

Development and characterization of myofibrillar protein film obtained from Nile tilapia mechanically separated meat (MSM)¹

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ABSTRACT - Processing residues are still one of the main obstacles in the fishing industry due to waste and environmental impact. With that, ways of reusing these residues are researched to generate new products with different applications. The objective of this work was to develop and evaluate protein films obtained from mechanically separated meat (MSM) of Nile tilapia (*Oreochromis niloticus*) filleting residues. To get the films, the casting technique was used, varying their composition in relation to the amount of protein (1.0, 1.5 and 2.0%) and glycerol (0.0, 0.1 and 0.2%), creating a 3² factorial plan, with 9 treatments. The films were characterized in terms of color, opacity, water solubility, mechanical properties and water vapor permeability (WVP). In the end, a yield of 18.70% of Nile tilapia myofibrillar proteins (MPs) and a post-lyophilization protein content of 87.33% were obtained. It was concluded that the film that presented the best result in all the characteristics analyzed was the one with 1.0% of MPs and 0.1% of glycerol.

Key words: Protein films. Water vapor permeability. Amino acid profile. Fish byproducts. Mechanical properties.

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INTRODUCTION

The waste of fishing industry processing, composed mainly of skin, viscera, heads, scales, pimples and eyeballs, are generated on a large scale and have caused serious environmental problems (ALCÂNTARA *et al.*, 2023). It is estimated that up to 62.5% of the raw material used in tilapia filleting, for example, is simply discarded (SOUSA *et al.*, 2022). In 2020, China alone produced 2 million t of tilapia, followed by Indonesia (1.4 million t), Egypt (1 million t) and Brazil (534 thousand t) (FAO, 2022).

To minimize environmental impacts, fish waste could be used in the formulation of by-products, such as mechanically separated meat (MSM) and surimi, that have high nutritional value (ROSSETTO; SIGNOR, 2020); in the extraction of myofibrillar proteins and in the elaboration of films and coatings with nutritional and technological quality (GÓES-FAVON *et al.*, 2021).

Fish residues can also be used to extract biocompounds of enormous applicability and high added value (ALCÂNTARA *et al.*, 2023) in the manufacture of biodegradable protein-based packaging, as an alternative to the use of non-degradable polymers (SUPUT *et al.*, 2017), as well as in the manufacture of edible films and coatings used to increase the shelf life of foods, including fish (MIRZAPOUR-KOUHDASHT; MOOSAVI-NASAB, 2019).

Fish meat is highly perishable, which, due to the biochemical reactions and microbial metabolism of the postmortem stage, result in rapid deterioration of its edible quality (YU; REGENSTEIN; XIA, 2019). Studies show that films and coatings based on gelatin extracted from fish can delay the multiplication of deteriorating and pathogenic microorganisms, improving the quality and shelf life of packaged foods (ABDELHEDI *et al.*, 2019). In the food industry, gelatin is already used as an ingredient to improve the elasticity, consistency, and stability of foods and to protect against dehydration, light and oxygen (MARTINS *et al.*, 2018).

Thus, fish waste that is discarded represents a huge risk to the environment and a loss of by-products that could be used to add nutritional quality to other food products. Based on these facts, this research had as main objective to protein films from the lyophilized MPs fraction of Nile tilapia filleting MSM, and to evaluate its amino acid profile and physical-chemical and mechanical properties.

MATERIAL AND METHODS

The materials used in this work were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge

(SisGen) under the number registered on the SISGEN platform with the code A058F53.

Obtaining the raw material and preparing the film of MPs from fish waste

In this research, the MSM of Nile tilapia, from the fish processing industry in the city of Orós/CE, was used and transported in a thermal box with ice for the processing unit of the Fish Technology Laboratory (LATEPE), in the Federal University of Ceará (UFC).

To obtain myofibrillar proteins (MP) the method used by Monterrey-Quintero and Sobral (2000) was adapted. MSM was obtained after evisceration and filleting, passing through bone separator. The obtained pulp was washed in six cycles, using in each cycle a washing solution in the proportion of 2:1 (solution: pulp), at 5 °C, for 10 minutes. A solution of distilled water at 0.85% NaCl was used for the first, second and third washes, and the other washes a solution of NaCl at 0.1% was used.

The supernatant containing fat and soluble protein was discarded. The final slurry was sieved through a 1mm mesh metal sieve to remove connective tissue, then frozen at -18 °C for 24 hours in a freezer, and subsequently dehydrated in a lyophilizer for 96 h, obtaining the lyophilized myofibrillar protein used as a basis for obtaining the protein films. The extraction yield was calculated and expressed in grams (g) of protein for each 100 g of MSM used.

Analyzes of extracted material

The elemental analysis of the MSM and amino acid profile of the fraction of lyophilized MPs were carried out according to the official methodology of the Association of Official Analytical Chemists (AOAC, 2005), for each treatment in triplicate.

Elemental analysis of MSM

Moisture was determined by the gravimetric method in an oven at 100 °C; the total nitrogen content, by the Kjeldahl method; the crude protein content obtained by multiplying by the factor 6.25; the lipid content, by the Soxhlet method; the ashes, by gravimetric method in muffle at 550 °C; and carbohydrates, by the difference of nutrients, according to Equation (1):

$$\% \text{carbohydrates} = 100 - (\% \text{protein} + \% \text{moisture} + \% \text{ashes} + \% \text{lipid}) \quad (1)$$

Amino acid profile

The amino acid profile of the fraction of lyophilized MPs was performed on the Biochrom 20 system (Pharmacia-LKB, Sweden). First, 2 mg of lyophilized MPs was hydrolyzed with 2 mL of 6N HCl containing 1% phenol (mass:volume). The hydrolysis was carried out in glass ampoules, sealed under a

nitrogen atmosphere, and kept in an oven at 110 °C for 24 h. After hydrolysis, the ampoules were opened and HCl and phenol were eliminated under reduced pressure in the presence of 1 M NaOH. Then, the hydrolyzed residues were washed five times with ultrapure water (milliQ) and dried under reduced pressure in the presence of phosphorus pentoxide. After drying, the samples were redissolved in a sterile buffer solution of 0.1 M sodium citrate pH 2.2 and subjected to amino acid analysis.

Elaboration of protein films

To obtain the MPs films, the methodology described by Monterrey-Quintero and Sobral (2000) with adaptations was used. An experimental design of 3² was carried out, totaling 9 treatments: three of MPs percentage (1.0, 1.5 and 2.0%) and within the least treatments, three variations in the percentage of glycerol (0.0, 0.1 and 0.2%), which are detailed in Table 1.

The protein films were obtained by the casting technique according to the methodology mentioned by Souza *et al.* (2010). Initially, the MPs were dispersed in a solution with distilled water pH = 2.7 (adjusted with lactic acid). The aqueous dispersion was maintained under gentle and constant agitation for 30 minutes using a stirrer, with the temperature controlled at 90 °C by a heating mantle (Q215M, Quimis, Brazil), to hydrate the MPs. Before hydration, using a bench pH meter (K39-1420A, Kasvi, Brazil), maintaining constant agitation of the aqueous solution.

Then, glycerol was added, maintaining the pH at 2.7 with constant agitation and the temperature of the filmogenic solution at 90 °C for 30 minutes. Afterwards, the filmogenic solution (28 mL) was spread in Petri dishes, which were placed in an oven with air circulation at 35 °C for 24 h. After drying, the films were stored for 48 h in desiccators maintained at 25 ± 2 °C and relative humidity of 10 ± 2%, controlled using a saturated silica gel solution, until the beginning of the analyses.

Characterizations of protein films

Water vapor permeability (WVP)

Water vapor permeability (WVP) was determined by gravimetry based on the ASTM E96 method

(ASTM, 2016). The films were sealed at the top of the cell containing 50 mL of distilled water and placed in a desiccator at 25 °C and 10% relative humidity containing silica. Cells were weighed at 2 h intervals for 8 h. The slope of the curve that represents weight loss as a function of time was obtained by linear regression, and the WVP of the films was determined according to Equation 2:

$$WVP = \frac{(WVTXL)}{\Delta P} \quad (2)$$

where, WVT is time rate of water vapor transmission (g. m⁻².h⁻¹) calculated from the slope of the curve divided by the area of the film, L is the average thickness of the film (m) and ΔP is the difference in the partial pressure of water vapor (atm) on the two sides of the film.

Three replicates were performed for each type of film. The thickness of each sample was measured using a digital micrometer in 10 different positions on each sample.

Solubility in water

Water solubility was determined by the percentage of dry matter of the film after 24 h of immersion in distilled water. The initial dry matter of the films was determined by drying discs with a diameter of 2 cm at 100 °C in a vacuum oven for 24 h. Other discs were cut, weighed, and immersed in 50 mL of distilled water, with periodic stirring, for 24 h at 25 °C. After this period, the films were removed and dried at 100 °C for 24 h to quantify the final dry matter. The solubility was defined by the difference between the initial and final dry matter in relation to the initial dry matter, with three replications for each sample.

Tensile tests

The following tensile tests were performed for the mechanical characterization of protein films: strain at break (%), tensile strength (MPa), and elastic modulus (MPa). The specimens used for the mechanical tests were cut by a pneumatic stamping press, with an operating pressure of seven bar. In the tensile tests, the specimens with a length of 61.92 mm and a width of 12.37 mm were affixed by means of claws coupled to a mobile crosspiece (Universal Testing Machine, model DL3000, EMIC, Brazil), following the ASTM D882 guidelines (ASTM, 2018), with a load cell of 100 N, traction speed of 50 mm.min⁻¹ and initial distance between the claws of 30 mm.

Table 1 - Experimental design of treatments with different concentrations of protein and glycerol to produce films and coatings

Treatments	0.0% glycerol	0.1% glycerol	0.2% glycerol
1.0% MP	T ₁	T ₄	T ₇
1.5% MP	T ₂	T ₅	T ₈
2.0% MP	T ₃	T ₆	T ₉

MP = myofibrillar protein; T = treatments that vary the percentage of MPs and plasticizer. Source: The authors

The breaking strength was expressed in MPa and calculated by dividing the maximum force (N) required to break the specimen by the initial cross-sectional area (m²) of the sample. The strain at break was given by the ratio between the final length at the breaking point of the sample and the initial length of the sample (30 mm), expressed as a percentage. The tests were performed five times for each sample.

Color and opacity

The color and opacity of the films were determined with a digital colorimeter (model NR200, Shenzhen ThreeNH Technology CO. Ltd., China), using an L*, a*, b* scale of the CIELab system, developed by Hunter (1975), where L* ranges from black and white (0 to 100), a* ranges from green to red (-60 to + 60), and b* ranges from blue to yellow (-60 to + 60). Nine repetitions were performed for each film sample.

Opacity (Y) was calculated as the ratio between the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w). Nine replicates of each film sample were randomly determined for Y_b and Y_w and their average was used for calculations. The results were expressed in percentage, according to Equation 3:

$$\text{Opacity}(Y\%) = \frac{Y_b}{Y_w} \times 100 \quad (3)$$

Statistical analysis

In the results, the test of data normality was applied, followed by two factor analysis of variance (two-way ANOVA) and the Tukey test at 5% significance, using the STATISTICA 7.0 software (Statsoft Inc., US).

RESULTS AND DISCUSSION

Myofibrillar protein yield

The successive washings of the MSM removed fat, blood, minerals and sarcoplasmic proteins, and the sieving

removed the connective tissue. Even so, a considerable concentration of MPs was obtained, resulting in a satisfactory yield of 18.70% of MPs.

Vidal-Campello *et al.* (2021) obtained a residue yield of 18.34% when working with tilapia protein concentrate subjected to several cycles of water washing and a deodorization process using phosphoric acid (H₃PO₄ [0,05%]) to reach the isoelectric point of the myofibrillar protein (pH ± 5) and a washing cycle at the end of the step with ethanol to reduce the lipid content.

The isolation of MPs by successive washes ensures that sarcoplasmic proteins are eliminated, favoring the formation of a protein gel. Gelation occurs by its coagulation during heating, which ends up adhering to the myofibrillar proteins. Thus, the use of MPs in the elaboration of fish derivatives guarantees a greater variability of products from residues and with a higher quality (GÓES-FAVON *et al.*, 2021). The main importance of washing is the removal of sarcoplasmic proteins, soluble in water, which prevent the formation of gelling solutions (GAMEZ-VILLAZANA; MOLINA; OJEDA, 2021), so that there is then an increase in the concentration of MPs (CANADA MILLAN *et al.*, 2021). The stability of emulsification made from myofibrillar proteins is directly related to the pH range, in which this range is above the isoelectric point of MPs, make them acquire negative charges, making the nonpolar part of the protein create an adhesion to the fat globules and the polar part to the water, preventing the fusion of the proteins (GAMEZ-VILLAZANA; MOLINA; OJEDA, 2021). Wang *et al.* (2022) claim that MPs has low solubility in water, corroborating the fact that some methods, such as washing, dilution and acid or alkaline solubilization, among others, do not guarantee the good solubilization of these proteins.

Elemental analysis of MSM and MPs

Table 2 contains the results referring to the elemental analysis of the MSM of Nile tilapia and the and fraction of lyophilized MPs of the MSM obtained.

Table 2 - Proximate composition (mean ± standard deviation) of Nile tilapia MSM and MPs

	MSM (%)	MP (%)
Moisture	75.90 ± 0.12	-
Proteins	12.92 ± 0.16	87.33 ± 0.77
Ashes	0.96 ± 0.021	9.46 ± 0.26
Lipids	9.80 ± 0.29	1.68 ± 0.57
Carbohydrates	0.64 ± 0.05	-

- Not quantified Source: The authors

Of the centesimal composition of DMI obtained in this study, most of it was moisture, with 75.90%, followed by protein content (12.92%), lipid fraction (9.80%), ash (0.96%) and carbohydrates (0.64%). This CMS protein content was lower than that found by Fogaça *et al.* (2015), working with surimi (14.81%) and MSM (15.87%) of tilapia, but they were higher than the lipid contents (7.15 and 7.62%, respectively). Moreover, Guimarães *et al.* (2018), studying MSM and surimi of snapper (*Pagrus pagrus*), obtained different results for proteins (69.76 and 88.01%), lipids (22.08 and 8.97%) and ashes (4.88 and 5.77%), respectively. Bacelar *et al.* (2021) found maximum values for proteins, lipids and ash of 19.1, 11.6 and 2.6%, respectively, in the preparation of nuggets using MSM from tilapia filleting residues, with different formulations.

The protein content was also much lower than the result of Marasca, Nogueira and Martins (2020), who obtained 90.8% protein from hake (*Cynoscion guatacupa*) cuttings. And, much higher, in relation to the lipid content (4.1%) of these same authors. They attributed their good results, respectively, to the method of separating proteins from hake cuttings and to the process of separating the lipid content along with the insoluble fraction during the protein concentration phase.

As for the fraction of MPs, obtained from the MSM of Nile tilapia and lyophilized, in this research, it presented a protein content of 87.33%, proving the efficiency of the method used. According to Wang *et al.* (2022), MPs account for approximately 50% of the total protein content and the remainder are sarcoplasmic and matrix proteins. The result of this study was higher than the values found by Scudeler *et al.* (2020), who obtained 85.99% of isolated Nile tilapia MSM proteins; and by Pongsetkul, Kingwascharapong and Senphan (2022), who found 84.43 mg/g of MPs in Nile tilapia samples kept under ice flakes and ice suspension.

The lipid content (1.68%) of the MPs isolated in this research was slightly higher than that obtained by Marasca, Nogueira and Martins (2020), of 1.5% in protein isolated from hake trimmings. However, it was much lower than that found by Scudeler *et al.* (2020), who obtained 10.96% (dry basis) of lipids in isolated Nile tilapia protein. According to these authors, this result was due to the removal of most of the lipids along with the insoluble protein portion during the centrifugation process. And for ash, the content obtained here was 9.46%, while Marasca, Nogueira and Martins (2020) and Scudeler *et al.* (2020), obtained only 1.6 and 0.69%, respectively. According to Guimarães *et al.* (2018), the protein concentrate washing process is the step responsible for removing ash and minerals from it. Thus, the high ash value was possibly due to salt (NaCl) residues (HENRIQUE; CEREDA; SARMENTO, 2008), used during the washing process.

However, in general, the results found in this work indicate that the MPS extraction process used was carried out properly and that Nile tilapia processing residues have a high potential as a protein source.

MP amino acid profile

The results of the profile of total amino acids present in the lyophilized myofibrillar protein of *O. niloticus* are described in Table 3 in comparison with the required amounts of essential amino acids by Food and Agriculture Organization of the United Nations and World Health Organization (FAO; WHO, 2002).

In general, the amino acid profile found in Nile tilapia myofibrillar proteins were like those reported for piramutaba skin gelatin (OLIVEIRA *et al.*, 2019), tilapia, grass carp and silver carp skin (TANG *et al.*, 2015), and Nile tilapia MPs (MONTERREY-QUINTERO; SOBRAL, 2000).

The result of the amino acid profile found in MPs is considered satisfactory, since all essential amino acids, such as histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, were present at satisfactory levels and greater than the FAO and WHO (2002) determination.

The highest concentrations were of glutamic acid (12.18%), aspartic acid (12.05%), lysine (12.42%) and proline (8.92%). The higher the amino acid content, the greater the stability of the helix through interchain hydrogen bonds and, therefore, greater gel strength, due to direct bonding between hydrogen and a water-binding molecule, and because of the hydrogen bonds to the carbonyl group (OLIVEIRA *et al.*, 2019).

The lowest concentration was of cysteine (0.7%) and tryptophan was not quantified. According to Silva, Lourenço and Pena (2017) amino acids like tryptophan and cysteine are normally absent in conventional gelatin. However, the presence of cysteine in low concentration in MPs was like that reported by these authors, both in skin and in Gilthead Bream (*Brachyplatystoma rousseauxii*) gelatins.

According to Rebouças *et al.* (2012), the main essential amino acids found in aquatic organisms are arginine, leucine and lysine. In this study, in addition to these, all essential amino acids were also observed.

Arruda *et al.* (2006), studying the silage from *O. niloticus* processing residues, determined the potential of this material, rich in proteins and lipids, to reduce costs and improve production efficiency, as well as to minimize the problems of environmental pollution, generated by the lack of a suitable destination for this material.

Considering the results of this work and others, therefore, it is understood that the MPs are rich in amino acids in their composition and structure.

Table 3 - Amino acid profile (mean \pm standard deviation) presents in Nile tilapia MPs compared to those required by FAO/WHO (2002) for adults

Amino acids	mg g ⁻¹ protein	FAO/WHO standard (mg g ⁻¹ protein)
alanine	53.20 + 0.12	-
glutamic acid	121.80 + 0.04	-
aspartic acid	120.50 + 0.05	-
serine	44.30 + 0.06	-
glycine	43.80 + 0.10	-
histidine**	26.30 + 0.07	15.00
arginine**	27.40 + 0.05	-
threonine**	46.90 + 0.08	23.00
tyrosine	34.20 + 0.03	-
tryptophan*	-	6.00
valine**	63.70 + 0.12	39.00
methionine**	31.70 + 0.03	16.00
cysteine	7.00 + 0.02	6.00
isoleucine**	59.30 + 0.06	30.00
leucine**	84.10 + 0.01	59.00
phenylalanine**	42.00 + 0.06	30.00***
lysine**	104.20 + 0.04	45.00
proline	89.20 + 0.09	-

* Not determined. ** Essential amino acids. *** phenylalanine + tyrosine Source: The authors

Acquisition and characterization of the film

All MPs filmogenic solutions showed excellent fluidity, that is, without any characteristic of agglomeration, being easily manipulable and presenting a coloration close to white. The films made with MPs of Nile tilapia proved to be flexible and easily manipulated, with a good appearance. Figure 1 shows examples of films made from MPs and plasticizers.

It was observed during production that films with glycerol are more flexible. This fact could be observed in its removal from the acrylic plates, however, the greater the amount of protein in the treatments, the lower the risk of rupture. Also, it was observed that with the addition of plasticizer (glycerol), changes in the properties of the films may occur in relation to the control group (no coating).

Glycerol has a hydrophilic character due to the presence of hydroxyl groups in the chain, allowing greater interaction (TARIQUE; SAPUAN; KHALINA, 2021). Although plasticizers are good film additives, they improve film flexibility and facilitate handling; its use should be analyzed, because it alters the barrier properties, such as permeability to water vapor and solubility (SOUZA *et al.*, 2010).

Figure 1 - Films made with MPs and glycerol

Source: The authors

The elaboration of edible films and coatings from MPs has been widely reported in studies, as proteins from fish have numerous advantages, such as, ability to form skins, plasticity, and elasticity, as well as a good barrier to oxygen (LACROIX; VU, 2014).

Water vapor permeabilities (WVP), solubility and thickness

The results obtained for water vapor permeability, solubility and thickness determined for each of the film formulations produced with MPs and different concentrations of glycerol can be seen in Table 4.

The values found for WVP of the protein films ranged from 0.61 to 1.04 g/m.day.atm with a significant difference ($p < 0.05$) between the values of the treatments. The addition of 0.5% MPs in the preparation of films from 1.5 to 2.0% made it possible to identify a direct relationship between protein concentration and WVP. It was found that the higher the incorporated protein concentration, the greater the water vapor permeability for the 1.0, 1.5, and 2.0% MPs films. Silva *et al.* (2018) state that reducing gelatin and glycerol concentrations produces a lower water vapor permeability, increasing tensile strength.

When analyzing the glycerol level for the protein films produced, its influence on WVP was observed. For the formulations of 1.0, 1.5, and 2.0% of proteins with the increase in the incorporation of 0.1 to 0.2% of glycerol, there was an increase in the WVP with a statistically significant difference ($p < 0.05$). It was also observed that the lowest concentration of 0.1% glycerol did not affect the loss of water vapor in the 1.5 and 2.0% MPs films.

According to Souza *et al.* (2010) the use of glycerol can alter the WVP of the films, as it is hygroscopic. The hydrophilic character of glycerol reduces intermolecular forces per polymer chain, facilitating the migration of water vapor molecules and, therefore, it may result in higher permeability values for films produced with a higher concentration of MPs and plasticizer (LACROIX; VU, 2014).

The increase in water vapor permeability with the addition of increasing glycerol to Nile tilapia MPs films has already been reported in a study carried out by Sobral, Monterrey-Quintero and Habitante (2002), with Nile tilapia myofibrillar proteins with concentrations of 15 and 65% of glycerol, there was a statistically significant difference ($p < 0.05$) in the two concentrations of glycerol. Glycerol increased the water loss of the 1.0, 1.5 and 2.0% protein films, so the plasticizer influences WVP.

Zavareze *et al.* (2012), studying different concentrations of myofibrillar proteins of croaker (*Micropogonias furnieri*) at 3, 4 and 5% observed an increase in WVP with the addition of MPS of croaker. The authors determined that the WVP of the films can increase with increasing amounts of protein, being directly related to the number of polar groups (side groups of polar amino acids) available in the protein chains ($-OH$, $-COOH$ and $-NH_2$).

The values found for solubility of protein films ranged from 17.09 to 22.27%, with a significant difference ($p < 0.05$) between treatments. These results agree with the range found by Cortez-Veja *et al.* (2013) from 18.1 to 27.6% in corvina (*Micropogonias furnieri*) protein isolate biofilms. And most are higher than the values of Monterrey-Quintero and Sobral (2000), which ranged from 12.3 to 19.5% MPs in films based on Nile tilapia, these lower values being attributed to the use of high molecular weight protein fractions.

Here, when analyzing the nine films, it was noticed that the increase in the protein and glycerol content resulted in an increase in material solubility with a statistically significant difference ($p < 0.05$) in all studied concentrations. According to Zavareze *et al.* (2012),

Table 4 - Values (mean \pm standard deviation) of water vapor permeability (WVP), solubility and thickness of MPs films (1, 1.5, and 2%) with or without addition of glycerol in the concentrations of 0.0, 0.1, and 0.2%

Concentration	WVP (g/m.day.atm)	Solubility (%)	Thickness (mm)
1% MP 0.0% G	0.61 \pm 0.49 a	17.09 \pm 0.32 a	0.084 a
1% MP 0.1% G	0.66 \pm 0.27 a	17.93 \pm 0.24 a	0.086 a
1% MP 0.2% G	0.71 \pm 0.73 a	17.98 \pm 0.13 a	0.109 a
1.5% MP 0.0% G	0.86 \pm 0.96 ab	18.09 \pm 0.32 ab	0.166 bc
1.5% MP 0.1% G	0.87 \pm 0.82 ab	18.13 \pm 0.39 b	0.162 b
1.5% MP 0.2% G	0.90 \pm 0.22 bcd	19.27 \pm 0.23 b	0.167 bc
2% MP 0.0% G	0.93 \pm 0.12 bcd	20.09 \pm 0.25 c	0.178 bc
2% MP 0.1% G	0.94 \pm 0.24 bcd	21.03 \pm 0.14 c	0.168 bc
2% MP 0.2% G	1.04 \pm 0.53 de	22.27 \pm 0.09 c	0.186 bc

MPs = myofibrillar proteins; G = glycerol. Means \pm standard deviation with different letters has statistically significant differences between them by Tukey's test (ANOVA $p < 0.05$). Source: The authors

the solubility of the films depends on the components of their structure, and proteins with high molecular weight are generally insoluble or poorly soluble in water. The film solubilities reported by them ranged from 27-28% when working with croaker (*Micropogonias furnieri*) MPs. And glycerol also has great influence on the solubility of films, due to its hydrophilic nature, being widely used in edible coatings to improve the polymer structure (ALCÂNTARA *et al.*, 2022).

The thickness of MPs films with different proportions of glycerol plasticizer ranged from 0.084 to 0.186 mm. According to the thickness values, it was observed that the films containing 1.0% of MPs showed a significant difference ($p < 0.05$) from the films with 1.5 and 2.0% of myofibrillar proteins. There was an increasing behavior of the thickness of the films in relation to the higher concentration of MPs, as well as in relation to the incorporation of glycerol. Lim *et al.* (2020) also observed an increase in thickness in cassava starch films when the glycerol proportion increased.

In view of the data, the three films of lower protein concentration (1% MP), and different glycerol concentrations, were the ones that had the best results in relation to barrier properties (PVA, solubility and thickness).

Mechanical characterization of films

The mechanical properties (strain at break, tensile strength and elastic modulus) determined for each of the film formulations produced with myofibrillar proteins and different concentrations of glycerol are shown in Table 5.

In strain at break, values ranged from 0.81 to 177.14 MPa, with a significant difference ($p < 0.05$) in some treatments, being the highest value for 1% MP 0.1% G, without statistical difference ($p > 0.05$) between this and 1% MP 0.2% G and 1.5% MP 0.2% G. For 1 and 1.5% MPs there was a significant increase in their values with the addition of the tested glycerol levels. However, in films with 2.0% MPs, the increase in strain at break with increasing addition of glycerol did not show continuous growth. According to Sobral (2000), the strain at break presents great dispersion, resulting in very low correlation coefficients, and does not have a linear behavior, generating great difficulty in adjusting statistical models to the results of strain at break. In general, the most resistant biofilms are less flexible, that is, they present less deformation at rupture, and they show an increase in mechanical resistance as there is an increase in the thickness of the biofilms (SOBRAL, 2000). However, with the incorporation of a plasticizer, the strain at break increases, because the plasticizer decreases the density of protein-protein interactions, making the films less resistant and more elastic (CUQ *et al.*, 1995).

The tensile strength also did not show a pattern in the results. Values ranged from 4,40 (1% MP 0,2% G) to 13,61 MPa (2% MP 0,2% G), with statistical difference ($p < 0.05$) in many treatments. And for glycerol, in general, a decrease was observed with increasing concentration of this, except for the 2% PM film, which increased, showing a statistical difference ($p < 0.05$) between most solutions. In recent studies, Kaewprachu *et al.* (2022) observed that there was a decrease in the tensile strength of the film as the proportion of glycerol increased.

Table 5 - Values (mean \pm standard deviation) of strain at break, tensile strength and elastic modulus in MPs films (1, 1.5, and 2%) with or without addition of glycerol in concentrations of 0.0, 0.1, and 0.2%

Concentration	Strain at break (MPa)	Tensile strength (MPa)	Elastic modulus (MPa)
1% MP 0.0% G	77.45 \pm 0.44 a	10.86 \pm 0.99 a	86.18 \pm 0.13 a
1% MP 0.1% G	177.14 \pm 0.23 b	4.64 \pm 0.89 b	29.13 \pm 0.09 b
1% MP 0.2% G	155.11 \pm 0.78 b	4.40 \pm 0.23 b	28.19 \pm 0.44 b
1.5% MP 0.0% G	1.68 \pm 0.13 c	12.01 \pm 0.76 ace	874.39 \pm 0.58 c
1.5% MP 0.1% G	47.37 \pm 0.69 d	7.44 \pm 0.77 d	377.7 \pm 0.28 d
1.5% MP 0.2% G	163.69 \pm 0.59 be	4.94 \pm 0.23 bd	33.83 \pm 0.53 e
2% MP 0.0% G	0.81 \pm 0.78 fc	6.91 \pm 0.55 d	600.09 \pm 0.24 f
2% MP 0.1% G	6.12 \pm 0.77 fc	10.20 \pm 1.66 ae	434.84 \pm 0.23 g
2% MP 0.2% G	1.51 \pm 0.29 fc	13.61 \pm 0.93 acef	105.51 \pm 1.03 h

MPs = myofibrillar proteins; G = glycerol; means plus standard deviation with different letters show statistically significant differences between them by Tukey's test (ANOVA $p < 0.05$). Source: The authors

Regarding the elastic modulus, the values ranged from 29.13 to 874.39 MPa, with a significant difference ($p < 0.05$) in most treatments, being the highest value for 1.5% MP 0.0% G. In general, the higher the elastic modulus, the lower the strain at break. Lacroix and Vu (2014) states that MPs films are stronger than most biopolymer films, but less elastic. The characteristics of tensile strength and elongation are highly associated with the amount of plasticizer, nature of the film-forming material and cohesion of the polymeric matrix structure (MOIA *et al.*, 2021). There are several strategies to improve the mechanical properties of edible films, the main one being the addition of a plasticizer. PMs produce brittle films with low mechanical strength, so it is necessary to add a plasticizer to reduce the fragility of the films, increase their flexibility and allow its use in the production of packaging films (ALCÂNTARA *et al.*, 2022; TARIQUE; SAPUAN; KHALINA, 2021).

Based on these three mechanical tests, it is not possible to choose a single film as being the best, as each mechanical property resulted in a different protein and plasticizer concentration.

Color and opacity of protein films

The brightness (L^*), a^* , b^* and opacity values for each of the film formulations produced with MPs and plasticizers are represented in Table 6.

Different concentrations of protein films with glycerol combinations ranged from 85.44 to 95.45 for brightness. The 1%MP films showed significantly lower ($p < 0.05$) brightness values when compared to the 1.5 and 1.5%, thus protecting food differently against light and its penetration. The increase in glycerol concentration did not provide a statistical

difference ($p < 0.05$) in brightness values between treatments with concentrations of 1.0 and 1.5% MP.

The color of the gelatin depends on the raw materials used and the extraction method, however, in general, the color does not affect the functional properties (SILVA; LOURENÇO; PENA, 2017). For a^* values (green - red axis), there was a statistical difference ($p < 0.05$) in most films. These positive values indicate a greater predominance of the red color. The increase in the glycerol concentration did not provide a difference for the a^* values for the 2.0% MPs films studied, although there was negative b^* values that indicate the predominance of the bluish color. The similarity of behavior and the proximity of the values of a^* and b^* with the work developed by Sobral, Monterrey-Quintero and Habitante (2002) with proteins from the muscle of *O. niloticus* suggest that the films presented a white to yellowish color.

The opacity of protein films ranged from 273.07 to 300.96%, with statistical difference ($p < 0.05$) for different protein levels, but no difference ($p > 0.05$) for glycerol concentrations. Opaque films make it difficult to transmit light. However, transparency is an important feature in some applications, such as food packaging. Thus, it is important that the films have low opacity (ZAVAREZE *et al.*, 2012). In this study, a greater opacity was observed in protein films with higher protein concentration than in films with lower concentrations, probably due to the characteristic coloration of MPs. A similar result was also found by Zavareze *et al.* (2012).

Given these results, the use of films with 1.0% MPs is recommended, especially regarding lightness and opacity data.

Table 6 - Values (mean \pm standard deviation) of brightness (L^*), a^* , b^* and opacity for MPs films (1, 1.5, and 2%) with or without addition of glycerol at concentrations of 0.0, 0.1, and 0.2%

Concentration	L^*	a^*	b^*	opacity (%)
1% MP 0.0% G	85.64 \pm 0.12 a	1.57 \pm 0.45 a	0.31 \pm 0.34 a	273.07 \pm 0.64 a
1% MP 0.1% G	85.44 \pm 0.19 a	1.24 \pm 0.23 b	0.30 \pm 0.44 a	273.18 \pm 0.38 a
1% MP 0.2% G	86.09 \pm 0.67 a	1.57 \pm 0.32 ac	0.31 \pm 0.34 a	275.40 \pm 0.54 a
1.5% MP 0.0% G	88.45 \pm 0.55 b	1.74 \pm 0.34 acd	0.35 \pm 0.67 a	284.65 \pm 0.22 b
1.5% MP 0.1% G	88.45 \pm 0.44 b	1.74 \pm 0.45 de	0.35 \pm 0.12 a	284.65 \pm 0.56 b
1.5% MP 0.2% G	88.42 \pm 0.33 b	1.76 \pm 0.34 def	0.29 \pm 0.18 a	283.23 \pm 0.22 b
2% MP 0.0% G	90.67 \pm 0.89 c	1.90 \pm 0.67 defg	-1.33 \pm 0.98 b	300.46 \pm 0.94 c
2% MP 0.1% G	95.45 \pm 1.34 d	1.89 \pm 0.56 defg	-1.33 \pm 1.04 b	296.01 \pm 1.04 c
2% MP 0.2% G	90.67 \pm 1.34 ce	1.90 \pm 0.45 defg	-1.33 \pm 1.04 b	296.01 \pm 0.97 c

MP = myofibrillar proteins; G = glycerol; L^* = brightness; a^* = red/green coordinate (-); b^* = yellow/blue coordinate (-). Source: The authors

CONCLUSIONS

1. Through the methodology used in this research, it was possible to obtain a yield of 18.70% of myofibrillar proteins (MPs) from Nile tilapia CMS and a post-lyophilization protein content of 87.33%;
2. From the MPs, it was possible to elaborate films with different protein concentrations (1.0, 1.5, and 2.0%) and different glycerol concentrations (0.0, 0.1, and 0.2%), being the filmogenic solutions of 1.0% of PMs and 0.1 and 0.2% of glycerol that apparently seem to have better performances considering the compilation of most of the physical-chemical and mechanical properties (WVP, solubility, thickness, strain at break, brightness and opacity).

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