incubation process in a thermostatic bath, with the use of crude papain as an unspecific proteolysis, applying a digestion rate of 10% (w w^{-1}) of the fish material, in a system of 100 mM sodium acetate buffer (pH 5.0) added of 5 mM EDTA, and 5 mM cysteine, whose the extraction condition followed at 60°C with duration of 24 h.

After enzyme-contact period, the digested tissue-resulting mixture was continually filtered using a nylon net and the supernatants were saved and centrifugated (9.560 × *g* for 20 min). On.skinGAGs that were present in recovered medium were precipitated with 10 mL of 10% cetylpyridinium chloride (CPC) solution at room temperature (25-28°C) for 24 h period. The mixtures were then centrifuged at 9.560 × *g* for 20 min and the *pellet*s containing the On.skinGAGs further washed with 100 mL of 0.05% CPC solution, dissolved (under mechanical stirring for 20 min) in 100 mL of a 2 M NaCl: ethanol (100:15 ratio, v:v) solution, and, then, combined with a 92.8% ice-cold alcoholic precipitation (24 h, 4°C) with addition of 100 mL at commercial level. Later, the On.skinGAGs were centrifugated (9.560 × *g* for 20 min), washed twice with 100 mL of

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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80% alcohol, and once with the same volume of 92.8% commercial alcohol. After each centrifugation (9.560 × *g* for 20 min) among the steps, the tilapia skin-derived material was dried using an oven drying with air circulation (60°C, 24 h) to obtain the raw masses from the fresh or dehydrated skin samples, which were named fOn or dOn.(freshwater or mesohaline)skinGAGs, respectively.

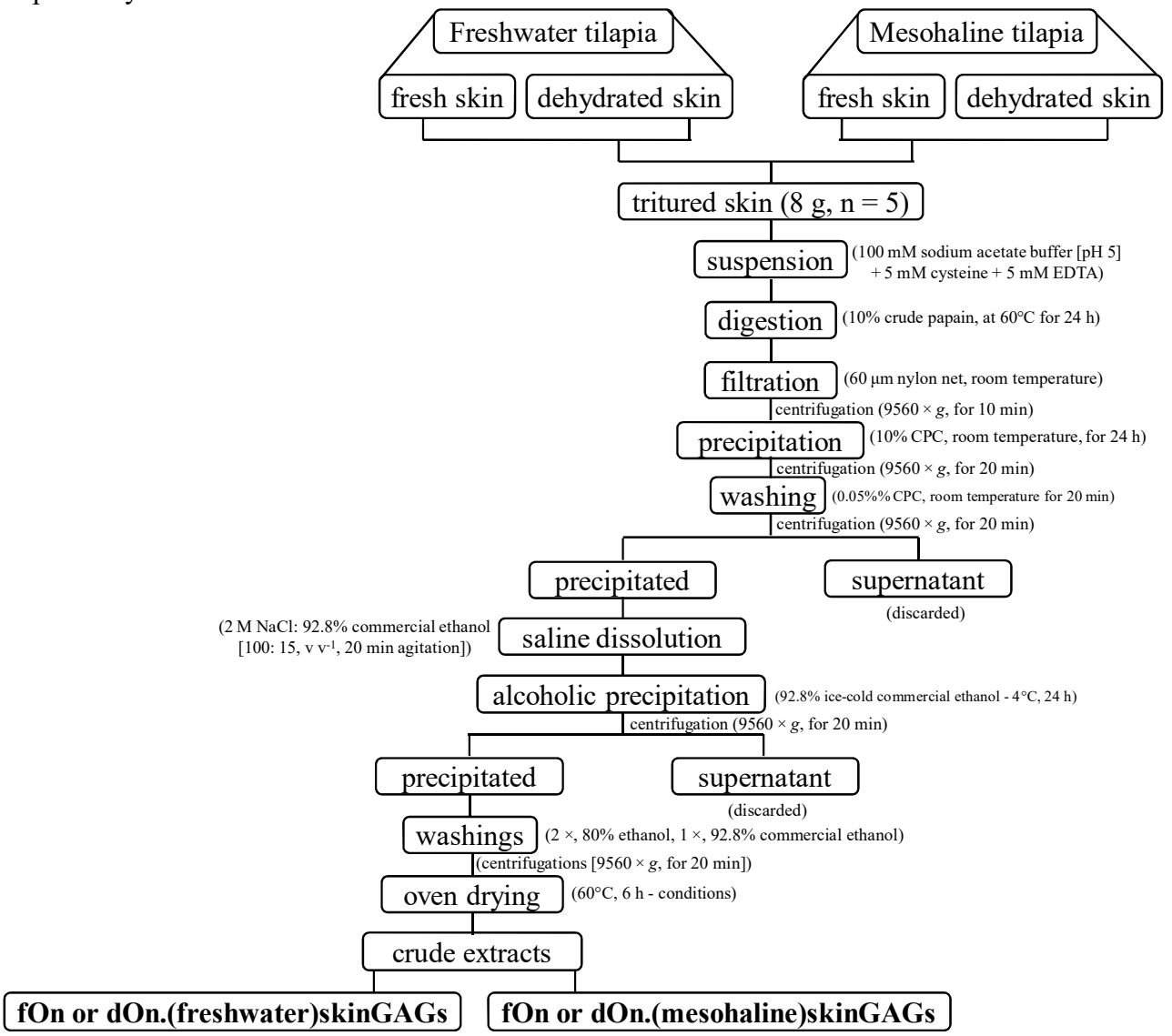

Figure 2. Scheme of obtaining of crude GAGs from the skin of Nile tilapia obtained from local market.

On the basis of the extractable material, the yield was further calculated according to *Eq.* (*3*) and expressed as the percentage (w w^{-1} %, n = 3) of the fresh or dehydrated matter (g).

$$\text{Yield (\%)} = \text{OnGAGs F or DM}^{-1} \times 100 \quad (3)$$

where: OnGAGs the dry weight of crude GAGs and F or DM the raw weight of fresh or dehydrated matter (freshwater or mesohaline tilapia skin).

Analysis of the Nile tilapia GAGs from skin samples

The influence of water source (0 *vs.* 10‰ mesohaline) on the composition of Nile tilapia skin was examined by partial biochemical analyses for fish waste-derived GAGs (Fernandes et al.,

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
DOI: 10.18817/repesca.v16i4.4256

2025; Rodrigues et al., 2025). For each extracted material, a test sample was previously prepared and analyzed for metachromasy and Fourier Transform Infrared (FT-IR) spectroscopy.

Metachromatic property

This assays was performed with a known solution and three aliquots (9, 18 or 27 µg, w v^{-1}) were checked, step-by-step, for metachromasy, in the presence of 1,9-dimethylmetilene (DMB) blue dye as an indicator stain of colorimetric (violet) reaction based on complex formed in sample (Farndale et al., 1976). The *in vitro* method was conducted, in tripicate, following Fernandes et al. (2025)' protocol for fish GAGs, using glass tubes and the property specific for sulfated GAGs visualized from the test sample preparation. The assay-related images were recorded by photography from a portable device after the experiment.

FT-IR spectroscopy

The functional groups present in matrix GAGs extracted from the fresh or mesohaline water-derived tilapia skin samples were recorded by FT-IR using a spectrometer (IRPrestige-21 Shimadzu, Japan). The spectral measurements were obtained from ~10 mg of the test sample prepared in potassium bromide (KBr) *pellets*, at a resolution profile of 4 cm^{-1}, with 64 scans min^{-1} at 500-4000 cm^{-1}. The spectra values and the graphicals were assigned and represented by mean of the Origin software version 8.0 as the Statistical Analysis Software (USA). All the graphicals were separatelly origined and then saved in Windows file to contruct the integrate figure.

Statistical analyses

All experimental values were expressed as mean ± standard deviation (n = 3 or 5). The skin parameter analyses (fresh or dehydrated waste and content or water loss) were analyzed by one-way ANOVA, followed by Tukey' test or *t*-Student' test, with $p < 0.05$ as statistically significant. For values retaled to extraction yield were applied the one-way ANOVA, followed by Tukey' test, considerating $p < 0.05$ as statistically significant. The analyses were done using the GraphPad Prism® version 5.01 for Windows (GraphPad Software, 1992-2007, San Diego, CA; www.graphpad.com).

Results and Discussion

Zootechnical aspects of freshwater or mesohaline Nile tilapia from local market

The biometric analysis to an isosmotic mesohaline condition (0 *vs*. 10‰) for marked specimens of Nile tilapia was compared with the available zootechnical indicators, and it was observed a significant difference between both sampled individual groups, as listed in table 2.

Table 2. Lenght (cm), height (cm), weight (g) and weight to lenght ratio values by fish group.

fish group (n = 10)	salinity (‰)	lenght (cm)	height (cm)	weight (g)	weight / lenght ratio
freshwater	0	28.87 ± 0.30^{a}	9.81 ± 0.30^{a}	430.80 ± 27.26^{a}	14.92 ± 0.30^{a}
mesohaline	10	31.88 ± 1.66^{b}	10.64 ± 0.66^{b}	580.30 ± 75.83^{b}	18.15 ± 0.56^{b}

Different letters show difference between groups at level of 5% (*t*-Student' test, $p < 0.05$).

Mesohaline Nile tilapia showed average lenght (31.88 ± 1.66 cm), height (10.64 ± 0.66 cm), weight (580.30 ± 75.83 g) and weight to lenght ratio (18.15 ± 0.56) values differing ($p < 0.05$) for those from freshwater specimens (Table 2), suggesting the hyphotesis that it cultured at salinity up to 16‰ had no negative effect on its performance, therefore, with a positive allometry regarding fish growth rate, when exposed to low salinity (Souza et al., 2019). Palmer et al. (2024) cultured

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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Nile tilapia at different temperature (14 or 22°C) and salinity (0, 16 or 34‰) and found better fish response in intermediate conditions, without cause sub-lethal damage. Our results indicated that commercially-obtained mesohaline *O. niloticus* has been considered a reality with appreciable animal size for human consumption (Moreira et al., 2001).

***In natura* or dehydrated skin yield of freshwater or mesohaline Nile tilapia from local market**

Although showing zootechnical differences between groups (Table 2), there was not an important relation with biomass per filleted fish in terms of fresh skin yield (%, w w^{-1}) that resulted 2.60 ± 0.12% and 2.68 ± 0.11% for fresh / mesohaline water tilapia, respectively ($p > 0.05$, Table 3).

Table 3. Total biomass, fresh skin mass, biomass to fresh skin ratio and fresh skin yield values by fish group.

fish group (n = 10)	salinity (‰)	total biomass (kg)	total fresh skin (kg)	biomass / skin	fresh skin (%, w w^{-1})*
freshwater	0	4.380	0.113	38.76	2.60 ± 0.12[a]
mesohaline	10	5.803	0.154	37.68	2.68 ± 0.11[a]

*Yield calculated from the whole filleted fish with head. Similar letters no indicating difference between groups at level of 5% (*t*-Student' test, $p > 0.05$).

These results between both Nile tilapia groups from a local market unit generated total biomasses unrelated by fish' size and weight for fresh skin obtaining (Table 3), since the human method and equipament, as well as fish anatomy could reflect in the filleting process of *O. niloticus*, which has a skin yield of ~5% in industrial terms (Moreira et al., 2001). These similar *in natura* skin yields suggested that in 10‰ salinity had no impact on *O. niloticus* skin growth, considerating the conditions and available body area for the filleted individuals so far (Franco et al., 2013). Suresh & Lin (1992) reviewed that the Nile tilapia has an optimal for growth in a range of 10-20‰.

As the skin represents an important volume of waste generated from tilapia processing, social/economic/environmental impacts can also be caused when it inappropriately discarded (Moreira et al., 2001; Fernandes et al., 2025). Further step was to analyze *in natura vs*. dehydrated skin of freshwater or mesohaline Nile tilapia with basis of average skin biomass of sampled individuals (n=5 per group), as shown in figure 3. Considerating Nile tilapia-removed fresh skin, both groups showed the same level of average yield of raw first-matter (8.13 ± 0.64 *vs*. 8.26 ± 0.76 g, respectively, $p > 0.05$) independent of water source (Figure 3A), therefore, no increasing in average skin biomass produced from tilapia exposure at 10‰ salinity, suggesting as a primary tolerance response to fresh tissue specific-isotonic condition (Palmer etal., 2024). However, when both dehydrated skins, there was a difference between average amounts (3.33 ± 0.25 *vs*. 4.97 ± 0.35 g, respectively, $p < 0.05$) from the respective groups (Figure 3A), leading to observation that tilapia' skin matrix could be maskered by constitution water in terms of raw yield, whose condition would decrease microbial charge for eco-friendly GAGs extraction and, obviously, an alternative approach to use the higher skin volume of environmental discard for bioeconomy (Fernandes et al., 2025).

To support these observations, raw skin by-product data were analyzed and compared in terms of percentage of the total fish and skin relative mass between both tilapia groups. From graphical analysis (Figure 3B), Nile tilapia filleting-dehydrated skin showed difference in relative percentage comparing with the freshwater and mesohaline *O. niloticus*, not only from the total fish (0.77 ± 0.04 *vs*. 3.93 ± 1.88%, respectively, w w^{-1}, $p < 0.05$), whose overall result was until 1.18-fold higher than that found for experimentally-monocultured Nile tilapia by Fernandes et al. (2025); but also from the fresh skin (41.00 ± 0.35 vs. 60.56 ± 1.46%, w w^{-1}, $p < 0.05$) that confirmed the effect of 10‰ salinity on the total skin biomass gain (Figure 3A). More relevant was to what occurred in mesohaline tilapia-derived skin yield where this mass gain resulted about 1.47-fold

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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higher *vs*. freshwater tilapia (Figure 3B), suggesting its rusticity by physical barrier to salinity stress (Rodrigues et al., 2009), but without losing energy to fish grow in isosmotic water (Souza et al., 2019; Palmer et al., 2024). Studies on the biomechanical properties of mesohaline tilapia skin deserve be still explored (Franco et al., 2013) for clinical evaluation (Alves et al., 2015) and animal/human nutrition, since the fillet with skin is also popuraly consumed (Moreira et al., 2001).

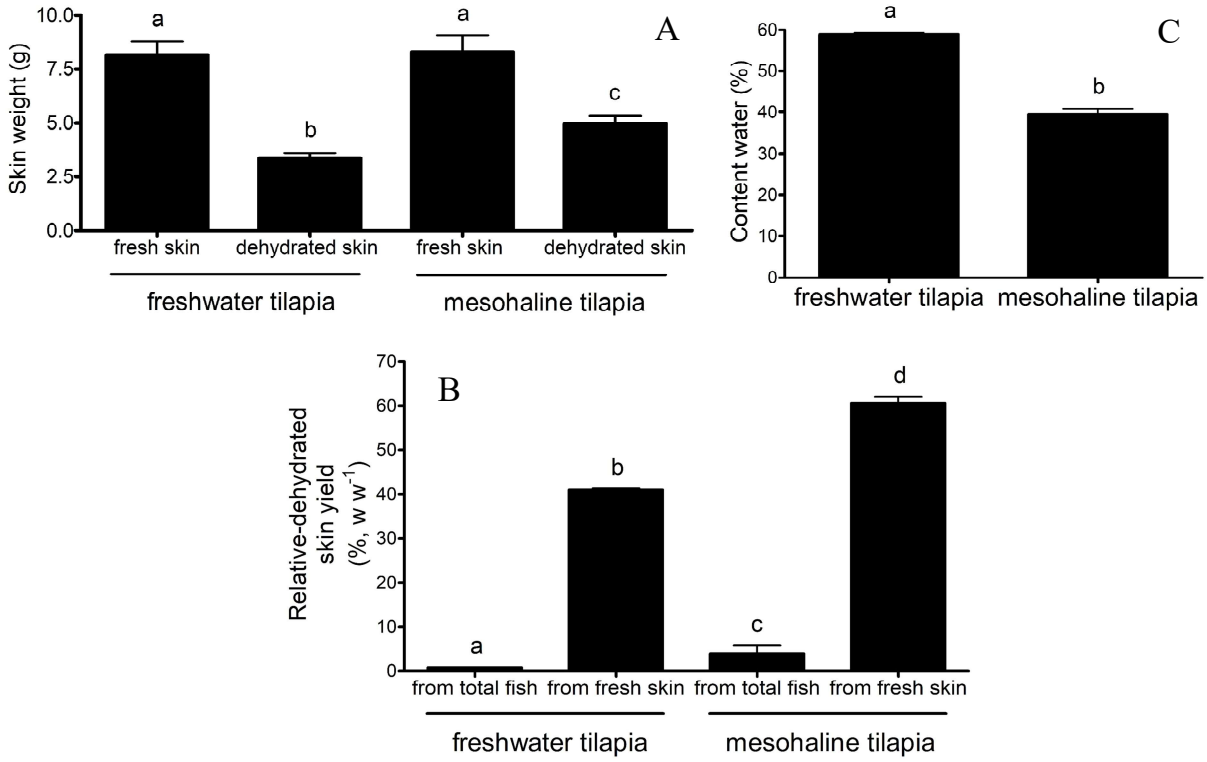

Figure 3. Average total fresh or dehydrated skin biomass (A), relative dehydrated skin yield (B) and water content of oven-dehydrated skin (C) of freshwater *vs*. mesohaline Nile tilapia, *O. niloticus*. Letters on the bars indicate significant differences at level of 5% (ANOVA, Tukey's test or *t*-Student test, $p < 0.05$, $n = 5$ group^{-1}).

As can be seen (Figure 3), the Nile tilapia exposed to 10‰ salinity stress could generate any consequence on its health status in terms of skin matrix water rate, therefore, leading to physiological changes. Mohamed et al. (2021) found harmful effects in organs (gill, liver and kidney) of salinity stressed tilapia. A primary response during stress is the decreased immunity of fish when it vulnerable to opportunistic disease (Moreira et al., 2001) because the handling also arises infections in seawater salinities (Suresh & Lin, 1992).

Thus, it was analyzed the overall constitution water results based on dehydrated skin and, after period of tissue dehydration (24 h, 45°C), mesohaline tilapia-obtained skins (39.40 ± 1.45%, w w^{-1}) showed a comparatively lower water content ($p < 0.05$) than that found for freshwater tilapia ones (58.95 ± 0.34%, w w^{-1}) (Figure 3C), postulating that the fish lost water through osmoregulation under saline condition of 10‰ water, which could explain the relative skin biomass gain (Figure 3B), thus strengthening its physical barrier when exposured to saline stress (Mohamed et al., 2021), but without compromising its growth performance (Suresh & Lin, 1992; Souza et al., 2019; Palmer et al., 2024). Collectivelly, these observations were interesting since the Nile tilapia change its skin matrix water composition as a dehydration mechanism to saline adaptation; and limited data are described in the scientific literature on the values related to water content in fish skin, where according to the Franco et al. (2013)' study, Nile tilapia showed a water percentage of 67.14% (w w^{-1}) in *in natura* skin indicating technological potential for making gloves and clothing.

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
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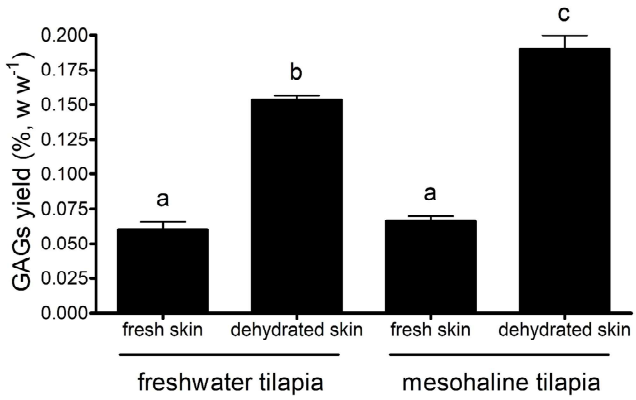
ARTIGO REVISTA BRASILEIRA DE ENGENHARIA DE PESCA

On the basis of these compositional variation, the following question was asked: would the biochemical composition of GAGs also change in the extracellular matrix of the skin of Nile tilapia subject to the mesohaline condition? Thus, subsequent analysis was to determine the percentage of Nile tilapia skin matrix-derived GAGs, when obtained from both water sources.

Yield of Nile tilapia GAGs from skin samples and metachromasy checking

From previously quantified total biomass (Figure 3), freshwater or mesohaline Nile tilapia-derived skin samples were prepared and then tritured tissue (fresh and dehydrated) overnight digested with crude papain, followed by both CPC and 92.8% ice-cold alcohol precipitations resulted in a significant difference of *O. niloticus* skin matrix-extracted crude GAGs yield depending on the water source and pretreated tissue (Figure 4). Skin GAGs content of Nile tilapia varied ($p < 0.05$) from 0.06 ± 0.00% for both freshwater samples to 0.15 ± 0.00/0.19 ± 0.01% (w w^{-1}) for those mesohaline samples (Figure 4A), respectively, with a relative difference of, at least, 2.5-fold higher regarding the dehydrated skin-derived yield that corresponded to a level of mass from 1.53 ± 0.03 to 1.91 ± 0.08 mg g $tissue^{-1}$ ($p < 0.05$, tabulated Figure 4B) from the dehydrated fist-matter digested by papain (Figure 2) considerated as an eco-friendly method (Liu et al., 2025).

A



B

water source	fresh skin (mg g $tissue^{-1}$)	dehydrated skin (mg g $tissue^{-1}$)
freshwater tilapia	0.60 ± 0.057[a]	1.53 ± 0.03[b]
mesohaline tilapia	0.66 ± 0.03[a]	1.91 ± 0.08[c]

Figure 4. Yield of crude GAGs extract (A) and respective GAGs mass per gram of fresh or dehydrated skin (B) of Nile tilapia, *O. niloticus*. Letters on the bars or between lines/columns indicate significant differences at level of 5% (ANOVA, Tukey's test, $p < 0.05$, n = 3 from raw skin mass of 5 individuals $group^{-1}$ - n = 1-5 for fresh skin / n = 6-10 for dehydrated skin).

Papain-assisted incubation has been applied for various aquatic organism' polysaccharide extraction (Dellias et al., 2004; Souza et al., 2007; Fernandes et al., 2025; Rodrigues et al., 2025). In this study, use of two skin preparations (fresh or dehydrated) distinctly determined that the better way to obtain tilapia GAGs derived from the reduction of water content than in *in natura* tissue valorization (Figures 3B, C) (Fernandes et al., 2025), although showing rates of crude GAGs extracts very low, as already expected (0.09-0.22%, w w^{-1}) using dehydrated skin of this species in different age and weight (Rodrigues et al., 2011; Salles et al., 2017; Nascimento et al., 2021; Pereira et al., 2021; Fernandes et al., 2025). Obviously, hydrated skin matrix led to an amount of higher fresh mass due to water present in the *in natura* tissue (Figure 3A) (Moreira et al., 2001), but not in relative terms of GAGs (Figure 4) obtained by protease from the fish mucosal site (Fernandes et al., 2025).

In fact, dehydrated skin samples of mesohaline Nile tilapia fish would be more susceptible to proteolytic action capable of extracting a significant level of GAGs *vs*. those from freshwater individuals based on external barrier of the marked animal (Figure 4). On this basis, it was speculated that the tilapia skin matrix overproduced GAGs by epidermal tissue in which mucous glands displayed a physiological response to 10‰ salinity (Moreira et al., 2001). This phenomenon

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
DOI: 10.18817/repesca.v16i4.4256
ARTIGO REVISTA BRASILEIRA DE ENGENHARIA DE PESCA

of majority availability in skin GAGs was curious, since no biochemical information of these compounds by bony fish against saline stress has been reported so far based on some marine organisms, which they change its physiological composition based on environmental variations, such as in seaweeds, seagrass and invertebrates (Pomin & Mourão, 2008).

Owing to this scenario on the production of mesohaline tilapia skin GAGs, indirect analysis by metachromasy assay concerning the influence of water source on the anionic composition in the skin matrix of *O. niloticus* was tested *in vitro* in the presence of DMB dye based on Fernandes et al. (2025). As expected by Fardale et al. (1976)' method, the figure 5 revealed that the metachromatic property was distinctly checked for the presence of sulfated GAGs in all test samples related to skin waste (fresh and dehydrated) removed from tilapia body. The positive reaction was concentration-dependent by a specific DMB dye-binding ability showing that the sulfated GAGs composition progressively changed the intensity in terms of violet property (Fernandes et al., 2025; Rodrigues et al., 2025). Thus, between both skin preparations confirmed those results found in figure 4 that demonstrated the dehydrated skins as the largest sources of sulfated GAGs, characterizing biochemical profiles of sulfated GAGs for both freshwater *vs*. mesohaline *O. niloticus* groups.

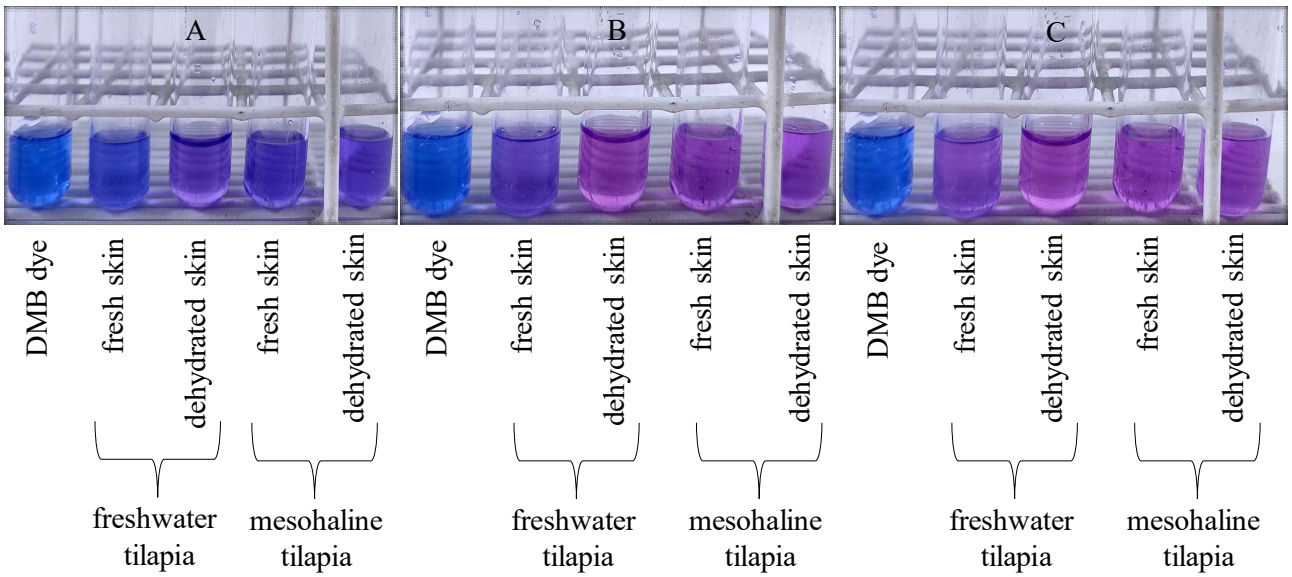


Figure 5. Positive reaction for metachromasy using 9 (A), 18 (B) or 27 (C) µg of freshwater or mesohaline *O. niloticus* (fresh or dehydrated) skin GAGs by checking with the DMB dye.

The morphological architecture of Nile tilapia skin is well-known (epidermis, dermis and hypodermis) (Franco et al., 2013; Alves et al., 2015). The epidermis-based physical barrier has glands that produces mucus which was subjected to papain digestion releasing sulfated GAGs from the mucosal site to the external surface of the fish body that act as physical-chemical immunological defense (Moreira et al., 2001), since the tilapia fish is sensitive to secondary infections in seawater salinities (Suresh & Lin, 1992).

Figures 4 and 5 suggested that the Nile tilapia exposured to 10‰ was isosmotic for it (Souza et al., 2019; Palmer et al., 2024) stimulating the mucous glands to oversecrete GAGs, as an osmorregulatory capacity to saline stress-induced fish (Boeuf & Payan, 2001) to maintain the ionic balance without compromising its growth (Souza et al., 2019; Mohamed et al., 2021). In fact, mesohaline tilapia-derived samples suggested a more intense detection for sulfate groups present in the chemical structures with a domain in charge density *vs*. for those from the freshwater tilapia (Figure 5C). Collectivelly, observations bring the hypothesis for a better biochemical understanding of the Nile tilapia, including it still in higher salinities with different phases of fish associated to

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
DOI: 10.18817/repesca.v16i4.4256
ARTIGO REVISTA BRASILEIRA DE ENGENHARIA DE PESCA

technological advancement (Suresh & Lin, 1992; Rodrigues et al., 2009; Souza et al., 2019), since the 10‰ stressed animals presented consequences on their physiological status related to their skin matrix water rate connected to sulfated GAGs content (Figures 3, 4, 5).

Studies on the distribution of GAGs in the extracellular matrix of fish organs have been evaluated (Souza et al., 2007; Fernandes et al., 2025). As the metachromatic profile showed a heterogeneity in charge composition of GAGs from the tilapia skin matrix (Figure 5), the electrostatic barrier of GAGs generated for the external surface of fish body against low salinity could be the result of intrinsic structural variations between the interface of these molecules and salinity. So, by the complexity of these physiological events led us to analyze the samples by FT-IR spectroscopy.

Salinity stressed Nile tilapia skin-extracted crude GAGs investigated by FT-IR

A comparative structural feature analysis by means of FT-IR was conducted among the GAGs-containing samples from the different Nile tilapia (fresh or dehydrated) skin preparations derived from both freshwater and mesohaline origins. The profiles of FT-IR, in the spectral window of 4000 to 500 cm^{-1}, characterized the preponderance of structural signals related to the presence of sulfated GAGs of hybrid chain or not within samples (Figure 6).

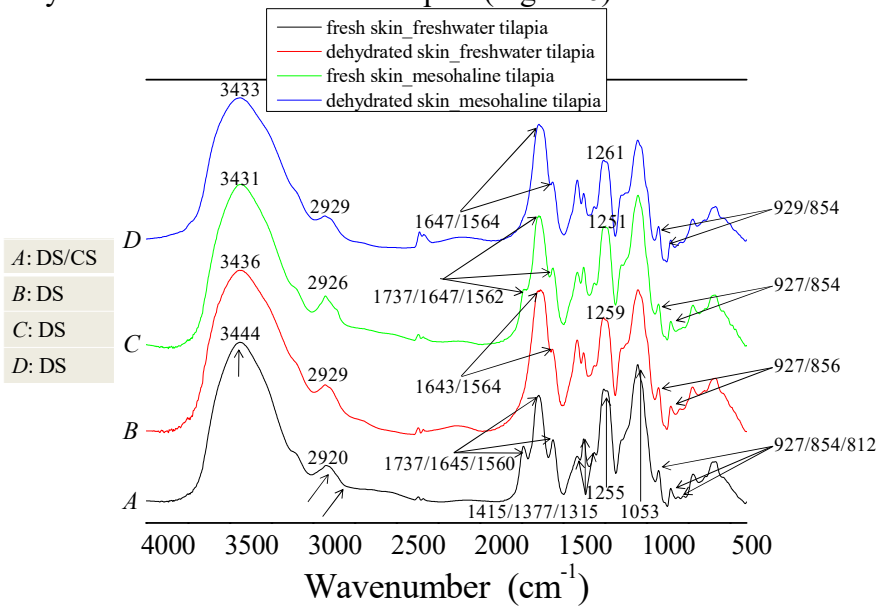


Figure 6. FT-IR spectra of (fresh-*A*,*C vs*. dehydrated-*B*,*D*) skin GAGs obtained from marked Nile tilapia (fresh-*A*,*B vs*. mesohaline-*C*,*D* water), using kBr samples at 500-4000 cm^{-1}. In left, tabulated classes of GAGs found for the respective skin preparations.

Skin of Nile tilapia showed in all the samples common characteristics for DS-type GAGs at 1200-900 cm^{-1}, as well as at 3431-3444 and at 2920-2929 cm^{-1} corresponding for -OH and C-H, respectively, signals of total sulfation at 1251-1261 cm^{-1} (S=O), of uronic acid at 1413-1415 cm^{-1}; and of absorptions related to amide I at 1643-1647 cm^{-1} (C=O/N-H), amide II at 1562-1564 cm^{-1} (N-H) and amide III at 1313-1375 cm^{-1} (N-H) (Pereira et al., 2021; Fernandes et al., 2025), but some structural variations were noted between both first-matter origins (Figure 6).

Curiously, such differences occurred around amide I and in the presence or not of signals related to the chemical class of GAGs, showing common three bands for respective fresh skin GAGs (at 1737/1647/1560-1562 cm^{-1}) (Figures 6A, C), as well as suggesting a DS/CS hybrid chain (at 854 and 812 cm^{-1}, sulfation at 4- and 6-carbon, respectively) from freshwater tilapia skin-derived GAGs (Figure 6A) (Pereira et al., 2021), but the absence in the mesohaline tilapia skin of signal

Rev. Bras. Eng. Pesca (2025) 16 (4): 11-24
DOI: 10.18817/repesca.v16i4.4256

around 820 cm^{-1} (sulfation at 6-carbon) for the sulfated GAGs-containing samples, respectively (Figure 6C). For dehydrated skin samples, it uniquely was found DS based on influence of papain digestion causing a spectral signal displacement at 856 cm^{-1} (Figures 6B, D) (Fernandes et al., 2025). These combined results led to the observation that this marked species contained different complex structures of GAGs, in hybrid chain or not, in its skin matrix when exposed or not to 10‰ salinity, where the overproduction of DS-type sulfated GAGs was preponderant to mesohaline individuals (Figures 4, 5, 6), since the variable position of sulfation on polysaccharides occurs in nature from species to species depending on the water source, as well as some variation occur in their glycosidic chains found in different tissues (Pomin & Mourão, 2008), as also postulated in the present study.

These observations raise the hypothesis that the Nile tilapia skin had DS/CS/amide I-related signals representing their original spatial conformation hydrating the freshwater fish-extracellular matrix (Figure 6A) (Liu et al., 2025) and, when it stressed by saline effect, its matrix would thicken, accumulating GAGs overproduced by the mucous glands of the epidermis (Moreira et al., 2001), protecting the tissue against osmotic desiccation to maintain ionic balance with the external environment, without harming zootechnical performance (Suresh & Lin, 1992; Souza et al., 2019), supporting already mentionated results (Figures 3, 4, 5).

Although showing an important molecular difference at level of sulfation degree/position between freshwater and mesohaline tilapia (Figure 6) as could occur in nature among different species (Dellias et al., 2004), the protection of the skin matrix by an antioxidant mechanism could not be discarded by sulfated GAGs acting as proton-donating substrate and, those signals (around 820 cm^{-1}) did not observe in the spectra of mesohaline tilapia-derived skin GAGs samples could be the result of primary sulfation sites involved in the sequestration of free radicals as demonstrated *in vitro* (Nascimento et al., 2021), possibly these radicals are origined during the fish's osmoregulation process, leading to an immunological defense effect by skin GAGs as observed in fish cryopreservation biology (Nascimento et al., 2021; Pereira et al., 2021).

The GAGs from Nile tilapia skin have also already been studied as *in vitro* anticoagulant agents (Rodrigues et al., 2011; Salles et al., 2017), opening new frontiers for the biotechnological use of waste generated by the processing of *O. niloticus* cultured in saline waters (Franco et al., 2013; Alves et al., 2015; Liu et al., 2025); besides, to the prospect of deepening biochemical research into the participation of GAGs in the osmoregulation process of the species when in higher salinities (Suresh & Lin, 1992; Rodrigues et al., 2009; Souza et al., 2019; Mohamed et al., 2021). A balanced diet containing probiotics and/or immunostimulants could be suggested due to the animal's energy expenditure during the transfer process to saline water, mapping, in parallel, the biochemistry of the skin matrix.

Conclusion

Commercially available freshwater or 10‰ mesohaline Nile tilapia (*Oreochromis niloticus*) had morphometry and body biomass unrelated to the yield of *in natura* skin. However, the comparative analysis by means of filleting-obtained fish skin revealed difference between raw fresh *vs*. dehydrated biomass yield, especially in dehydrated mass from the total fish and respective fresh skin. These combined results indicated a water loss by mesohaline fish skin after its dehydration in an oven that suggested as a compensatory effect of bony fish by a greater production of glycosaminoglycans during its osmoregulation process without loss of zootechnical performance. The structural variation of glycosaminoglycans by infrared analysis suggested that the dermatan/chondroitin hybrid chain found in freshwater tilapia fresh skin could have overproduced by mesohaline tilapia skin matrix as a proton-donating substrate in the antioxidant process, immunologically protecting the fish matrix against the adverse effects of salinity.

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