

ORIGINAL ARTICLE

Effect of *Alpinia zerumbet* essential oil on the shelf life of tambaqui fillets during short-term refrigerator storage

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ABSTRACT

We investigated the effect of *Alpinia zerumbet* essential oil on the quality and shelf life of tambaqui (*Colossoma macropomum*) fillets stored under refrigeration (10.0 ± 0.5 °C) for 14 days. The treatments were *A. zerumbet* essential oil at 0.75% v v⁻¹ (AEO 0.75%), *A. zerumbet* essential oil at 1.5% v v⁻¹ (AEO 1.5%) and a control (no essential oil). The sample quality and shelf life were determined by the total psychrotrophic count (TPC) and chemical parameters (pH, total volatile basic nitrogen, centesimal composition and thiobarbituric acid reactive substances - TBARS) at zero, seven and 14 days of storage time. The TPC decreased significantly ($p < 0.05$) with an *A. zerumbet* essential oil level of 1.5% until seven days of storage. The concentration of *A. zerumbet* essential oil at 0.75% resulted in lower pH, TBARS, and TVBN values in comparison with the other treatment and the control. Thus, *A. zerumbet* essential oil was efficient in extending the shelf life of refrigerated tambaqui fillets up to approximately seven days.

KEYWORDS: antioxidant, psychrotrophic count, quality, fish storage

Efeitos do óleo essencial de *Alpinia zerumbet* sobre a vida útil de filés resfriados de tambaqui

RESUMO

Investigamos o efeito do óleo essencial de *Alpinia zerumbet* sobre a qualidade e vida útil de filés de tambaqui (*Colossoma macropomum*) armazenados sob refrigeração ($10,0 \pm 0,5$ °C) durante 14 dias. Os tratamentos foram óleo essencial de *A. zerumbet* (OEA) a 0,75% v v⁻¹ (OEA 0,75%) e 1,5% v v⁻¹ (OEA 1,5%), além de um controle (sem OEA). A qualidade e vida útil das amostras foram determinadas por contagem psictrófica total (CPT) e parâmetros químicos (pH, bases nitrogenadas voláteis totais, composição centesimal e substâncias reativas ao ácido tiobarbitúrico - SRATB), nos dias zero, sete e 14 de armazenamento. O TPC diminuiu significativamente ($p < 0,05$) com óleo essencial de *A. zerumbet* a 1,5% por até sete dias de armazenamento. O tratamento contendo OEA a 0,75% resultou em menores valores de pH, SRTAB e BNV-T em comparação ao outro tratamento e ao controle. Portanto, o óleo essencial de *A. zerumbet* foi eficiente para prolongar a vida útil do filé de tambaqui por aproximadamente sete dias.

PALAVRAS-CHAVE: antioxidante, contagem de psictróficos, qualidade, estocagem de pescado.

INTRODUCTION

Fish is the main source of animal protein in the human diet due to its high nutritional value (FAO 2016). Fish is both a subsistence food for many populations, especially in developing countries, and a generator of wealth derived from fishing and aquaculture, as it one of the most traded food commodities worldwide and is worth approximately US\$ 142 billion in 2016

(FAO 2018). It is estimated that fish could represent 50% of the animal protein consumption in 2030 (FAO 2018).

However, fresh and minimally processed fish are susceptible to microbial spoilage and lipid oxidation due to their high water and unsaturated fat contents (Iturriaga *et al.* 2012; Secci and Parisi 2016), which result in an undesirable off-flavor and a reduced shelf life. Traditionally, synthetic additives with antimicrobial

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(e.g., potassium sorbate) and antioxidant actions (e.g., BHT and EDTA) have been used to increase the shelf life of food and sustain consumer acceptance (Carocho *et al.* 2014; Honold *et al.* 2016). However, as the excessive consumption of synthetic food additives is related to gastrointestinal, respiratory, dermatological and neurological disorders (Randhawa and Bahna 2009; Wilson and Bahna 2005), their use is restricted by regulations to minimize health risks (Leuschner and Zamparini 2002). Therefore, the demand for natural alternatives, such as essential oils (EOs) extracted from plants, has been increasing (Pawar *et al.* 2012). In Europe and the USA, EOs are used as food additives (CBI 2018; FDA 2017). In Brazil, ANVISA regulates the use of essential oils by Resolution RDC nr. 2 (15 January 2007), and the list of approved EOs for use in food is the same as that used by the FDA and the EU (ANVISA 2007).

In addition to flavoring properties, EOs have shown strong antioxidant and antimicrobial properties (Pesavento *et al.* 2015; Jouki *et al.* 2014; Ye *et al.* 2013; Brenes and Roura 2010; Burt 2004), limiting the growth of food pathogens and increasing the shelf life of some foods (Gómez-Estaca *et al.* 2010; Ojagh *et al.* 2010). For example, an oregano EO showed an antimicrobial effect, emerging as an alternative to preserve sliced ham (Galindo 2017) and chicken pate (Moraes-Lovison 2017); a thyme EO reduced the total psychrotrophic count and extended the shelf life of fillets of trout (*Oncorhynchus mykiss*) (Chamanara *et al.* 2013); a tangerine peel EO (*Citri reticulatae pericarpium*) reduced the degradation process of bream (*Megalobrama amblycephala*) fillets during iced storage (He and Xiao 2016); lemon and thyme EOs maintained grass carp (*Ctenopharyngodon idella*) fillet quality (Cai *et al.* 2017); and a clove EO decreased the microbial growth and lipid oxidation of tuna (*Thunnus thynnus*) fillets during 17 days of storage (Echeverría *et al.* 2018).

Among the species of plants used in the extraction of EOs with potential for use in food is *Alpinia zerumbet*. It is an Asian plant with antimicrobial properties (Victório *et al.* 2009) against *Staphylococcus aureus* (Castro *et al.* 2016). The extract of *A. zerumbet* had antioxidant and antimicrobial activities in minced meat by inhibiting lipid oxidation and increasing microbial stability (Cheah and Gan 2000). *Alpinia zerumbet* is widely cultivated and distributed in tropical and semitropical areas, including Brazil (Thenmozhi *et al.* 2011). It is a very popular medicinal plant in Brazil (Costa *et al.* 2016). *Alpinia zerumbet* EO does not present cytotoxic or genotoxic action *in vivo* or *in vitro* (Oliveira 2008). It is used in traditional Japan cuisine and herbal medicine, and there is no data on the maximum amounts tolerated by humans without signs of toxicity (Teschke and Xuan 2018).

Tambaqui (*Colossoma macropomum*) is an Amazonian fish that has an efficient performance in intensive farming, and it reaches high market values (Val *et al.* 2000). However, the method

of storing raw fish in ice used in the current distribution system causes quality loss and fast spoilage (Li *et al.* 2013). Accordingly, the shelf life of raw tambaqui is approximately 20 days (Borges *et al.* 2013; Silva *et al.* 2018), and its fillets have a shelf life of approximately 6 days in iced storage (Bottino *et al.* 2017).

Therefore, based on the antimicrobial properties presented by *A. zerumbet* and its oriental cuisine uses, we tested *A. zerumbet* EO as a natural additive for fish fillets to promote a longer shelf life. The present study investigated the effect of *A. zerumbet* EO on the quality and shelf life of tambaqui fillets during 14 days of refrigerated storage.

MATERIAL AND METHODS

Preparation and analysis of essential oil

Alpinia zerumbet leaves were harvested at Parnaíba, Piauí, Brazil (03°01'27.5"S, 41°44'53.5"W) in the morning (until 9 am). The essential oil (EO) was extracted by hydro-distillation in the Clevenger system for 3 h. The *A. zerumbet* essential oil (AEO) was analyzed using a gas chromatography-mass spectrometry (GC-MS) instrument (Varian GC-450/MS-240, Palo Alto, CA, USA) according to Castro *et al.* (2016). The GC-MS analysis identified 23 volatile compounds, of which p-cymene (32.72%), 1,8-cineole (24.05%) and 4-terpineol (20.23%) were the major components.

Fish samples

Farmed tambaqui (45 fishes; six months old; mean weight = 560 g) were purchased at an aquaculture station at Ilha Grande, Piauí, Brazil, stored immediately on ice and transported in polystyrene boxes to the laboratory of Food Analysis and Technology, Parnaíba-PI. The fish were gutted and filleted manually. The resulting fillets were divided into three different batches of 15 units each.

Sample preparation

Initially, a primary aqueous solution with 1% glacial acetic acid was prepared. The control solution was prepared by adding 1 L of distilled water in the primary solution to a maximum of 2 L. Two treatment solutions were composed of 1% acetic acid, 0.2% Tween-20 (Merck, Darmstadt, Germany), and AEO (0.75% and 1.5%, respectively), which were homogenized under magnetic stirring at 800 rpm at 25 °C.

The fish fillets were randomly placed into three batches (control, AEO 0.75% and AEO 1.5%). The batches did not differ significantly in size and weight of fillets (Table 1). Each fillet was immersed for 30 s in 600 mL of the solution assigned to its respective treatment, and, after two minutes, immersed a second time for 30 s (Chamanara *et al.* 2013). The fillets were then glazed with cold water (2 °C), packaged in polyethylene bags and stored in a refrigerated incubator at 10 °C during 14 days. The chemical and

Table 1. Characteristics of the tambaqui (*Colossoma macropomum*) fillets used in the experiment [total weight (g), width (cm), height (cm) and thickness (cm)]. Values are the mean \pm SD of 15 replicates, without significant differences between the treatments ($p < 0.05$).

Group	Weight (g)	Width (cm)	Height (cm)	Thickness (cm)
Control (I)	104.6 \pm 23.9	22.9 \pm 2.1	13.3 \pm 1.2	2.7 \pm 0.2
AEO 0.75% (II)	99.5 \pm 23.3	23.3 \pm 1.5	13.2 \pm 1.0	2.6 \pm 0.2
AEO 1.50% (II)	96.1 \pm 15.5	22.6 \pm 1.2	13.4 \pm 0.8	2.7 \pm 0.5

microbial analyses were performed at three time points: day zero (after treatment and prior to refrigeration), and at day 7 and 14 of the refrigeration period, to assess the overall quality of the fish fillets.

Chemical analyses

The proximate composition analysis was performed according to standard analytical methods (AOAC 2005). Five samples were taken from each treatment at each time point and analyzed in triplicate. The moisture content was determined as the weight difference for 2 g of muscle before and after heating in an oven (SOLAB, Piracicaba, Brazil) for 24 h at 105 °C. The total nitrogen content (CP) was determined by the Kjeldahl procedure, and the protein content was estimated using a conversion factor of 6.25. Ash content was determined as the weight difference after heating at 550 °C for 6 h in a muffle furnace (Quimis, São Paulo, Brazil), and lipid content was quantified by extraction with petroleum ether in a Soxhlet apparatus.

The pH was measured using a digital pH meter (Del Lab model DLA-pH, São Paulo, Brazil). Muscle samples (10 g) were homogenized with 40 mL of distilled water, and the homogenate was used for pH determination according to the Kirschnik *et al.* (2013) method.

Lipid oxidation was evaluated through the formation of thiobarbituric acid reactive substances (TBARS), according to Vyncke (1970), in samples of 10 g of muscle. A 5-ml aliquot of the distillate was used for color development and was measured at 532 nm using a spectrophotometer (HACH, model 2800, Germany). The results were expressed as mg of malondialdehyde (MDA) per kg of muscle. The oxidation potential was calculated from the standard curve $y = 0.2727x - 0.2007$ ($r^2 = 0.991$).

The total volatile base nitrogen (TVB-N) determination was carried out using a methodology adapted from Savay da Silva *et al.* (2008), in which 50 g of muscle was homogenized in 150 mL of 5% trichloroacetic acid for the precipitation of protein nitrogen. After filtration, the amount of low-molecular-weight nitrogen compounds soluble in TCA was measured using the semi-macro Kjeldahl method, and the soluble nitrogen content was estimated using a conversion factor of 6.25.

Psychrotrophic bacteria count

The total psychrotrophic count (TPC) was determined through the pour plate method using plate count agar (HIMEDIA®, LBS Marg, Mumbai, India) as a medium at days zero, 7 and 14 of storage (Evancho *et al.* 2001). We homogenized 10 g of the anterior dorsal region of the fillets in 90 mL of 0.1% peptone solution. In addition, serial decimal dilutions were prepared from a 10^{-1} dilution, as necessary. The plates were incubated at 10 °C for seven days. Two replicates in each group were analyzed. The bacterial count results were expressed as \log_{10} .

Statistical analysis

All measurements were carried out in triplicate and mean values per sample were used in the analyses. The differences among the treatments and storage times were determined by the Kruskal-Wallis test (one-way ANOVA) followed by the Dunn's *post hoc* test. The Bonferroni correction was applied to adjust the significance level of multiple test results. The interactions between the treatments and storage times were determined by the Tukey-Kramer test. Significance was defined at $p < 0.05$. The analyses were carried out with the ASSISTAT version 7.7 software package.

Ethical and legal aspects

The Ethics Committee on the Research Animals Use (CEUA) of Embrapa Meio Norte (protocol 02/2014) approved the study. The use of *Alpinia zerumbet* was authorized by CGEN through the Embrapa special authorization for genetic heritage access for scientific research purposes (nr. 002/2008, IBAMA process 2001.001558/2006-21).

RESULTS

Proximal composition

The proximate composition of the fillets varied among control and treatments (Figure 1). Moisture varied significantly among all groups at all time-points, with highest values for AEO 1.5% at day 14 (Figure 1a). The ash content was significantly higher in the fillets with AEO compared to the control group (Figure 1b). There was no significant effect of storage time on moisture and ash content. CP was significantly lower in the treatments until day 7, but significantly higher in AEO 1.5% at day 14 (Figure 1c). Lipid contents tended to increased significantly in all groups throughout storage time, yet did not vary significantly among the groups at any time-point (Figure 1d).

pH

There was a significant effect of AEO at day 7 and day 14, and on the interaction of AEO x storage time on the pH for AEO 0.75%. At day zero, all samples had similar pH. At day 7, a significant decrease in pH was observed among the treatments and control

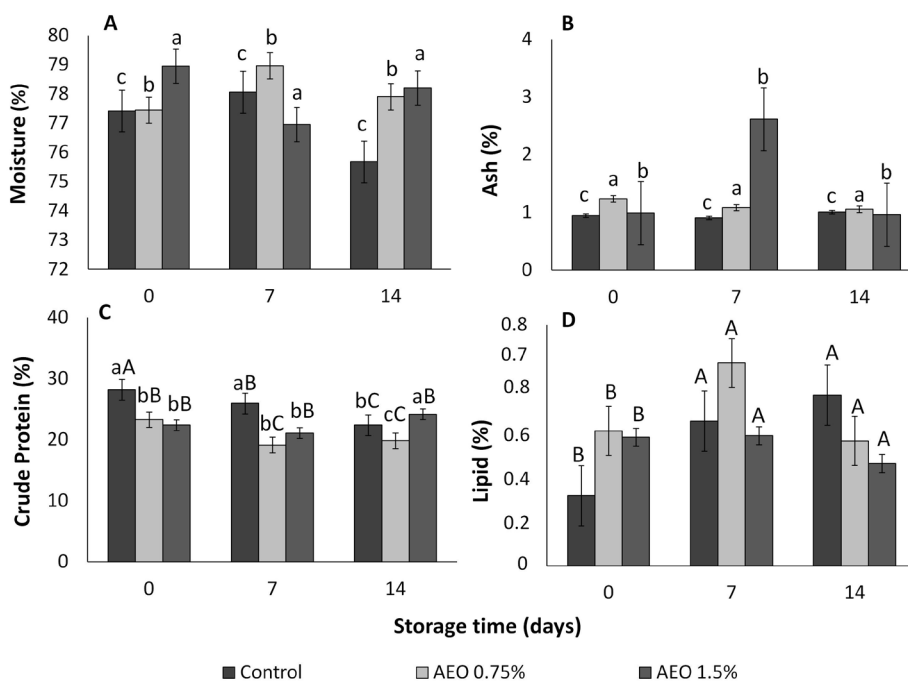


Figure 1. Changes in the proximate composition of control and AEO-treated tambaqui fillets during refrigerated storage (0 = day zero prior to refrigeration, 7 and 14 = after seven and 14 days of refrigeration, respectively). AEO 0.75% = *Alpinia zerumbet* essential oil at 0.75%; AEO 1.5% = *Alpinia zerumbet* essential oil at 1.5%. Columns represent the mean (N = 5), bars the standard error of the mean. The same small letters on the bars indicate no significant difference among the treatments within each time point. The same capital letters on the bars indicate no significant differences among time points within each treatment ($p < 0.05$).

(control > AEO 0.75% > AEO 1.5%). On day 14, there was a significant difference only between the control and AEO 0.75%, which showed lower pH values (Figure 2).

TBARS

TBARS were similar among the groups at day zero, but decreased in the treatments relative to the control at days 7 and 14 (Figure 3). Throughout storage time, the control showed higher values of TBARS (25.9% and 23.1% higher than AEO 0.75% and AEO 1.5%, respectively). At day 14 the lowest values of TBARS (1.5 mg MDA/kg fillet) were determined for 0.75%.

TVB-N

There was an effect of the AEO and storage time on TVB-N. The TVB-N values showed a similar behavior as did the TBARS at day zero, with no significant difference between the treatments. However, at day 7, the control showed lower values, followed by AEO 0.75% and AEO 1.5% (Figure 4). At day 14, there was no significant difference among samples.

Total bacterial count

AEO and storage time significantly affected TPC (Figure 5). At day zero, there was no significant difference among the groups. At day 7 AEO 1.5% showed a significantly lower bacterial count compared to the control and AEO 0.75%. At day 14 TPC increased significantly in all groups, when the fillets became unfit for consumption according to Brazilian law (Brazil 2001).

DISCUSSION

During cold storage, there are changes in the physicochemical properties of fish fillets, with a reduced water-holding capacity that promotes moisture decrease and nutrient loss (Aubourg 2001). Previous studies showed a positive effect of the essential oil of thyme (*Thymus daenensis*) and savory (*Satureja bachtiarica*) on moisture loss of trout (*Oncorhynchus mykiss*) fillets (Abedi *et al.* 2016). The major components of thyme oil are p-cymene (8.4%), γ -terpinene (30.9%) and thymol (47.6%) (Borugá *et al.* 2014), and the major components of savory oil are p-cymene (7.3%), γ -terpinene (23.9%), thymol (44.5%), β -caryophyllene (5.3%) and borneol (4.2%) (Sefidkon and Ahmadi 2000). Thymol and p-cymene increase superoxide dismutase and glutathione peroxidase activities, which have protective effects that maintain the integrity of muscle cells (Hashemipour *et al.* 2013). A similar effect was observed in our tambaqui fillets, with a lower moisture percentage in the control group, which indicates that the p-cymene (32.7%, Castro *et al.* 2016) in the AEO probably acted as a preservative, coating the fillets and keeping their water-holding capacity. The higher ash content in the fillets treated with AEO indicates a preservation effect on mineral content. Since AEO prevented water loss during storage, it also reduced the cellular rupture that promotes protein loss. Crude protein represents the main nutritional value of seafood and is the main factor responsible for its texture and water-holding capacity (Sikorski and Kolakowska 2016).

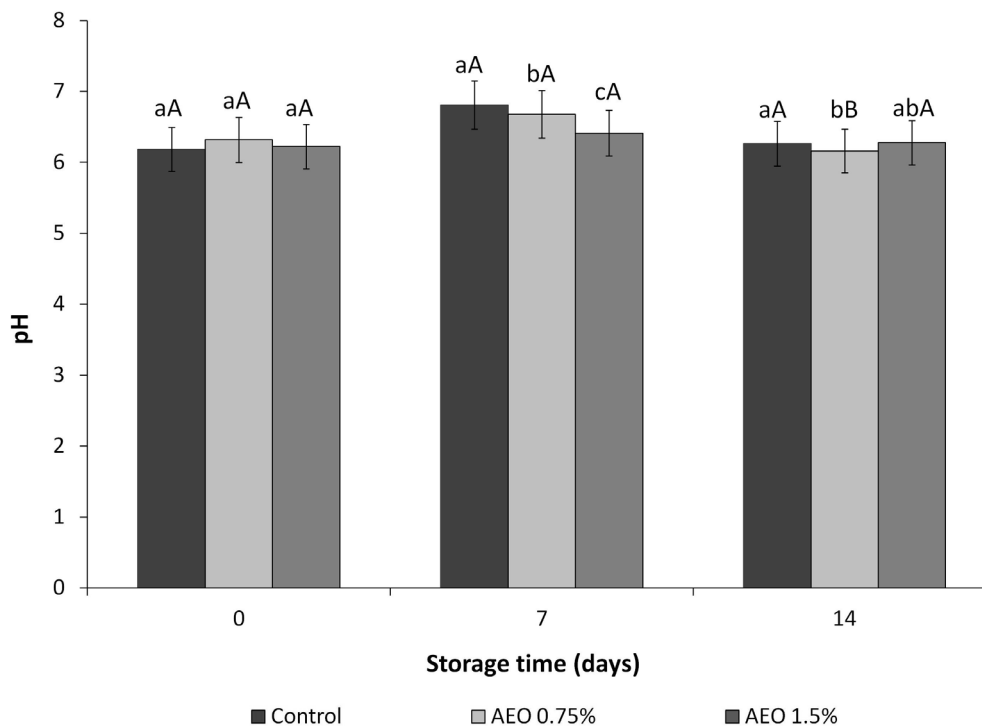


Figure 2. Changes in the pH of control and AEO-treated tambaqui fillets during refrigerated storage (0 = day zero prior to refrigeration, 7 and 14 = after seven and 14 days of refrigeration, respectively). AEO 0.75% = *Alpinia zerumbet* essential oil at 0.75%; AEO 1.5% = *Alpinia zerumbet* essential oil at 1.5%. Columns represent the mean (N = 5), bars the standard error of the mean. The same small letters on the bars indicate no significant difference among the treatments within each time point. The same capital letters on the bars indicate no significant differences among time points within each treatment ($p < 0.05$).

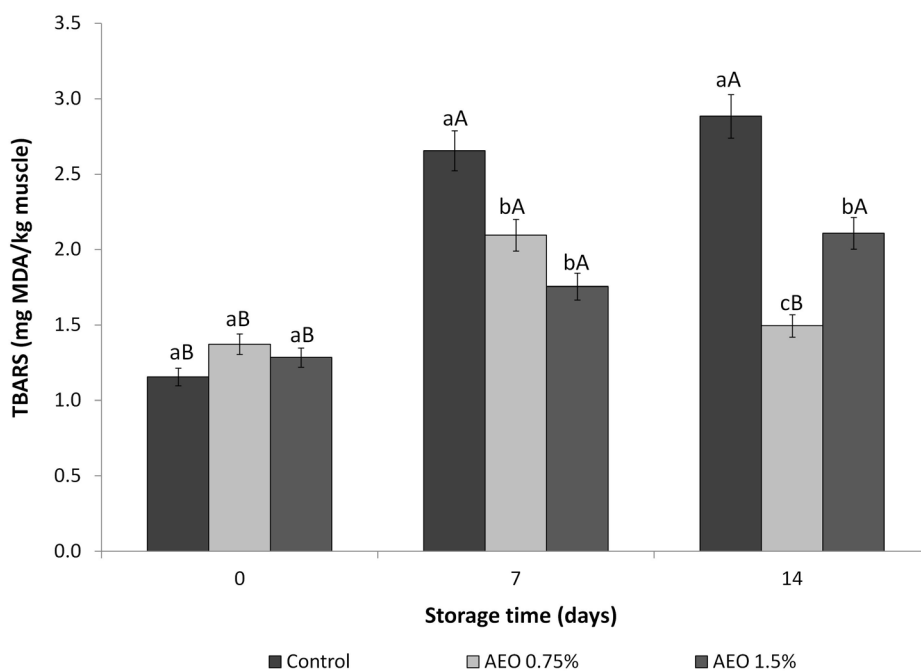


Figure 3. Changes in the TBARS of control and AEO-treated tambaqui fillets during refrigerated storage (0 = day zero prior to refrigeration, 7 and 14 = after seven and 14 days of refrigeration, respectively). AEO 0.75% = *Alpinia zerumbet* essential oil at 0.75%; AEO 1.5% = *Alpinia zerumbet* essential oil at 1.5%. Columns represent the mean (N = 5), bars the standard error of the mean. The same small letters on the bars indicate no significant difference among the treatments within each time point. The same capital letters on the bars indicate no significant differences among time points within each treatment ($p < 0.05$). Missing letters indicate that all comparisons were non-significant.

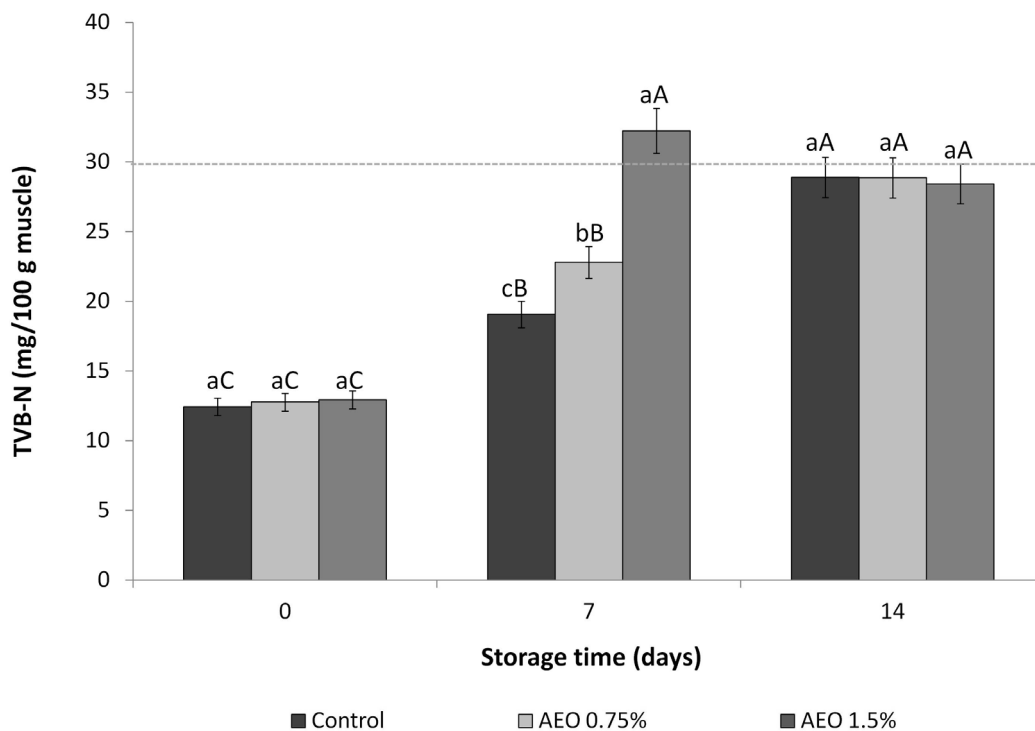


Figure 4. Changes in the TVB-N of control and AEO-treated tambaqui fillets during refrigerated storage (0 = day zero prior to refrigeration, 7 and 14 = after seven and 14 days of refrigeration, respectively). AEO 0.75% = *Alpinia zerumbet* essential oil at 0.75%; AEO 1.5% = *Alpinia zerumbet* essential oil at 1.5%. The same small letters on the bars indicate no significant difference among the treatments within each time point. The same capital letters on the bars indicate no significant differences among time points within each treatment ($p < 0.05$). The horizontal line represents the limit established by Brazilian legislation.

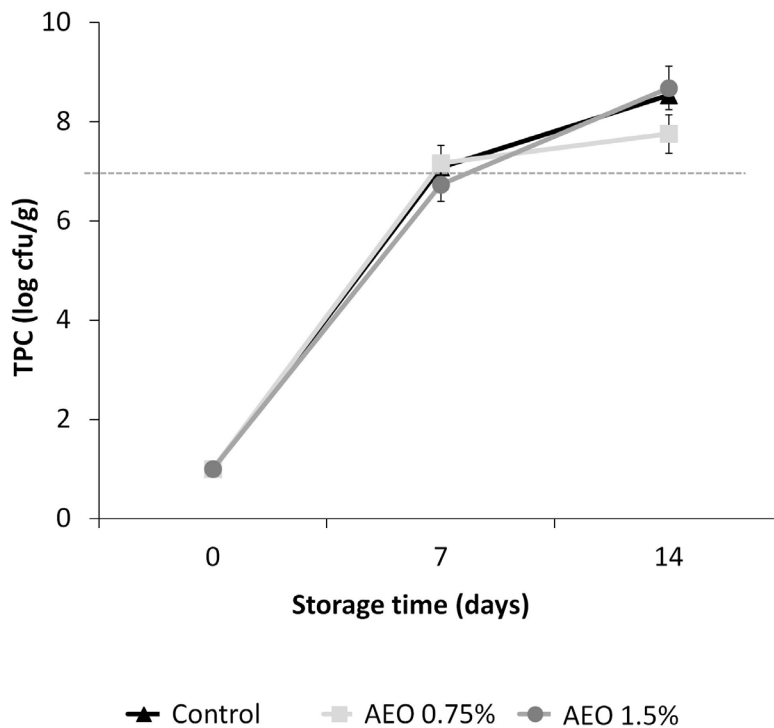


Figure 5. Development of the total bacterial count of control and AEO-treated tambaqui fillets during refrigerated storage. AEO 0.75% = *Alpinia zerumbet* essential oil at 0.75%; AEO 1.5% = *Alpinia zerumbet* essential oil at 1.5%. Bars represent the standard deviation. The horizontal line represents the limit established by Brazilian legislation.

Fat content is one of the most important indices for seafood quality determination (Abedi *et al.* 2016). A decrease in lipid content could be related to lipid oxidation and enzymatic degradation of the muscle phospholipids (Hendriks *et al.* 2006). The increase in lipid content observed in this study may be related to the decrease observed in crude protein content, since centesimal composition involves a relation among moisture, fat, crude protein and ash contents (Sikorski and Kolakowska 2016) where the sum of components must be approximately 100%.

The effect of AEO on the relative proportions of the components of centesimal composition may have been related to the pH changes during storage time. In general, an increase in pH may be due to the production of volatile bases or microbial enzyme action (Li *et al.* 2012). The decrease in pH observed in our treatments might have resulted from the antioxidant and antibacterial characteristics of the AEO, which inhibits the activity of the endogenous proteases and contributes to the extension of the fish sample preservation (Fan *et al.* 2008). Similar results were observed for silver carp (*Hypophthalmichthys molitrix*) fillets coated with *Pimpinella affinis* essential oil (PAO), although the fillets were coated with twice the concentration of essential oil (1.5% v/v) (Ariaii *et al.* 2015).

Essential oils are lipophilic, thus they can easily enter cells and disrupt and/or permeabilize the cell membrane (Perricone *et al.* 2015), which can cause depolarization of mitochondrial membranes, influence the Ca^{2+} channels, and reduce the pH gradient (Bakkali *et al.* 2008). In some cases, EOs can cause the coagulation of the cytoplasm as well as damage to lipids and proteins (Burt 2004). This may explain the differences observed between control and treatments in lipid and crude protein contents and pH.

TBARS are the predominant products of secondary lipid oxidation and are considered good chemical indicators of quality assurance and estimations of the extent of lipid oxidation during chilled storage (Jeon *et al.* 2002; Goulas and Kontominas 2007). Therefore, the lower TBARS level in AEO 0.75% indicates that this treatment was the most efficient in lipid oxidation preservation of the tambaqui fillets. The use of an EO to protect food against lipid oxidation has been reported elsewhere (e.g., Lee *et al.* 2005; Lu *et al.* 2010; Ojagh *et al.* 2010). In silver carp, EO resulted in a reduction of 18% in TBARS (Ariaii *et al.* 2015). In our study, the reduction in TBARS in AEO 0.75% was approximately 51%, suggesting that AEO has a significant potential to be used as an antioxidant in fish.

In addition to lipid oxidation, fish decomposition implies progressive proteolysis by the action of microorganisms and, to a lesser degree, by autolytic enzymes (Howgate 2010; Ocaño-Higuera *et al.* 2011), such as TVB-N. Therefore TVB-N has traditionally been used as a quality indicator in refrigerated aquatic products and includes trimethylamine, dimethylamine, ammonia and other compounds, which come from the degradation of proteins and nonprotein nitrogenous compounds by endogenous

enzymes (Li *et al.* 2013). The Brazilian legislation sets a maximum acceptable limit for fish of 30 mg TVB-N/100 g of product (Brasil 2001). According to the quality index, a TVB-N value of less than 25 mg is very good, 30 mg is good, 35 mg is marketable, and more than 35 mg is considered unconsumable (Varlık *et al.* 2000; Cakli 2007; Dogan and Izci 2016).

In rainbow trout treated with EO levels of approximately 0.2% to 1%, the treatments using rosemary or thyme EO at 1% resulted in lower TVB-N values relative to the control (Erkan 2012; Yildiz 2015). The inhibition of TVB-N production in fish may be attributed to the effects of the phenolic and organosulfur constituents of EO (Burt 2004), that react with the sulfhydryl and amino groups of proteins. In our study, the high TVB-N for AEO 1.5% at day 7 was likely related to the significant antibacterial activity detected in this treatment at the same time-point, and points to a capacity of AEO to reduce bacterial growth, which causes the oxidative deamination of nonprotein nitrogen compounds (Banks *et al.* 1980).

Fish and seafood in general are highly perishable food products, mainly due to microbiological growth and lipid oxidation, which are known to be the principal causes of the quality deterioration in these products (Hassoun and Çoban 2017). The antimicrobial activity of some common EOs in fish, either alone or in combination with other preservative systems, depends on the oil type and its concentration of antimicrobial compounds, in addition to the amount used in bioactive packaging. For example, *Lippia multiflora* EO was only effective in inhibiting microbial development in smoked mackerel (*Scomber scombrus*) at concentrations greater than 0.5% (Cyrille *et al.* 2017). Rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum verum*), fennel (*Foeniculum vulgare*), and cardamom (*Elettaria cardamomum*) EOs also reduced the initial total bacterial count, psychrophilic bacteria, and total mold and yeast as well as prolonged the shelf life of fillets of carp (*Cyprinus carpio*) during cold storage at 4°C (Hasan *et al.* 2017). These oils have common components (cineole, α -pinene, α -terpineol, sabinene and limonene) with proven antimicrobial effects (Abdelwahab *et al.* 2017; Chegini and Abbasipour 2017; Takayama *et al.* 2016; Owolabi *et al.* 2009; García-Jiménez *et al.* 2000). Our results for tambaqui are probably related to the presence of 1.8-cineole (24.1%) and 4-terpineol (20.2%) as major components in AEO, which likely mediated the lower bacterial count at day 7 in the treatment with highest EO level. At day 14, all treatments had CFU/g values above the maximum count for fish of 10^7 CFU g^{-1} recommended by FAO (Huss 1997).

Considering the different groups of chemical compounds present in EOs, it is very likely that the antibacterial and antioxidative activity of EOs is attributable to mechanisms targeting different cell structures and processes, such as the cytoplasmic membrane, perturbations on the proton-proton force, electron flow, and transport and coagulation of cell

contents (Burt 2004). This diffuse action mode could explain why the lower AEO level (0.75%) was more efficient in preventing lipid oxidation and the higher AEO level (1.5%) was more efficient in preventing bacterial growth, while both levels preserved the nutritional value of the tambaqui fillets in comparison to the control.

CONCLUSIONS

Treatment with 1.5% *Alpinia zerumbet* essential oil (AEO) reduced the bacterial growth in tambaqui fillets refrigerated at 10 °C and prolonged the shelf life of the fillets for up to seven days. AEO 1.5% is recommended as an antimicrobial agent in tambaqui fillets during short-term refrigerator storage. Lower levels of AEO (0.75%) presented antioxidant effects.

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