PAPER

COLD AND HOT SMOKED NILE TILAPIA FILLETS: QUALITY AND YIELD OF PIGMENTED AND UNPIGMENTED FILLETS

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ABSTRACT

Aiming evaluate the effects of smoking techniques on the quality of tilapia fillets, a 2x2 factorial scheme experiment was conducted comprising two smoking techniques (hot and cold) and two pigmentation (with and without). Cold smoked fillets and fillets with pigmentation demonstrated a greater yield. Hot smoked fillets were tenderer and presented lower moisture and greater ash and protein contents. The pigmentation did not influence the smoked fillet composition, but ash content was greater in fillets without pigmentation. The sensory acceptance of hot smoked fillets was better. The pigmentation influenced the color and appearance; however, fillets without pigmentation gave better flavor.

Keywords: benzo(a)pyrene, chemical composition, *Oreochromis niloticus*, organoleptic aspects, smoked yield

1. INTRODUCTION

Smoking is a technique that has been used since antiquity in order to preserve food from the effects of natural degradation and oxidation (VARLET *et al*., 2007). The degree of conservation of the fish depends on the synergistic actions between the addition of salt, the preservative effects from smoke compounds (phenols, aldehydes, and organic acids), and the dehydration that occurs during the smoking process (Fuentes *et al*., 2010). However, smoking is currently more utilized for its organoleptic qualities, as it is a process that provides fewer preservative benefits but more sensory qualities such as aroma, flavor, color, and it also adds value to the product (CARDINAL *et al*., 2006). Smoked fish is a highly nutritional food that contains polyunsaturated fatty acids, fat-soluble vitamins, essential minerals, and essential amino acids for humans (BILGIN *et al*., 2008).

Traditionally, there are two methods of smoking: hot and cold; these differences are obtained by temperature changes in the smoking chamber. Cold smoking is done at 33°C so that the intense thermal treatment is avoided and the nutrient structure is preserved (ARVANITOYANNIS and KOTSANOPOULOS, 2012). As a result, cold smoking does not offer adequate protection against harmful microorganisms, thus decreasing the shelf-life of the cold smoked fish. In the hot smoking process, the temperature ranges from 70 to 80°C, which results in baking of the meat (ARVANITOYANNIS and KOTSANOPOULOS, 2012). The heat and dehydration reduce the water activity of the fish, thus limiting the growth of microorganisms and increasing the shelf-life (ABOLAGBA and OSIFO, 2004).

The smoking process occurs in three steps: salting, heating, and smoking. Salt is used to preserve and to enhance the flavor of the smoked fish (GUIZANI *et al*., 2014), to help in the dehydration process, inhibit microorganism growth, and extract the salt soluble protein (CHENG *et al*., 2007).

The appearance is the first factor that influences the consumer who is buying smoked products. To get a better color in smoked fish, artificial coloring can be added, or the smoking time can be extended, the latter of which would lead to more weight loss in the fish and possible economic losses (BERAQUET and MORI, 1984). In the smoking process, color can be added to either intensify or subdue the golden red color. Natural dyes in foods have been utilized to give or to intensify color as well as to restore the color in products after the smoking process. The artificial pigmentation can ensure greater color uniformity, and the product becomes more attractive, which can significantly influence its acceptability with consumers.

Nile tilapia (*Oreochromis niloticus*) is the fourth most farmed fish worldwide (FAO, 2018) and the most widely farmed in Brazil (PEIXEBR, 2019). Its meat has a high nutritional value, featuring good taste and texture, and its fillet provides a good acceptance (SOUZA *et al*., 2015).

Therefore, an experiment was conducted to evaluate the effects of hot and cold smoking techniques on the quality, yield, and organoleptic characteristics in fillets of *in natura* Nile tilapia (*Oreochromis niloticus*).

2. MATERIALS AND METHODS

2.1. Animals and experimental procedures

Method was carried out in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA).
We used 250 Nile tilapia (*Oreochromis niloticus*) and submitted them to depuration for 48 hours in tanks with running water and without feed. After the depuration, animals were euthanized by severing the spinal cord followed by hand filleting. The fillets were vacuum-packed and kept cooled for 12 hours until the smoking time. From the 500 fillets that were obtained, half were assigned to the cold smoking treatment while the other half was assigned to hot smoking. The two fillets from each fish were randomly distributed to be pigmented or not.
The smoking of the fillets was achieved by using an industrial smoker (model Arprojet, Arprotec, Valinhos, Brazil), with smoke produced via wood friction (wooden rafter from pink eucalyptus).
For the smoking process, the fillets (with and without pigmentation) were immersed in a 20% brine solution at a 2:1 ratio of brine solution (weight/volume) for 30 minutes. After this period, the fillets were washed in running water and were placed in the screen of the smoking cart.
For the pigmentation process, the fillets were submerged in a water solution (1 kg fillets/1 liter of solution) with annatto extract (6 mL/L) for 15 minutes. The pigmented and unpigmented fillets were taken to the cold chamber (0 to 1°C), where they remained for 7 hours to remove superficial water (drainage). Afterwards, the fillets were placed in the smoking chamber to achieve partial drying (cold smoking = 30ºC and hot smoking = 50ºC) for 60 minutes. After, smoke was added to the process; the temperatures in the hot smoking treatment ranged from 50ºC to 80ºC for 3 hours, while in the cold smoking treatment the temperatures ranged from 30ºC to 40ºC for 5 hours.
At the end of the smoking process, the fillets were taken to a cold chamber (0° to 1ºC), and after the cooling process, they were vacuum packed and individually labeled.

2.2. Scanning electron microscopy

Three samples from the dorsal part of the smoked fillets were removed for scanning electron microscopy analysis; they were fixed in 2.5% buffered glutaraldehyde and then received 1% osmium tetroxide for 2 hours. Afterwards, the samples were washed in phosphate buffer, dehydrated in ethanol, and dried at a critical point with CO_2. The specimens were metallized with gold-palladium ions and electron-micrographed with JEOL JSM-5410.

2.3. Fillet yields and areas

In order to determine the yield, 90 fillets per treatment were utilized, and each weight was multiplied by 2 (number of fillets per fish). All yield data was calculated in relation to the total animal weight. Fillet yields were analyzed for *in natura* and smoked treatments, and calculations were made to determine the losses that occurred during the smoking process (*in natura* fillet yield minus smoked fillet yield).
To determine the fillet area, the fillets were placed on parchment paper and were circumscribed by using a pencil. After, a geographic information system was utilized with

the Geocoded Information Processing System – Spring program (INPE, 1999), which was developed by the National Institute of Spatial Research – INPE in order to complete the calculation of the fillet areas.

2.4. Quality parameters in smoked fillets compared with in natura fillets

2.4.1 Chemical composition analysis

Four fillets per treatment collected before and after the smoking process were used for the chemical composition analysis. These samples were packed in plastic bags, identified, and stored at -18ºC until the analyses.
The samples of *in natura* and smoked fillets were ground using a multiprocessor to obtain a homogeneous sample. Aliquots from this sample were used for the chemical composition determinations (4 replications; moisture, ash, and crude protein) according to the official methodology of AOAC (2005), while total lipid determination was achieved based on the methodology described by BLIGH and DYER (1959).

2.5 Color, texture, and pH

Ten smoked fillets per treatment and three *in natura* fillets (control) were used for the evaluation of the color. The fillet color was determined using a portable colorimeter (MiniScan XE, HunterLab, Reston, VA, USA) with a D65 light source (6500ºKelvin), observation angle of 10º, and a 30 mm opening measurement cell while using the scale L*, a*, and b* of the CIELab system that was developed by Judd and Hunter (HUNTER, 1975).
The shear force was determined in 10 smoked fillets per treatment and 3 *in natura* fillets (control). The fillets were sheared with a Warner Bratzler catheter at 500 mm/min by using a texturometer (TA.XT2i, SMS, Surrey, England). The force as a function of the deformation was calculated using the average of 10 measures in different positions per fillet sample, which was expressed in kg using the program Texture Expert V.1.15 (SMS, Surrey, England).
The pH of the smoked fillets was determined using a portable pH meter (DM2, Digimed, São Paulo, Brazil) in 10 fillets per treatment after the end of the smoking process.

2.6. Organoleptic characteristics

For the sensory analysis, the method utilized to evaluate the acceptability of the products was the preference test, which represents the sum of all sensory perceptions and considers the opinions of the consumers. This test measures consumer preference in order to predict the acceptability of a product. Thus, the acceptance attributes test was conducted by using a 9-point hedonic scale varying from "extremely disliked" (1 point) to "extremely liked" (9 points), as according to DUTCOSKY (2007).
Forty non trained tasters were used for the sensory evaluation. Within 36 hours after the smoking process, the fillets were cut and the samples were standardized in terms of weight and portion of the fillet (25 g). Then, the samples were packed in aluminum papers and identified. The tasters randomly received the samples on plates coded with three random numbers, and a sheet for sensory analysis was provided to evaluate flavor, internal color, aroma, texture, salt content, and general acceptance. Also, entire fillets per treatment were evaluated in terms of appearance attributes and the superficial fillet color.

2.7. Benzo(a)pyrene production

Aliquots (n = 4) from samples of *in natura* and smoked fillets that were utilized in the chemical composition were also used to determine the benzo(a)pyrene content. The samples were subjected to saponification with methanol KOH, liquid-liquid extraction with cyclohexane and dimethylformamide water (9:1, v/v), and cleaning via column chromatography of silica gel. The determination of benzo(a)pyrene was performed using high performance liquid chromatography with fluorescence detection (HPLC).

2.8. Experimental design

The experiment was completely randomized in a 2 × 2 factorial design consisting of two smoking techniques (HS = hot smoking and CC = cold smoking) and two pigmentation treatments (FWP = fillet with pigmentation and FOP = fillet without pigmentation) with 90 replications per treatment to determine yield analysis and losses during processing. For the chemical composition (n = 4), color determination (n = 10) and texture (n = 10) characteristics of smoked fillets, *in natura* fillets (control) were added in the design. Three *in natura* replications were used for color and texture, while for the other measurements we used the same number of replications as the smoked fillets (n = 4). For the determination of fillet area (n = 20) the type of process was included in the analysis (*in natura* and smoked). For the determination of benzo(a)pyrene (n = 3), we compared the HS and CC fillets with the *in natura* fillets (control). The fillet was considered the experimental unit.

The results of the analyzed variables were submitted to variance analysis by GLM procedure from the statistical computer program Statistical Analysis System (SAS, 2005). and the means were compared using the Tukey test with a 5% probability level. For the sensory analysis, the Friedman test (Chi-square test) was utilized with the non-parameterized Tukey test ($\alpha = 0.05$).

3. RESULTS

3.1. Yield, area, and area losses due to processing

In natura fillets presented with a similar weight, while smoked fillets presented with an average weight of 73.77 g/fillet. The smoking and the pigmentation processes affected the smoked fillet weight (Table 1). The hot smoked fillets presented with a lower weight ($p<0.01$) than cold smoked fillets, while the pigmented fillets were heavier ($p<0.01$) than those without pigmentation. A significant difference was not observed in the fillet yields.

A significant interaction was observed ($p<0.01$) in the smoked fillet yield and the smoked fillet losses (Table 1, Fig. 1). We observed that the cold smoking technique demonstrated a greater yield independent of fillet pigmentation (fillets with pigmentation = 24.90%) and lack of pigmentation (fillets without pigmentation = 25.03%) (Fig. 1A). Considering hot smoked fillets, the fillets with pigmentation presented with a greater yield (23.85%) than fillets without pigmentation (22.20%).

Regarding the losses that occurred during the smoking process, an interaction was observed between smoking techniques and pigmentation (Table 1, Fig. 1B). Cold smoked fillets (fillets with pigmentation = 11.54% and fillets without pigmentation = 11.52%)

presented with lower losses during the smoking process than hot smoked fillets (fillets with pigmentation = 12.51% and fillets without pigmentation = 14.43%).

Table 1. Means of weight, yield, losses and areas that occurred during the processing of Nile tilapia fillets (*Oreochromis niloticus*) that were submitted to cold and hot smoking techniques, with and without pigmentation.

Factors of variation	Fillet weight (g)		Yield (%)		Smoked losses (%)
	in natura	Smoked	Filleting	Smoked	
Smoking technique (T)					
Cold (CC)	112.32±17.25 a	76.94±13.35 a	36.51±1.12 a	24.97±1.68	11.53±1.52
Hot (HS)	112.43±16.10 a	70.81±11.56 b	36.52±0.90 a	22.98±1.96	13.51±1.97
Pigmentation (P)					
With (FWP)	113.37±16.87 a	75.82±12.67 a	36.40±0.95 a	24.37±2.19	12.03±2.08
Without (FOP)	111.43±16.42 a	71.82±12.68 b	36.59±1.05 a	23.54±1.89	13.05±1.84
F test					
Technique (T)	0.01ns	14.67**	0.00 ns	77.42**	87.04**
Pigmentation (P)	0.83ns	5.84*	2.25 ns	11.71**	20.81**
Interaction T × P	0.02ns	3.55ns	0.36 ns	16.20**	21.63**

Means in the same column that are followed by different letters differed by Tukey test (P<0.05) ** Significant (p<0.01); ns- non-significant (p>0.05). (*) Means with the same lowercase letter for smoking technique within the kind of pigmentation, while uppercase letters for pigmentation within smoking technique did not differ by Tukey test (P>0.05). Data expressed as mean ± standard deviation.

Smoking technique significantly affected (P<0.01) the fillet area. The hot smoked fillets had a greater superficial area (41.92 cm^2) than cold smoked fillets (37.17 cm^2). The use of pigments did not affect the fillets' area, but a significant reduction (p<0.01) in area was observed when the fillet processing technique was evaluated (*in natura* = 41.99 cm^2 and smoked = 37.11 cm^2). The smoking process decreased the fillet area by 11.62% (4.88 cm^2) when compared to *in natura* fillets. The pigmentation did not interfere (p>0.05) in the loss of fillet area.

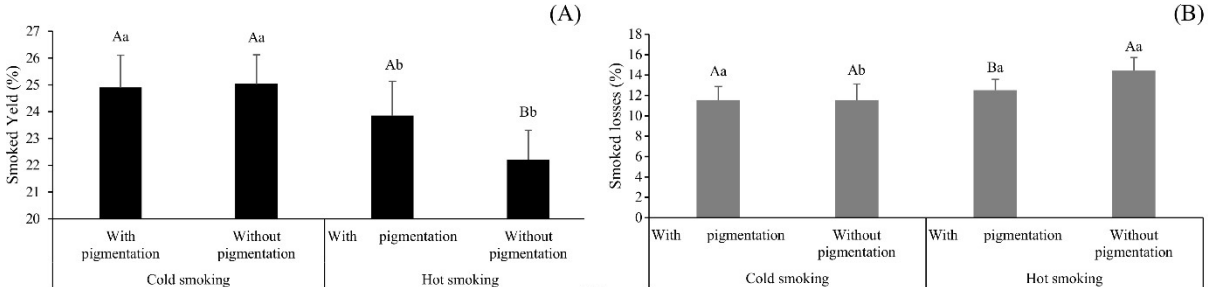

Figure 1. (A) Smoked yeld (%); (B) Smoked losses (%), unfolding of the interaction between Smoking Technique *x* Pigmentation in fillets of Nile tilapia (*Oreochromis niloticus*). (*) Means followed by the same lowercase to the factor Smoking Technique and uppercase to Pigmentation does not differ by Tukey test (p>0.05). Vertical bars represent the standard deviation of the mean.

3.2. Chemical composition

Variation occurred in the chemical composition of *in natura* fillets when compared to the final product (Table 2). Hot smoked fillets had a lower moisture content (68.90%) than cold smoked fillets (72.24%), while crude protein and ash contents were significantly greater (25.20 and 3.85% respectively) in hot smoking.
The smoking technique did not influence total lipid content (Table 2). It was observed that fillet pigmentation affected the ash and energy content. The pigmented fillets presented with lower ash contents (2.84%) than those without pigmentation (4.37%).

Table 2. Means of chemical composition[*] of Nile tilapia fillets (*Oreochromis niloticus*) submitted to cold and hot smoking techniques with and without pigmentation.

Factors of variation	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
Smoking technique (T)				
Cold (CC)	72.24±2.37 a[(1)]	22.50±2.30 b	2.10±0.25 a	3.19±0.87 b
Hot (HS)	68.90±2.29 b	25.20±1.71 a	2.08±0.99 a	3.85±0.91 a
Pigmentation (P)				
With (FWP)	71.45±2.60 a	23.60±1.98 a	2.19±0.98 a	2.84±0.43 b
Without (FOP)	69.67±2,92 a	24.07±2.87 a	2.04±0.26 a	4.37±0.58 a
Control = *in natura*	80.83±0.58	17.48±0.13	1.18±0.07	1.00±0.01
F test				
Control vs Fatorial	102.62**	45.36**	5.74*	227.61**
Smoking technique (T)	13.46**	10.31**	0.004 ns	23.59**
Pigmentation (P)	3.74 ns	0.45 ns	0.26 ns	103.60**
Interaction T x P	0.09 ns	0.31 ns	0.47 ns	0.32 ns

[(1)]For each factor, means of the same factor in a column that are followed by the same letter did not differ by Tukey test ($p > 0.05$). ns - Non-significant ($p > 0.05$) *Significant ($p < 0.05$) **Significant ($p < 0.01$)
(*) 2 replications per sample were used for protein, lipids, and ash content, while 3 replications were used for moisture content. Moisture content is showed in Franco *et al*. (2013). Data expressed as mean ± standard deviation.

3.3. Color, texture, and pH of the fillets

When analyzing the fillet color (Figure 2), we found that unpigmented *in natura* fillets had an average chroma value of a* and b* of -1.5 and 9.54, respectively. The values of a* and b* increased in function of the smoking technique and pigmentation. Pigmented fillets had values for a* and b* that were significantly greater than those without pigmentation, while the hot smoking process yielded greater chroma values than cold smoked fillets.
The unpigmented hot smoked fillets presented with a lightness (66.04) that was significantly greater ($p < 0.05$) than pigmented fillets (62.45). Cold smoked fillets with pigmentation (48.74) did not differ from those cold smoked fillets with pigmentation (49.46) in terms of lightness.

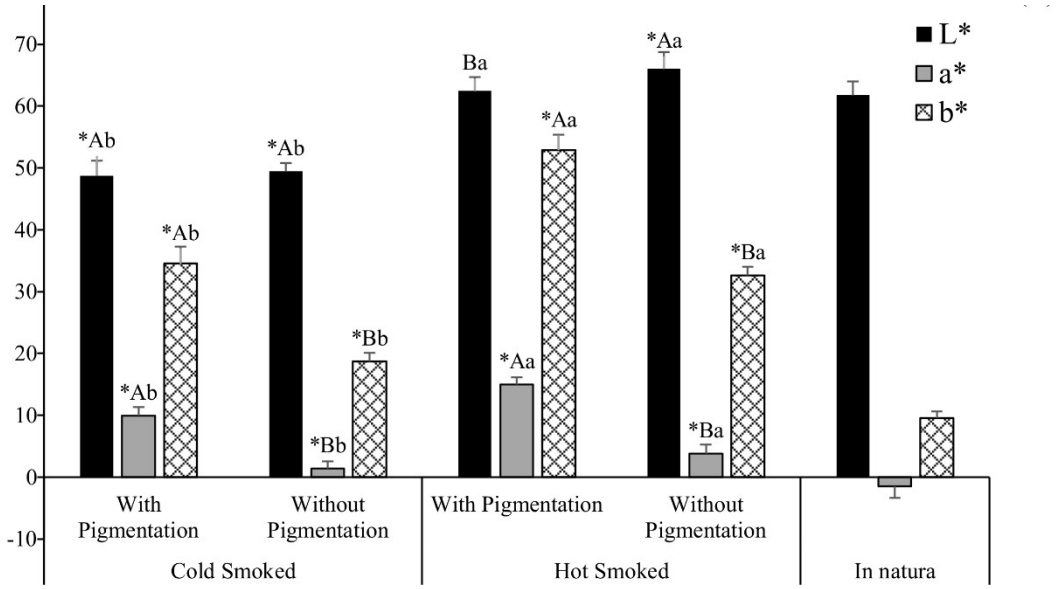

Figure 2. Means of color system (CIELab) for smoked and *in natura* fillets with interactions between smoking technique and fillet pigmentation. Means following the same uppercase letter (lowercase) for the factor pigmentation within each smoked technique (smoked technique within each pigmentation) did not differ by Tukey test at 5% of probability level. *indicates that for each smoking technique and pigmentation treatment, the mean differs from control (*in natura*). Vertical bars represent the standard deviation of the mean.

There was an interaction between smoking techniques and pigmentation for shear force of the fillets (Fig. 3A). Cold smoked fillet shear force values (with pigmentation = 6.40 kg and without pigmentation = 6.11 kg) were greater (p <0.05) than hot smoked fillets values (with pigmentation = 2.71 kg and without pigmentation = 3.09 kg). There was a significant difference between smoking techniques and pigmentation in fillets in comparison to *in natura* fillets.

Through scanning electron microscopy, we can observe the film surrounding the fillets (Fig. 3B). In cold smoked fillets, collagen fibers were observed (Fig. 3C).

Cold smoked fillets presented with a lower (p <0.01) pH value (6.43) than HS fillets (6.94). The fillets without pigmentation had a lower pH (6.59) in relation to fillets with pigmentation (6.78) due to dehydration in fillets without pigmentation. The pH changes that occurred in cold versus hot techniques are due to water loss during the process, with more water loss occurring in hot smoked fillets (pH = 6.94). The greatest smoking time decreased the pH values in cold smoked fillets (pH = 6.43).

3.4. Benzo(a)pyrene production

When analyzing the benzo(a)pyrene content in cold smoked fillets (0.45 μg/kg) and hot smoked fillets (0.49 μg/kg) and comparing them to *in natura* fillets (0.26 μg/kg), we observed that the smoking process affected the benzo(a)pyrene content, wich was significantly higher (p <0,05) in the fillets subjected to smoking (regardless of type, hot or cold) compared to fillets *in natura*.

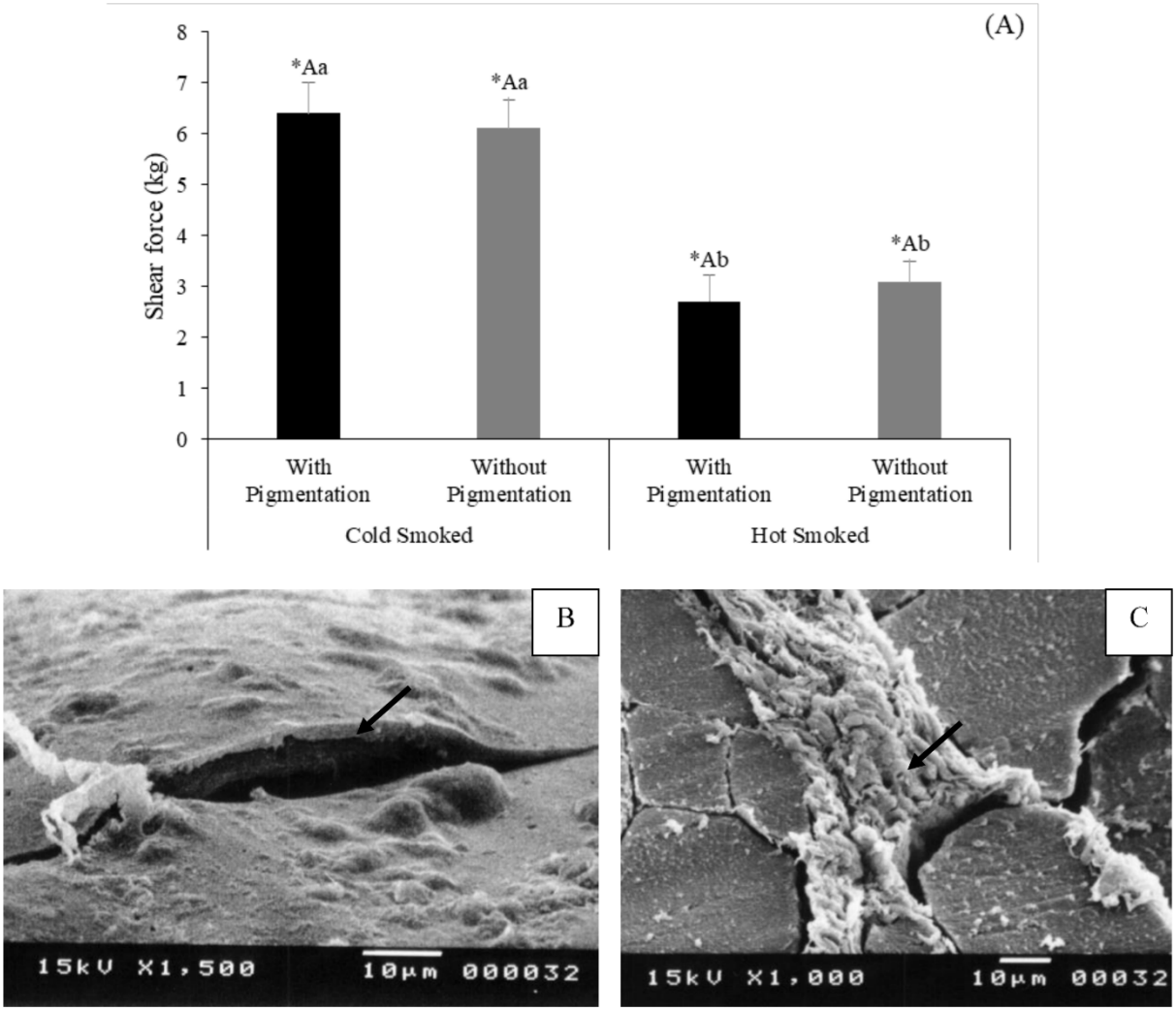

Figure 3. (A) Means of shear force (kg) of interaction when compared between smoking technique (cold and hot) and pigmentation (with = FWP and without = FOP). Means following the same uppercase (lowercase) letter for the factor pigmentation within of each smoking technique (smoking technique within each pigmentation) did not differ by Tukey test at a 5% probability level. * indicates that for each smoking technique and pigmentation treatment, the mean following the * differs from control (*in natura*). Vertical bars represent the standard deviation of the mean. (B) Electron-micrograph of smoked fillets depicting the film that is formed by protein denaturation and leaching of fat, which is associated with the compound action generated by organic matter pyrolysis. (C) Presence of collagen fiber bundles among muscle tissue of cold smoked fillets.

3.5. Organoleptic characteristics

There was a significant effect (p <0.05) of smoking technique on flavor, aroma, texture, salt content, superficial and internal color, and general acceptance. Hot smoked fillets were the best evaluated by tasters. The pigmentation had a significant effect on attributes such as appearance, flavor, superficial color, and salt content in smoked fillets. Fillets with pigmentation received greater scores than fillets without pigmentation for appearance, superficial and internal color, and flavor (Table 3).

There was a significant interaction (p<0.05) between smoking technique and pigmentation on salt content. Hot smoked fillets presented with greater scores by tasters when compared to pigmented and unpigmented cold smoked fillets in terms of salt content.

Table 3. Probability value for the Friedman Chi-square test, means and non-parameterized Tukey multiple comparison test values (α = 0.05) based on the scores assigned by tasters for Nile tilapia smoked fillets.

	Entire fillet			Portion fillet				
	Appearance[(1)]	Color	Internal color	Aroma	Flavor	Texture	Salt content	General acceptance
Smoking technique (T)								
Cold (CC)	6 a	6 b	5 b	5 b	4 b	4 b	5,5 b	4 b
Hot (HS)	6 a	7 a	8 a	7 a	8 a	8 a	8 a	7 a
Pigmentation (P)								
With (FWP)	7 a	7 a	6 a	6 a	6 b	6 a	6 b	6 a
Without (FOP)	5 b	6 b	6 a	6 a	7 a	6 a	7 a	7 a
F test								
Smoking technique (T)	0.6985	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Pigmentation (P)	0.0001	0.0001	0.3897	0.3481	0.0445	0.4320	0.0183	0.2893
Interaction T×P	0.8575	0.4652	0.4497	0.7237	0.1573	0.0947	0.0112	0.0679
Coeficiente of variation (%)	3.85	1.89	3.22	2.89	1.66	2.52	3.01	2.99

[(1)]For each factor, means in the same column that have the same letter did not differ significantly by the non-parameterized Tukey multiple comparison test (α = 0.05).

4. DISCUSSION

In the present study, the losses that occurred during the smoking process were greatest for hot smoked fillets (13.51%). According to SIGURGISLADOTTIR *et al*. (2000), fillet weight losses between 10 and 25% during the smoking process, due to dehydration and fat leaching in muscle tissues, is commonly observed.

A significant difference was not observed in the fillet yields. Thus, we can infer that fish weights were homogeneous, and that we used one filleting method and the same person to perform the filleting.

The cold smoking technique demonstrated a greater yield independent of fillet pigmentation and lack of pigmentation. Considering hot smoked fillets, the fillets with pigmentation presented with a greater yield than fillets without pigmentation. These results were also observed by FRANCO *et al*. (2010), who reported that Matrinxa fillets with skin (*Brycon cephalus*) had a lower hot smoked fillets yield (33.79%) when compared with cold smoking yields (34.46%).

Regarding the losses that occurred during the smoking process, cold smoked fillets presented with lower losses during the smoking process than hot smoked fillets. Although pigmentation did not interfere in the losses for cold smoked fillets, it did affect hot smoked fillets. This fact is due to the dehydration that occurred in the fillets, as hot

smoked fillets presented with lower moisture contents; despite the fact that there was no significant difference observed, the fillets with pigmentation had the lowest moisture content.
The smoking process decreased the fillet area by 11.62% when compared to *in natura* fillets. Thus, the losses that occurred during the smoking process are related to the weight, thickness, and area of the fillets. The hot smoked fillets demonstrated lower losses in fillet area compared to cold smoked fillets; the temperature used in the process led to greater losses in the product due to the greater reduction in moisture content of the fillet.
In the chemical composition, the greater values for crude protein and total lipids observed in smoked fillets in relation to *in natura* fillets are due to the effects of dehydration. These results were also observed by VASILIADOU *et al*. (2005) for hot smoked dourade (*Sparus aurata*); the total lipid concentration increased from 7.55% (*in natura* fish) to 12.92% (smoked fish), crude protein content ranged from 20.65% (*in natura*) to 25.67% (smoked), while moisture content decreased from 69.96 % to 57.45% after the smoking process.
The greater ash content in the *in natura* fillets compared to the smoked fillets may be due to sodium chloride absorption in the muscle tissue during the brining process of the smoked fillets. This result may also be due to the varied nutrient concentration, which is a side effect of moisture losses during dehydration, which takes place during the smoking process.
The pigmented fillets presented with lower ash contents, and this can be related to the loss of salt in the fillets due to the annatto extract solution that was used in the fillet pigmentation process.
When analyzing the fillet color, the values of a* and b* increased in function of the smoking technique and pigmentation. The processing technique (time x temperature) that was used influenced the chroma a* and b* and led to lower chroma values in cold smoked fillets. CARDINAL *et al*. (2001), when studying Atlantic salmon (*Salmo salar*), relayed that the temperature did not affect these two color parameters. BERAQUET and MORI (1984) observed that smoking time contributed intensively to color formation in smoked fishes (mackerel *Scomber japonicus*); in other words, cold smoked fishes (8 hours at temperature below 35ºC) presented with a golden yellow color that was more intense than hot smoked fishes due to the long exposure time of the fillets to smoke.
It was theorized that reactions between carbon compounds and proteins are responsible for color formation in the smoked fish surface, while absorbed phenolic compounds are deeply related to the flavor and aroma of the smoked product (HUDA *et al*., 2010).
There was an interaction between lightness (L*) and chroma a* and b*. Pigmented fillets had values for a* and b* that were significantly greater than those without pigmentation, while the hot smoking process yielded greater chroma values than cold smoked fillets.
The smoking process results in water losses of the meat, which decreases the fillet lightness (FUENTES *et al*., 2012). However, for hot smoked fillets in the present study, the increase in processing temperature led to a greater release of lipids, which resulted in brighter fillet surfaces independent of the pigmentation treatment, and consequently, caused an increase in the lightness.
Bixin, natural pigment annatto seed (*Bixa orellana* L.), is the compound responsible for food pigmentation, and it has a good thermal stability below 100ºC (GIRIDHAR *et al*., 2014). In both cold and hot smoking processes, the smoking chamber temperature did not exceed 100ºC, which therefore did not alter the fillet color. This can be demonstrated by the homogeneity of the results for chroma a* and b*.
The unpigmented hot smoked fillets presented with a lightness that was significantly greater than pigmented fillets. Cold smoked fillets with pigmentation did not differ from

those cold smoked fillets with pigmentation in terms of lightness. The lightness increased due to steam because actomyosin is denatured by heat. However, if the smoking process is independent of temperature, the lightness decreases once the smoking process deposits chemical compounds, which are produced naturally via wood pyrolysis, into the smoked product.

Hot smoked unpigmented fillets had greater values for a* and b* and yielded a golden red color; these values were greater than those of pigmented cold smoked fillets, whose red tone was also significantly less. The greater smoking time results in blackened fillets, so the dehydration and fillet color should be controlled to obtain a more acceptable final product (HASSAN, 1988).

Lysine participates with the ε-amina group in the initial steps of the Maillard reaction (Siskos *et al*., 2005), as seen in the active reaction of aldehyde (formaldehyde, glyoxal, furfural, coniferaldehyde, and sinapaldehyde) from the smoke with the amina group of the lysine. The smoking process increases the lysine content in the fish due to the Maillard reactions (AKINTOLA, 2015). Thus, this can justify the greater values (a* and b*) observed in hot smoked fillets when compared to cold smoked fillets. Despite the shorter smoking time in the hot smoking process (3h), the temperature provides greater amounts of dehydration and more reactions between lysine and smoke compounds, thereby obtaining a more intense coloration.

When we evaluated the shear force of the fillets, cold smoked fillet shear force values were greater than hot smoked fillets values. The salt that was used in the smoking process can affect the final texture of the smoked fillet, which can be seen in terms of water retention capacity, isoelectric point, and protein functionality. Thus, greater salt concentrations are responsible for a firmer texture (GALLART-JORNET *et al*., 2007), which then results in a better flavor and greater stability during storage. The production of a superficial film in smoked meat is due to the protein denaturation that occurs as a result of dehydration, which is associated with salt and heating (HASSAN, 1988). This film on the surface may prevent excessive leaching of fat or evaporation (SIGURGISLADOTTIR *et al*., 2000).

Through scanning electron microscopy, we can observe the film surrounding the fillets. In hot smoked fillets, this was very important, because they were tenderer than cold smoked fillets. Thus, they needed a more consistent structure to avoid disruption via touch or light pressure. This is important mainly for fillets or fish that are kept hanging inside of the smoking chamber in order to avoid falling during the smoking process.

Collagen is the largest component of the intramuscular connective tissue of fish, and it has an important role in maintaining fillet integrity and muscle cohesiveness (AUSSANASUWANNAKUL *et al*., 2012) since it contributes to meat stability and firmness. During the smoking process, the activity of endogenous proteases increases, and these enzymes hydrolyze muscle proteins, thereby breaking down the connective tissue (HULTMANN *et al*., 2004) and altering the texture of the smoked fillet.

Collagen contributes to the texture of *in natura* fishes, but it is not important in the texture of baked fish (HAARD, 1992). The textural resistance of baked muscle decreases with increasing moisture contents up to 79% (Lee and Toledo, 1976). Above 79% of moisture, the resistance decreases, thus reflecting the effects of shear force and compression. Morris *et al*., (2004) reported that hot smoking results in protein denaturation via heat, but there are no theoretical explanations for the phenomenon relating to the thermoviscoelastic properties of the muscle tissue. In hot smoked fillets, despite the lower moisture content (68.90%), a temperature between 50 and 80ºC was enough to alter the structure of collagen fibers and increase tenderness of the fillets, while the film on the fillet surface was responsible for keeping the surface intact until the end of the process. In cold smoked

fillets, collagen fibers were observed; therefore, the temperature utilized was not severe enough to denature the collagen fibers.

During the smoking process, polycyclic aromatic hydrocarbons (PAH) can be formed by organic matter from the wood (VISCIANO *et al*., 2008). Polycyclic aromatic hydrocarbons are a compound group that consists of three or more condensed aromatic rings, which are produced during the incomplete combustion process involving wood, coal, or oil, of which the benzo[a] pyrene is the most studied because it is highly carcinogenic (WRETLING *et al*., 2010).

Regarding the pH of the fillets, cold smoked fillets presented with a lower pH value than hot smoked fillets, and the fillets without pigmentation had a lower pH in relation to fillets with pigmentation, due to dehydration in fillets without pigmentation. The pH changes that occurred in cold versus hot techniques are due to water loss during the process, with more water loss occurring in hot smoked fillets. The greatest smoking time decreased the pH values in cold smoked fillets, and these changes are due to acid absorption from the smoke, moisture loss, as well as the reaction between phenol or polyphenol and carbonyls with proteins and amino groups, respectively (HASSAN, 1988).

When analyzing the benzo(a)pyrene content in cold smoked fillets and hot smoked fillets and comparing them to *in natura* fillets, we observed that the smoking process affected the benzo(a)pyrene content, wich was significantly higher in the fillets subjected to smoking (regardless of type, hot or cold) compared to fillets *in natura*. However, the mean values that we observed are considered low.

The temperature influences the amount of benzo(a)pyrene, as does the exposure time of the product to the smoke compound in pyrolysis. The smoked fish, in adequate conditions, normally present with a low amount of benzo(a)pyrenes, and the maximum level for benzo[a]pyrene in smoked fish and smoked fishery products is 5.0 μg/kg (WRETLING *et al*., 2010); the values in this study are within the range reported.

In sensory analysis, hot smoked fillets were the best evaluated by tasters. Fillets with pigmentation received greater scores than fillets without pigmentation for appearance, superficial and internal color, and flavor.

During the smoking process, the phenols contained in the smoke are responsible for conferring the desirable sensory properties as well as important antioxidants (STOŁYHWO and SIKORSKI, 2005). The lipids in the fish absorb the aromatic substances present in the smoke. We observed that cold smoked fillets had lower scores for flavor and aroma, which can be associated with the fat content in the fillets and the temperature used in the process. When the temperature is high (above 35ºC), the fat in the muscle moves to the surface; this improves the appearance and retention of aromatic substances in the fillets, which therefore creates a better aroma and flavor (BERAQUET and Mori, 1984).

Hot smoked fillets presented with greater scores by tasters when compared to pigmented and unpigmented cold smoked fillets in terms of salt content. This is due to the smoking process since moisture losses occurred in the hot smoked fillets. In the fillets without pigmentation, there was a consequent increase in the salt concentration in the smoked fillets, thus providing a tasty fillet in terms of salt content.

In temperatures above 30ºC, the fish acquires better flavor and coloration when compared to cold smoked products from the same time period (BERAQUET and MORI, 1984). This finding confirms the observations in the present study, where the flavor was significantly greater for hot smoked fillets than cold smoked fillets. The same phenomenon occurred in terms of superficial and internal color of the fillets, because the tasters attributed the greatest scores to hot smoked fillets when compared to cold smoked fillets.

Hot smoked Matrinxa fillets presented with the best results in terms of flavor, salt content, and acceptance, while cold smoked fillets had the best results for color and appearance (FRANCO *et al*., 2010). According to these authors, aroma and texture were not influenced by the smoking techniques.
The tasters believed that hot smoked fillets had a better texture (score 8) when compared to cold smoked fillets (score 4). This fact is due to the tenderness of the hot smoked fillets, as these fillets had a lower shear force. The tasters did not observe any difference in the texture of pigmented and unpigmented fillets, and when this characteristic was analyzed in terms of shear force, no difference was observed.

5. CONCLUSIONS

Time and temperature affected the fillet quality in terms of composition, sensory attributes, yield, area, salt content, color, and pH.
The pigmented hot smoked fillets presented with the best results regarding quality (chemical composition, salt content, color, texture, water activity, and pH) and organoleptic characteristics; however, they presented with the lowest yield in terms of processing, and consequently, had greater weight losses.
The smoking process altered the chemical composition of the smoked fillets by reducing moisture and providing a greater concentration of crude protein, total lipids, and ash. The hot smoked fillets presented with a lower moisture content, but had a greater crude protein and ash content. The pigmentation interfered with ash content.
The pigmentation improved the color and general acceptance of smoked fillets, and was also associated with a greater yield for hot smoked fillets. The reddish color (a*) and the yellow color (b*) produced a golden red fillet that was more pigmented in hot smoked fillets than the others; these fillets were considered to be the most adequate by tasters.
In terms of public health (or food safety), the smoking techniques utilized in this study were adequate because they produced smoked products with a benzo(a)pyrene content below the limit allowed by legislation.

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