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León, Julián Guillermo; López, Jairo Humberto; Lozano, José Manuel; Muñoz-Ramírez, Adriana Patricia

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# Cryoprotective effect of sorbitol on the muscle microstructure of yamú (*Brycon amazonicus*) during storage at 2 and -18 °C

## Efecto crioprotector del sorbitol en la microestructura del músculo del yamú (*Brycon amazonicus*) durante su almacenamiento a 2 y -18 °C

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Julián Guillermo León[1]*, Jairo Humberto López[2], José Manuel Lozano[3] and Adriana Patricia Muñoz-Ramírez[4]

## ABSTRACT

Although freezing is generally used to preserve the sensory and nutritional quality of fish and their products, it cannot mitigate physicochemical changes of the fish meat during storage. This study aimed to determine the cryoprotective effect of sorbitol incorporated into the yamú muscle (*Brycon amazonicus*), subjected to different storage times and temperatures. The methodology consisted of analyzing microstructural changes, protein profile, and physicochemical properties (texture, water holding capacity and pH) of the yamú's meat under two temperatures (2±2 and -18±2 °C), two storage times (24 and 48 h) and the incorporation or not of 5% (w/w) of a 60% sorbitol solution. The microstructural changes were analyzed by optical microscopy and scanning electron microscopy, and the protein profile was analyzed by SDS PAGE electrophoresis. The physicochemical properties evaluated in yamú's meat were affected mainly by the interaction between temperature and storage time. The myofibrillar proteins underwent a partial degradation, and changes in the connective tissue were observed concerning the loss of texture especially when the meat was not treated with sorbitol at freezing temperature (-18 °C). The use of sorbitol minimized the negative effects of freezing on the characteristics of the yamú muscle, maintaining the integrity of the muscular microstructure and generating a cryoprotective effect in comparison to untreated meat.

**Keywords:**
Cryoprotectant
Fish
Freezing
Myofibrillar proteins
Preservatives
Texture

## RESUMEN

Aunque la congelación es generalmente usada para preservar la calidad sensorial y nutricional del pescado y sus productos derivados, no permite mitigar los cambios fisicoquímicos de la carne del pescado durante su almacenamiento. Este estudio tuvo como objetivo determinar el efecto crioprotector del sorbitol incorporado al músculo de yamú (*Brycon amazonicus*), sometido a diferentes tiempos y temperaturas de almacenamiento. La metodología consistió en analizar cambios microestructurales, perfil proteico y propiedades fisicoquímicas (textura, capacidad de retención de agua y pH) de la carne bajo dos temperaturas (2±2 o -18±2 °C), dos tiempos de almacenamiento (24 y 48 h) y la incorporación o no de 5% (p/p) de una solución de sorbitol al 60%. Los cambios microestructurales fueron analizados por medio de microscopia óptica y electrónica de barrido, el perfil proteico se analizó por medio de electroforesis SDS PAGE. Las propiedades fisicoquímicas evaluadas en la carne de yamú se vieron afectadas principalmente por la interacción entre la temperatura y el tiempo de almacenamiento. Las proteínas miofibrilares sufrieron una degradación parcial y se evidenciaron cambios en el tejido conectivo, relacionados con la perdida de textura especialmente cuando la carne no fue tratada con sorbitol a temperatura de congelación (-18 °C). El uso de sorbitol minimizó los efectos negativos de la congelación sobre las características del músculo de yamú, manteniendo en un estado más integro de la microestructura muscular, generando un efecto crioprotector en comparación con la carne no tratada.

**Palabras clave:**
Crioprotector
Pescado
Congelamiento
Proteínas microfibrilares
Conservantes
Textura

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. AA 14490, Bogotá, Colombia.
[2] Instituto de Ciencia y Tecnología de Alimentos-ICTA. Universidad Nacional de Colombia. Carrera 30 No. 45-03, Bogotá, Colombia.
[3] Facultad de Ciencias. Universidad Nacional de Colombia. AA 14490, Bogotá, Colombia.
[4] Facultad de Medicina Veterinaria y de Zootecnia. Universidad Nacional de Colombia. AA 7495, Bogotá, Colombia.
* Corresponding author: <jgleonr@unal.edu.co>

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Freezing is generally used to preserve the sensory and nutritional quality of fish and their products (Gonçalves *et al*., 2012). However, it cannot mitigate physicochemical changes during storage (Benjakul and Visessanguan, 2011) due to a different freezing speed between the outside and the inside of the cell, the migration of moisture from the interior of the cell to the extracellular space is generated, resulting in the growth of ice crystals (Hunt and Park, 2014). The formation of ice crystals and temperature changes during frozen storage damage the structure of the meat, which causes alterations in the biochemical reactions that occur at the cellular level and affect its physical quality parameters (Velasco *et al*., 2010; Benjakul and Visessanguan, 2011; Leygonie *et al*., 2012; Lee *et al*., 2017). These alterations, including the denaturation of the myofibrillar proteins, are associated with the loss of functional, nutritional and sensory properties of the meat, including gelling, emulsification, viscosity, solubility, and water holding capacity (Jacobsen *et al*., 2010; Andersen and Jørgensen, 2004; Lund and Baron, 2010; Nikoo *et al*., 2016).

Myofibrillar proteins include contractile proteins such as myosin and actin, regulatory proteins such as tropomyosin and troponin, and other minor proteins (Harnedy and Fitzgerald, 2012). These are mainly responsible for the functional properties of meat, especially myosin (Watabe *et al*., 1992; Ramírez *et al*., 2000) and are prone to denaturation during long-term frozen storage (Goeller *et al*., 2004; Medina and Pazos, 2010). Changes that occur in fish muscle during frozen storage can be minimized using appropriate cryoprotective additives (Nikoo and Benjakul, 2015), which can protect tissue from freeze damage by mitigating the growth of ice crystals (Alvarez *et al*., 2010). It is known that cryoprotectants such as carbohydrates and polyols reduce denaturation of myofibrillar protein during frozen storage, maintaining functional properties in meat (Kittiphattanabawon *et al*., 2012; Nikoo *et al*., 2016).

The yamú (*Brycon amazonicus*) is the most common species among the bryconids, native from the eastern Colombian plains, and the most explored for fish farming due to its omnivorous feeding habit, its rapid growth and nutritional efficiency, optimum taste of its meat, and its special characteristics for sport fishing (Arias, 2006). Yamú muscle is highly susceptible to quality loss induced during frozen storage. However, there are no studies on the effect of cryoprotectants on the fish's meat during frozen storage. For that reason, this study aimed to determine the cryoprotective capacity of sorbitol when it is incorporated into the yamú muscle and subjected to different storage times and temperatures.

## MATERIALS AND METHODS

### Biological material

The yamú fish (*Brycon amazonicus*) were obtained from an aquaculture production farm located in the Municipality of Lejanías, Meta, Colombia (3°31'33.8"N, 74°01'20.9"W), with a variable temperature from 6 °C in the paramo to average temperatures of more than 24 °C in the plain. 24 specimens of approximately 500 g each, fed with an artisanal diet, were sacrificed by thermal shock in ice water, eviscerated and immediately sent to the laboratory, kept packed in plastic bags under refrigeration at 4 °C in polystyrene refrigerators with ice for 4 h. Upon arrival, the specimens were washed with cold water (5±1.5 °C), and two fillets were obtained from each one.

### Experimental treatments and sample preparation

The fillets were stored at 2±2 °C and -18±2 °C, for 24 and 48 h with the injection of a 60% sorbitol solution (Sorbitol USP 70%, Ciacomeq SAS) until achieving an absorption of 5% (w/w). In total, eight treatments with three repetitions each were performed (Table 1).

### Texture analysis

The texture was evaluated by a compression test using an electronic texturometer model Stable Micro System texture analyzer (TA.XT2, Surrey, England) (Larsson *et al*., 2014). All tests were done at refrigeration temperature (4 °C). Three cubes of 2 cm×2 cm×1 cm were taken from each fillet – the speed before the test: 1.00 mm $s^{-1}$, the speed of the test: 1.10 mm $s^{-1}$ and the speed after the test: 10.00 mm $s^{-1}$, the distance between the cylinder and the sample: 15.0 mm, and the sample compression: 40.0%.

### Water holding capacity (WHC)

The water holding capacity (WHC) of the yamú meat samples was carried out following the methodology proposed by Sánchez-Alonso *et al.* (2012). Three grams of the sample were wrapped in filter paper (two filter papers

Whatman No. 1, 110 mm diameter) previously weighed, then they were introduced in a falcon tube and centrifuged for 15 min at 3000 g. After centrifugation, the papers were carefully removed and weighed. The WHC was expressed as a percentage of water retained by the sample after centrifugation.

**Table 1**. Experimental design of the yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage.

| Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Sorbitol injection | without sorbitol | with sorbitol | without sorbitol | with sorbitol | without sorbitol | with sorbitol | without sorbitol | with sorbitol |
| Temperature | 2±2 °C | | -18±2 °C | | 2±2 °C | | -18±2 °C | |
| Storage time | 24 h | | | | 48 h | | | |

**pH measurement**

The pH of the resulting suspension was measured with a calibrated pH meter after homogenizing 10 g of the sample with 100 mL of distilled water according to the methodology described by Mohan *et al.* (2007).

**Optical microscopy**

Samples were immersed in formalin buffer at 4% and embedded in paraffin. The plates with the extended sample were stained with Masson's trichrome in order to highlight the collagen fibers. For this process, they were initially washed with distilled water and then immersed in Weigert's Hematoxylin for 5 min, then washed with distilled water and immersed in Ponceau fuchsin for 5 min, then immersed in phosphomolybdic acid for 5 min. This solution was transferred to a blue aniline solution for 5 min. Finally, the samples were analyzed with a microscope (Leica DM750 P, Leica Microsystems, Heerbrugg, Switzerland) and images were taken with different approaches (4X, 10X and 40X) (Castañeda *et al*., 2016).

**Scanning electron microscopy (SEM)**

The samples for this procedure were immersed in formalin buffer at 4%, then dried to critical point using the EK 3150 equipment for 15 min and then metalized with gold using an equipment Quorum Q150R ES, to proceed to the observation with a microscope SEM (FEI, Quanta 200 -r). The fish samples were mounted on individual supports. The images obtained were captured with magnitudes of 200X, 1000X and 4000X (Castañeda *et al*., 2016).

**Protein extraction and quantification**

Protein extraction was carried out by applying modifications to the method proposed by Cao *et al*. (2006). 1 g of sample was homogenized in an Ultra-turrax® IKA T-25 USA homogenizer with four volumes of distilled-deionized water for 30 s and centrifuged at 5000 g and 4 °C for 5 min, the supernatant was removed. The precipitate was washed with 50 mM sodium phosphate buffer (pH 7.5) for 30 s; then the homogenate was centrifuged at 5000 g and 4 °C for 5 min, the precipitate was suspended in 4 volumes of cold sodium phosphate buffer 50 mM (pH 7.5), the centrifugation process was repeated twice, the supernatant was discarded, and one last centrifugation was performed at 3000 g for 15 min. The final precipitate was diluted with 50 mM phosphate buffer and 500 mM NaCl pH 8.0. Each of the protein extracts was added with 5 µL 100 mM iodacetamide and 25 µL Ethylenediaminetetraacetic acid (EDTA) 50 mM as enzyme inhibitors.

Quantification of each protein extract was carried out to standardize the amount of protein in each sample. Quantification of protein concentration was performed following the bicinchoninic acid (BCA) method, using a Pierce ™ BCA® Thermo Scientific protein determination kit for total protein counting. This method combines the protein reduction of cupric ($Cu^{+2}$) to cuprous cations ($Cu^{+1}$) in an alkaline medium and the reaction of green to purple color by the chelation of the BCA molecules with the cuprous ions. This water-soluble complex exhibits a strong absorbance at 562 nm that is almost linear with increasing protein concentrations in a range from 20 to 2000 µg $mL^{-1}$.

### Electrophoretic mobility profiles by SDS-PAGE

For the identification of myofibrillar protein profiles of yamú meat, polyacrylamide gel electrophoresis was carried out with an electrophoresis system (Mini-PROTEAN® Tetra Vertical Electrophoresis Cell, Bio-Rad, 25 USA). The separation gel was 7%, the concentration gel was 5% (w/v), the run buffer used was Tris-glycine pH 8.3. 30 µg of total protein of the protein extracts obtained from each sample were used. The separation was performed at a constant voltage of 120 V for the concentration gel and 100 V for the resolution gel. A molecular weight standard from 12 to 225 kDa was used, and the staining was performed with colloidal blue G-250 (CBB), which was prepared by diluting 125 g of CBB in 125 mL of isopropanol, then 50 mL of glacial acetic acid was added, and a volume of 500 mL was completed with deionized distilled water (Laemmli, 1970).

### Statistical analysis

A descriptive analysis was made by treatment to the data obtained for texture, water retention capacity, and pH, to explore measures of central tendency, dispersion, and atypical observations in the data. The eight treatments were analyzed according to a completely randomized design in a factorial arrangement $2^3$, where the factors were storage temperature, time and use of sorbitol. Each treatment had three repetitions. An ANOVA was performed to determine whether there was a significant difference among the levels of each of the factors. When a difference was found between the levels of the factors, a Tukey test was performed to find which of the treatments had a significant difference. An ANOVA was performed for each response to establish the difference and interaction among factors. In the presence of interaction, the interaction graphs were constructed to be able to conclude on the best treatments. The R Studio 1.1.383® program (2017) was used.

## RESULTS AND DISCUSSION

### Texture analysis

The variance analysis performed for the texture parameters obtained indicate that there was a significant difference between treatments ($P$<0.05). The Tukey test suggests that the treatments subjected to the incorporation of sorbitol -18 °C for 24 and 48 h (T4 and T8) obtained the highest compression force value and did not present significant difference among them (Figure 1).

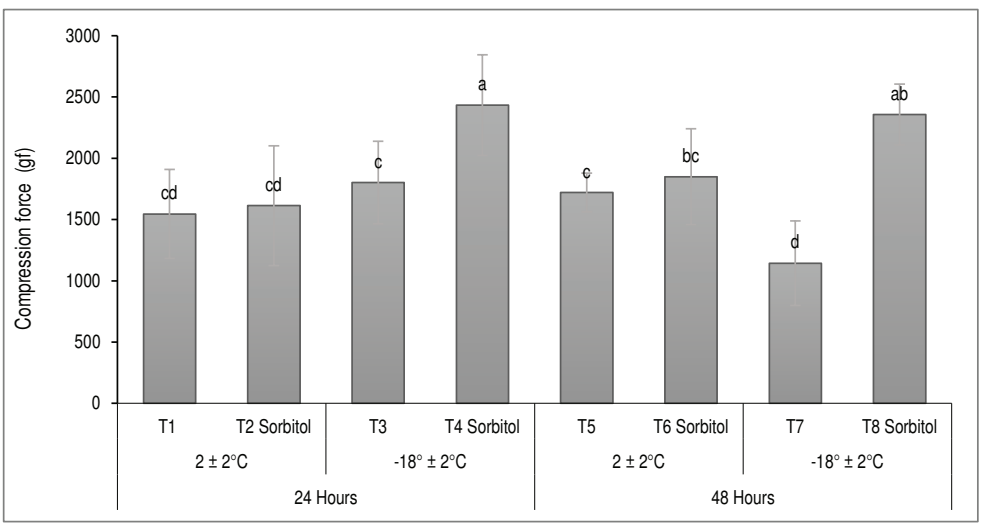


**Figure 1.** Variation of the compressive force of the yamu´s meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage. Different letters represent significant differences.

The incorporation of sorbitol and the storage time caused a significant difference ($P$<0.05) on the values of the texture of yamú's meat, separately ($P$<0.05). In turn, its values presented a significant difference ($P$<0.05) due to the interaction between the incorporation of the sorbitol and the storage temperature. It is noteworthy that the texture values are higher when the meat is treated with sorbitol and stored at -18 °C regardless the time it was stored

because the cryoprotectant acts only at freezing temperature, preventing the formation of intracellular or intercellular ice crystals that may affect the texture. The interaction ($P$<0.05) of the incorporation of sorbitol with the storage time made possible to observe that the texture of the meat is higher in all cases, in which sorbitol is incorporated, especially when compared with the meat that was stored for 48 h without the incorporation of the cryoprotectant (Figure 2).

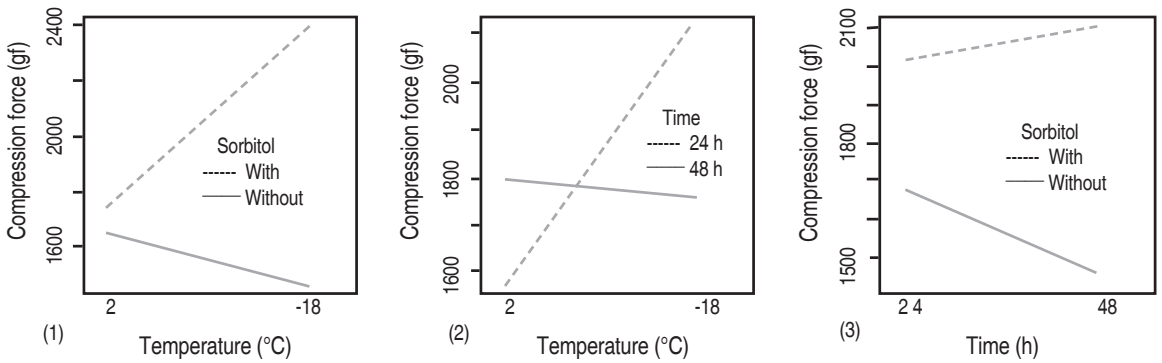


**Figure 2.** Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) on the variable texture.

The presence of interaction ($P$<0.05) between temperature and storage time indicates that the freezing temperature maintains a greater texture in the meat during 24 h of storage in comparison with the decrease in texture observed at 48 h of frozen storage, independently of the use or not of sorbitol. The effect of sorbitol is not visible when the meat is stored at a temperature of 2 °C regardless of the storage time, 24h (T2) and 48 h (T6). Besides, significant texture loss was obtained in the treatments that did not use the sorbitol incorporation at 2 °C for storage time of 24 h (T1) and 48 h (T5); the same occurred to the 24 h storage at -18 °C without sorbitol (T3), and with an even higher loss when the meat was stored for 48 h at -18 °C without sorbitol (T7). However, when the incorporation of sorbitol was used at -18 °C for 24 h (T4), the highest compressive strength was obtained, although this is diminished at the 48 h (T8) of storage.

The results in the compression test show higher values in all treatments than those reported for fillets of the same species stored at -8 °C for 61 h (Castañeda *et al*., 2016) and for *Brycon cephalus* stored at -3 °C for 12 h (Suárez Mahecha *et al*., 2006). These results may be influenced by the time elapsed from slaughter to storage, storage time, diet and treatment with sorbitol. However, the data are similar to those reported for *Urophycis chuss* treated with the incorporation of sorbitol and sodium tripolyphosphate (Bigelow and Lee, 2007).

The loss of texture is evident when the meat is subjected to -18 °C without the incorporation of sorbitol due to damages generated by the formation of intracellular and intercellular ice crystals that damage the muscular structure (Tomaniak *et al*., 1998; Leygonie *et al*., 2012); there is a higher damage as storage time elapses. On the other hand, the incorporation of sorbitol in the meat is effective when it is stored at a temperature of -18 °C, thus obtaining values of compressive strength above other treatments, with similar results to those obtained in *Onchorynchus mykiss* fillets, immersed in an 8% solution of sorbitol and sucrose and stored at -20 °C (Jittinandana *et al*., 2003).

**Water holding capacity and pH**

The analysis of variance performed for the WHC parameters obtained shows that there was a significant difference between treatments ($P$<0.05). The Tukey test indicates that the treatment subjected to 24 h at 2 °C without the incorporation of sorbitol (T1) obtained the highest WHC (70.4%), while the treatments T3 (-18 °C for 24 h) , T5 (2 °C for 48 h), and T7 (-18 °C for 48 h), that also did not include the incorporation of sorbitol,

presented statistical similarity among them and low WHC with values of 63.05, 65.7 and 62.01%, respectively. Being the lowest value, the one obtained for T7. The treatments that used the sorbitol incorporation T2 (2 °C for 24 h), T4 (-18 °C for 24 h), T6 (2 °C for 48 h), and T8 (-18 °C for 48 h) had WHC values of 66.30, 66.47, 63.46, and 66.92, respectively; these treatments were statistically similar except for T6 (Figure 3).

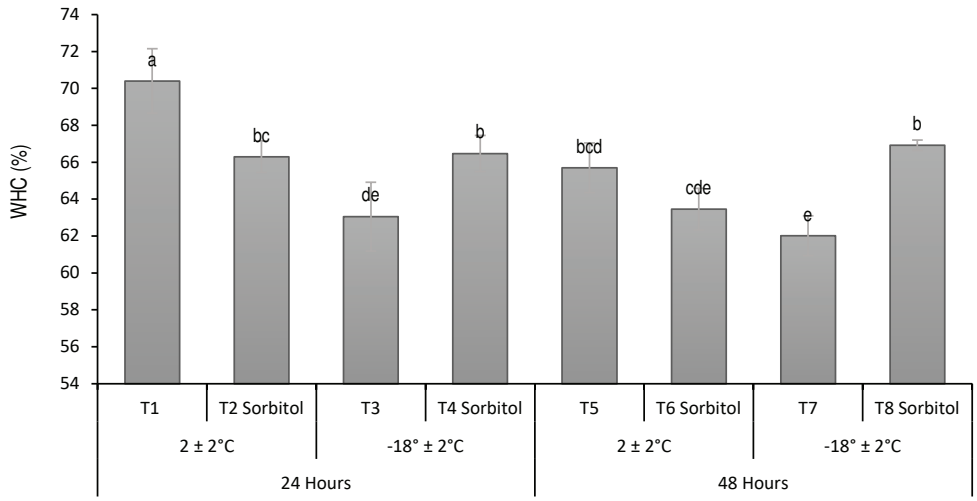


**Figure 3.** Variation of WHC of yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different storage times and temperatures. Different letters represent significant differences.

The interaction of the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) affected the variable WHC (Figure 4). These values were influenced by temperature and storage time ($P$<0.05); the interaction between the incorporation of sorbitol with storage temperature also presented a significant effect ($P$<0.05) because the WHC presented the lowest value when the meat was stored at -18 °C without the incorporation of the cryoprotectant, while this characteristic remained when the meat was treated with sorbitol. The interaction between temperature and storage time ($P$<0.05) showed that, independently of the storage temperature, WHC decreases after 48 h for all treatments, especially for those who were not treated with sorbitol.

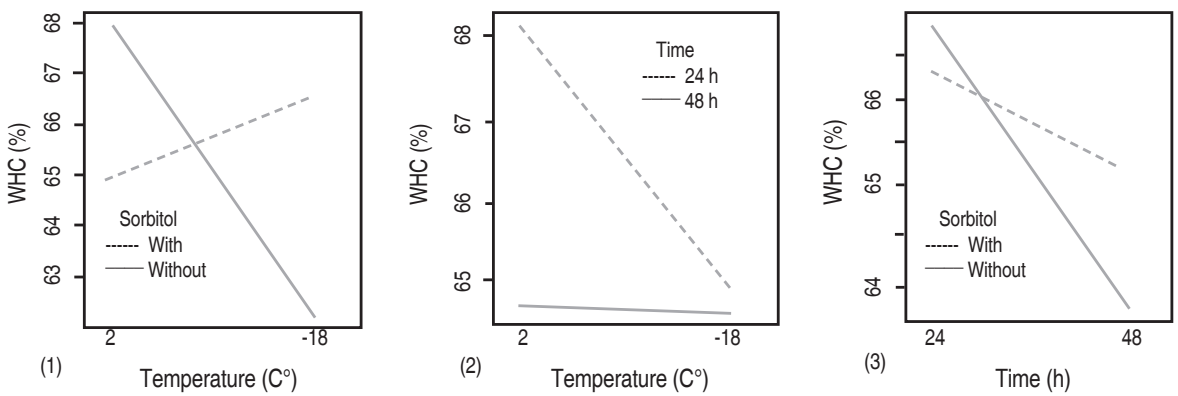


**Figure 4.** Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of meat of yamú (*Brycon amazonicus*) on the variable water holding capacity.

WHC reported a significant reduction in all treatments, decreasing even more in those that did not have the inclusion of sorbitol. This reduction is explained by the injection of 5% (w/w) of the sorbitol solution that increased the moisture of the treated fillets. This decrease in WHC is due to the loss of functionality of cell membranes, which suffer irreversible damage during freezing, losing their rehydration capacity due to loss of lipid components (Seidel, 2006), and denaturation of myofibrillar proteins such as actin and myosin (Andersen and Jørgensen, 2004; Lund and Baron, 2010). The results of water retention capacity are related to the results obtained in the compression test, where the treatments that maintained the values of WHC (Figure 3) and obtained the highest values of compressive strength (Figure 1) were the same subjected to -18 °C under the incorporation of sorbitol after 24 and 48 h of storage.

The pH variation of yamú's meat was subjected to the incorporation or not of sorbitol at different storage times and temperatures (Figure 5). The Tukey test showed that the treatment subjected to 2 °C for 24 h with the incorporation of sorbitol (T2) obtained the highest pH (6.17), followed by the treatment subjected to 2 °C for 48 h with the incorporation of sorbitol (T6) with a pH value of 6.08. The treatment subjected to -18 °C for 48 h without the incorporation of sorbitol (T6) was the one that obtained the lowest pH (5.93), which showed statistical similarity with the treatment without sorbitol incorporation at -18 °C for 24 h (T3), presenting a pH of 5.95.

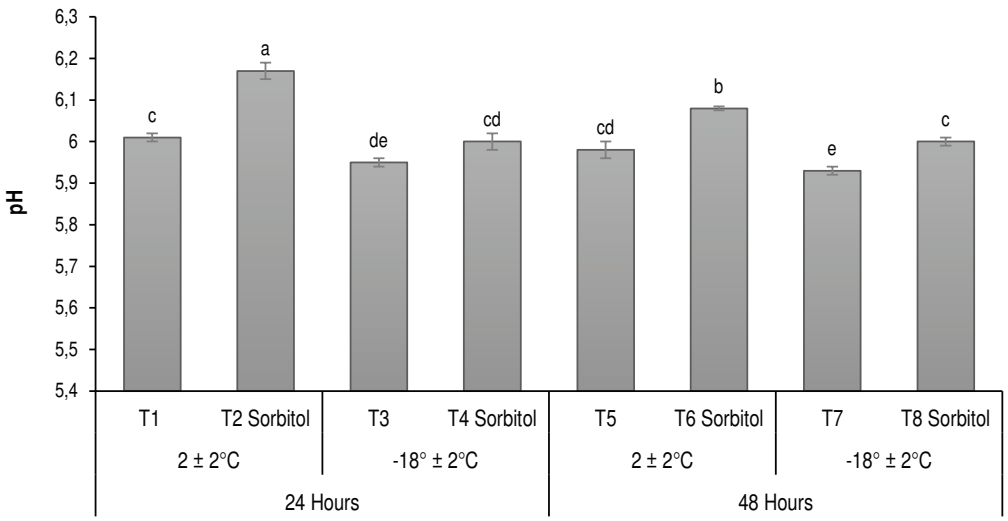


**Figure 5.** Variation of the pH of the yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage. Different letters represent significant differences.

Low pH was evidenced for the treatments without the incorporation of sorbitol at 2 °C and -18 °C for 24 h of storage (6.01 and 5.95 for T1 and T3 respectively) which decreases after 48 h of storage (5.98 and 5.93 for T5 and T7 respectively). The pH for the treatments stored at -18 °C for 24 and 48 h with the incorporation of sorbitol (T4 and T8) was 6.00 (Figure 6), showing a statistical similarity between them.

The pH is higher when sorbitol is added to the yamú meat, regardless of the storage time. However, in the absence of sorbitol, storage at -18 °C causes a reduction in pH, especially when this is prolonged by 48 h. The decrease in pH during storage indicates the transformation of muscle glycogen into lactic acid during the post-mortem stage (Einen *et al*. 2002). Similarly, a low pH is directly related to the loss of texture (Ang and Haard, 1985; Kiessling *et al*., 2004) due to the weakening of connective tissue and protein denaturation (Lavéty *et al*., 1988). The pH remains stable during storage for 48 h when sorbitol is incorporated in the meat subjected to -18 °C.

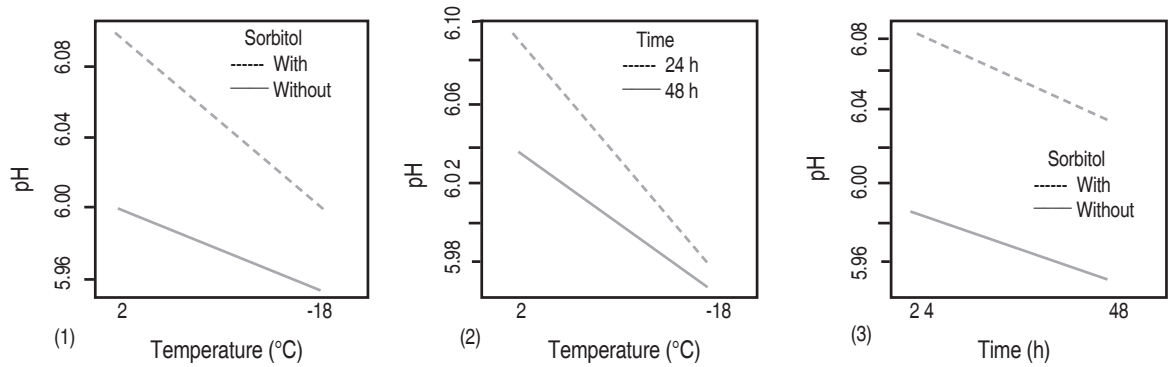


**Figure 6.** Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) on the variable pH.

## Scanning electron microscopy

The treatments stored for 24 h at 2 °C with or without the incorporation of sorbitol (T1 and T2) showed the myofibrillar structure in its integral state without the separation of the myotomes, while a slight separation of them is observed in the treatments stored for 24 h at -18 °C (T3 and T4). Besides, the changes in the organization of muscle fibers were evidenced in treatments stored at 2 °C for 48 h (T5 and T6). For the treatment stored at -18 °C for 48 h (T7) showed a greater separation of myotomes and myofibrils than the treatment that had the incorporation with sorbitol at the same temperature and storage time conditions (T8), where the separation of the myofibrils was not so evident (Figure 7).

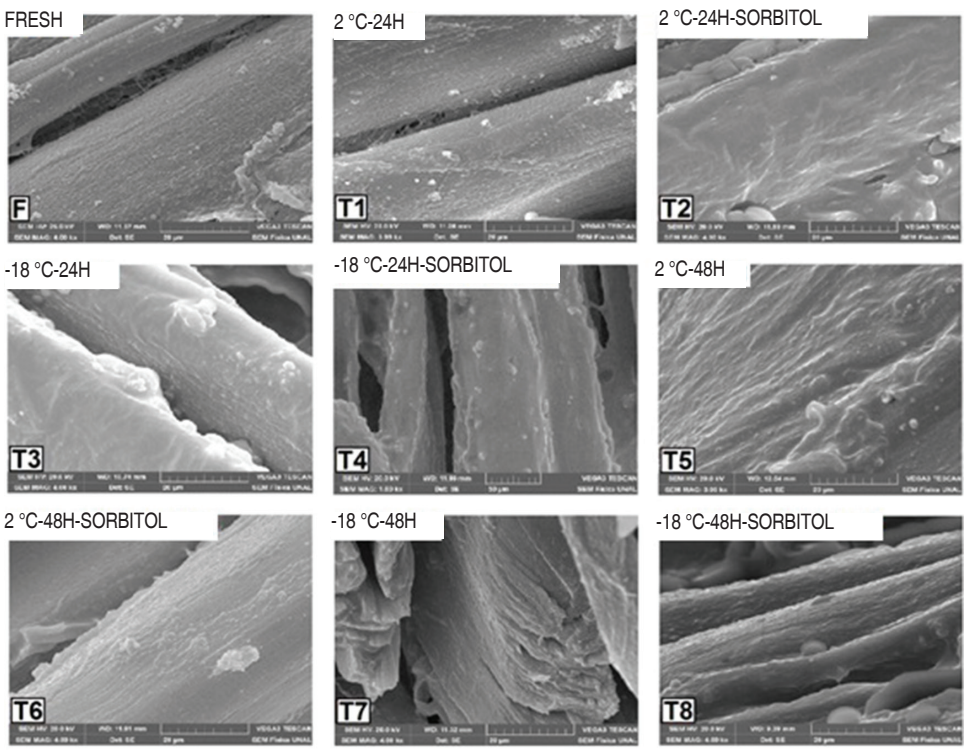


**Figure 7.** Scanning electron microscopy (SEM) images of yamú meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage times and temperatures.

**Optical microscopy**

The cross sections of the muscle fibers show slight separation of the endomysium in treatments stored for 24 or 48 h at 2 °C not treated with sorbitol (T1 and T5), whereas treatments with sorbitol incorporation under the same conditions did not present this separation. The structure of the endomysium was affected in larger areas in treatments subjected to -18 °C and not treated with sorbitol regardless of the storage time (T3 and T7) in addition to the presence of interfibrillar separations. Treatments subjected to -18 °C with sorbitol incorporation showed a lower separation of endomysium and interfibrillar spaces (T4), although slightly higher when storage was prolonged for 48 h (T8) (Figure 8).

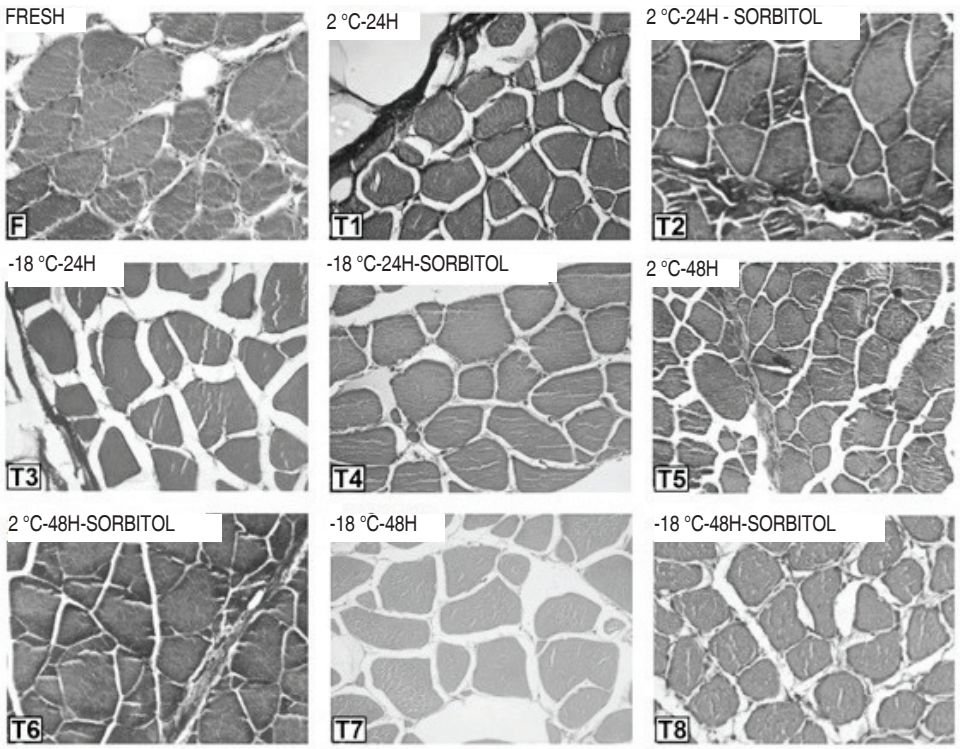


**Figure 8.** Optical microscopy analysis of yamú meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage imes and temperatures.

The results obtained by optical microscopy and scanning electron microscopy showed the degradation of the connective tissue due to storage at low temperatures; especially at -18 °C, where the loss of interfibrillar tissue, spaces in the myocomata and the separation between myotomes are evident. Those changes in the microstructural tissue are associated with the detachment of the endomysium already reported for other species (Shigemura *et al.,* 2003) and are independent of the storage time (Figure 4 and 5) . Similar results are reported for fillets obtained from the same species and stored at a temperature of -8 °C (Castañeda *et al*., 2016). These changes in muscle structure indicate the degradation of collagen due to storage at low temperatures, which has been reported for several species (Ando *et al*., 1991; Sato, *et al*., 1991; Sato *et al*., 1997; Shigemura *et al*., 2003; Larsson *et al*., 2014). The formation of ice crystals during freezing damages the ultrastructure of the muscle of fish, which causes alterations in the biochemical reactions that occur at the cellular level and influence the physical quality parameters of the meat (Leygonie *et al*., 2012). However, the muscle structure was maintained in a better condition when the meat was treated under the same storage conditions and with the incorporation of sorbitol, finding less separation between myotomes and greater integrity of the interfibrillar tissue.

**Electrophoretic mobility profiles by SDS-PAGE**

Up to 14 bands were detected in polyacrylamide gel electrophoresis (Figure 9). The bands with the highest molecular weight (243.54 and 220.93 kDa) were found in all treatments, with a higher intensity in treatments stored for 48 h (T5, T6, T7, and T8). In addition, bands with lower molecular weight (156.08 kDa, 104.39 kDa, 99.43 kDa, 79.09 kDa, 76.72 kDa, 71.31 kDa, 67.51 kDa, 60.93 kDa, and 57.68 kDa) were found in all treatments, especially in those subjected to -18 °C (T3 and T4) and with a slightly higher intensity in treatments subjected to -18 °C for 48 h without sorbitol incorporation (T7 and T8). Bands with a molecular weight of 45.76 and 43.96 kDa with low intensity were presented for treatments stored at 2 °C for 24 h (T1 and T2). The same bands were slightly more intense for the treatments stored at -18 °C for 24 h (T3 and T4) and a higher intensity for the treatments stored at 2 and -18 °C and with (T6 and T8) or without (T7).

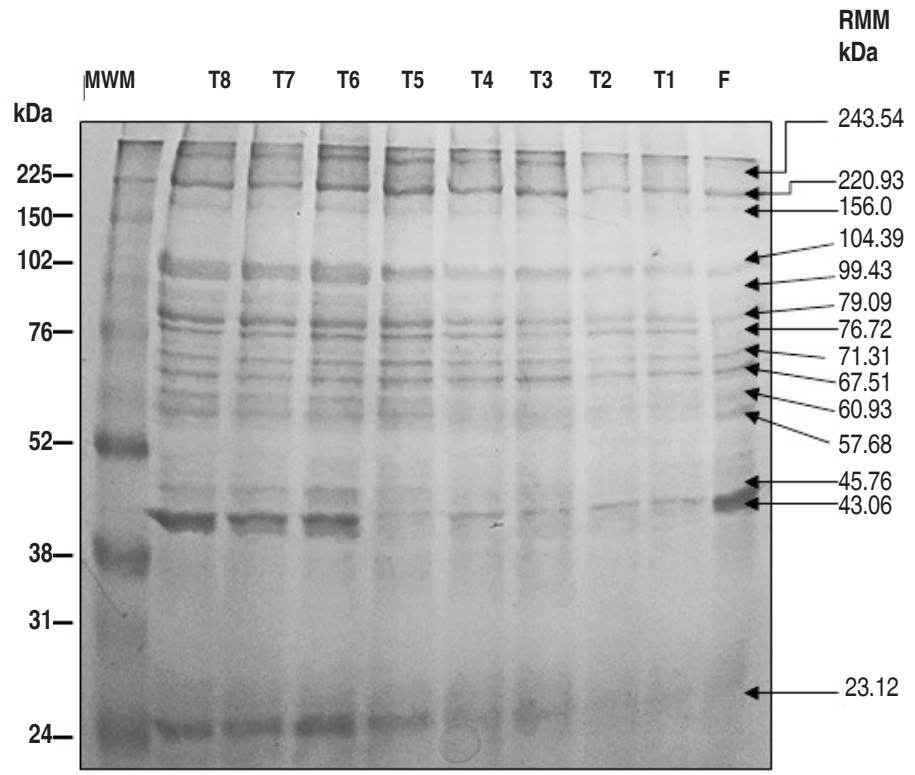


**Figure 9.** SDS-PAGE gel electrophoresis of 7% acrylamide of protein extracts of yamú's meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage times and temperatures. MWM line: Molecular Weight Marker; RMM: Relative Molecular Mass.

Myofibrillar proteins include contractile proteins such as myosin and actin, regulatory proteins such as troponin, and other minor proteins (Harnedy and Fitzgerald, 2012). Other authors have reported the molecular weight of some proteins present in fish meat, such as long chain myosin 200 kDa (Liu *et al*. 2013), α-actin 105 kDa, troponin 78 kDa (Ladrat *et al.* 2003) and actin 45 kDa (Cao *et al*. 2006). Bands with similar molecular weights can be observed in all treatments with a greater intensity in treatments stored for 48 h (T5, T6, T7, and T8) where the bands with similar molecular weights to heavy chain myosin (220.93 kDa), α-actin (104.39 kDa), troponin (79.09 kDa). and actin (45.76 and 43.06 kDa) were maintained. On the other hand, bands with higher (243.54 kDa) and smaller molecular weight (156.08, 99.43, 76.72, and 23.12 kDa) were also found and are not present in the line corresponding to the analyzed protein profile of the fresh meat (F), and they have greater intensity at 2 °C and -18 °C for 48 h of storage with the addition or not of sorbitol (T5, T6, and T7). It can be inferred that these bands are protein aggregates or partially degraded myofibrillar

protein fragments, which are largely responsible for the functional properties of meat during refrigerated or frozen storage (Mackie, 1993). The fragmentation or aggregation of the myofibrillar proteins generates losses of their functionality and solubility generating softening (Medina and Pazos, 2010; Nikoo *et al*. 2016; Leygonie *et al*. 2012), being lower in the treatment subjected to -18 °C for 24 h with the incorporation of sorbitol (T4).

## SUMMARY

The yamú's meat suffered a partial degradation of its myofibrillar proteins such as myosin, α-actin and actin, especially when it was not treated with sorbitol at freezing temperature (-18 °C), besides there were changes in the connective tissue, related to the loss of texture. The physicochemical properties evaluated in yamú's meat were affected mainly by the interaction between temperature and storage time, while the incorporation of sorbitol and the storage temperature had a significant effect on the texture of yamú's meat. The water holding capacity was significantly affected by the time and temperature of storage, and the pH of the meat was significantly affected by all the evaluated factors.

## CONCLUSIONS

The main damages caused by the freezing of the muscular structure of the yamú's meat are presented by the loss of the connective tissue and denaturation of the myofibrillar proteins, which generates the loss of functional characteristics such as texture and water holding capacity. The use of sorbitol at -18 °C minimized the negative effects of freezing on the characteristics of the yamú's muscle, maintaining the muscular microstructure in a better condition, generating a cryoprotective effect compared to untreated meat.

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