Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.00336

The effect of chitosan ice glazing on the quality of frozen tilapia fillets: microbiological, physicochemical, and sensory characteristics

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Abstract

We evaluated the effects of adding chitosan to the glazing solution for tilapia fillets on the microbiological, physicochemical, and sensory attributes of these fillets. We froze fresh tilapia fillets and then glazed them with a solution containing chitosan (0.75, 1.50, or 2.25%) or water (control treatment). Hence, there were a total of four treatments, with 52 fillets per treatment. At various times during frozen storage (0, 30, 60, 90, 120, 150, and 180 days), we analyzed pH, color, and thiobarbituric acid reactive substances (TBARSs). We examined the percentage of coating incorporation as well as the centesimal composition and sensory attributes. We observed a linear increase in the incorporation of the coating as the chitosan level increased. The pH showed a significant difference after 30 days, and it showed a linear increase as the chitosan concentration increased. For the color of the fillets, after storage for 30 days, only the intensity of red showed a linear decrease as the chitosan concentration increased, and after storage for 90 days, the luminosity of the fillets decreased linearly. At 90 and 120 days, there was a linear increase in the yellowness of the fillets. Chitosan had no effect on TBARS formation. As the chitosan concentration increased, there was a linear decrease in heterotrophic bacteria in the fillets, regardless of the storage time. The moisture content increased linearly, while the protein decreased linearly, as the chitosan concentration increased. The ether extract and ash contents were not different between the treatments. In the sensory analysis, there was no regression effect for the evaluated attributes; however, the general acceptability of the fillets of the control treatment was superior to glazing with chitosan. We conclude that a glazing solution containing a high chitosan concentration effectively increases coating incorporation and fillet moisture and decreases the presence of bacteria in fillets, without greatly affecting pH or color during freezing for up to 6 months.

Keywords: chitosan; ice glazing; *Oreochromis niloticus*; tilapia fillets; shelf life.

Practical Application: Chitosan reduces moisture loss, discoloration, and bacterial presence of frozen tilapia fillets.

INTRODUCTION

Fish is a highly perishable food due to its biological and chemical composition (Ramezani et al., 2015). The high protein content, a pH that is close to neutral, high water activity, and the presence of proteolytic enzymes are factors that intensify the degradation process (Liu et al., 2010). Deterioration begins quickly after the death of the animal (Ghaly et al., 2010) due to a complex combination of physical, chemical, and biochemical properties and microbiological processes (Netam et al., 2018), resulting in lipid oxidation, protein degradation, and loss of other valuable nutrients (Addis, 2015). In addition, the production of undesirable compounds such as trimethylamine and low-molecular-weight volatile nitrogenous bases directly affects the quality and shelf life of the fish (Ocaño-Higuera et al., 2011). In view of this, the use of conservation techniques is essential.

One of the most used techniques for fish is lowering the temperature through refrigeration and freezing (Netam et al., 2018). Freezing inhibits enzymatic activity and delays the growth of microorganisms (Soares et al., 2016). Methods that use low temperatures are more suitable for storing food for relatively long periods of time, which is why they are the most common in the food industry (Zhu et al., 2019). However, it is common that during the storage period, direct contact with very cold temperatures and occasional temperature fluctuations cause moisture loss by sublimation and drying on the surface of the meat, leading to an effect called freezer burn (Soares et al., 2016). In addition, freezing and frozen storage also influence the texture, color, flavor, and nutritional value of the meat (Leygonie et al., 2012). As a way to avoid and reduce these effects in frozen products, a method called glazing can be applied after freezing. The surface of an already frozen product is coated with ice by immersion or spraying with a coating solution (Gonçalves, 2021). Glazing minimizes quality loss resulting from low storage temperatures, incorrect transport, the freezing and thawing rate, and temperature fluctuations (Soares et al., 2016). This is one of the most used processes in fish processing industries because it is one of the least expensive (Soares et al., 2017).

Received: 26 April, 2024.
Accepted: 16 June, 2024.
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Conflict of interest: nothing to declare.
Funding: Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (Termo de Outorga: 133/2023 /SIAFEM: 33108).

Generally, the glazing coating solution consists of water alongside additives with a thickening and antioxidant function, such as phosphates (Gonçalves, 2021; Netam et al., 2018). However, interest in natural preservatives has increased, providing the opportunity for “green labeling” and, consequently, attracting consumers interested in this type of product (Zhou et al., 2010). According to Ramezani et al. (2015), natural preservatives that extend the shelf life and that have antioxidant and antibacterial activities are the most desirable.

Chitosan, derived from chitin, is a versatile biomaterial due to its antioxidant, bioactivity, non-toxicity, biocompatibility, biodegradability, and low allergenicity. Moreover, chitosan has a large surface area; high porosity, tensile strength, and conductivity (Cheung et al., 2015); and strong antimicrobial activity (Soares et al., 2016). Several studies using chitosan in the fish glazing solution have confirmed its efficiency in maintaining the quality of fillets during storage (Fan et al., 2009; Ojagh et al., 2010; Shi et al., 2019; Soares et al., 2016). However, there have been no studies evaluating the effectiveness of chitosan in glazing Nile tilapia (*Oreochromis niloticus*) fillets, the most cultivated species in Brazilian fish farming (Peixe BR, 2024). Thus, we evaluated the effects of chitosan in the glazing solution on the microbiological, physicochemical, and sensory attributes of Nile tilapia fillets frozen for up to 6 months. We hypothesize that the addition of chitosan to the glazing solution improves the quality of the fillets during the freezing period, via antibacterial and antioxidant protection, with possible beneficial effects in decreasing fillet dehydration and preventing discoloration.

2 MATERIALS AND METHODS

2.1 Experimental design

The experiment was carried out in a completely randomized design, with three chitosan concentrations (0.75, 1.50, and 2.25%) and a control treatment (fillets glazed with water only). Hence, there were a total of four treatments, with 52 fillets per treatment. At each freezing storage time point (0, 30, 60, 90, 120, 150, and 180 days), three fillets were removed per treatment to analyze pH, color, and lipid oxidation, and one fillet was removed for microbiological characterization. The sensory analysis was performed after storage for 120 days (10 fillets per treatment). Finally, the centesimal composition was determined after storage for 150 days (14 fillets per treatment).

2.2 Obtaining the fillets

A total of 104 Nile tilapia were used. They were cultivated in excavated ponds in the city of Dourados (Mato Grosso do Sul, Brazil) and had an average weight of 700 g. The use of animals was approved by the Ethics Committee on the Use of Animals of the Universidade Federal da Grande Dourados, under protocol number 19/2019. After harvesting, the tilapia was desensitized by sectioning the spinal cord and placed in Styrofoam boxes with ice. The fish was subjected to desquamation, evisceration, skin removal, and manual filleting. The fillets were washed in chlorinated water and subjected to individual freezing (-18°C) for 20 h, after which the glazing process was performed.

2.3 Preparing the glazing solutions

The chitosan used had a degree of deacetylation of 85% (Polymar, Fortaleza-CE, Brazil). The different chitosan solutions (0.75, 1.50, and 2.25%) were prepared in a 1% acetic acid solution; they were subjected to constant stirring on a heating plate at 45°C until complete dissolution. After cooling, the solutions were stored at 5 ± 2°C until their use to glaze the tilapia fillets. Before starting the glazing, the solutions were cooled to 1°C and maintained at this temperature by placing ice around the flask.

2.4 Application of the glazing solutions to the fillets

After freezing, the fillets were weighed individually and submitted to the glazing process, which consisted of immersing the frozen fillets in different glazing solutions (maintained at 1–3°C) for 10 s. The glazing process was carried out individually for each fillet. For the control treatment, the fillets were immersed twice in water (maintained at 1–3°C) for 10 s each. All fillets were frozen for 5 min and then weighed again to verify the incorporation of the glazing. The fillets were packed individually in labeled plastic bags and stored in a freezer at -18°C. To carry out the analyses described below, the samples were thawed under refrigeration (5 ± 2°C) for approximately 16 h.

2.5 Incorporation of glazing

After glazing, the percentage of glazing incorporation was determined with the Equation 1:

$$\text{Glazing incorporation (\%)} = \frac{Pgi - Pf}{Pf} \, X \, 100, \qquad (1)$$

Where:

Pf: the fillet weight before glazing;

Pgi: the fillet weight after the initial glazing.

2.6 pH

The pH was measured three times per fillet, in three fillets per treatment and per storage time (0, 30, 60, 90, 120, 150, and 180 days), using a portable digital potentiometer (Testo model 205, Testo, Lenzkirch, Germany) that had an insert for meat. In addition, the pH of each solution was measured three times.

2.7 Color

Fillet coloring was evaluated on the ventral side of the fillet, taking six different reading points per sample, in three fillets per treatment and storage time (0, 30, 60, 90, 120, 150, and 180 days). The luminosity value (L*) was determined using a colorimeter (Minolta model CR-10, Konica Minolta, Japan) under a 90° angle and at room temperature. L* defines the luminosity (L* = 0 black and L* = 100 white), while chroma a* represents the red-green component and chroma b* represents the yellow-blue component. The color of the glazing solutions was determined three times per solution.

2.8 Microbiological analysis

The presence of heterotrophic bacteria in the tilapia fillets was evaluated. For each storage time (0, 30, 60, 90, 120, 150, and 180 days), one fillet per treatment was analyzed in triplicate. Each sample was homogenized for 90 s in a stomacher (Lab-Blender 400, PBI, Milan, Italy), and the dilutions were prepared in a sterile saline solution. Afterward, the dilutions were plated on plates containing counting agar and incubated for 24 h at 37°C (Serio et al., 2018). After incubation, the microorganisms were counted.

2.9 Centesimal composition

Analyses were performed in duplicate, on 14 fillets per treatment, after 120 days of frozen storage. The moisture, ash, and lipid contents were determined according to AOAC methodology (2005). The crude protein content was determined by the semimicro Kjeldahl method, as described by Silva and Queiroz (2002).

2.10 Lipid oxidation

Lipid oxidation was evaluated at each storage time (0, 30, 60, 90, 120, 150, and 180 days) in three fillets per treatment (in triplicate). Lipid oxidation was evaluated by determining thiobarbituric acid reactive substances (TBARSs), using the methodology proposed by Wrolstad et al. (2005). For this purpose, a 5-g sample was mixed with 10 mL of 10% (w/v) trichloroacetic acid and homogenized in a processor. After filtration, 2 mL of the filtrate was added to 2 mL of 0.02 M aqueous thiobarbituric acid (TBA) in a test tube. The assay tubes were incubated at 100°C for 15 min; then, the absorbance was measured at 532 nm in a spectrophotometer. The TBARS value was calculated from the standard curve and is expressed as milligrams of malondialdehyde per kilogram of fish.

2.11 Sensory profile

Sensory analysis was approved by the Ethics Committee for Research with Human Beings of the Universidade Federal da Grande Dourados (CEP/UFGD), under Protocol No. 5644903.

The sensory profile of the fillets was analyzed after storage for 120 days. It involved 100 untrained tasters. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

For the sensory evaluations of the fillets, they were initially thawed under refrigeration (5 ± 2°C) for approximately 16 h. Fillets free of trimmings were cut into cubes (approximately 3 g), packed in aluminum foil, and placed in an oven at 180°C for 10 min. One fillet cube of each treatment was offered per taster, using disposable material free of any strange odors. The samples were offered to the tasters under white light.

Along with the sensory analysis form, a glass containing water and two saltine crackers were provided. Each taster was instructed to eat a piece of cracker and drink water between each sample.

Ten sessions were held, each with 10 different tasters. Each taster evaluated four samples labeled with a random three-digit code that corresponded to the treatment. Samples were served in a randomized design to avoid order and transposition effects (MacFie et al., 1989). The tasters were asked to taste and rate each sample on the acceptability of four attributes (color, texture, juiciness, and overall acceptability) using a 9-point scale, ranging from 1 (dislike extremely) to 9 (like extremely) (Dutcosky, 2007). The average scale was not included, as described by Font I Furnols et al. (2008).

2.12 Statistical analysis

The data are presented as the mean ± standard error of the mean. The results were submitted to regression analysis at 5% significance by using the regression procedure of STATISTICA 7.1® (Statsoft Inc., Tulsa, OK, USA). Analysis of variance was performed to compare the glazing with solutions containing chitosan with the control treatment. If there was a significant difference ($P < 0.05$), then the Dunnet post hoc test was applied.

3 RESULTS AND DISCUSSION

3.1 Analysis of the glazing solutions

The pH and yellowness intensity (chroma b*) increased linearly in the glazing solution as the chitosan concentration increased (Table 1). On the contrary, the luminosity and the intensity of red (chroma a*) decreased linearly as the chitosan concentration increased. All solutions containing chitosan showed significantly different pH, chroma a*, and chroma b* values compared to the control solution.

The lower pH of the chitosan-containing solutions was probably the result of the 1% acetic acid used for the dilution as chitosan is only soluble in an acidic aqueous medium (Rinaudo,

Table 1. pH and color of the chitosan-containing glazing solutions.

Parameter	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
pH	6.61 ± 0.04	4.39 ± 0.02*	4.73 ± 0.00*	5.02 ± 0.02*	< 0.0001 [A]
L*	83.42 ± 1.45	85.77 ± 0.10	84.49 ± 0.07	81.07 ± 0.38	< 0.0001 [B]
a*	-2.03 ± 0.03	-1.86 ± 0.02*	-2.29 ± 0.03*	-2.58 ± 0.03*	< 0.0001 [C]
b*	0.05 ± 0.09	1.84 ± 0.15*	5.25 ± 0.34*	9.23 ± 0.53*	< 0.0001 [D]

The data are expressed as the mean ± standard error of the mean; $P < 0.05$ indicates a significant difference compared to the control solution (Dunnet's test); [A] Linear regression between the chitosan concentrations: $y = 0.3167x + 4.0778$, $R^2 = 0.9982$; [B] Equation of the line: $y = -2.35x + 88.473$, $R^2 = 0.9354$; [C] Equation of the line: $y = -0.36x - 1.5233$, $R^2 = 0.9876$; [D] Equation of the line: $y = 3.6933x - 1.9489$, $R^2 = 0.998$.

2006). As the chitosan concentration increased, the pH increased, indicating a balance between the acid and the amount of chitosan. According to Hamdine et al. (2005), depending on the concentrations and type of acid used in the dilution, chitosan can directly affect the pH of the solution.

Regarding the coloring, when diluted, chitosan may present a yellowish to clear color. After total dilution, solutions with higher concentrations of chitosan showed a greater yellow intensity. This effect may have resulted from the degree of deacetylation of the chitosan used in this study (85%). According to Verlee et al. (2017), changes in the molecular weight and degree of deacetylation alter the chemical and physical structure. Indeed, there is a wide variety of chitosan available on the market. The use of chitosan degradation products such as oligosaccharides, which have smaller chains and consequently have better solubility and lower viscosity under physiological conditions, could represent an alternative to the use of chitosan in glazing solutions (Zou et al., 2016).

3.2 Coating incorporation

There was a linear increase in the incorporation of the coating (% glazing) from 9.79 to 23.92% as the chitosan concentration increased from 0.75% to 2.25% (Figure 1).

This behavior was probably caused by the increase in viscosity as the chitosan concentration increased. Intermediate materials, which are between a liquid and a solid, are composed of proteins, polysaccharides, or a combination of both. They result from the complex interaction between solvents and the molecular network (Oakenfull et al., 1997). According to Sathivel et al. (2007), chitosan solutions present characteristic macromolecular behavior, where the polymer molecules intertwine with each other. This tangle of molecules is responsible for conferring the viscoelastic character of the solution (Oakenfull et al., 1997). In addition, longer chitosan chains impact the solubility of chitosan and lead to higher viscosity under physiological conditions (Zou et al., 2016). These characteristics make it more difficult to apply the coating and drain the excess solution. The increase in viscosity is directly proportional to the chitosan concentration. A more viscous solution provides superior adhesion between the fillet and the solution as it has greater resistance to movement. Thus, the final coating is thicker (Soares et al., 2016).

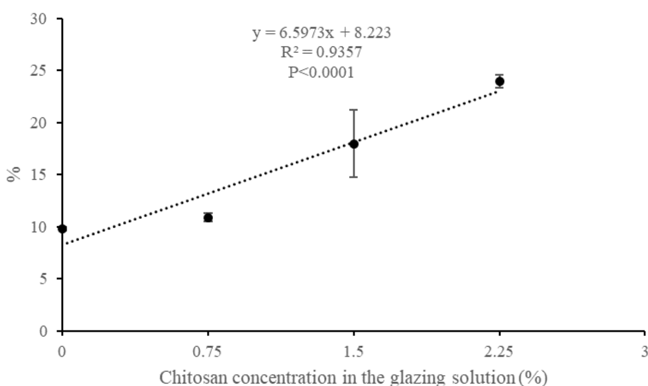

Figure 1. Incorporation of the coating on tilapia fillets glazed with chitosan-containing solutions and stored frozen. The vertical bars indicate the standard error of the mean.

Brazilian legislation establishes that the maximum limit for the amount of glazing with or without additives is 12% on the surface of the fish (Brasil, 2017). Thus, only the 0.75% chitosan concentration, presenting an incorporation of 10.89%, could be legally commercialized. Hence, there is a need to study methods to apply the coating or to change the dilution process to make the other solutions viable treatments.

3.3 pH

After 30 days of storage under freezing conditions, the pH of the fillets increased linearly as the chitosan concentration increased from 0.75 to 2.25% (Table 2). The mean pH of the fillets glazed with chitosan-containing solutions was significantly different from the control treatment only at days 30 and 60 ($P < 0.05$).

The pH reduction after 30 days could be directly related to the coating solution. This effect may be related to the migration of the coating, which has an acidic pH, into the fish muscle (Soares et al., 2013). This phenomenon explains the fact that only the fillets glazed with the solution containing 0.75 or 1.50% chitosan had a lower pH than the control fillets, indicating that these fillets incorporated the characteristics of the solutions. However, after 60 days, the fillets glazed with each chitosan-containing solution showed a significantly reduced pH compared to the control fillets. This outcome could be due to the fact that chitosan had reached its maximum degree of interaction with the fillet, and thus, there was stability between the coating and the fillet. At the subsequent time points, the pH

Table 2. pH of tilapia fillets glazed with chitosan-containing solutions and stored frozen.

Time (days)	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
0	6.39 ± 0.15	6.35 ± 0.05	6.22 ± 0.03	6.31 ± 0.12	0.7520
30	6.12 ± 0.02	5.80 ± 0.06*	5.92 ± 0.01*	6.00 ± 0.02	0.0064 [A]
60	6.13 ± 0.07	5.86 ± 0.02*	5.95 ± 0.03*	5.89 ± 0.02*	0.5103
90	6.20 ± 0.01	6.13 ± 0.15	5.97 ± 0.07	5.99 ± 0.05	0.3537
150	6.18 ± 0.11	6.09 ± 0.05	6.12 ± 0.05	6.04 ± 0.03	0.4070
180	6.08 ± 0.07	6.13 ± 0.06	6.16 ± 0.21	6.10 ± 0.04	0.8643

The data are presented as the mean ± standard error of the mean; *$P < 0.05$ compared to the control treatment (Dunnet's test); [A]Linear regression between different chitosan concentrations: $y = 0.0967x + 5.7148$, $R^2 = 0.9844$.

of the fillets glazed with chitosan-containing solution increased gradually and did not differ from the control fillets. This is a common effect resulting from the action of freezing and thawing (Sathivel et al., 2007).

Brazilian legislation establishes a maximum pH of 7.00 for the muscle portion of the frozen fish to be fit for human consumption (Brasil, 2017). All samples showed a pH below this value.

3.4 Color

After storage for 30 days, only the intensity of red (a*) presented a linear decrease based on the chitosan concentration (Table 3). The color of the fillets seems to have a direct relationship with the coating solution, as demonstrated by the pH changes. The decrease in the intensity of red after 30 days may have been the result of the interaction between the coating and the fillet as the fillet took on the more yellowish hue characteristic of the chitosan-containing solution.

After storage for 90 days, the fillets showed a linear increase in the intensity of yellow (b*) as the chitosan concentration in the glazing solution increased. This behavior also occurred at 120 days, confirming the theory that the fillets tended to incorporate the color characteristics of the coating solution.

After storage for 90 days, the luminosity (L*) of the fillets decreased linearly as the chitosan concentration increased — the same behavior observed in the solutions. This fact may also be related to the thickness of the coating, which becomes less translucent due to the modification of the refractive index of the surface layer (Cardoso et al., 2016).

The average luminosity did not differ between the treatments at any time point ($P > 0.05$). However, the average intensity of red (a*) differed significantly between the control and chitosan treatments after storage for 90 days ($P < 0.05$). In addition, after storage for 30 days, the mean intensity of red for the 2.25% chitosan treatment differed significantly from the control treatment ($P < 0.05$). For the intensity of yellow, the only significant difference occurred after 90 and 120 days: It was significantly higher for the 2.25% chitosan treatment compared to the control ($P < 0.05$). This difference probably occurred due to the characteristic color of chitosan becoming more pronounced due to the high chitosan concentration, resulting in a reduction in the intensity of red and an increase in the intensity of yellow.

Table 3. Color of tilapia fillets coated with chitosan-containing solutions and stored frozen.

Time (days)	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
Luminosity (L*)					
0	39.39 ± 0.52	40.05 ± 0.56	39.19 ± 0.65	39.89 ± 0.63	0.8592
30	47.20 ± 0.19	51.34 ± 0.82	50.48 ± 1.40	49.81 ± 0.84	0.3077
60	49.56 ± 0.56	49.17 ± 0.68	49.36 ± 0.88	49.37 ± 0.43	0.8294
90	46.20 ± 0.52	47.10 ± 0.70	47.43 ± 0.89	46.14 ± 0.85	0.4228
120	45.97 ± 0.38	49.82 ± 1.81	48.39 ± 0.61	46.13 ± 0.63	0.0469 [A]
150	46.55 ± 1.10	47.01 ± 0.42	46.50 ± 0.44	46.69 ± 0.03	0.5339
180	46.93 ± 1.02	49.09 ± 0.81	50.60 ± 0.22	48.54 ± 0.98	0.6689
Intensity of red (a*)					
0	-0.29 ± 0.13	-0.21 ± 0.04	-0.60 ± 0.12	-0.50 ± 0.17	0.1719
30	-0.06 ± 0.22	-0.60 ± 0.33	-1.05 ± 0.27	-1.62 ± 0.16*	0.0206 [B]
60	-0.70 ± 0.06	-0.98 ± 0.30	-1.09 ± 0.17	-0.63 ± 0.31	0.3810
90	-0.75 ± 0.29	-1.58 ± 0.19*	-1.76 ± 0.14*	-1.55 ± 0.12*	0.8947
120	-1.33 ± 0.12	-1.64 ± 0.22	-1.66 ± 0.13	-1.14 ± 0.16	0.0927
150	-1.12 ± 0.15	-1.53 ± 0.07	-1.32 ± 0.28	-1.37 ± 0.25	0.5930
180	-0.77 ± 0.18	-1.30 ± 0.25	-1.46 ± 0.33	-1.21 ± 0.30	0.8358
Intensity of yellow (b*)					
0	-1.44 ± 0.16	-0.98 ± 0.31	-1.92 ± 0.03	-1.21 ± 0.19	0.6200
30	-0.51 ± 0.23	-0.26 ± 0.36	-0.78 ± 0.26	-0.85 ± 0.30	0.2023
60	-2.66 ± 0.56	-3.00 ± 0.37	-3.18 ± 0.70	-1.29 ± 0.72	0.1082
90	-1.10 ± 0.49	-0.88 ± 0.09	-0.15 ± 0.35	0.27 ± 0.13*	0.0061 [C]
120	-0.79 ± 0.13	-0.51 ± 0.16	-0.66 ± 0.01	0.94 ± 0.19*	0.0114 [D]
150	-0.34 ± 0.17	-0.54 ± 0.58	-0.52 ± 0.92	0.73 ± 0.66	0.2445
180	-1.00 ± 0.18	-0.49 ± 0.68	-0.23 ± 0.87	1.03 ± 1.02	0.2319

The data are expressed as the mean ± standard error of the mean; *$P < 0.05$ compared to the control treatment (Dunnet's test); [A]Linear regression between different chitosan concentrations: $y = -2.4626x + 51.81$, $R^2 = 0.9834$; [B] Equation of the line $y = -0.6778x - 0.07$, $R^2 = 0.9954$; [C]Equation of the line $y = 0.7715x - 1.4104$, $R^2 = 0.9772$; [D] Equation of the line $y = 0.9652x - 1.5276$, $R^2 = 0.6728$.

Although we observed variation in color, Soares et al. (2015) found that glazing salmon fillets with chitosan-containing solution did not lead to significant differences in color. They even reported greater color stability after glazing with a chitosan-containing solution. However, they used chitosan with a degree of deacetylation of 91%, probably resulting in a clearer solution that had less effect on the fillets.

3.5 Lipid oxidation

The chitosan concentration had no significant effect ($P > 0.05$) on the formation of TBARS at any storage time compared to the control treatment (Table 4).

These results indicate that the chitosan coating did not have a significant impact on the lipid oxidation of tilapia fillets. We did not expect this outcome given that chitosan is known for its antioxidant characteristics (Cheung et al., 2015). The absence of a difference in lipid oxidation might be because that tilapia is a lean fish, with a lipid content of around 1%. Thus, lipid oxidation may not play an important role in deterioration of this fish. In previous studies that analyzed lipid oxidation (with the TBARS method) of fatty fish, chitosan did exert an antioxidant effect: gelatin applied to rainbow trout (Nowzari et al., 2013), edible film applied to salmon (Sathivel et al., 2007), and a coating to salmon (Hammond & Skonberg, 2012). Chitosan probably provides an oxygen barrier that delays lipid oxidation (Cardoso et al., 2016). By contrast, a study carried out by Soares et al. (2013) with samples of salmon glazed with chitosan and frozen for 6 months, submitted to analysis to determine the TBA value, did not show significant differences in lipid oxidation. However, we cannot directly compare out study to that one because unlike TBARS, the TBA value only considers the amount of malondialdehyde and not the general extent of lipid oxidation (Cardoso et al., 2016).

3.6 Microbiological analysis

After storage for 30, 60, 90, and 120 days, there was no bacterial growth in the fillets glazed with solution containing 1.5 or 2.25% chitosan. However, after storage for 150 and 180 days, heterotrophic bacteria had grown in these fillets. Moreover, at all storage times, heterotrophic bacteria had grown in tilapia fillets glazed with solution containing 0.75% chitosan (Table 5). In general, as the chitosan concentration increased, there was a linear decrease in heterotrophic bacteria in fillets, regardless of the storage time.

This decrease in heterotrophic bacteria in fillets containing higher chitosan concentrations is possibly due to the characteristic antibacterial effect of chitosan (Fan et al., 2009). Several studies have reported that chitosan inhibits microbial growth in frozen fish (Fan et al., 2009; Nowzari et al., 2013; Soares et al., 2015, 2017). Furthermore, when chitosan is a component of the coating solution, it is more available to act against microbes, whereas when it is part of a solid structure, such as an edible film, it is more retained (Nowzari et al., 2013), facilitating the antibacterial action.

Chitosan inhibits gram-positive and gram-negative bacteria as well as fungi (Verlee et al., 2017). Moreover, it can be bacteriostatic or bactericidal depending on the pH and degree of deacetylation (Li et al., 2016). In the present study, chitosan showed bacteriostatic activity, given that at 150 and 180 days there was an increase in the amount of heterotrophic bacteria in the fillets glazed with 1.50 and 2.25% chitosan. This may be due to the degree of deacetylation of the chitosan used in the present study (85%), which is relatively low: Other studies have used chitosan with a degree of deacetylation of ≥ 91% (Soares et al., 2013, 2015).

3.7 Centesimal composition

The centesimal composition of the fillets (Table 6) revealed that the moisture content increased linearly, while the crude protein content decreased linearly as the chitosan concentration in the glazing solution increased. The ether extract and ash contents did not differ between the treatments. The fillets

Table 5. Growth of heterotrophic bacteria in tilapia fillets glazed with chitosan-containing solutions and stored frozen.

Time (days)	Control	Chitosan concentration in the glazing solution (%)		
		0.75	1.50	2.25
0	4.47	4.42	4.42	4.42
30	3.72	2.52	0	0
60	3	2.3	0	0
90	3	2.82	0	0
120	3.36	3.22	0	0
150	3.98	3.82	3	2.82
180	4.02	3.9	3.22	2.82

The data are presented as log colony-forming units/g.

Table 4. Thiobarbituric acid reactive substances (milligrams of malondialdehyde/kilogram of sample) in tilapia fillets glazed with chitosan-containing solutions and stored frozen.

Time (days)	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
0	16.02 ± 0.27	14.73 ± 0.46	13.40 ± 0.87	13.46 ± 0.47	0.2125
30	13.71 ± 0.27	13.41 ± 0.59	15.01 ± 0.07	14.25 ± 0.48	0.2717
60	9.99 ± 0.38	12.03 ± 0.80	11.10 ± 0.45	11.57 ± 1.70	0.6678
90	11.09 ± 0.16	15.56 ± 1.74	13.34 ± 1.04	12.83 ± 0.42	0.1826
120	12.78 ± 0.82	17.01 ± 0.99	14.73 ± 0.55	17.18 ± 1.12	0.9123
150	10.71 ± 0.05	14.64 ± 0.06	14.27 ± 1.17	13.38 ± 1.32	0.3752

The data are presented as the mean ± standard error.

glazed with the solution containing 1.50% chitosan presented significantly different moisture, protein, and ash contents compared to the control fillets ($P < 0.05$). The fillets glazed with the solution containing 2.25% chitosan had significantly different moisture, protein, and ether extract contents compared to the control fillets ($P < 0.05$).

The increase in moisture may have been caused by the high degree of incorporation of the coating: Fillets glazed with solutions containing more chitosan also had a higher percentage of coating. During storage, coating moisture is lost rather than fillet moisture; hence, the coating serves as a protective barrier to water loss. Low temperatures during freezing cause moisture loss via sublimation and drying on the surface of the meat (Soares et al., 2016). Therefore, glazing is a way to prevent the sublimation of the water contained in the fillets. This sacrificial effect delays the loss of moisture from the fillet until the coating layer is exhausted by evaporation (Sathivel et al., 2007).

There is an inversely proportional relationship between the moisture and lipid and protein contents (Ogawa & Maia, 1999). We also observed this relationship in the present study for the moisture and protein contents: As the moisture content increased, there was a proportional decrease in the protein content. Thus, although the protein and lipid parameters showed a significant difference, the effect was possibly not directly caused by the chitosan itself, but rather by changes in the moisture content due to greater incorporation of the coating.

3.8 Sensory profile

In the sensory analysis (Table 7) of the fillets glazed with solutions containing different chitosan concentrations, there was no regression effect ($P > 0.05$) for the evaluated attributes. However, when comparing the means of the control treatment with the others, we observed differences ($P < 0.05$). Only the fillets glazed with the solution containing 0.75% chitosan had a significantly lower flavor score than the control fillets ($P < 0.05$). The fillets glazed with the solution containing 0.75 or 2.25% chitosan had significantly lower texture scores than the control fillets ($P < 0.05$). Finally, the control fillets showed a significantly higher general acceptability score ($P < 0.05$).

After cooking the fillets for sensory analysis, we noticed that the coating solutions formed a gel on the fillets; this phenomenon was more noticeable in fillets that had been glazed with solution containing 1.50 and 2.25% chitosan. This viscosity, resulting from the high concentration of coating incorporated during glazing, may have been responsible for the lower general acceptability of the samples glazed with chitosan relative to the control.

Although chitosan treatments were not well received in this work, Soares et al. (2017) reported glazing salmon fillets with chitosan and freezing them for 6 months did not negatively influence the sensory attributes of the frozen, thawed, and cooked samples. Indeed, glazing even improved the preservation of color, odor, texture, and general appearance. Considering only the attributes of flavor and texture, the 1.50% chitosan concentration presented the most satisfactory results as the means were similar to the means of the control fillets.

Based on these findings, additional research is needed to investigate the effects of the chitosan molecular weight and degree of deacetylation on coating solutions. These factors can influence color and sensory characteristics, which are decisive for the commercialization of fish, and the incorporation of the coating, which is restricted by law. In the future, chitosan could become a more viable and safe coating for tilapia fillets stored for long periods of time.

Table 6. Centesimal composition of tilapia fillets glazed with chitosan-containing solutions and stored frozen for 150 days.

Parameter	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
Moisture	78.32 ± 0.18	79.28 ± 0.37	80.23 ± 0.40*	80.69 ± 0.29*	0.0221 [A]
Protein	21.08 ± 0.27	20.09 ± 0.37	19.22 ± 0.33*	18.60 ± 0.36*	0.0184 [B]
Ether extract	1.80 ± 0.20	1.29 ± 0.17	1.37 ± 0.13	1.17 ± 0.14*	0.6047
Ash	1.09 ± 0.02	1.06 ± 0.03	1.00 ± 0.02*	1.08 ± 0.02	0.0628

The data are expressed as the mean ± standard error of the mean; *$P < 0.05$ compared to the control treatment (Dunnet's test); [A]Linear regression between different chitosan concentrations: $y = 0.7041x + 78.661$, $R^2 = 0.9615$; [B] Equation of the line: $y = -0.7435x + 20.789$, $R^2 = 0.9907$.

Table 7. Sensory attributes of tilapia fillets glazed with chitosan-containing solutions and stored frozen for 120 days.

Attribute	Control	Chitosan concentration in the glazing solution (%)			P-value
		0.75	1.50	2.25	
Odor	6.24 ± 0.18	6.09 ± 0.19	6.23 ± 0.19	6.20 ± 0.19	0.6736
Color	7.10 ± 0.16	6.91 ± 0.16	6.50 ± 0.18	6.86 ± 0.16	0.8261
Flavor	7.27 ± 0.16	6.46 ± 0.18*	6.85 ± 0.18	6.72 ± 0.20	0.3259
Texture	7.52 ± 0.12	6.91 ± 0.17*	6.98 ± 0.17	6.79 ± 0.19*	0.6250
General acceptability	7.38 ± 0.12	6.52 ± 0.17*	6.78 ± 0.17*	6.75 ± 0.18*	0.3444

Hedonic scale between 1 (dislike extremely) and 9 (like extremely). The data are expressed as the mean ± standard error of the mean; *$P < 0.05$ compared to the control treatment (Dunnet's test).

4 CONCLUSION

We conclude that glazing tilapia fillets with a high chitosan concentration (1.50% and 2.25%) increases the incorporation of the coating and the moisture of the fillets and decreases the presence of bacteria. Moreover, these solutions do not greatly affect the pH and fillet color during frozen storage for up to 6 months. Based on the sensory results, the use of 1.50% chitosan is indicated to improve the quality of tilapia fillets that are stored frozen.

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