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Effect of natural and synthetic antioxidants on oxidation and storage stability of mechanically separated tilapia meat

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ABSTRACT

The effects of sodium tripolyphosphate, ascorbic acid, sodium erythorbate, and natural antioxidants — green tea extract and propolis extract — on the physicochemical stability of mechanically separated meat (MSM) of tilapia stored for 180 days at −18 °C were investigated. The proximate composition, lipid oxidation (thiobarbituric acid reactive substances, TBARS), pH, water activity and color (L*, a*, b*, c* and °h) were analyzed. Frozen storage affected the proximate composition of the MSM by reducing the protein content and increasing the lipid and moisture contents. All color parameters showed significant differences ($p \leq 0.05$) throughout the 180 days of storage. The addition of antioxidants to MSM resulted in a reduction in a* value, which indicated that oxidation of myoglobin occurred during storage. At the end of 180 days of storage, the TBARS values for the treatments with sodium erythorbate and ascorbic acid were 33.55 and 20.81% lower than that of the control, respectively. These findings can be used by tilapia processors to maintain MSM quality for longer storage period. The green tea and propolis extracts did not have a significant effect on delaying lipid oxidation.

1. Introduction

In the last two decades, there has been a great expansion in the culture of tilapia, in which global cultivated production jumped from 383.654 t in 2000 to 5.714.901 t in 2017, representing 10.14% of the total production of farmed fish (El-Sayed, 2019). Tilapia is mainly commercialized through filleting, and this species has a low fillet yield of only approximately 30% (Monteiro et al., 2014). Thus, with the increase in production, there is a significant increase in the amount of byproduct of filleting generated, which is commonly underutilized, used as animal feed, or discarded (Bessa et al., 2016). The use of this byproduct is an alternative for the development of new, lower-production-cost products and for the reduction of organic residue discarded in the environment (Magalhães, Mársico, Soares, & Monteiro, 2019).

In this sense, several technological strategies have been adopted in recent years to increase the consumption and application of byproducts from food production, such as the consumption of products obtained by the separation of meat adhered to bones and skin. The product resulting from this process is called mechanically separated meat (MSM), which, according to Borgogno, Husein, Secci, Masi, and Parisi (2017), derives from the removal of the remaining meat from the bones by applying low ($\leq 10^4$ kPa) or high pressure ($>10^4$ kPa). The nutritional characteristics of MSM show great variability as a function of the differences in its production process and the raw material used (Hac-Szymanczuk, Cegielka, Karkos, Gniewosz, & Piwowarek, 2019; Signor, Simoes, Coldebella, Signor, & Boscolo, 2019). However, a factor common to MSM is high susceptibility to lipid oxidation, an especially notable trait of MSM obtained from fish, in which most lipids contain highly unsaturated fatty acids (Palmeira, Marsico, Monteiro, Lemos, & Conte, 2016).

The onset of lipid oxidation in meat products can be delayed by the use of antioxidant compounds (Bakalivanova & Kaloyanov, 2014). In addition to increasing the stability of food components, especially polyunsaturated lipids, they prevent degradation, discoloration, and oxidative rancidity and maintain the sensory characteristics (Lorenzo et al., 2018). Synthetic antioxidants have been used as food additives for years; however, the demand for natural antioxidants has recently increased due to the potential toxicity and carcinogenicity commonly attributed to synthetic compounds. The trends in the processing of meat products are directed towards the use of antioxidants such as β-carotene, α-tocopherol, sodium erythorbate and ascorbic acid (Granato, Nunes, & Barba, 2017; Bakalivanova & Kaloyanov, 2014).

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Natural sources of antioxidants with high levels of phenolic compounds, such as propolis and green tea, have been shown to reduce oxidation as effectively as synthetic antioxidants (Vargas-Sanchez, Ibarra-Arias, Torres-Martinez, Sanchez-Escalante, & Torrescano-Urrutia, 2019). Propolis is a resinous substance collected and processed by Western honey bees (*Apis mellifera*) from flower buds, stems, leaves, and cracks in various species of trees and plants that are mixed with beeswax. In addition to propolis extract having potential beneficial health effects, it has antimicrobial and antioxidant activity and can be used in fish products to avoid oxidative processes (Vargas-Sanchez, Ibarra-Arias, et al., 2019). Green tea (*Camellia sinensis*) is among the medicinal plants best known for being rich in phenolic compounds, such as flavonoids, flavonols, and phenolic acids, and plays an important role as an antioxidant (Jayawardana, Warnasooriya, Thotawattage, Dharmasena, & Liyanage, 2019).

Although green tea and propolis extracts have been studied as natural antioxidants in various food products, to date, no reports have been found in the literature of their applications in fish MSM. Thus, the present study aimed to evaluate the oxidative stability and physical and physicochemical properties of tilapia MSM supplemented with different antioxidants during six months of storage.

2. Materials and methods

2.1. Materials

Tilapia carcasses (100 kg) resulting from the evisceration (without all viscera) and filleting processes (without head, skin and gills) were obtained from a local fish processing company, Cia do Peixe (Lavras, Brazil). Tilapias were slaughtered with 600 ± 100 g (20 ± 5 cm length) and the study was conducted during the spring. The carcasses were transported frozen to the Fish Farming sector of the Federal University of Lavras (UFLA, Lavras, Brazil), where they were washed in running water and the dorsal and caudal fins were mechanically removed with a table bandsaw (CAF Máquinas 1.69).

Green tea (*C. sinensis*) powder extract and aqueous propolis extract were purchased from local pharmacies (Lavras, Brazil).

2.2. Production and storage of tilapia MSM

MSM was obtained by processing the carcasses in an electric deboner (High Tech HT- 100C). Samples of approximately 1000 g were packed in polyethylene plastic bags and immediately transferred to the Fish Processing Pilot Plant of the Department of Food Science. One group was separated as a control treatment containing only minced fish (MF) without antioxidant and other five groups were added of 0.50% sodium tripolyphosphate (TPP); 0.50% sodium tripolyphosphate and 0.25% sodium erythorbate (TPP + SE); 0.50% sodium tripolyphosphate and 0.05% ascorbic acid (TPP + Asc); sodium tripolyphosphate and 0.05% green tea extract (TPP + GTE); and 0.50% sodium tripolyphosphate and 0.10% propolis extract (TPP + PE).

The doses used in the study were based on previous studies that evaluated the effect of antioxidants in controlling lipid oxidation in meat products. The concentration selected for tripolyphosphate was based on a previous study by Kirschnik, Trindade, Gomide, Moro, and Viegas (2013). The concentrations of sodium erythorbate (New Max Industrial, Americana, MG, Brazil) and ascorbic acid were based on studies by Schmidt et al. (2016) and Sánchez-Escalante, Djenane, Torrescano, Beltran, and Roncales (2003), respectively. The concentrations of green tea (*C. sinensis*) and propolis extracts were defined based on a previous study by Pereira (2009, p. 128). The additives were directly added to the MSM and carefully homogenized in a cutter (R5 Plus model, Robot Coupe, Rio de Janeiro, Brazil) for a sufficient period of time to ensure a uniform distribution. The control sample was also homogenized with cutter equipment, ensuring the study's standardization.

After the addition of antioxidant sources, the samples were divided into 84 portions [6 treatments x 7 evaluation times (0, 30, 60, 90, 120, 150, 180) x 2 (repetition twice)] each of 70 ± 10 g in individual polyethylene plastic bags. Twelve plastic bags were grouped and repackaged in another polyethylene plastic bag to be removed at each evaluation interval. The samples were frozen within 24 h after production and stored at −18 °C in a vertical freezer (GTPC555 model, Gelopar, Chapada Araucária, Brazil) for 30, 60, 90, 120, 150, and 180 days before analysis.

2.3. Chemical and physicochemical analyses

The analyses of moisture (method No. 967.08), lipids (method No. 2003.06), ash (method No. 942.05), and protein (method No. 988.05) were performed according to the methodologies described by the Association of Official Analytical Chemists (AOAC, 2005). The pH was determined in a Tecnal digital potentiometer (model Tec-3MP, Tecnal, São Paulo, Brazil) with an immersion electrode. The samples were homogenized in advance at a ratio of 1:2 (5 g MSM and 10 mL distilled water) in a polytron. Water activity was measured using an Aqualab model 4 TE device (Decagon Devices, Washington, USA) at a temperature of 25 °C (±0.5 °C). Lipid oxidation was evaluated by the determination of TBARS by the spectrophotometric method described by Vyncke (1970) at a fixed wavelength of 532 nm, and the results were expressed in mg malondialdehyde (MDA)/kg sample.

2.4. Physical analysis

The color of the samples was determined with a Nix Color Sensor Pro colorimeter (NPRO; Nix Sensor, Ltd, Burlington, Ontario, Canada) using the CIE Lab system and by defining the chromatic space in rectangular coordinates (L*, a*, b*). The values of L* (brightness, black 0/white 100), a* (intensity of red, (−) green/(+) red) and b* (intensity of yellow, (−) blue/(+) yellow) were obtained. The chroma or saturation (c*) and hue angle (°h) values were calculated, where $c^* = [(a^{*2} + b^{*2})\ 1/2]$ and °h = tangent arc (b*/a*). In total, four measurements were performed on the surface of each sample.

2.5. Statistical analyses

A two-way analysis of variance (ANOVA) was used to determine the effects of natural and synthetic antioxidant treatments (control (MF), TPP, TPP + SE, TPP + Asc., TPP + GTE and TPP + PE) and storage time (0, 30, 60, 90, 120, 150 and 180 days) on the oxidative stability, physical and physicochemical properties of mechanically separated tilapia meat. The proximate composition and water activity (a_w) were determined at the beginning (day 0) and end of storage (180 days), while the analyses of pH, color, and thiobarbituric acid reactive substances (TBARS) were performed at intervals of 30 days. The experiments were replicated twice and all parameters were measured in duplicate (n = 4). The experiment was conducted with a completely randomized statistical design (CRD) and the results were evaluated using SISVAR software version 5.6. Scott-Knott's test was used to verify the existence of significant differences between the mean values ($p \leq 0.05$).

3. Results and discussion

3.1. Proximate composition

Tilapia was chosen in this study because it is the second most cultivated freshwater fish worldwide and produces a considerable amount of byproduct of filleting (Sangkharak, Paichid, Yunu, & Klomklao, 2020). In addition, tilapia is one of the preferred aquaculture fish for consumption due to its flavor, appearance and ease of cooking (Chikowi, Ochieng, & Jumbe, 2020). Table 1 shows the changes in the proximate composition of tilapia MSM supplemented with different antioxidants during the frozen storage period.

Table 1
Proximate composition (g/100 g on a wet basis) of tilapia MSM with added antioxidants.

Treatments	Proximate composition	
	Moisture (g/100 g)	
	0	180
MF[(1)]	63.71 ± 0.62[(2) Aa]	62.13 ± 0.17[Db]
TPP	62.76 ± 0.29[Ba]	63.19 ± 0.42[Ca]
TPP + SE	62.25 ± 0.15[Ba]	62.80 ± 0.59[Ca]
TPP + Asc	62.72 ± 0.13[Bb]	64.22 ± 0.53[Ba]
TPP + GTE	62.62 ± 0.24[Bb]	64.87 ± 0.38[Aa]
TPP + PE	62.39 ± 0.33[Ba]	62.14 ± 0.49[Da]
	Crude protein (g/100 g)	
MF	11.29 ± 0.79[Aa]	9.16 ± 0.51[Bb]
TPP	11.27 ± 0.18[Aa]	9.65 ± 0.26[Bb]
TPP + SE	10.79 ± 0.50[Aa]	9.09 ± 0.35[Bb]
TPP + Asc	11.47 ± 1.02[Aa]	9.91 ± 0.78[Ba]
TPP + GTE	11.10 ± 0.71[Aa]	9.27 ± 0.54[Bb]
TPP + PE	10.72 ± 0.24[Aa]	10.58 ± 0.71[Aa]
	Lipids (g/100 g)	
MF	23.35 ± 1.31[Ab]	28.79 ± 0.95[Aa]
TPP	23.35 ± 0.58[Ab]	24.49 ± 0.74[Ba]
TPP + SE	23.41 ± 0.55[Aa]	23.35 ± 0.75[Ca]
TPP + Asc	23.75 ± 0.55[Ab]	25.26 ± 0.78[Ba]
TPP + GTE	23.09 ± 0.29[Aa]	23.27 ± 0.56[Ca]
TPP + PE	23.18 ± 1.01[Aa]	23.32 ± 0.20[Ca]
	Ash (g/100 g)	
MF	0.74 ± 0.30[Bb]	0.93 ± 0.03[Ba]
TPP	1.27 ± 0.08[Aa]	1.36 ± 0.05[Aa]
TPP + SE	1.40 ± 0.03[Aa]	1.42 ± 0.03[Aa]
TPP + Asc	1.34 ± 0.03[Aa]	1.34 ± 0.07[Aa]
TPP + GTE	1.35 ± 0.02[Aa]	1.35 ± 0.09[Aa]
TPP + PE	1.36 ± 0.02[Aa]	1.34 ± 0.01[Aa]

[(1)] MF: minced fish (control); TPP: MF + 0.5% sodium tripolyphosphate; TPP + SE: 0.5% sodium tripolyphosphate + 0.25% sodium erythorbate; TPP + Asc: 0.5% sodium tripolyphosphate + 0.05% ascorbic acid; TPP + GTE: 0.5% sodium tripolyphosphate + 0.05% green tea extract; TPP + PE: 0.5% sodium tripolyphosphate + 0.1% propolis extract.
(2) Data represent mean (n = 4) ± standard deviation. Different letters within the same sampling day (A, B) and throughout storage (a, b) differ significantly ($p \leq 0.05$) by Skott-Knott's test.

At time 0, no significant differences ($p \leq 0.05$) was observed in terms of lipid and protein content. On the other hand, the control treatment differed significantly ($p \leq 0.05$) from the other treatments in relation to moisture content and ash content. According to Oliveira Filho (2009), the addition of additives and salts to products contributes to an increase in ash content. This increase, in turn, has an inverse correlation with the moisture content. This relationship occurs because as certain components of the proximate composition (such as water) decrease in relation to the total (100%), the content of other components increases. Variance analysis of proximate composition values revealed the significant ($p \leq 0.05$) impacts of antioxidants sources and freezing storage time (180 days), along with significant interactions between these two factors.

Magalhães et al. (2019), Signor et al. (2019), Bessa et al. (2016) and Kirschnik et al. (2013) reported varying values of moisture (67.38–86.14%), protein (4.78–20.73%), lipids (2.34–17.77%) and ash (0.43–1.62%) for tilapia MSM, which demonstrates the effect of variations in production process on the nutritional characteristics of carcasses and consequently on the resulting MSM.

Comparing the proximate composition of tilapia MSM with other fish species, the data obtained are in agreement with prior findings, except for the lower protein content. Ahmed, Liaquat, Shah, Abedl-Farid, and Jahangir (2020) found significant differences ($p \leq 0.05$) between the proximate composition of five fish species, including Mahseer (*Tor putitora*), Silver carp (*Hypophthalmichthys molitrix*), Common carp (*Cyprinus carpio*), Thela fish (*Catla catla*) and Rainbow trout (*Oncorhynchus mykiss*). Moisture ranged between 70.2 and 77.7%, ash between 1.0 and 1.4%, crude protein between 18.5 and 22.3%, and carbohydrates between 0.9 and 5.9%. According to the study, fish species that presents lower fat content always contain high water content. Rodrigues, Monteiro, Canto, Costa, and Conte-Junior (2020) analyzed proximate composition of fish fillets from four fish species *Piaractus mesopotamicus*, *Colossoma macropomum*, *Piaractus mesopotamicus* X *Colossoma macropomum* and *Piaractus brachypomus* raised under the same farming conditions and fed with the same diet. Differences were observed ($p \leq 0.05$) for moisture (70.47–79.25%), ash (0.97–1.28%), crude protein (16.79–25.00%) and lipid (0.84–9.23%) contents. According to Sgnaulin et al. (2020), the nutritional characteristics of fish vary according to diet. Thus, diets with unbalanced energy and protein levels significantly affect fat accumulation in carcasses.

Although the low protein content, the MSM preparation process was properly conducted, and the use of this coproduct may be a viable alternative for the development of practical products with lower production costs. Some consumers may feel discouraged from consuming fish due to the time required for preparation, leading to a preference for fish products that are more convenient or ready for consumption. However, ready-to-eat products available on the market are expensive, and major barriers to consumption seem to still exist (Palmeira et al., 2016). In this sense, several studies (de Lima et al., 2017; Lago et al., 2019; Netto, de Oliveira, Lapa-Guimaraes, & Viegas, 2020; Signor et al., 2019) have focused on the preparation of nutritious and practical foods from fish byproducts, which will promote the availability of products with more accessible prices on the market.

3.2. Lipid oxidation

The most serious quality problem that affects fish MSM is lipid oxidation. The presence of polyunsaturated fatty acids (PUFAs) and heme pigments make fish meat more susceptible to oxidation than other meats, especially when cell structures are disrupted and lipids are exposed to hemoglobin and other pro-oxidants present during MSM processing (Rundblad, Holven, Ottestad, Myhrstad, & Ulven, 2017). Although tilapia, like other vertebrate fish species, are not able to biosynthesize PUFAs, such as linoleic acid (18:2 n-6) or linolenic acid (18:3 n-3), these fatty acids can be found in the species due to dietary supplementation with oils rich in omega-3 and omega-6 fatty acids (Sangkharak et al., 2020).

The processing of carcasses in MSM further promotes oxidation by increasing the surface area of the fish muscle in contact with oxygen in the air (de Lima et al., 2017; Rundblad et al., 2017). As many of the secondary oxidation products are volatile compounds, they are responsible for the rancid odor and taste (Sveinsdottir et al., 2020). In food, the evolution of lipid oxidation can be measured by the quantification of MDA and other substances that react with TBA, called TBARS (Hac-Szymanczuk et al., 2019). Information related to TBARS and MDA content is reported in the literature as one of the most appropriate metrics for predicting oxidative stability in fish and fish-based products (Aldomás, Giannini, Ciarlo, & Boeri, 1986).

The evolution of the TBARS values of tilapia MSM during storage is shown in Fig. 1. The addition of antioxidants and storage time affected the TBARS values ($p \leq 0.05$). As expected, no significant difference ($p \leq 0.05$) was observed between treatments on day 0, which was similar to the results for up to 30 days of storage. After 30 days of storage, the TPP + SE and TPP + Asc treatments showed greater lipid stability than the other treatments ($p \leq 0.05$). At the end of 180 days of frozen storage, the TBARS values for the TPP + SE and TPP + Asc treatments were 33.55 and 20.81% lower than those of the control, respectively. The application of 0.50% sodium tripolyphosphate in tilapia MSM had no significant effect on the inhibition of lipid oxidation. In contrast, the TPP treatment showed a TBARS value 23.29% higher than that of the control at the end of the storage period, which indicates that the additive may have had a pro-oxidant effect. Fig. 1 also shows a gradual evolution of TBARS values during storage, although there was a stabilization stage between 60 and 120 days. The data presented here are in agreement with prior findings. Netto et al. (2020) observed TBARS values ranging

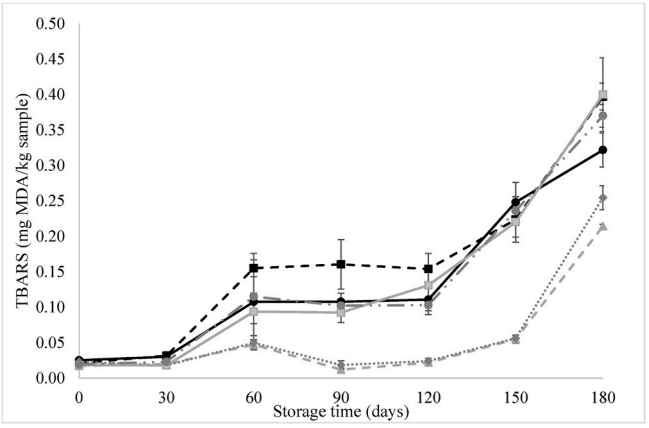

Fig. 1. Thiobarbituric acid reactive substances (TBARS) in tilapia MSM during freezing storage (−18 °C) for 180 days. Vertical bars represent the standard deviation of the mean.
(1) MF: minced fish (control, –●–); TPP: MF + 0.5% sodium tripolyphosphate (-■-); TPP + SE: 0.5% sodium tripolyphosphate + 0.25% sodium erythorbate (-▲-); TPP + Asc: 0.5% sodium tripolyphosphate + 0.05% ascorbic acid (··◆··); TPP + GTE: 0.5% sodium tripolyphosphate + 0.05% green tea extract (–■–); TPP + PE: 0.5% sodium tripolyphosphate + 0.1% propolis extract (- ·●- ·).

from between 0.017 and 0.400 mg MDA/kg sample throughout the storage of snacks prepared with MSM tilapia. Kirschnik et al. (2013) found TBARS values ranging from 0.20 to 0.65 mg malonaldehyde/kg in Nile tilapia MSM.

Sodium erythorbate has a strong antioxidant effect, which prevents the development of oxidative rancidity when applied at concentrations above 100 ppm; at lower concentrations, it can act as a pro-oxidant (Gray & Pearson, 1987). Kirschnik et al. (2013) also confirmed the effectiveness of lipid oxidation inhibition in tilapia MSM supplemented with sodium erythorbate (0.10%) and sodium tripolyphosphate (0.50%) for 180 days under frozen storage (- 18 °C), although these additives did not completely prevent the action of endogenous enzymes and microbiological activity. The perishability of fish meat is directly related to its low muscle glycogen reserves that result in pH values close to neutrality after rigor mortis. This accelerates the action of endogenous enzymes (such as cathepsins, lipoxygenases, peroxidases) and favors the growth of microorganisms. Both endogenous and microbial enzymes can degrade fish meat through the oxidation of fatty acids (Palmeira et al., 2016).

Ascorbic acid is an isomer of erythorbic acid, which is the acid corresponding to erythorbate. Ascorbic acid is one of the most commonly used water-soluble antioxidants against reactive oxygen species (Silva & Lidon, 2016). In the study by Abilmazhinova, Vlahova-Vangelova, Dragoev, Abzhanova, and Balev (2020) on horse MSM, an increase in ascorbic acid concentration from 0.01 to 0.03% in a parabolic way decreased the TBARS value. Primary antioxidants, such as sodium erythorbate and ascorbic acid, are radical scavengers. Conversely, secondary antioxidants, such as polyphosphates, are chelating agents. Therefore, different types of antioxidants have different inhibitory functions within each food matrix.

Polyphosphates, such as sodium tripolyphosphate, also act as cryoprotectants by preventing protein denaturation in foods. The cryoprotective effect occurs when low-molecular-weight compounds thermodynamically favor the maintenance of proteins in their native conformation. Tripolyphosphates prevent the aggregation of actomyosin, increase the water retention capacity of MSM and sequester metal cations, which contributes to the inhibition of lipid oxidation and protein denaturation. Tripolyphosphates are widely used in fish because muscle proteins are less stable than proteins from poultry, cattle and pigs (Kirschnik et al., 2013). When evaluating the stability of karmout (*Claries lazera*) MSM frozen for 180 days, Abdel-Aal (2001) found that the addition of 0.50% sodium erythorbate reduced oxidation during storage, while the addition of 0.50% sodium tripolyphosphate did not delay oxidation.

The green tea and propolis extracts had no significant effect on lipid oxidation delay. Surprisingly, the treatments with added natural extracts (TPP + GTE and TPP + PE) showed the highest TBARS values ($p \leq 0.05$), which were 24.22 and 14.90% higher than those of the control, respectively, at the end of 180 days of storage. It is possible that the concentrations of the natural extracts were not sufficient to neutralize the oxidizing factors present in the tilapia MSM and the extracts may have acted as pro-oxidants. The data presented here are in agreement with formerly reported data for GTE in mackerel (*Scomber scombrus*) minced fish (Alghazeer, Saeed, & Howell, 2018; Ozen & Soyer, 2018). According to Ozen and Soyer (2018), the GTE-added sample had higher peroxide value than control samples during storage. The authors stated that the large amount of chlorophyll pigments in green tea extract can result in a pro-oxidant effect when it is used in higher concentrations (500 ppm). Ferreira et al. (2012) also reported a pro-oxidant effect of propolis extract (0.1 g/L) as its addition induced increased TBARS results in fish.

In general, the addition of tripolyphosphate and sodium erythorbate and tripolyphosphate and ascorbic acid was effective in controlling lipid oxidation, differently from what was observed in the samples with the other additives (tripolyphosphate, tripolyphosphate and propolis extract, tripolyphosphate and tea extract green). There are several factors that affect the effectiveness of antioxidants, such as their molecular structure (molecular weight, number and position of OH/OCH_3 radicals, etc.), polarity (hydrophilic or hydrophobic compounds), and concentration (Lorenzo et al., 2018). Furthermore, Capecka, Mareczek, and Leja (2005) report that the antioxidant activity of natural extracts depends on the number of polyphenolic compounds. Although natural antioxidants have the potential to inhibit oxidation, their addition may represent a challenge in terms of sensory acceptance, especially if they are added at high concentrations (Damerau et al., 2020).

Several studies have reported the efficacy of natural antioxidants in meat products, both in delaying lipid oxidation and inhibiting microbial growth. The addition of 0.5 and 0.75% marcela (*Achyrocline satureioides*) extract to tilapia sausage MSM significantly decreased lipid oxidation and microbial growth in the product (de Lima et al., 2017). Oregano (*Origanum vulgare* L.) essential oil added to chicken MSM slowed lipid oxidation and bacterial growth (Hac-Szymanczuk et al., 2019). The inclusion of 1% powdered green tea (*C. sinensis*) leaves (GTP) to lamb sausage reduced lipid oxidation by 36–40% and the total bacterial count by 31–49%. However, the addition of GTP negatively affected the sensory quality because it reduced the acceptability score of the product (Purnamayanti, Jamhari, Hanim, & Irawan, 2020).

Due to the complexity of lipid oxidation, there is no universal limit for measured TBARS values that correspond to unpalatable levels of rancid taste or odor (Sveinsdottir et al., 2020). However, fish can be considered inappropriate for consumption when presenting values above 3 mg MDA/kg sample (Al-Kahtani, Abu-Tarboush, & Bajaber, 1986). Terra, Cichoski, and de Freitas (2006) stated that TBARS values up to 1.59 mg MDA/kg sample are considered to be too low to be perceived by sensory analysis. All evaluated treatments had TBARS values much lower than those reported above. The TPP + SE treatment presented the lowest TBARS value ($p \leq 0.05$), which showed that this combination of additives was the most efficient in delaying lipid oxidation. The average values and adjusted models with the respective coefficient of determination (R^2) for the results of TBARS and pH are shown in Table 2.

3.3. pH and water activity

The pH of the tilapia MSM without additives (MF) was lower than the pH values observed by Signor et al. (2019) and Angelini et al. (2013), which were 7.13 and 6.79, respectively. According to Signor et al.

Table 2
Regression equations for predicting the lipid oxidation and pH of tilapia MSM with added antioxidants.

Variable	Regression equations	R^2	P > F
TBARS	$y = 0.00001x^2 - 0.00057x + 0.03660$	0.9066	0.0000
pH	$y = -0.0000001x^3 + 0.000076x^2 - 0.00093x + 6.66869$	0.8983	0.0000

(2019), the pH value obtained in their study was explained by a high percentage of water in the meat, which makes the product highly perishable. In addition, the pH observed for the MSM (MF) control sample was lower than those observed in the other treatments with added antioxidants ($p \leq 0.05$) (Fig. 2). This result was similar to the results of the study by Kirschnik et al. (2013) on tilapia MSM. The addition of sodium tripolyphosphate is responsible for an increase in pH due to the alkalinity of the phosphate group (Kirschnik et al., 2013). According to Konno (1992), sodium tripolyphosphate has a cryoprotective effect by maintaining a pH of approximately 7, at which proteins are relatively stable.

In all samples, there was a pH increase until 120 days of storage and a subsequent pH reduction until 180 days. The pH changes in fish result from enzymatic and bacterial activities, which affect the concentration of free hydrogen and promote the decomposition of molecules. Therefore, an increase in pH indicates protein degradation with the production of metabolites such as ammonia and other amines due to microbial action and endogenous enzymes (Palmeira et al., 2016; Contreras-Guzmán, 2002). Enzymatic reactions may occur due to a small proportion of water still remaining in the thawed state (Gokoglu & Yerlikaya, 2015). According to Kirschnik and Macedo-Viegas (2009), the behavior of pH during storage under freezing also depends on the storage temperature, salt composition, physiological state, and buffering power of proteins. After 120 days of storage, an inverse relationship was observed between the pH and TBARS values. Witte, Krause, and Bailey (1970) found a correlation coefficient of −0.245 between pH and TBARS values, in which each increase of one pH unit resulted in an approximately 0.14-unit reduction in the TBARS value.

Regarding water activity, the values observed were less than 0.99, as found by Signor et al. (2019). The MSM and the treatments supplemented with natural extracts (TPP + GTE and TPP + PE) showed higher values of a_w ($p \leq 0.05$) than the other treatments (Table 3). The storage

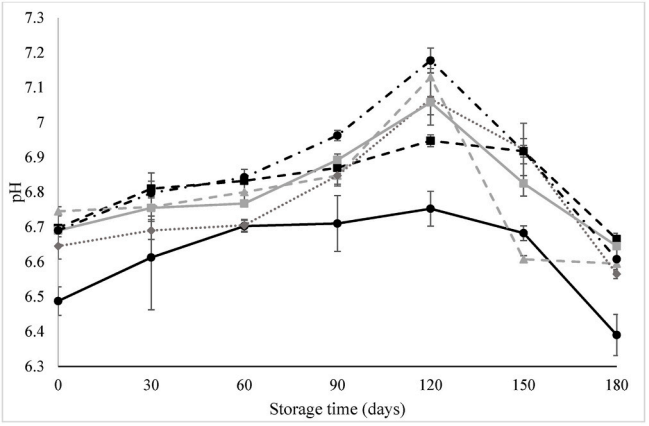

Fig. 2. Mean pH values of tilapia MSM with added antioxidants during freezing storage (- 18 °C) for 180 days. Vertical bars represent the standard deviation of the mean.
(1) MF: minced fish (control, –●–); TPP: MF + 0.5% sodium tripolyphosphate (-■-); TPP + SE: 0.5% sodium tripolyphosphate + 0.25% sodium erythorbate (-▲-); TPP + Asc: 0.5% sodium tripolyphosphate + 0.05% ascorbic acid (··◆··); TPP + GTE: 0.5% sodium tripolyphosphate + 0.05% green tea extract (–■–); TPP + PE: 0.5% sodium tripolyphosphate + 0.1% propolis extract (- ·●- ·).

Table 3
Mean a_w values of minced tilapia add of antioxidants stored at freezing temperature (- 18 °C) at day 0 and day 180.

Treatments	Storage time (days)		Mean
	0	180	
MF(1)	$0.966 \pm 0.002^{(2)Ab}$	0.945 ± 0.003^{Ba}	0.955 ± 0.011
TPP	0.962 ± 0.002^{Aa}	0.942 ± 0.002^{Bb}	0.952 ± 0.011
TPP + SE	0.963 ± 0.001^{Aa}	0.939 ± 0.001^{Bb}	0.951 ± 0.013
TPP + Asc	0.960 ± 0.002^{Aa}	0.939 ± 0.001^{Bb}	0.950 ± 0.011
TPP + GTE	0.967 ± 0.005^{Ab}	0.940 ± 0.001^{Bb}	0.954 ± 0.015
TPP + PE	0.970 ± 0.002^{Ab}	0.942 ± 0.002^{Bb}	0.956 ± 0.015
Mean	0.964 ± 0.004	0.941 ± 0.003	

(1) MF: minced fish (control); TPP: MF + sodium tripolyphosphate 0.5%; TPP + SE: sodium tripolyphosphate 0.5% + sodium erythorbate 0.25%; TPP + Asc: sodium tripolyphosphate 0.5% + ascorbic acid 0.05%; TPP + GTE: sodium tripolyphosphate 0.5% + green tea extract 0.05% and TPP + PE: sodium tripolyphosphate 0.5% + propolis extract 0.1%.
(2) Data represent mean (n = 4) ± standard deviation. Different letters within the same sampling day (A, B) and throughout storage (a, b) differ significantly ($p \leq 0.05$) by Skott-Knott's test.

time affected ($p \leq 0.05$) the a_w of the tilapia MSM, since the treatments with added antioxidants showed lower values of a_w than the control (MF) after 180 days of storage at −18 °C. Water activity is one of the main obstacles to microbial stability and safety of fish and fish products. Water activity refers to the water content available for enzymatic and biochemical reactions and microorganism growth. As fish and fish products have an average water activity close to 0.9, spoilage processes occur at high rates. Thus, measuring water activity can be an important indicator of the susceptibility of these products (Hall, 2012; Tsironi, Houhoula, & Taokis, 2020). For this reason, it is essential that tilapia MSM be used immediately after being processed or be stored under freezing. Although microbiological analyzes have not been performed during storage as this was not the main objective of the present study, these analyzes are essential to assess the overall quality of mechanically separated tilapia meat.

3.4. Physical characterization

The processing of tilapia meat can strongly affect its color and influence consumer acceptance. Thus, it is important to study the color behavior in fish MSM intended for human consumption to predict the changes that may occur in the final product (Bessa et al., 2016). The color changes of the tilapia MSM were monitored during the 180 days of storage under freezing, as shown in Table 4. The average values and adjusted models with the respective coefficient of determination (R^2) for the results of instrumental color are shown in Table 5. CIE Lab values may present values darker than the sample appears to the eye due to color analysis methods based on the reflectance of materials with a certain translucency do not reflect all the light emitted by the analysis instrument.

The results showed that the effect of treatment versus storage time was significant ($p \leq 0.05$). All color parameters showed significant differences ($p \leq 0.05$) throughout storage and indicated changes in color. However, the initial values of L*, b* and °h were not affected by the incorporation of antioxidants. In turn, the addition of antioxidants to MSM resulted in a significant reduction ($p \leq 0.05$) in a* values compared to those of the control sample (a* = 8.71) (day 0), especially in the TPP + Asc treatment, which had the lowest mean (a* = 4.24). Similar behavior was also reported by Magalhães et al. (2019) for extruded snacks prepared with tilapia MSM flour and broken rice grains, in which the incorporation of antioxidants (propolis ethanol extract, ascorbic acid, and butylated hydroxytoluene — BHT) decreased the presence of metmyoglobin compared to the control. Other extracts rich in phenolic compounds, such as teas, spices, and fruit extracts, were also related to red color changes in meat samples during refrigerated storage

Table 4
Measured instrumental color of tilapia MSM during freezing storage (180 days).

Item	Day	Treatments					
		MF	TPP	TPP + SE	TPP + Asc	TPP + GTE	TPP + PE
L*	0	57.84^{Aa}	58.78^{Aa}	56.83^{Aa}	59.97^{Aa}	54.72^{Ab}	57.25^{Ab}
	30	57.76^{Aa}	61.27^{Aa}	63.17^{Aa}	60.62^{Aa}	63.32^{Aa}	62.28^{Aa}
	60	58.45^{Aa}	60.01^{Aa}	64.02^{Aa}	60.84^{Aa}	61.73^{Aa}	62.62^{Aa}
	90	50.25^{Bb}	58.42^{Aa}	59.62^{Aa}	55.47^{Aa}	52.25^{Bc}	57.80^{Ab}
	120	51.07^{Bb}	56.27^{Aa}	60.97^{Aa}	51.62^{Bb}	51.37^{Bc}	52.97^{Bc}
	150	46.05^{Ac}	47.27^{Ab}	50.42^{Ab}	44.92^{Ac}	45.10^{Ad}	47.30^{Ad}
	180	45.35^{Ac}	45.90^{Ab}	49.37^{Ab}	42.80^{Ac}	41.32^{Ad}	44.35^{Ad}
a*	0	8.71^{Aa}	5.98^{Ba}	5.44^{Ba}	4.24^{Ca}	6.16^{Ba}	5.88^{Ba}
	30	8.44^{Aa}	3.35^{Dc}	4.87^{Ca}	2.79^{Db}	4.43^{Cb}	6.03^{Ba}
	60	4.99^{Ab}	3.48^{Bc}	5.23^{Aa}	4.41^{Aa}	3.11^{Bc}	3.18^{Bb}
	90	4.90^{Ab}	2.35^{Cc}	2.65^{Cb}	1.98^{Cb}	4.60^{Ab}	3.45^{Bb}
	120	5.00^{Ab}	3.47^{Bc}	4.35^{Aa}	1.92^{Cb}	5.65^{Aa}	3.17^{Bb}
	150	3.50^{Bc}	5.05^{Ab}	5.25^{Aa}	1.50^{Cb}	5.55^{Aa}	3.65^{Bb}
	180	3.17^{Bc}	6.30^{Aa}	5.17^{Aa}	1.57^{Cb}	5.58^{Aa}	3.42^{Bb}
b*	0	17.25^{Aa}	16.77^{Aa}	16.11^{Ab}	15.95^{Aa}	16.42^{Aa}	16.72^{Ab}
	30	16.93^{Ba}	16.08^{Ba}	17.47^{Aa}	16.39^{Ba}	16.88^{Ba}	17.80^{Aa}
	60	14.93^{Ab}	15.16^{Ab}	16.00^{Aa}	15.74^{Aa}	16.12^{Aa}	15.74^{Ab}
	90	7.60^{Bc}	8.62^{Bc}	8.17^{Bd}	8.02^{Bb}	10.20^{Ab}	9.60^{Ac}
	120	8.82^{Bc}	9.25^{Bc}	9.77^{Ac}	8.42^{Bb}	10.42^{Ab}	8.47^{Bc}
	150	8.65^{Ac}	8.57^{Ac}	8.63^{Ad}	8.27^{Ab}	9.42^{Ab}	8.80^{Ac}
	180	8.90^{Ac}	9.12^{Ac}	8.85^{Ad}	8.42^{Ab}	9.82^{Ab}	8.62^{Ac}
C*	0	19.33^{Aa}	17.87^{Ba}	17.00^{Bb}	16.51^{Ba}	17.54^{Ba}	17.74^{Bb}
	30	18.93^{Aa}	16.44^{Ba}	18.19^{Aa}	16.65^{Ba}	17.47^{Ba}	18.99^{Aa}
	60	15.99^{Ab}	15.55^{Ab}	16.83^{Ab}	16.35^{Aa}	16.35^{Ab}	15.74^{Ac}
	90	9.72^{Bc}	9.45^{Bc}	8.90^{Bd}	8.57^{Bb}	11.27^{Ac}	10.57^{Ad}
	120	10.17^{Bc}	10.02^{Bc}	11.05^{Ac}	8.77^{Cb}	11.92^{Ac}	9.07^{Ce}
	150	9.35^{Bc}	10.00^{Bc}	11.03^{Ac}	8.40^{Cb}	10.92^{Ac}	9.50^{Be}
	180	8.90^{Bc}	9.92^{Bc}	11.00^{Ac}	8.67^{Bb}	11.15^{Ac}	9.42^{Be}
°h	0	63.22^{Aa}	69.88^{Aa}	71.34^{Aa}	75.19^{Aa}	69.44^{Aa}	70.73^{Aa}
	30	63.63^{Ba}	78.30^{Aa}	74.11^{Aa}	80.37^{Aa}	75.50^{Aa}	69.71^{Ba}
	60	71.53^{Aa}	77.04^{Aa}	72.39^{Aa}	74.84^{Aa}	79.02^{Aa}	78.31^{Aa}
	90	59.93^{Ba}	74.47^{Aa}	71.32^{Aa}	77.25^{Aa}	66.32^{Bb}	75.57^{Aa}
	120	60.50^{Aa}	67.95^{Aa}	66.60^{Aa}	59.90^{Ab}	61.35^{Ab}	69.40^{Aa}
	150	67.77^{Ba}	59.52^{Bb}	58.05^{Bb}	79.40^{Aa}	59.62^{Bb}	67.50^{Ba}
	180	67.76^{Aa}	56.35^{Bb}	52.20^{Bb}	72.62^{Aa}	53.42^{Bb}	67.60^{Aa}

(1) MF: minced fish (control); TPP: MF + sodium tripolyphosphate 0.5%; TPP + SE: sodium tripolyphosphate 0.5% + sodium erythorbate 0.25%; TPP + Asc: sodium tripolyphosphate 0.5% + ascorbic acid 0.05%; TPP + GTE: sodium tripolyphosphate 0.5% + green tea extract 0.05% and TPP + PE: sodium tripolyphosphate 0.5% + propolis extract 0.1%.
(2) Data represent mean (n = 4) ± standard deviation. Different letters within the same sampling day (A-C) and though storage (a-e) differ significantly ($p \leq 0.05$) by Skott-Knott's test.

Table 5
Regression equations for predicting the instrumental color of tilapia MSM with added antioxidants.

Variable	Regression equations	R^2	P > F
L*	$y = -0.00074x^2 + 0.04572x + 59.14172$	0.9216	0.0000
a*	$y = 0.00018x^2 - 0.04250x + 6.02648$	0.9229	0.0000
b*	$y = 0.00024x^2 - 0.09763x + 18.06090$	0.8086	0.0000
C*	$y = 0.00026x^2 - 0.10064x + 19.04941$	0.8378	0.0000
°h	$y = 0.00001x^3 - 0.00370x^2 + 0.25107x + 69.86848$	0.9158	0.0030

(Vargas-Sanchez, Ibarra-Arias, et al., 2019).

Throughout the storage period, the values of the parameters L*, b* and c* significantly decreased ($p \leq 0.05$), which indicated that the samples became darker. The heme pigments present in MSM are initially in oxygenated form due to the incorporation of air during the meat deboning process and form the oxymyoglobin complex, which provides a bright light red color. However, the stored oxygen allows the heme group to undergo redox reactions and electron transfer with the addition of antioxidants, which react with oxygen and reduce the color of the meat (Brewer, 2004).

In the control treatment, the a* value decreased significantly after 60 days of storage, which can be attributed to the oxidation of myoglobin and accumulation of metmyoglobin (de Lima et al., 2017). According to Selani et al. (2011), pigment oxidation may be related to the lipid oxidation process since free radicals formed from fatty acids can react with the iron atoms of myoglobin and thus change the color of food during its storage. According to Araújo (2011), peroxides or byproducts of their degradation can interact with proteins and amino acids and form darker pigments. This statement corroborates the results observed in this study, in which a reduction in L* was observed, which indicates a darkening of the tilapia MSM; the reduction was especially notable after 150 days of storage, which coincided with the highest TBARS values.

4. Conclusions

The samples added with antioxidants showed higher ash content. Higher lipid content and lower protein content were observed after 180 days of frozen storage. An increase in pH was observed until 120 days of storage for all samples, after this period an inverse relationship between pH and TBARS values was established.

MSM fish samples became darker over storage, probably due to myoglobin oxidation. The addition of sodium tripolyphosphate (0.50%) + sodium erythorbate (0.25%) and sodium tripolyphosphate (0.50%) + ascorbic acid (0.05%) reduced the lipid oxidation of tilapia MSM during freezing storage. In contrast, the addition of green tea and propolis

extracts was not efficient in reducing lipid oxidation, and the extracts may have acted as pro-oxidants.

CRediT authorship contribution statement

Ana Cláudia Silveira Alexandre: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Francielly Corrêa Albergaria:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft. **Lara Maria dos Santos Ferraz e Silva:** Investigation, Data curation, Writing – original draft. **Luíza Aparecida Carneiro Fernandes:** Investigation, Data curation, Writing – original draft. **Maria Emília de Sousa Gomes:** Methodology, Project administration, Formal analysis, Supervision, Funding acquisition, Resources, Writing – review & editing. **Carlos José Pimenta:** Methodology, Funding acquisition, Resources.

Declaration of competing interest

The authors declare no conflict of interest.

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