Instrumental color and oxidative stability of light and dark muscles of Nile tilapia

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ABSTRACT: *The aim of the present study was to compare the color and oxidative stability of light (LM) and dark (DM) muscles of Nile tilapia (Oreochromis niloticus) stored at 4 °C for eight days. Five different trials of LM and DM samples were analyzed for instrumental color attributes (lightness, redness and yellowness), including the surface color stability through ratio of reflectance at 630/580 nm (R630/580), myoglobin concentration, total lipid content, fatty acid profile, metmyoglobin reducing activity (MRA), pH, lipid oxidation and protein oxidation. Results of the present study indicated that DM of Nile tilapia (Oreochromis niloticus) present lower oxidative and color stability during refrigerated storage than LM.*
Key words: *Oreochromis niloticus, myoglobin, lipid oxidation, protein oxidation, fatty acid profile.*

Cor instrumental e estabilidade oxidativa de músculos claros e escuros de tilápia do Nilo

RESUMO: *O objetivo do presente estudo foi comparar a estabilidade de cor e oxidativa de músculos claros (LM) e escuros (DM) de tilápia do Nilo (Oreochromis niloticus) armazenados a 4° C por oito dias. Cinco repetições diferentes de amostras de LM e DM foram analisadas quanto aos atributos instrumentais de cor (luminosidade, teor de vermelho e teor de amarelo), incluindo a estabilidade de cor na superfície através da razão de reflectâncias nos comprimentos de onda 630 e 580 nm (R630/580), concentração de mioglobina, lipídios totais, perfil de ácidos graxos, atividade de redução da metamioglobina (MRA), pH, oxidação lipídica e oxidação proteica. Os resultados do presente estudo indicam que músculos escuros (DM) de tilápia do Nilo (Oreochromis niloticus) apresentam menor estabilidade oxidativa e de cor durante o armazenamento refrigerado, quando comparados aos músculos claros (LM).*
Palavras-chave: *Oreochromis niloticus, mioglobina, oxidação lipídica, oxidação proteica, perfil de ácidos graxos.*

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most farmed freshwater species in the world mainly due to their genetic and reproductive characteristics, reaching a global production of 1.6 million tonnes in 2016 (FAO, 2016). In Brazil, tilapia leads the fish farming with approximately 239 thousand tonnes in 2015, representing 47.1% of the national aquaculture (IBGE, 2016). This fish species exhibits valuable sensory characteristics such as a firm texture and easily removable fish bones (CASTAGNOLLI, 1992; FITZSIMMONS et al., 2011), and also demonstrates a great market acceptance (MONTEIRO et al., 2013).

Meat color is the main sensory parameter for the consumer purchase decision in retail markets (MANCINI & HUNT, 2005). The Nile tilapia (*Oreochromis niloticus*) presents dark muscle along the lateral line, which contains a large amount of myoglobin (Mb) that can oxidize and favor the occurrence of premature browning due to the

accumulation of metmyoglobin over storage period (CHOW et al., 2009a), leading to product rejection. Mb is the main pigment responsible for meat color, which can also act as a pro-oxidant factor (CHAIJAN et al., 2008). Thus, the concentration and redox state of the Mb impact directly on the color stability of these muscles (SUMAN & JOSEPH, 2013). The Mb susceptibility to oxidation is determined by secondary structures of globin and different amino acid sequences, which varies among species. Besides, the combination of other intrinsic factors, such as the concentration of lipids and some specific enzymes, and the presence of lipid oxidation secondary compounds can also influence the Mb and color stability (LEE et al., 2010; MANCINI & HUNT, 2005).

Different muscle types present distinct oxidative stabilities, potentially due to their specific composition and metabolism that affect their biochemical characteristics (GLANCY & BALABAN, 2011; KIESSLING et al., 2006). In accordance, researches in yellowtail (*Seriola quinqueradiata*), chub mackerel (*Scomber japonicus*), frigate mackerel (*Auxis thazard*), catfish (*Clarias macrocephalus*) and saithe (*Pollachius virens*) demonstrated that light and dark muscles of the same fish species present different composition and oxidative behavior (BAE & LIM, 2012; CHAIJAN et al., 2013; KARLSDOTTIR et al., 2014; SOHN et al., 2005). These facts can contribute for different color stability in these muscles, potentially interfering on the application in the food industry and the development of novel fish products (CHAIJAN et al., 2004; SÁNCHEZ-ZAPATA et al., 2011).

Once tilapias are mainly distributed as skinned fillets packed in transparent plastic bags (CHOW et al., 2009b), color changes can impair on the commercial value of these products. Despite the relevance of Nile tilapia (*Oreochromis niloticus*) to global aquaculture, there is no information regarding these changes on dark and light muscles in this species when subjected to conditions similar to the retail display. Therefore, the aim of the present study was to investigate the color and oxidative stabilities of light and dark muscles of Nile tilapia (*Oreochromis niloticus*) during 8 days of refrigerated storage (4 °C).

MATERIALS AND METHODS

Experimental design

Fifteen kilograms of fresh Nile tilapias (*Oreochromis niloticus*) were purchased from a farm located in Cachoeiras de Macacu (22° 27' 46" S, 42°

39' 10" W), Rio de Janeiro, Brazil. Live weight and length of the fish were approximately 640 ± 52.4 g and 29.9 ± 1.8 cm, respectively. They were all raised under the same production conditions and feed management (32% of protein feed in fattening stage).

The fish were slaughtered according to the Aquatic Animal Health Code, and then were beheaded and gutted. The carcasses were immediately transported in filtered ice (1 °C) to the meat laboratory at Universidade Federal Fluminense within 2 h. The fish were randomly allocated to form five different repetitions (n = 5), samples were stored under refrigeration (4 °C) for eight days, and analyzed on days 0 and 8 of storage.

The fish were kept under refrigeration and light (LM) and dark (DM) muscles were excised on day 0 for myoglobin concentration, total lipid content and fatty acids profile determination. Instrumental color parameters (L^*, a^*, b^* values, and R630/580), metmyoglobin reduction activity (MRA), pH, lipid oxidation (2-thiobarbituric acid reactive substances - TBARS) and protein oxidation (carbonyl content) were evaluated on days 0 and 8 of refrigerated storage. All the analyses were performed in duplicate.

Myoglobin concentration

Myoglobin concentration analysis was carried out according to FAUSTMAN & PHILLIPS (2001) method, with slight modifications. Briefly, a sample aliquot of 5 g was homogenized with 45 ml of sodium phosphate (40 mM) buffer at pH 6.8 and, then, the homogenate was filtered through Whatman no. 41 filter paper, followed by an additional filtration in a 0.22 µm pore size polyvinylidene difluoride membrane. The absorbance of the resulting filtrate was read at 525 nm (A_{525}) utilizing a sodium phosphate (40 mM) buffer as the blank on the UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The following equation was used to calculate myoglobin concentration:

Myoglobin (mg/g of protein) = $(A_{525} / (7.6$ mM^{-1} cm^{-1} × 1 cm)) × 17 × 10

Where: 7.6 mM^{-1} cm^{-1} = millimolar absorptivity coefficient of myoglobin at 525 nm; 1 cm = light path length of cuvette; 17 kDa = average molecular weight of myoglobin and 10 = dilution factor.

Total lipid content

The determination of total lipid content in the samples was performed according to the method described by BLIGH & DYER (1959) with slight modifications proposed by CONTE-JUNIOR et

al. (2007). Total lipid content was extracted through sample homogenization with methanol / chloroform / water (2:2:1, v/v/v) (MONTEIRO et al., 2017). Results were expressed as percentage of total lipids (%).

Fatty acids profile

The extraction of the total lipid content was performed as previously described. The lipid fraction was methylated with 10% HCl solution in methanol (CONTE-JUNIOR et al., 2007). Two microliters of the methylated fatty acids were analyzed using the Clarus 400 gas chromatograph coupled with a flame ionization detector (Perkin Elmer, Massachusetts, USA) and the separation was performed using a polyethylene glycol column of 30 m long, 0.32 mm internal diameter and 0.25 μm film thickness (Sulpeco, Philadelphia, USA). The carrier gas used was helium at a flow rate of 1.8 mL/min. The injector (10 psi) and detector temperatures were set at 260 °C and 280 °C, respectively. The oven temperature and time conditions were based on CANTO et al. (2015a), with modifications. Initially, the temperature of the oven was set to 110 °C, increasing 40 °C/min until 233 °C, where was maintained for 2 minutes. Thereafter, the temperature was raised to a rate of 1 °C/min, until it reaches 240 °C, where it was held for 21 minutes. Methylated fatty acids were identified by the comparison of their retention time to those of 37 fatty acid methyl esters from commercial standard mixtures (Sulpeco, Philadelphia, USA).

Muscle pH

The pH values of LM and DM were determined using a portable pH meter HI 99161 (Hanna Instruments US Inc., Woonsocket, USA), with a penetration probe previously calibrated to pH 4.01 and 7.01 (KRAUSE et al., 2011). On each sampling day, the probe was inserted directly in two different points in each muscle type.

Instrumental color evaluation

The instrumental color readings were measured at two random locations in both muscle types using a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan) with measuring aperture diameter of 8 mm, illuminant A and 10° standard observer. Results were expressed as the average values of L^* (lightness), a^* (redness) and b^* (yellowness). Additionally, the surface color stability was indirectly estimated through ratio of reflectance at 630/580 nm. A low ratio of R630/R580 indicates a high metmyoglobin formation (AMSA, 2012).

Metmyoglobin reducing activity (MRA)

MRA evaluation was performed utilizing the method proposed by SAMMEL et al. (2002). In order to induce myoglobin oxidation, cubes (15 g) of LM and DM samples were submerged in solution of sodium nitrite (0.3%) solution during 20 min at room temperature. Subsequently, the excess of solution was blotted dry, the samples were vacuum packed and then were subjected to reading at a range of reflectance from 400 to 700 nm on the light-exposed surface, using a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc., Osaka, Japan). Further, the samples were incubated at 30 °C for 2 h to induce the reduction of metmyoglobin and rescanned. The percentage of metmyoglobin produced on meat surface at pre-incubation and post-incubation was calculated using formulas established by KRZYWICKI (1979).

A = log 1/R, where A = reflex attenuance, and R = reflectance at 525, 572 and 700 nm expressed as a decimal.

% Metmyoglobin = $(1.395 - [(A572 - A700 / (A525 - A700)]) \times 100$, where 1.395 = constant, A572 = reflex attenuance at 572 nm, A525 = reflex attenuance at 525 nm, and A700 = reflex attenuance at 700 nm.

Then, the percentage of metmyoglobin reducing activity was determined using the following equation (AMSA, 2012):

Metmyoglobin reducing activity (MRA) = ((pre-incubation surface metmyoglobin – post-incubation surface metmyoglobin) / pre-incubation surface metmyoglobin) × 100

Lipid oxidation

The 2-thiobarbituric acid reactive substances (TBARS) technique (YIN et al., 1993) was performed in order to evaluate the lipid oxidation. For samples extraction, the homogenization of LM and DM (5 g) with 22.5 mL trichloroacetic acid (TCA, 11%) were executed, with successive filtration using Whatman no. 1 filter paper. Then, 1 mL of each filtrate was mixed with 1 mL of aqueous thiobarbituric acid (TBA, 20 mM), followed by incubation in dark conditions for 20 h at 25 °C. The blank was obtained by mixture of 1 ml of TCA (11%) with 1 mL of TBA (20 mM) and subjecting it to the same analytical conditions. The results were expressed as absorbance values recorded at 532 nm in an UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Protein oxidation

Protein oxidation was analyzed utilizing the methodology described by MERCIER et al.

(1998) with modifications (ARMENTEROS et al., 2009). The protein carbonyl groups were detected and measured by reaction with 2,4 dinitrophenylhydrazine (DNPH). Briefly, for protein extraction LM and DM (3 g) were homogenized with 30 mL of KCl solution (0.15M) and for each sample, two equal eppendorfs containing 100 µL of the sample were added to 1000 µL of trichloroacetic acid (TCA; 10%). Eppendorfs were centrifuged at 5,000 x g for 5 min at 4 ˚C, the supernatant was disposed, at the first eppendorf was added 1000 µL of HCl (2M), and in the second was added 1000 µL of 10 mM DNPH in 2M HCl, followed by incubation at room temperature for 1 h, with vortexing every 15 min. Then, without pour off the supernatant, 500 µL of TCA (20%) was added to the mixture and centrifuged at 11,000 x g for 10 min at 4 ˚C. The supernatant was slowly discarded, and the precipitates were washed three times with 1000 µL of ethanol:ethyl acetate (1:1 vol/vol) to remove unreacted DNPH. The final protein pellet was dissolved in 1 mL of guanidine hydrochloride (6 M) in sodium phosphate (20 mM) at pH 6.5 and centrifuged at 11,000 x g for 10 minutes at 4˚C to remove insoluble particles. The absorbance of the samples was measured at 370 nm for carbonyl content, and 280 nm for protein content using UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) against a blank of guanidine hydrochloride (6M) in sodium phosphate (20 mM). Results were expressed as nmol carbonyl/mg protein, based on the molar extinction coefficient of 21,000 M^{-1} cm^{-1}.

Statistical analysis

The statistical software XLSTAT version 2014.6.01 (Addinsoft, Paris, France) was used to perform the statistical analysis. Differences between physicochemical parameters from both muscle types (LM and DM) and storage days (0 and 8) were evaluated separately utilizing one-way ANOVA through Student's t-test at 5% significance level ($P<0.05$). Principal component analysis (PCA) was performed to verify the main influencing parameters for each muscle type and for each storage day, while Pearson correlation was used to correlate the attributes. Both PCA and Pearson's correlation were determined at 5% level of significance ($P<0.05$).

RESULTS AND DISCUSSION

Myoglobin concentration (MC) and total lipid content (TLC)

DM demonstrated greater ($P<0.05$) values for both parameters (MC: 7.58 ± 1.48 mg/g; TLC:

6.24 ± 1.54 %) than LM (MC: 1.51 ± 0.15 mg/g; TLC: 1.60 ± 0.76 %). The difference on MC and TLC between the two muscles is potentially associated mainly to muscle fiber type constitution, which varies according to the muscle function and can present oxidative or glycolytic metabolism (GLANCY & BALABAN, 2011). The major part of fish skeletal muscle is composed of light muscle, constituted by white fibers, which are responsible for high swimming speeds and present glycolytic properties, low lipid content and oxygen exigency, and thus low myoglobin concentration (KIESSLING et al., 2006; LEE et al., 2010). Nonetheless, fish dark muscle is commonly located between the lateral line and the vertebral column, and is constituted by red fibers, which maintain the constant low speed swimming (OCHIAI et al., 2001; TSUKAMOTO, 1981). These fibers exhibit an oxidative metabolism leading to increased energy requirements, and thus high lipid and oxygen consumption rates, with high myoglobin concentration (FAUSTMAN et al., 2010).

Similar results of myoglobin concentration were reported by RICHARDS & HULTIN (2002) in light and dark muscles of rainbow trout (*Oncorhynchus mykiss)* and atlantic mackerel *(Scomber scombrus).* In addition, SOHN & OHSHIMA (2010) also documented that dark muscle samples of skipjack tuna (*Katuwonus pelamis)* presented higher lipid content than their white counterparts.

Fatty acid profile

The fatty acids (FAs) profile analysis of light and dark muscles of Nile tilapia (*Oreochromis niloticus*) resulted on the identification of 19 fatty acids, with five saturated fatty acids (SFAs), six monounsaturated fatty acids (MUFAs) and eight polyunsaturated fatty acids (PUFAs), which are presented in table 1. In addition, there was observed the pattern PUFAs > MUFAs > SFAs. Moreover, the predominant FAs observed in both muscles were respectively linoleic acid (LA, C18:2n6), oleic acid (OA, C18:1n9) and palmitic acid (PA, C16:0). FAs profile on fish highly depends on the dietary lipid sources (BLANCHET et al., 2005; KARAPANAGIOTIDIS et al., 2006; SHARMA et al., 2010). Commercial feed used in fish farming are rich in vegetable oils, which result in a large content of LA and OA as well (ALASALVAR et al., 2002; GRIGORAKIS et al., 2002). Similar pattern of FAs families (PUFAs >MUFAs >SFAs) were reported by JUSTI et al. (2003) on farmed Nile tilapia (*Oreochromis niloticus*) fillets. In agreement with our findings, KULAWIK et al. (2013) demonstrated

Table 1 - Fatty acid profile (%) of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at day 0 of refrigerated storage (4 °C).

Fatty acids	---Muscle type---	
	LM	DM
C14:0	1.49 ± 0.17^a	1.60 ± 0.02^a
C14:1	0.03 ± 0.01^b	0.06 ± 0.01^a
C16:0	13.23 ± 0.52^a	11.46 ± 0.73^b
C16:1	10.13 ± 1.80^a	10.18 ± 0.91^a
C18:0	1.27 ± 0.05^a	0.84 ± 0.10^b
C18:1n7	4.89 ± 0.55^a	6.00 ± 0.72^a
C18:1n9	14.59 ± 1.31^b	17.67 ± 0.82^a
C18:2n6	29.95 ± 1.05^a	30.66 ± 0.68^a
C20:0	3.43 ± 0.26^a	3.56 ± 0.64^a
C18:3n6	2.80 ± 0.15^a	2.77 ± 0.24^a
C20:1	1.43 ± 0.23^b	2.58 ± 0.12^a
C18:3n3	0.17 ± 0.04^a	0.33 ± 0.12^a
C20:2	3.43 ± 0.49^a	3.09 ± 0.38^a
C22:1n9	0.19 ± 0.01^b	0.30 ± 0.03^a
C20:3n3	0.18 ± 0.04^b	0.37 ± 0.10^a
C22:2	1.49 ± 0.29^a	1.29 ± 0.19^a
C24:0	2.00 ± 0.08^a	1.75 ± 0.09^b
C20:5n3	1.57 ± 0.20^a	0.93 ± 0.07^b
C22:6n3	7.75 ± 1.20^a	4.57 ± 0.74^b
Σ SFA	21.41 ± 0.33^a	19.20 ± 0.57^b
Σ MUFA	31.26 ± 2.90^b	36.79 ± 0.66^a
Σ PUFA	47.33 ± 2.62^a	44.00 ± 1.21^a

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

Results are expressed as mean ± standard deviation (n = 5).

Means without common superscripts (a and b) in a row are different ($P<0.05$).

similar predominant FAs in fresh tilapia (*Oreochromis niloticus*); however, they observed a greater amount of MUFA when compared to PUFA. Additionally, TANAMATI et al. (2008) documented high levels of LA, OA and PA in common commercial freshwater fish feed reinforcing the results of the present study.

Regarding the differences between both muscle types, DM presented greater ($P<0.05$) amount of C14:1, C18:1n9, C20:1, C22:1n9 and C20:3n3 when compared to LM. Additionally, LM demonstrated higher ($P<0.05$) levels of C16:0, C18:0, C24:0, C20:5n3 and C22:6n3 than DM ones. Despite the higher ($P<0.05$) levels of C20:5n3 and C22:6n3 in LM, there was no difference between the PUFA content of both muscle types. Similar results were observed in light and dark muscles of rainbow trout (*Oncorhynchus mykiss*) by INGEMANSSON et al. (1993), except for C14:1, C20:1, C20:3n3 and C24:0, which were not observed in their study.

The differences ($P<0.05$) on individual FAs were related to their distinct energy metabolism and contributed to the results of total SFA and MUFA, which were greater ($P<0.05$) in LM and DM, respectively. MUFA is more susceptible to oxidation than SFA (PAOLA & ISABEL, 2015); therefore, DM presented higher oxidative propensity than LM, which in association with the abundant presence of other pro-oxidants such as myoglobin (SÁNCHEZ-ZAPATA et al., 2011), can cause increased lipid oxidation levels. Moreover, the secondary products of this oxidation process can react with the proteins at the muscle, leading to the formation of carbonyl groups and increased protein oxidation levels that directly impair the color stability (LUND et al., 2011). Similarly to our findings, UNDELAND et al. (1998) observed the same trend for SFA and MUFA and, consequently, for lipid oxidation values on light and dark muscle portions of herring (*Clupea harengus*).

Muscle pH

DM demonstrated greater ($P<0.05$) pH values than LM on day 0 of storage (Table 2). This result is potentially related to differences in the metabolic processes of both muscle types where dark muscles usually present a minor concentration of glycolytic enzymes compared to light muscle, resulting in a lower conversion rate of glycogen into lactic acid thereby leading to a lower initial acidification in dark muscle (MOYES & GENGE, 2010). Nonetheless, pH values of DM and LM did not demonstrate difference ($P>0.05$) at day 8 of refrigerated storage (Table 2) possibly due to difference in muscle buffering capacity and glycogen content between both muscles (RAWDKUEN et al., 2008). In partial agreement with our findings, CHAIJAN et al. (2005) evaluated sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) and observed that dark muscle exhibited slightly greater pH values than light muscle on day 0 of storage, whereas no differences were observed between both muscle types on days 6, 9, 12 and 15.

Furthermore, pH values of LM and DM increased ($P<0.05$) over storage time (Table 2) potentially due to an enzymatic activity in fish tissue during the post-rigor period. During fish deterioration process, the action of endogenous and microbial enzymes promotes protein degradation, releasing secondary alkaline compounds such as ammonia, which increases muscle pH values (RODRÍGUEZ et al., 2004; UCHIYAMA et al., 1966). In agreement with the present research, KO et al. (2016) and SUBBAIAH et al. (2015) observed similar pattern of pH values in tilapia (*Oreochromis niloticus*) stored under refrigerated and frozen conditions, respectively.

Instrumental color

LM demonstrated greater ($P<0.05$) L^* values than DM in both storage days (0 and 8) (Table 2). ONYANGO et al. (1998) established an inverse relation between L^* values and myoglobin content, corroborating our findings. Similarly to our results, SÁNCHEZ-ZAPATA et al. (2010) reported lower L^* values for dark muscle compared to white muscle in bluefin tuna (*Thunnus thynnus)*. Regardless of the muscle type, L^* values were not affected ($P>0.05$) by the storage period (Table 2). In accordance with results of the present study, MANTILLA et al. (2008) and PIVARNIK et al. (2013) reported the same trend in L^* values during refrigerated storage of tilapia (*Oreochromis* spp.) fillets.

Moreover, DM presented greater ($P<0.05$) a^* and b^* values than LM (Figure 1; Table 2). The greatest a^* values observed in dark muscle can be explained due to large amount of myoglobin present in this type of muscle (CHAIJAN et al., 2010), which was observed in the present study. Furthermore, due to its higher lipid content, the deposition of liposoluble yellow carotenoids from the corn of dietary origin is higher on dark muscle than on their light counterpart, which potentially provides an increased yellow color,

Table 2 - Physicochemical parameters of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at days 0 and 8 of refrigerated storage (4 °C).
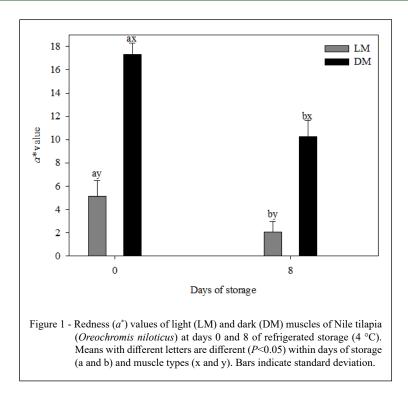
Parameters	Muscle type	------Days of storage------	
		0	8
Meat pH	LM	5.97 ± 0.08^{by}	6.25 ± 0.05^{ax}
	DM	6.16 ± 0.02^{bx}	6.30 ± 0.04^{ax}
L^* value	LM	61.40 ± 0.73^{ax}	58.28 ± 3.16^{ax}
	DM	47.52 ± 2.68^{ay}	42.90 ± 7.66^{ay}
b^* value	LM	6.87 ± 0.97^{ay}	3.93 ± 0.96^{by}
	DM	10.40 ± 0.87^{ax}	8.75 ± 0.97^{bx}
TBARS [†]	LM	0.01 ± 0.00^{bx}	0.02 ± 0.00^{ay}
	DM	0.02 ± 0.01^{bx}	0.10 ± 0.01^{ax}
Carbonyl content (nmol/mg)	LM	0.84 ± 0.25^{by}	1.66 ± 0.43^{ay}
	DM	1.95 ± 0.63^{ax}	2.18 ± 0.14^{ax}

[†]2-thiobarbituric acid reactive substances. Results expressed as absorbance values at 532 nm.

Results are expressed as mean ± standard deviation (n = 5).

Means without common superscripts (a and b) in a row are different ($P<0.05$).

Means without common superscripts (x and y) within a parameter in a column are different ($P<0.05$).

Figure 1 - Redness (a^*) values of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at days 0 and 8 of refrigerated storage (4 °C). Means with different letters are different ($P<0.05$) within days of storage (a and b) and muscle types (x and y). Bars indicate standard deviation.

justifying our results of b^* values (HERATH et al., 2016; KHALIFA et al., 2018). SOHN et al. (2005) also reported greater redness and yellowness in dark muscle compared to white ones in yellowtail (*Seriola quinqueradiata*). In addition, a^* and b^* values of LM and DM demonstrated a decrease ($P<0.05$) over storage at 4 °C (Figure 1; Table 2). The decrease on a^* values during storage suggested a browning of the fish, due to the myoglobin oxidation into metmyoglobin mainly caused by the inactivation of reductase enzymes over storage time (CHIOU et al., 2001). Additionally, a decrease in b^* values is potentially associated to carotenoids bleaching induced by oxidation reactions (BRITTON & KHACHIK, 2009; GOBANTES & CHOUBERT, 1998).

In agreement with our results, PIVARNIK et al. (2013) noticed a decrease on a^* values during refrigerated storage of tilapia (*Oreochromis* spp.) fillets. Moreover, similar trend for a^* and b^* values were reported by MANTILLA et al. (2008) in tilapia (*Oreochromis niloticus*) fillets over refrigerated storage.

Lipid oxidation

DM exhibited greater ($P<0.05$) TBARS values than LM at days 0 and 8 (Table 2), which can be attributed to dark muscle composition with large amount of total lipids, MUFA and myoglobin leading to faster lipid oxidation (SÁNCHEZ-ZAPATA et al., 2011). PETILLO et al. (1998) reported that the great amount of lipid soluble antioxidants content in dark muscles was not enough to hinder the lipid oxidation process favored by the pro-oxidant activity of myoglobin due to the high content of this heme protein in dark muscles. Myoglobin pro-oxidant activity mainly derives of the release of the iron from the heme group, enhancing lipid oxidation (FAUSTMAN et al., 2010). In agreement with our findings, SHAHIDI & SPURVEY (1995) and SOHN et al. (2005) observed similar pattern of TBARS values between dark and light muscles of mackerel (*Scomber scombrus*) and yellowtail (*Seriola quinqueradiata*), respectively.

Moreover, both muscle types exhibited an increase ($P<0.05$) in TBARS values over storage time (Table 2). Fish muscle presents a great amount of polyunsaturated fatty acids, which are more susceptible to lipase and lipoxygenase activity during lipid degradation process, leading to increase of secondary compounds formation during storage (HULTIN, 1994; MEMON et al., 2011). In accordance with our results, RAWDKUEN et al. (2008) and KARLSDOTTIR et al. (2014) reported an increasing trend of lipid deterioration in giant catfish (*Pangasianodon gigas*) and saithe (*Pollachius virens*) muscles during cold storage, respectively.
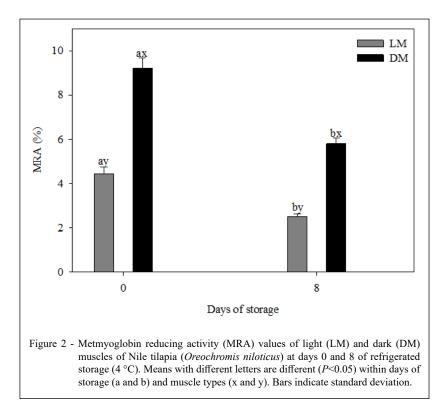
Protein oxidation

DM exhibited greater (*P*<0.05) carbonyl content than LM in both storage days (0 and 8) (Table 2). These results are potentially due to the different composition of both muscles. As demonstrated by the present study, dark muscle presents greater amount of myoglobin concentration, MUFA and total lipids, where myoglobin can act as pro-oxidant factor increasing both protein and lipid oxidations (FAUSTMAN et al., 2010; LUND et al., 2011). Furthermore, lipids are substrates to lipid oxidation leading to an accumulation of secondary compounds, that interact with proteins, producing carbonyl derivatives (FILGUERAS et al., 2010; LUND et al., 2007; TOKUR & KORKMAZ, 2007). Due to lack of studies regarding protein oxidation on dark and light muscles in fish species, our results were compared to previous report in dark and light chicken meat cuts (SOYER et al., 2010), which also observed greater carbonyl content in leg meat (dark muscle) than in breast meat (light muscle).

During storage period, protein oxidation demonstrated an increasing trend in both muscles types (Table 2). These results are potentially associated to post-mortem biochemical changes such as the release of catalytic compounds and oxidizing enzymes that promote the formation of protein carbonyls (ESTÉVEZ, 2011). BARON et al. (2007) and KINOSHITA et al. (2007) also observed this pattern during refrigerated storage on rainbow trout (*Oncorhynchus mykiss*) and bonito (*Katsuwonus pelamis*) unseparated muscles, respectively. The data of the present study reinforce the well-recognized connection between lipid and protein oxidation mechanisms (EYMARD et al., 2009).

Color stability

In order to investigate the color stability of DM and LM, the metmyoglobin reducing activity (MRA) and the ratio of reflectance of 630 nm (oxymyoglobin) to 580 nm (metmyoglobin) were evaluated (JOSEPH et al., 2012). Greater (*P*<0.05) values of MRA and R630/580 were observed for DM on days 0 and 8 separately, which was expected due to the higher myoglobin concentration in this muscle type. However, the difference (Δ) between the final (day 8) and initial (day 0) values of these parameters revealed that the surface discoloration was more pronounced in DM (Δ R630/580 = -0.9 and Δ MRA = -3.42) than in LM (Δ R630/580 = -0.15 and Δ MRA = -1.93) indicating a lower color stability in dark muscle type (Figures 2 and 3).

Figure 2 - Metmyoglobin reducing activity (MRA) values of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at days 0 and 8 of refrigerated storage (4 °C). Means with different letters are different (*P*<0.05) within days of storage (a and b) and muscle types (x and y). Bars indicate standard deviation.
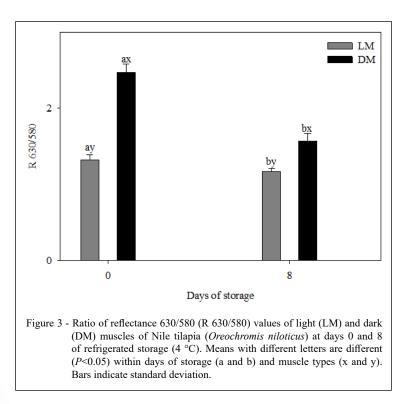
DM behavior may be associated with its higher myoglobin content, which can act as a pro-oxidant (TOKUR & KORKMAZ, 2007); and with its greater levels of lipid and protein oxidation, which may lead to the production of secondary compounds that affect color stability by binding to myoglobin histidine residues (SUMAN & JOSEPH, 2013). In addition, light muscles are rich in white fibers, which present a glycolytic metabolism, whereas dark muscle present large amount of red fibers with oxidative metabolism (GLANCY & BALABAN, 2011; KIESSLING et al., 2006; OCHIAI et al., 2001). Glycolytic muscles present greater amount of antioxidant enzymes that can act stimulating the production of NADH, which is an important cofactor in the enzymatic and non-enzymatic metmyoglobin reduction (CANTO et al., 2015b; ECHEVARNE et al., 1990). Moreover, dark muscles also present greater content of mitochondria than light muscles (PETILLO et al., 1998; SHINDO et al., 1986). The higher mitochondrial respiration rate reduces the penetration of oxygen in the muscle, favoring the formation of deoxymyoglobin on the meat surface, which is more susceptible to oxidation than oxymyoglobin (RAMANATHAN et al., 2008). Therefore, the increased consumption of oxygen in dark muscle and the unbalance among pro and antioxidant factors is responsible for their lower color

stability (MCKENNA et al., 2005; UNDELAND et al., 1998). Similarly, SOHN et al. (2010) observed analogous trend on color stability comparing light and dark muscles of skipjack tuna (*Katuwonus pelamis*).
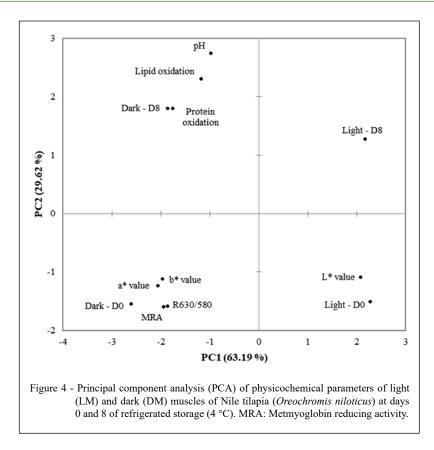
Furthermore, a decrease ($P<0.05$) on MRA and R630/580 values of LM and DM were observed during storage period (Figures 2 and 3). These results suggested metmyoglobin accumulation, which can be explained by the reduction in activity of the metmyoglobin reductase system enzyme combined with the increase on oxidative compounds concentration derived of lipid and protein oxidation in both muscles through storage time (CHIOU et al., 2001; PONG et al., 2000). Therefore, the results of the present study demonstrated that the oxidative reactions overcame the reductive mechanisms of both muscles through storage time. In agreement with our findings, THIANSILAKUL et al. (2010) also reported an accumulation of metmyoglobin in seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus × O. niloticus*) muscles over 15 days of refrigerated storage.

Principal component analysis (PCA)

This multivariate analysis explained 92.81% of the total data variance (Figure 4). The first principal component (PC1) contributed with 63.19% of this variance, and separated LM from DM,

Figure 3 - Ratio of reflectance 630/580 (R 630/580) values of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at days 0 and 8 of refrigerated storage (4 °C). Means with different letters are different ($P<0.05$) within days of storage (a and b) and muscle types (x and y). Bars indicate standard deviation.

Figure 4 - Principal component analysis (PCA) of physicochemical parameters of light (LM) and dark (DM) muscles of Nile tilapia (*Oreochromis niloticus*) at days 0 and 8 of refrigerated storage (4 °C). MRA: Metmyoglobin reducing activity.

independently of the storage day. This component divided the muscle types based on square cosines (> 0.6) of a^* and b^* values, R630/580, MRA and protein oxidation, which were greater in DM.

The second principal component (PC2) contributed with 29.62% of the variance and separated the storage points (day 0 and day 8), regardless muscle type. This categorization was based on square cosines (> 0.6) of pH and oxidation parameters (lipid and protein), which presented greater values at D8. In addition, the combination of PC1 and PC2 resulted in four groups: DM—D0; LM—D0; DM—D8 and LM—D8, emphasizing the difference of LM and DM during refrigerated storage based on physicochemical parameters evaluated in this study.

Regarding Pearson's correlation, the color parameters were highly (r > 0.9) correlated (*P*< 0.05), between them as following: R630/580 ratio and MRA (r = 0.97), R630/580 ratio and a^* values (r = 0.97), and MRA and a^* values (r = 0.99). In accordance with the present study, OCHIAI et al. (1988) evaluated two myoglobin extracts of bluefin tuna (*Thunnus thynnus)* with different concentrations, and documented high correlation between a^* values and percentage of metmyoglobin (r = -0 .999 at myoglobin concentration of 1.1 mg/ml; r = -0.985 at 0.7 mg/ml).

CONCLUSION

The data of the present study demonstrated that DM presented lower oxidative stability than LM during refrigerated storage time. These results can be explained by the greater lipid and protein oxidation on DM, induced by the higher lipid, MUFA and myoglobin contents, which leads to an increase on metmyoglobin accumulation and, consequently, lower color stability. The differences between DM and LM should be considered in order to evaluate strategies to improve the quality and commercial value of fillets and other products of Nile tilapia (*Oreochromis niloticus*), such as isolated or combined use of active packaging system and antioxidants.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

We authors of the article entitled " Instrumental color and oxidative stability of light and dark muscles of Nile tilapia" declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation to the Ethics Committee of the Universidade Federal do Rio de Janeiro, but we are aware of the content of the Brazilian resolutions of the National Council for Control of Animal Experimentation - CONCEA "http://www.mct.gov.br/index.php/content/view/310553.html" if it involves animals.

Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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