

## EFFECTS OF FREEZE-DRYING ON THE STRUCTURE OF NILE TILAPIA OREOCHROMIS NILOTICUS FILLETS

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**Pauliana Leão de Souza<sup>1</sup>, Leonardo Balcewicz Junior<sup>2</sup>, Daniel da Silva Ladislau<sup>3</sup>,  
Adriano Teixeira de Oliveira<sup>4</sup>, Eduardo Luis Cupertino Ballester<sup>5</sup> and Altevir Signor<sup>6</sup>**

### ABSTRACT

This study aims to investigate the effects of freeze-drying on the structure of freeze-dried *Oreochromis niloticus* tilapia fish at different processing times. Foram analyzed water activity (Aw), texture, core and Varredura Electron Microscopy (MEV). The results demonstrate alterations in the theory of mass and structure of two fibers over a long period of time, with the formation of fissures and detachment of muscle fibers. The effects of the process are greater than we file them with less mass theory (2.5 kg) freeze-dried for 36 hours. The use of freeze-drying for dehydration of tilapia fish has been shown to be effective, providing the removal of water theory from two tissues. Meanwhile, its use causes alterations in the physical-chemical and structural properties of the food. For this reason, even sensory analysis is necessary, aiming to better understand the effects of the process on the other characteristics and oilability of the product, such as the flavor of the filé.

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<sup>1</sup> MSc in Fisheries Resources and Engineering  
State University of Western Paraná  
ORCID: <https://orcid.org/0000-0003-1985-7322>  
LATTES: <http://lattes.cnpq.br/6911195798439428>  
E-mail: paulianaleao@gmail.com

<sup>2</sup> PhD in Innovative Extension and Sustainable Rural Development  
State University of Western Paraná  
ORCID: <https://orcid.org/0000-0002-0418-7701>  
LATTES: <http://lattes.cnpq.br/3496552546615582>  
E-mail: lbjr1266@hotmail.com

<sup>3</sup> PhD in Fisheries Resources and Engineering  
State University of Western Paraná  
ORCID: <https://orcid.org/0000-0002-0467-6353>  
LATTES: <http://lattes.cnpq.br/8098824072487689>  
E-mail: danielladislau@gmail.com

<sup>4</sup> PhD in Biological Diversity  
Federal Institute of Education, Science and Technology of Amazonas  
ORCID: <https://orcid.org/0000-0003-4988-9878>  
LATTES: <http://lattes.cnpq.br/9164471794674935>  
E-mail: adriano.oliveira@ifam.edu.br

<sup>5</sup> PhD in Biological Oceanography  
Federal University of Paraná  
ORCID: <https://orcid.org/0000-0001-5199-1754>  
LATTES: <http://lattes.cnpq.br/7710902249475122>  
E-mail: elcballester@ufpr.br

<sup>6</sup> PhD in Animal Science  
State University of Western Paraná  
ORCID: <https://orcid.org/0000-0002-4659-6466>  
LATTES: <http://lattes.cnpq.br/4844380942902865>  
E-mail: altevir.signor@gmail.com

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## INTRODUCTION

Aquaculture and fishing have guaranteed food security and nutrition for the populations of several countries, especially those with developing economies such as Brazil (Andrade et al., 2024; Oliveira et al., 2023; Rodrigues et al., 2024). In 2022 alone, global aquaculture production reached a record 185 million tonnes, of which 51% (94 million tonnes) came from aquaculture. This growth was driven by increased global aquatic food consumption (20.7kg per capita in 2022) (FAO, 2024).

In 2023, Brazil reached the historic mark of 655.3 tons in the production of farmed fish, resulting in a production value of 6.7 billion reais, with tilapia *Oreochromis niloticus* being the main species cultivated in the country and the state of Paraná being the largest producer of the species in the national territory (PPM, 2023). The choice of tilapia by Brazilian producers is related to its rusticity, genetics, easy reproduction and market potential, and white meat and mild flavor (Pedroza Filho et al., 2020; Góes-Favon et al., 2021). In the national market, tilapia is sold mainly as fresh or frozen fillets (Silva et al., 2022).

Currently, different techniques are used to preserve foods of aquatic origin, from more traditional methods, such as drying (natural, by salting or with hot air), to those with high-performance technology (microwaves, heat pump, combined drying and vacuum freeze-drying) (Zeng et al., 2024). Freeze-drying is a dehydration process based on the sublimation of ice to remove liquids from foods or products (Harguindeguy and Fissore, 2020). The use of this method has been growing within the food industry because it generates high-quality products with low loss of food characteristics, such as flavor and aroma, being used in different types of food (Zeng et al., 2024; Oyinloye and Yoon, 2020). Thus, it is becoming a new technological tool companies use in food. It provides added value to the product, increased production and reduced costs with equipment, energy, waste, transportation and storage (Pisano et al., 2014).

Although freeze-drying causes little loss of food characteristics, depending on the process conditions, interference may occur mainly in structural properties, such as denaturation and oxidation of proteins, changes in density, porosity and shrinkage, in addition to changes in the color and texture of food (Nowak and Jakubczyk, 2020; Lee et al., 2024). In this case, monitoring parameters such as water activity ( $A_w$ ), color, texture and Scanning Electron Microscopy (SEM) are essential to determine food quality. Water activity ( $A_w$ ) helps prevent and limit microbial growth in food, being a necessary parameter for

inhibiting microbial growth and thus ensuring shelf stability of food based on its moisture content (Barbosa-Cánovas et al., 2007; Santos et al., 2012; Lemos et al., 2015; Magro et al., 2016). Appearance (color) and texture are among the main factors of food quality, influencing consumer preference, and are evaluated in different food processing operations (Rustagi, 2020). Scanning Electron Microscopy (SEM) has been very important in the food industry, as it makes it possible to observe the damage caused to the structure of foods (Dalvi-Isfahana et al., 2019).

In Brazil, only some studies still involve the freeze-drying of aquatic foods. Some studies seek to evaluate the physical-chemical composition of freeze-dried flour based on waste from native species, such as the pirarucu *Arapaima gigas*, aiming at adding value to new products (Barbosa et al., 2021). Others have developed freeze-dried frog meat soups aimed primarily at consumers on hypoallergenic diets (Andrade et al., 2022). However, research on the freeze-drying process and its effects on fish meat is still in its infancy (Paula da Costa et al., 2023; Souza et al., 2024). Therefore, the objective of the present study was to investigate the effects of the freeze-drying process on the structure of tilapia fillets from the fishing industry.

## MATERIALS AND METHODS

### FREEZING AND FREEZE-DRYING OF TILAPIA FILLETS

For the experiment, 50 kg of fresh tilapia fillets were used, which were initially frozen at -30 °C for 24 hours in a freezer (Dynamic 1,030 L). A prototype vertical freeze dryer (BR 10 2020 010874 3) was used to freeze-dry the fillets.

After freezing, the samples were randomly arranged on the freeze dryer shelves in non-standard sizes until reaching the desired weight. Four times and three processes with different weights for each time were established for dehydration (Table 1).

Table 1: Freeze-drying time for Nile tilapia *Oreochromis niloticus* fillets and the three processes with different initial masses.

Time (Hours)	Mass (kg)		
18	2,500	3,500	4,500
24	2,500	3,500	4,500
30	2,500	3,500	4,500
36	2,500	3,500	4,500

## WATER ACTIVITY ( $A_w$ )

The  $A_w$  analyses were performed using an Aqualab 4 TE food water meter. Initially, the samples were placed in polyethylene containers for later reading on the device. The reading time varied between 10 and 20 minutes, and the temperature was maintained at 25°C.

## TEXTURE

The texture of the fillets was determined according to the shear force exerted to cut the sample using a TA HD Plus texturometer equipped with a WarnerBratzler triangular cutting blade, 3.0 mm thick and 70 mm wide. The samples were cut into 2 cm<sup>3</sup> cubes, always in the transverse direction of the muscle fibers.

## COLOR

To determine the color standards, the guidelines described by the CIEL<sup>\*</sup>a<sup>\*</sup>B<sup>\*</sup> model recommended for colorimetric food tests were followed. The results were expressed in terms of lightness—L<sup>\*</sup> (black-white), hue—a<sup>\*</sup> (red-green) and chromaticity—b<sup>\*</sup> (yellow-blue) (1). The analyses were performed using a Konica Minolta Chroma Meter model CR400 colorimeter.

## SCANNING ELECTRON MICROSCOPY (SEM)

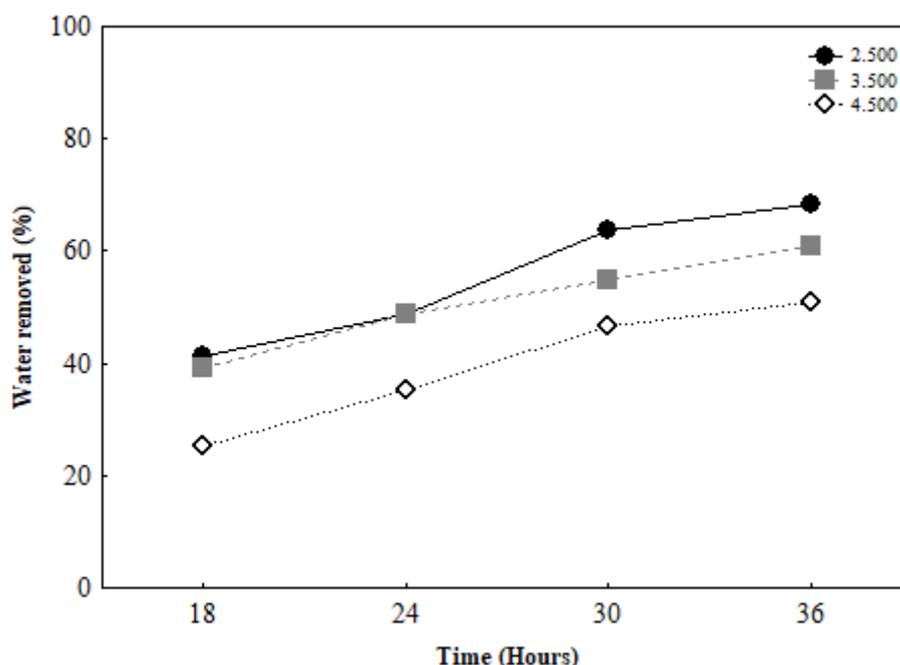
Observations of the microstructures of the fillets were obtained using a scanning electron microscope (SEM), TESCAN brand, model VEGA 3 with a resolution of 3nm and magnification capacity of up to 300kX. Initially, samples were collected from the dorsal region of the fillets after freeze-drying in 2 cm cubes and fixed in FAA (formaldehyde 50 ml (37%), acetic acid 50 ml and ethyl alcohol 900 ml (63%) for 24h. After the 24-hour period, the samples were transferred to vials with 70% alcohol. Subsequently, the samples were subjected to the LEICA EM CPD300 critical point, where the material was dehydrated in a carbon dioxide (CO<sub>2</sub>) bath and fixed on stubs, with the aid of double-sided carbon tape and coated with a 5nm layer of gold Denton Vacuum Desk V Standard metalizer.

## RESULTS

### FREEZE-DRYING, ACTIVITY ( $A_w$ ), TEXTURE AND COLOR OF FILLETS

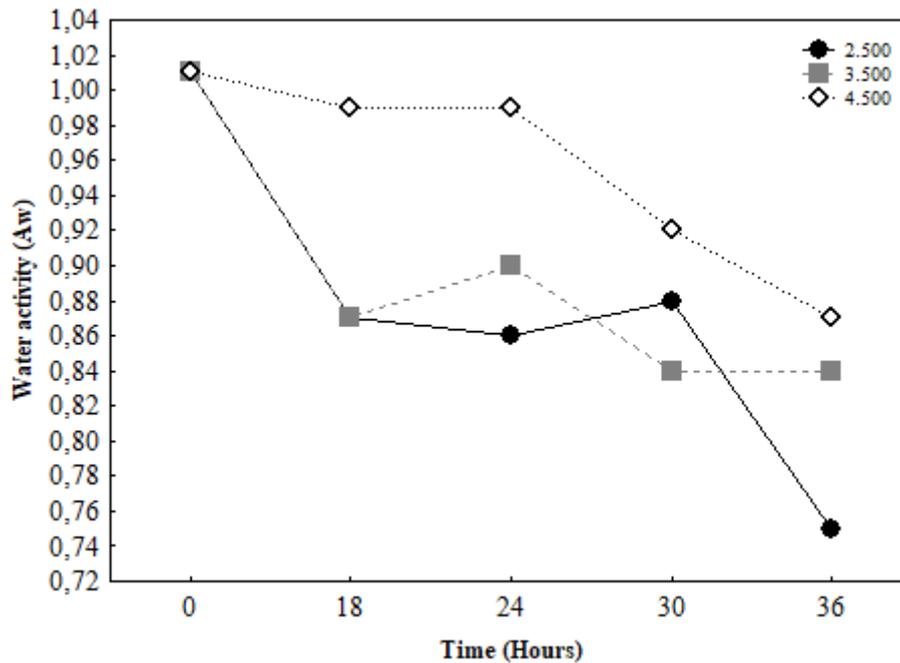
The water loss percentages increased exponentially during the process times for all weights. However, fillets weighing 2,500 kg freeze-dried for 30 and 36 hours were those that presented the highest percentages of water loss during the freeze-drying process (63.6 and 68.3%, respectively) (Figure 1). Meanwhile, fillets with higher weights (4,500 kg) subjected to the same processing time obtained lower water loss percentages (Figure 1). Among the different weights, the lowest percentages of water loss recorded were for 4.500 kg fillets with 18 and 24 hours of freeze-drying (25.3 and 35.4%, respectively) (Figure 1).

Figure 1. Fillet water content ( $H_2O$ ) changes during freeze-drying at different times for Nile tilapia *Oreochromis niloticus*.



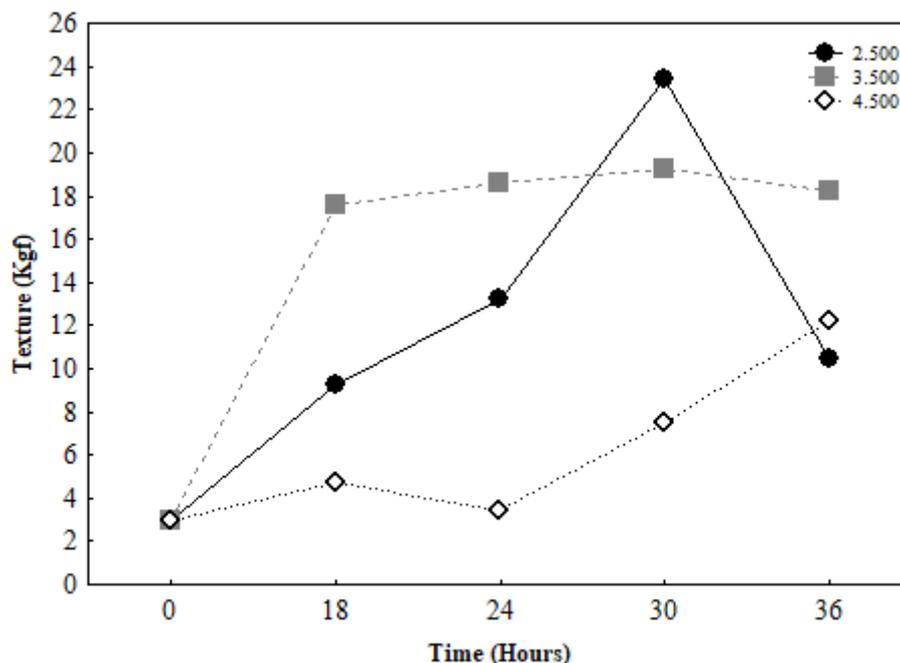
The water activity indexes ( $A_w$ ) of the fillets reduced throughout the process (Figure 2). In general, fillets weighing 4.500 kg presented the highest water activity indexes ( $A_w$ ) at the different process times, especially at 18 and 24 hours of the process (0.99 each) (Figure 2). The lowest water activity value observed was for fillets weighing 2.500 kg freeze-dried for 36 hours (0.75) (Figure 2).

Figure 2. Water activity ( $A_w$ ) of fillets during freeze-drying at different times for Nile tilapia *Oreochromis niloticus*.



The texture was below 13 kgf for fillets weighing 4,500 kg at the different processing times (Figure 3). On the other hand, these values ranged between 17.6 (18 hours) and 18.2 kgf (36 hours) in fillets weighing 3,500 kg (Figure 3). The highest value of this parameter was recorded for fillets weighing 2,500 kg processed for 30 hours (23.4 kgf) (Figure 3).

Figure 3. Texture of fillets during freeze-drying at different times for Nile tilapia *Oreochromis niloticus*.



Color measurements demonstrated that the luminosity indexes ( $L^*$ ) gradually increased during the freeze-drying of fillets with different weights, consistently above the scale's center (50) at different process times. However, the highest indices ( $L^*$ ) were observed in fillets freeze-dried for 36 hours, mainly in fillets weighing 3.500 kg, indicating a light gray coloration. A pattern is similar to that observed in fillets weighing 2.500 kg after 30 hours and fillets weighing 3.500 kg after 18 hours (Table 2). Regarding hue ( $a^*$ ), positive values were observed in fillets weighing 3.500 kg at 24 hours and 2.500 kg at 18 hours. No negative values of  $b^*$  were found in the chromatic coordinate for fillets of either size (Table 2).

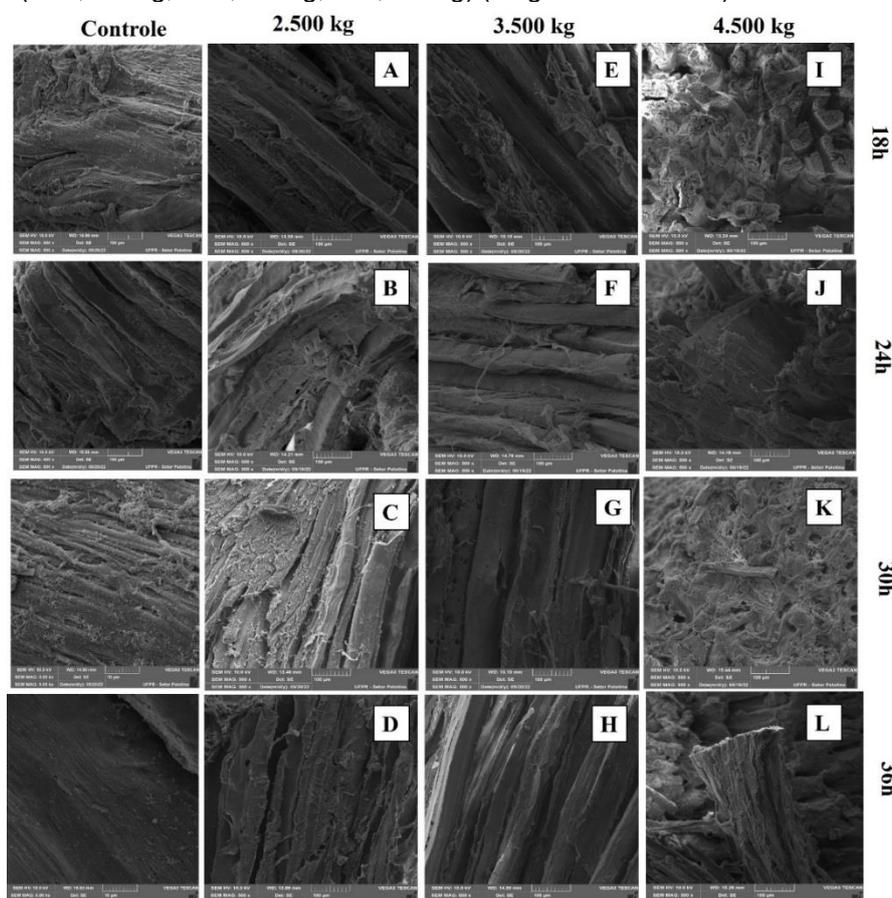
Table 2: Color of Nile tilapia *Oreochromis niloticus* fillets freeze-dried at different times. A) Fillets weighing 2.500 kg, B) Fillets weighing 3.500 kg and C) Fillets weighing 4.500 kg.  $L^*$  = luminosity (black to white),  $a^*$  = hue: red ( $+a^*$ ) to green ( $-a^*$ ) and  $b^*$  = chromaticity: yellow ( $+b^*$ ) to blue ( $-b^*$ ).

Color of freeze-dried tilapia fillets													
Freeze-drying time (hours)													
		18h			24h			30h			36h		
	Mass (kg)												
	ontrol	.500	.500	.500	.500	.500	.500	.500	.500	.500	.500	.500	.500
*	1.23	2.92	2.78	6.45	0.51	1.20	8.15	5.85	4.12	2.93	2.70	6.63	4.11
	2,80	9.11	6.92	11.05	8.40	4.75	4.86	3.43	6.36	4.03	4.28	4.52	7.75
*	0.01	1.71	1.04	.04	1.85	.98	1.07	2.61	1.41	0.86	0.98	1.86	0.45
	1.21	1.06	0.89	4.45	0.45	2.39	0.97	0.47	1.20	1.80	1.92	0.84	1.80
*	.04	3.53	6.96	7.36	4.54	8.19	5.35	3.12	7.04	5.82	6.23	6.91	8.00
	1.01	3.07	2.00	2.79	1.86	1.44	1.40	1.30	1.46	1.38	1.68	1.21	1.98
*	.13	3.68	7.02	7.79	4.66	8.33	5.42	3.39	7.16	5.92	6.35	7.03	8.08
	0.88	3.05	1.92	3.22	1.86	1.60	1.39	1.21	1.43	1.42	1.64	1.17	1.97
*	0.75	7.66	3.84	8.39	7.30	7.38	4.19	01.43	4.93	3.38	3.89	6.38	1.66
	10.02	4.55	3.79	13,07	1.92	7.06	3.69	2,78	4.26	6.09	6.86	2.99	5,78

## SCANNING ELECTRON MICROSCOPY (SEM)

Using scanning electron microscopy, some changes were observed in the muscle microstructure of the fillets after the freeze-drying process (Figure 4). In processes involving 4,500 kg fillets for 18 hours, the muscle fibers are complete and surrounded by the perimysium, with pores between the myofibrils that remain surrounded by the endomysium (Figure 4I). These aspects were different from those observed with 2,500 and 3,500 kg fillets freeze-dried for the same time, which did not present differences in microstructure, and the fibers were detached and entangled with the perimysium, presenting fissures between them (Figure 4A and E). On the other hand, cracks were lower in 4.500 kg fillets processed for 24 hours, where the muscle fibers were denser than those observed in fillets of the same mass processed for a shorter time (18 hours) (Figures 4J and I). Similar microstructural characteristics can be considered for the other fillets processed for 24 hours (2.500 and 3.500 kg), where complete muscle fibers were observed, but with the perimysium breaking (Figures 4B and F).

Figure 4. Microphotograph of Nile tilapia *Oreochromis niloticus* fillets freeze-dried at different times: 18h (A: 2,500 kg; E: 3,500 kg; I: 4,500 kg); 24h (B: 2,500 kg F: 3,500 kg J: 4,500 kg); 30h (C: 2,500 kg; G: 3,500 kg; K: 4,500 kg); 36h (D: 2,500 kg; H: 3,500 kg; L: 4,500 kg) (magnification: 500x).



In 4,500 kg fillets freeze-dried for 30 hours, the presence of voids in the tissue microstructure was observed, as well as a compressed and tangled appearance, possibly due to the formation of ice crystals during the dehydration stages (Figure 4K). At the same time, muscle fibers in the 2,500 and 3,500 kg fillets were also observed to be deformed, ruptured or detached due to dehydration (Figure 4C and G). When 4,500 kg fillets were subjected to longer freeze-drying times (36 h), the muscle fibers appeared compressed and deformed with the perimysium peeling (Figure 4L). The same was true for the 2,500 and 3,500 kg fillets (36 h), where fissures and ruptures were observed between the compressed muscle fibers (Figure 4D and H).

## DISCUSSION

All fillets showed a gradual increase in water loss throughout the process. However, the dehydration percentages were higher in fillets with smaller masses (2,500 kg) freeze-dried for 30 and 36 hours. Different factors can influence the results of food freeze-drying, such as heating temperature, use of pre-treatments, equipment load capacity, degree of vacuum and freezing, which can interfere with the quality of the product, whether in appearance, moisture content or nutritional composition (Nowak and Jakubczyk, 2020).

The present study shows probable differences between fillet dehydration rates, which may have been influenced by heat transfer rates between different fillet masses. Sample thickness and the cellular structure of the material can interfere with sublimation rates, restricting the mass transfer coefficient and decreasing dehydration rates (Oyinloye and Yoon, 2020).

Processing time is also a factor that can contribute to changes in the quality of the freeze-dried product. For example, Paula da Costa *et al.* (2023) noted that freeze-drying time significantly influenced the quality ( $A_w$ , moisture, and yield) of *Gymnura altavela* skate meat. It requires a drying time of 24 hours to reach a moisture content of 3.7%, which is considered ideal for inhibiting the proliferation of microorganisms.

Knowing the water activity ( $A_w$ ) is essential for preserving dried fish and other foods, as it regulates the products' microbial load (Nguyen *et al.*, 2014; Tapia *et al.*, 2020; Fitri *et al.*, 2022). Foods with high water content, such as fish, with water activity ( $A_w$ ) values greater than 0.90 become prone to microbiological contamination (Abbas *et al.* 2009). Therefore, for dried fish, water activity ( $A_w$ ) values are expected to be between 0.60 and 0.85 (Fitri *et al.*, 2022). In the present study, only smaller fillets (2.500 kg) processed for 36

hours reached  $A_w$  values within the recommended range for dried fish (0.75). Although there is little change in the number of bacteria during freeze-drying due to the low drying temperature, some pathogen spores can survive the process stages if they are present in the raw material or acquired through contamination during processing (Citrakar et al., 2019).

Texture, like color, is among food's most important physical properties, directly influencing consumer preference (Baingana, 2024; Kamei et al., 2024). During the drying process in food, significant textural changes occur due to reduced water and moisture content, resulting in muscle contraction and increased rigidity and porosity (Nowak and Jakubczyk, 2020; Agregán et al., 2024). These findings are consistent with the results of our study, where fillets that presented higher percentages of water loss were also those that obtained greater hardness. Tests with different drying methods on the yellow croaker *Larimichthys polyactis* also demonstrated more rigid textural properties using the freeze-drying method than the others (Kim et al., 2020). In addition to water content, the concentration of lipids and proteins in the muscle, the drying methods and parameters used, the type of raw materials, fat composition, pH, genetics and proteolytic potential are factors that can also contribute to the occurrence of changes in the texture of dehydrated foods (Nguyen et al., 2014).

The texture results demonstrated that the fillet tissue stiffened during the freeze-drying process. This is similar to that described by Nie *et al.* (2022), who noted an increase in the hardness and thickness of vacuum-freeze-dried tilapia skins (60 and 150 minutes). According to the researchers, these effects were attributed to the processing time, which directly affected the skin properties by removing water (bound, retained, and free) during drying. In freeze-dried products, porosity is an essential element for predicting quality since the distribution and size of pores especially influence crispness (Nowak and Jakubczyk, 2020). Therefore, different methods have emerged to evaluate the textural changes in fish fillets affected by vacuum freeze-drying. For example, researchers Ma *et al.* (2017) used hyperspectral imaging models (400 nm to 1000 nm) to simultaneously predict textural changes in *Ctenopharyngodon idella* grass carp fillets subjected to vacuum freeze-drying, including characteristics such as hardness, chewiness and gumminess.

The appearance of the fillets changed during the process, especially with increasing  $L^*$  (luminosity) and with the samples darkening. Fish muscle discoloration can be affected by different factors: drying, postmortem storage, microbial and biochemical changes, salting

and brining, modified atmosphere packaging using CO<sub>2</sub> and thermal processing (Singh et al., 2022). Compared to other dehydration methods, freeze-drying has proven to be efficient in preserving aquatic foods' physical and chemical qualities, especially their color. An example of this can be observed between freeze-drying and hot air drying in the processing of *Penaeus vannamei* shrimp, where it was noted that the use of the first method provides better conditions for controlling lipid oxidation rates and preserving the color of the product (Li et al., 2020). In the study by Zhu et al. (2022), the authors employed different drying methods on *Takifugu obscurus* pufferfish fillets and noted a low color change in vacuum freeze-dried fillets when compared to the other methods; in addition, the respective treatment was the only one in which the oxidation of fatty acids and the Maillard reaction during the process was absent, events attributed to the low temperatures that are generally employed in this type of drying.

The formation of empty spaces in the muscles of the freeze-dried fillets was observed, as well as the deformation of the muscle fibers. According to Lee et al. (2024), during the freeze-drying of meat and aquatic products, denaturation and oxidation of proteins can occur, thus altering the sensory and nutritional attributes of the food and affecting the quality of the product. For this reason, the authors recommend better planning in the choice of the type of sample and in the operational conditions of the freeze-drying process, aiming to obtain high-quality products. In addition, other relevant factors in the drying of meat and fish are the fibrils (thin fibers of the connective tissue) and the sarcolemma (thin layer of connective tissue wrapped around the muscle fiber), essential for the diffusion of water during the process (Harguindeguy and Fissore, 2020).

Unlike our findings, Paula da Costa et al. (2023) did not notice differences in the structure of freeze-dried (16-24 hours) *G. altavela* skate meat. In this case, such differences may be associated with the type of raw material and processing time used in the experiment, the latter being shorter than that used in the present study. In food freeze-drying, controlling the freezing rate is also essential since the shape and position of ice crystals directly affect the properties and structures (Nowak and Jakubczyk, 2020; Tan et al., 2021). In addition to the morphological characteristics of the products, in freeze-drying, the shape and size of the ice can also interfere with the sublimation rates (Petzold and Aguilera, 2009). Among the main physical changes caused by freezing on the microstructure of food tissues are recrystallization (or maturation), cryoconcentration, cryodeformation and freezer burn (Dalvi-Isfahana et al., 2019).

In the literature, some studies also demonstrate the effects of dehydration methods on fish fillets' structural characteristics, including freeze-drying. For example, in the research conducted by Luo *et al.* (2021), the researchers noted that the concentration of free water in the muscles of tilapia fillets increased with the rupture of cell membranes during the different drying stages. Meanwhile, Zhu *et al.* (2022) reported that the use of vacuum freeze-drying preserved the myofibril and maintained lower shrinkage in semi-dry fillets of *T. obscurus* pufferfish compared to other drying methods.

## **CONCLUSION**

It is concluded that freeze-drying is an effective method for dehydrating tilapia fillets. However, its use resulted in changes in the structural characteristics of the fillet muscles, as evidenced by the analyses performed in the study. Such changes can directly influence the final quality of the product, such as appearance, texture or flavor. Therefore, to better elucidate the effects of the process on the characteristics of tilapia fillets, it would be important to include sensory analyses in future studies to observe whether the process also causes changes in the flavor of the food.

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