Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v25e-76774E

e-ISSN 1809-6891
Veterinary Medicine | Research article

Effect of *Mentha piperita* essential oil in the conservation of refrigerated tambatinga hybrid fish meat

Efeito do óleo essencial de *Mentha piperita* na conservação de carne refrigerada do peixe híbrido tambatinga

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Abstract: The search for safer foods has led to increased research attention to discover natural alternatives to synthetic additives that are used in the food industry. Natural preservatives, such as essential oils (EOs) from plants, could increase fish conservation and even positively affect human health. Therefore, the objective of the study was to evaluate the effect of *Mentha piperita* EO on the physicochemical characteristics and concentration of microorganisms in chilled tambatinga (*Colossoma macropomum* × *Piaractus brachypomum*) meat. *Mentha piperita* EO was prepared at three concentrations (0%, 0.25% and 0.50%) in a solution containing distilled water, propylene glycol and Tween. The meat samples remained immersed in this solution for 60 min; then, they were packed in plastic packages and stored under refrigeration (± 0.4 °C) for 14 days. During this storage, pH, total volatile nitrogenous bases (TVB-N), peroxides, thiobarbituric acid reactive substances (TBARS) and counts of strict and facultative aerobic mesophilic microorganisms were determined. The major constituents found in *M. piperita* EO were geranial (32.28%), neral (18.64%) and geranic acid (6.98%). None of the EO concentrations affected TVB-N, but there were some changes to the pH. Both 0.25% and 0.50% EO reduced the formation of peroxides and TBARS. The growth of microorganisms was reduced by treatment with 0.50% EO. Based on the findings, 0.50% EO was more effective in reducing the deterioration of meat kept refrigerated for up to 14 days.

Keywords: Fish meat; lipid oxidation; microbial spoilage; natural preservatives; shelf life

Resumo: A busca por alimentos mais seguros levou a uma maior atenção da pesquisa para encontrar alternativas naturais aos aditivos sintéticos usados na indústria alimentícia. Os conservantes naturais, como os óleos essenciais (OE) de plantas, podem aumentar a conservação dos peixes e até afetar positivamente a saúde humana. Portanto, o objetivo do estudo foi avaliar o efeito do OE de *Mentha piperita* nas características físico-químicas e concentração de microrganismos em carne resfriada de

Received: July 12, 2023. Accepted: October 2, 2023. Published: December 20, 2023.

tambatinga (*Colossoma macropomum* × *Piaractus brachypomum*). O OE de *M. piperita* foi preparado em três concentrações (0%, 0,25% e 0,50%) em solução contendo água destilada, propilenoglicol e Tween. As amostras de carne permaneceram imersas nesta solução por 60 min; em seguida, foram acondicionados em embalagens plásticas e armazenados sob refrigeração (± 0,4°C) por 14 dias. Durante esse armazenamento foram determinados pH, bases nitrogenadas voláteis totais (BNVT), peróxidos, substâncias reativas ao ácido tiobarbitúrico (TBARS) e contagens de microrganismos mesófilos aeróbios estritos e facultativos. Os principais constituintes encontrados no OE de *M. piperita* foram geranial (32,28%), neral (18,64%) e ácido gerânico (6,98%). Nenhuma das concentrações de OE afetou as BNVT, mas houve algumas alterações no pH. Tanto 0,25% quanto 0,50% de OE reduziram a formação de peróxidos e TBARS. O crescimento de microrganismos foi reduzido no tratamento com 0,50% de OE. Com base nos resultados, a concentração de 0,50% de OE foi mais eficaz na redução da deterioração da carne mantida refrigerada por até 14 dias.

Palavras-chave: Carne de peixe; oxidação lipídica; deterioração microbiana; conservantes naturais; vida de prateleira

1. Introduction

Fish, crustaceans and molluscs are nutritionally valued foods because they contain high-quality proteins, lipids, vitamins and minerals. The consumption of these foods is correlated with the improvement in health and life expectancy of populations[1,2]. In Brazil, among the most bred species are *Oreochromis niloticus* (Nile tilapia) and native species such as *Colossoma macropomum* (tambaqui), *Piaractus mesopotamicus* (pacu) and *Arapaima gigas* (pirarucu)[3]. Tambatinga, the species used in this study, is a hybrid developed from the crossing of the native species *C. macropomum* and *Piaractus brachypomus* (pirapitinga) that exhibits higher growth and productivity rates than its parents. As such, it has become an important species in the scenario of fish farming in North and Northeast Brazil[4,5].

Fish meat, sold in different ways, is the final product of fishing and fish farming, requiring care from the capture of the fish to its distribution, with special attention to sanitary aspects, storage conditions and conservation of the product[6]. Fresh fish is a very perishable meat and has a short shelf life as several processes such as protein degradation, fat oxidation and decomposition by microbes or by endogenous enzymes contribute to the deterioration of meat[7,8]. For this reason, one or more adequate preservation methods are required in order to maintain the safety and quality and extend the shelf life of such products[9]. In this sense, the use of processes that reduce oxidative events (formation of hydroperoxides, aldehydes, alkanes, conjugated dienes) and fish deterioration (formation of biogenic amines, sensory changes, discoloration, loss of nutrients and water) can improve their conservation[10,11,12]. The application of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), sorbate and benzoate, commonly used to increase the oxidative stability of fish and derived products, is now being discouraged due to the risk of diseases that they can cause[12,13]. On the other hand, substances of natural origin (extracted from spices or herbs) such as essential oils (EOs) have received special attention in the food industry and in human nutrition as they present molecules capable of reducing the chemical

and microbiological decomposition of fish meat and derived formulations[10,2]. Phenolic compounds such as thymol, eugenol, rosmarinic acid, carvacrol, gingerol, menthol and citral, among other molecules present in EOs, have antimicrobial and antioxidant action that is of interest to the food industry[13,14].

In some studies, the main constituents found in the EO of *Mentha piperita* were menthol, menthone and menthyl acetate, and these molecules revealed significant antimicrobial activity against bacteria and pathogenic fungi in humans[15] and also against bacteria and parasites in fish[16,17]. Spearmint essential oil applied to fillets of *Sciaenops ocellatus* in the form of steam prevents the breakdown of proteins and the formation of biogenic amines and decreases the development of microorganisms in refrigerated fish[18]. Thus, these natural preservatives such as EOs could perfectly meet the increasing consumer demand for clean-label products that are fresh and free of chemical additives.

Therefore, in the present study, we aim to evaluate the effect of the EO of *M. piperita* in solution applied to fillets of tambatinga, a freshwater fish, on the physicochemical characteristics and total concentration of microorganisms in the meat kept refrigerated.

2. Material and methods

All procedures adopted in the study involving animals were approved by the Animal Use Ethics Committee of the Federal University of Pampa (approval no. 004/2019).

2.1 Essential oil

The EO of *M. piperita* used in this study was supplied by Vimontti (Santa Maria, Rio Grande do Sul, Brazil). The oil was analysed on a gas chromatograph coupled to a mass spectrometer (GCMS; QP2010 Ultra system, Shimadzu Corporation, Tokyo, Japan), equipped with GCMS-Solution software. The identification of the components was based on the time and linear retention index (series of n-alkanes C8–C40), on the interpretation and comparison of the mass spectra obtained with the Adams[19], NIST[20] and FFNSC 2 libraries as described in detail by Fernandes[21].

2.2 Fish and sample preparation

The study was carried out at the Fisheries Laboratory of the Federal University of Maranhão, Chapadinha Campus. Tambatinga specimens (310 ± 11.4 g and 26.05 ± 3.15 cm) were kept in a tank for depuration (density of 11.7 kg m^{3-1}) for 48 h. In this process, the average water renewal rate was 6 L min^{-1}. In addition, dissolved oxygen and water temperature were analysed daily, and the mean values found were 5.87 ± 0.05 mg L^{-1} and 28.50 ± 0.86 °C, respectively.

At the end of the depuration period, the fish were slaughtered by hypothermia (water:ice 1:1) and weighed to calculate weight loss (%). There was a 4.9% reduction in fish weight. The fillet was removed and weighed to calculate the yield (fillet weight/total fish weight × 100). A fillet yield of 34.8% was obtained. The fillet was washed in running water and then in water containing 10 ppm of chlorine.

2.3 Preparation and application of EO to fish meat samples

Fillet samples were separated (750 g treatment^{-1}, in duplicate) and exposed to a solution containing different EO concentrations: 0% (control), 0.25% or 0.50%. The EO, which had a density of 0.85 g mL^{-1}, was diluted and emulsified in a solution containing 80% distilled water, 20% propylene glycol and 0.05% Tween. The meat samples remained immersed in 1.5 L of solution for 60 min. This time interval was chosen based on the study by Dang[22]. After exposure, the samples were drained on racks for 5 min, packed in plastic packages and stored under refrigeration at ± 0.4 °C for future analysis of meat quality parameters.

2.4 Physicochemical analysis

On days 0 (initial), 7 and 14 days of refrigeration (± 0.4 °C), the pH (hydrogen potential) of the samples was analysed using a previously calibrated portable meat pH meter (Akso Electronic Products, Rio Grande do Sul, Brazil). Total volatile nitrogenous bases (TVB-N) were determined as described by Savay da Silva[23]. Protein nitrogen was precipitated with trichloroacetic acid. The filtered material containing the volatile nitrogen was alkalised, received in a solution of boric acid and mixed indicator (methyl red and bromocresol green) and titrated with standardised sulphuric acid solution.

Peroxides were measured according to Chapman and Mackay[24]. Fat was extracted from fillets and dissolved (200 µL) in a benzene:methanol solution (70:30, v/v), followed by the addition of 30% ammonium thiocyanate (10 µL) and ferrous chloride (10 µL). The samples were incubated (50 °C for 2 min) and absorbance was assessed at 520 nm. The results were calculated from the standard curve of iron solution (0.7 to 7.1 µmol).

Thiobarbituric acid reactive substances (TBARS) were evaluated according to Buege and Aust[25]. The sample (1 g) was homogenised in 1.15% potassium chloride solution (1:5, w/v) and centrifuged at 3000 rpm for 10 min. The supernatant (0.75 mL) was incubated in a water bath (100 °C for 15 min) with 30% trichloroacetic acid solution and 0.67% thiobarbituric acid. Next, n-butyl alcohol (1.5 mL) was added to extract the coloured product. Absorbance was measured at 535 nm. The results were calculated from the standard curve of malondialdehyde (MDA) solution (0.6 to 12 nmol).

2.5 Microbiological analysis

The standard count of strict and facultative aerobic mesophilic microorganisms in the fillets was conducted according to the methodology proposed by the Brazilian Ministry of Agriculture, Livestock and Supply[26]: 25 g of sample was diluted and homogenised in 0.1% peptone saline solution (1:10 w/v). A sample (1 mL) of each dilution (10^{-1}, 10^{-2} and 10^{-3}) was pipetted onto the surface of the plates containing the agar culture medium for counting (PCA), in duplicate. The plates were incubated for 48 h at 36 °C and then the colonies formed were counted. The results are expressed in colony-forming units (CFU) g^{-1}.

2.6 Statistical analysis

The data were subjected to the normality test, followed by two-way analysis of variance (ANOVA) (the factors were essential oil concentration and refrigeration time). Means were compared using Tukey's test ($p < 0.05$), using the statistical program Statistica 7.0.

3. Results

3.1 Chemical composition of EO

By analysing the chemical composition, it was possible to identify the major constituents of the EO, highlighting citral, which is a mixture of neral (18.51%) and geranial (32.05%) isomers, which together represented 58% of the composition. It was also possible to identify a mixture of neranic acid and geranic acid (about 7.83%). Low concentrations of piperitone (0.44%), 6-methyl-hep-5-en-2-one (0.13%) and sabinene (0.09%) were found. Due to the high concentration of citral in the EO, there is a strong indication that its biological activity is attributed to this mixture of isomers.

3.2 Physicochemical analysis

There was a reduction in pH in fillet samples exposed to 0.25 and 0.50% EO at the initial evaluation period (Table 1). After seven days, this reduction was only observed in the samples exposed to 0.25% EO. Fillet samples exposed to control (0%) and 0.50% EO had a higher pH. After 14 days of refrigeration, samples treated with 0.50% EO had a lower pH; it did not differ significantly from the control samples, but was significantly lower than the samples treated with 0.25% EO (Table 1).

Table 1. pH of tambatinga fillets kept under refrigeration, after application of *Mentha piperita* essential oil (EO)

Refrigeration time (days)	EO concentration		
	0%	0.25%	0.50%
0	6.36 ± 0.04^{bB}	6.28 ± 0.03^{aA}	6.23 ± 0.06^{aA}
7	6.29 ± 0.04^{abA}	6.23 ± 0.01^{aA}	6.30 ± 0.01^{bB}
14	6.24 ± 0.01^{aA}	6.35 ± 0.01^{bB}	6.21 ± 0.01^{aA}

The results are expressed as the mean ± standard deviation (n = 4). Lowercase letters indicate significant differences between the treatments within the storage period (refrigeration days) by Tukey's test ($p < 0.05$). Uppercase letters indicate significant differences between storage periods within the same treatment by Tukey's test ($p < 0.05$).

The concentration of TVB-N was not altered in fillets exposed to EO in relation to meat from the control treatment (0% EO), in any of the evaluated refrigeration periods. The TVB-N concentration also did not change in the treatments with the passage of refrigeration time (Figure 1). At baseline, values ranged from 8.68 to 9.57 mg $\%^{-1}$. After seven days under refrigeration, the TVB-N content varied from 8.97 to 10.89 mg $\%^{-1}$ and in the final evaluation, at 14 days, the values varied from 9.12 to 10.33 mg $\%^{-1}$.

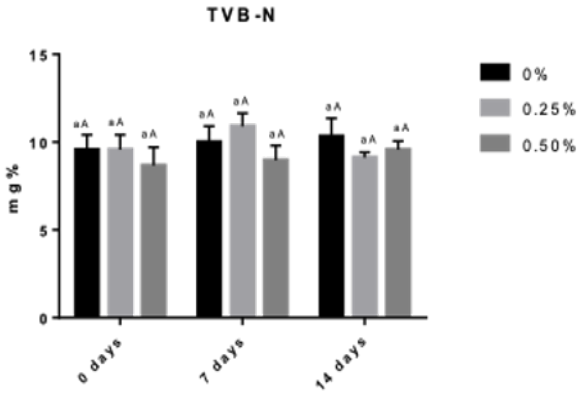

Figure 1. TVB-N (B) of tambatinga fillets kept under refrigeration, after application of Mentha piperita essential oil (EO). The results are expressed as mean ± standard deviation (n = 4). Lowercase letters indicate significant differences between the treatments within the storage period (refrigeration days) by Tukey's test (p < 0.05). Uppercase letters indicate significant differences between storage periods within the same treatment by Tukey's test (p < 0.05).

In relation to the products of fat oxidation, the peroxide content was reduced in meat exposed to both concentrations of EO compared to samples from the control treatment (0% EO), in the initial period and after 7 and 14 days under refrigeration (Figure 2A). In the initial evaluation, the peroxide content found in the control treatment was 0.96 ± 0.11 mEq kg lipid^{-1}. In meat exposed to EO, values of 0.61 ± 0.06 mEq kg lipid^{-1} (0.25% treatment) and 0.57 ± 0.14 mEq kg lipid^{-1} (0.50% treatment) were observed. In general, these molecules increased with the storage time of the meat, with the exception of the evaluation at seven days of refrigeration, when lower values of peroxides were found in all treatments (around 0.20 mEq kg lipid^{-1}).

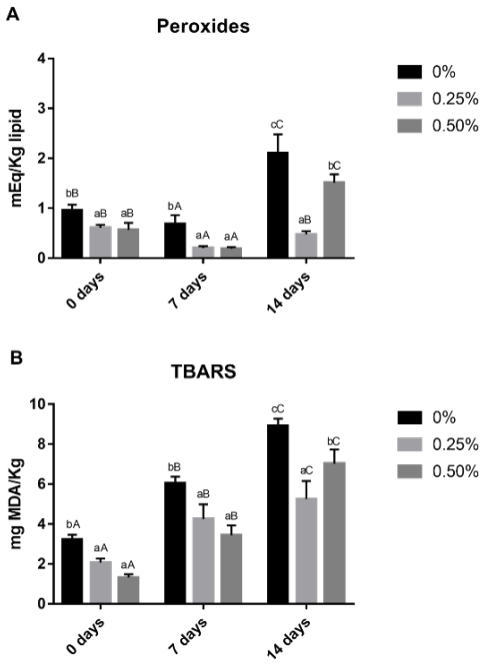

Figure 2. Peroxides (A) and TBARS (B) of tambatinga fillets kept under refrigeration, after application *Mentha piperita* essential oil (EO). The results are expressed as mean ± standard deviation (n = 6). Lowercase letters indicate significant differences between the treatments within the storage period (refrigeration days) by Tukey's test (p < 0.05). Uppercase letters indicate significant differences between storage periods within the same treatment by Tukey's test (p < 0.05).

Likewise, the concentration of TBARS in the tambatinga fillets was reduced on exposure to EO, for all refrigeration periods evaluated (Figure 2B). In the initial evaluation, the values found in the meat (mg MDA kg^{-1}) were: 3.21 ± 0.25 for the control treatment; 2.07 ± 0.20 for the 0.25% EO treatment; and 1.32 ± 0.17 in the 0.50% EO treatment. As the refrigeration period went on, the concentration of TBARS increased in all treatments, but in a less accentuated way in the fillets exposed to the EO of *M. piperita* (Figure 2B).

3.3 Microbiological analysis

There were fewer colonies in fillets exposed to 0.50% EO during all periods of refrigerated storage (Table 2).

Table 2. Counts of strict and facultative aerobic mesophilic microorganisms in tambatinga fillets kept under refrigeration, after application of *Mentha piperita* essential oil (EO)

Refrigeration time (days)	EO concentration		
	0%	0.25%	0.50%
0	2900	2675	605
7	3500	1550	448
14	3125	4750	1675

Microorganism count data is presented as colony-forming units g^{-1} (n = 2).

4. Discussion

The major constituents found in the EO of *M. piperita* used in the present study were geranial, neral and geranic acid. Neranic acid, piperitone and sabinene were present in low concentrations. The oil was extracted from a plant grown in southern Brazil (RS) and the characterization of the constituents and their concentration differ from those found in the EO of two varieties of *M. piperita* grown in northeastern Brazil (Ceará). For the chocolate mint variety, the major constituents found were menthofuran (23.7%), menthone (17.2%), D-neo isomenthol (14.3%) and pulegone (10.7%). For the grapefruit mint variety, linalyl acetate (51.3%) and linalool (25.4%) were identified in higher concentrations[27]. The same authors also observed that other *Mentha* species (*M. aquatica*, *M. spicata* (menthol mint), leaf spearmint, homegrown mint varieties) had carvone (31–58%) and limonene (20–37%) as major constituents. In another study, Chagas[28] reported higher concentrations of menthol (33.8%), menthone (15.2%), menthyl acetate (13%) and pulegone (8.3%) in the EO of *M. piperita* cultivated in north Brazil (Manaus), whereas low concentrations of geranial (1.1%), neral (1.3%), piperitone (1.6%) and sabinene (0.2%) were observed. The authors report that environmental factors such as altitude, soil, temperature, light, fertilization and interactions with predators are conditions that influence metabolic pathways in plants and can change the composition of the EO[29,30]. In addition, aspects such as plant geographical origin, genotype, age and vegetative cycle, as well as the plant organ and extraction method used, may cause significant variations in the composition found[31].

Geranial (alpha-citral) and neral (beta-citral) are the two isomeric aldehydes that make up citral, a monoterpene that occurs naturally in herbs, plants and citrus fruits[32,33]. Citral (3, 7-dimethyl-2,6-octadienal) is found in the EO of several botanical species such as *Cymbopogon flexuosus*[34], *Aloysia triphylla*, *Lippia alba* and *Zingiber officinale*, among others[17]. In plants such as lemongrass and verbena, strong antibacterial activity is associated with a high citral content (above 35%). In the EO of these plants, antifungal and antioxidant action has also been found[35].

The main physicochemical parameters used to determine the degree of freshness of fish are pH and TVB-N[36]. Brazilian legislation[37] establishes that fish considered fresh must have a pH below 7.0 and a TVB-N of up to 30 mg%. Fish with an excellent state of freshness should have between 5 and 10 mg% TVB-N. In this sense, our results showed that the tambatinga fillets were in an excellent state of freshness until the end of the refrigeration period (14 days). *Mentha piperita* EO did not affect the TVB-N content, but there were specific changes in the pH of the fillets. The main reasons may be related to the low pH of the meat after *rigor mortis*, resulting from the anaerobic breakdown of glycogen into lactic acid[38, 39], in addition to the adequate process of fish slaughter and meat processing and the evaluated storage time. However, specific changes observed in the pH of tambatinga fillets during refrigeration may be associated with autolytic and bacterial degradation processes, which lead to the generation of alkaline molecules and, consequently, increased the pH of the meat[38, 39, 40]. Rampelotto[34] did not observe any effects of *C. flexuosus* EO (containing 45.6% geranial and 32.1% neral) on the pH of *Rhamdia quelen* fillets stored frozen for 12 months. The fish had been fed diets containing 1 or 3 mL EO kg^{-1} diet for 20 days before slaughter. The authors reported that the initial pH of the fillets was 6.36, reaching 6.68 after nine months of storage.

Lipid oxidation is one of the main non-microbial changes in meat and leads to changes in sensory properties (colour, flavour, odour and acceptability) and shelf life[8, 11]. Fish meat is especially susceptible to lipid oxidation due to its high content of unsaturated fatty acids. In this sense, *M. piperita* EO proved to be efficient in delaying lipid oxidation, as evidenced by the lower formation of peroxides and TBARS in tambatinga meat stored refrigerated for up to 14 days. According to Lis-Balchin[35] and Chandra and Farook Ah[41], the EO of plants such as *C. flexuosus* and verbena, species that have citral as one of the main components of their EO, show antioxidant activity *in vitro*. The isolated constituent (citral) was also tested and showed an increase in antioxidant activity, determined by the Ferric Reducing Antioxidant Power (FRAP) method, as the concentration increased (5–40 mg mL^{-1})[41]. Therefore, according to these findings, our hypothesis is that citral is mainly responsible for the antioxidant action of *M. piperita* EO on tambatinga meat.

In a previous study, supplementation with *C. flexuosus* EO, containing around 77% citral, directly in the fish diet did not show a protective effect against lipid oxidation (based on the content of conjugated dienes, peroxides and hexanal) or protein oxidation (total content of sulphhydryl groups reduced and carbonyl protein) in silver catfish meat[34]. The authors observed a variable response according to the EO concentration used: fish fed with 1 mL EO kg^{-1} showed a slight increase in the lipid oxidation of the meat over 12 months under freezing. In contrast, the meat of fish fed 3 mL EO kg^{-1} showed an increase in protein oxidation over the storage period.

In addition to freshness and oxidative stability analyses, microbiological evaluation of fish meat is important to demonstrate its quality and durability, because spoilage in fish occurs mainly as a result of bacteriological activity[42]. The use of 0.50% *M. piperita* EO kept the microorganism count in tambatinga meat low during the entire refrigeration period. The count for this treatment was 2–4-fold lower compared with the fillets treated with 0% or 0.25% EO. According to *in vitro* studies, the high antibacterial and antifungal activity of *C. flexuosus* is related to the strong activity of citral, which is the main monoterpene in its EO[35,41]. The antimicrobial action of *M. piperita* EO found in the present study is consistent with this information.

5. Conclusion

Mentha piperita EO displayed the constituent forms of citral (geranial and neral) as the most abundant molecules. Treating tambatinga fillets with this EO helped reduce lipid oxidation and the development of microorganisms. The concentration of 0.50% of this EO in solution was more effective in reducing the deterioration of meat kept refrigerated.

Conflict of interests

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: A. Pretto. *Formal analysis*: A. Pretto. *Investigation*: A. Pretto, I. R. Marinho, I. P. Doihara, T. S. Costa and O. S. Monteiro. *Project administration*: A. Pretto and J. M. Lopes. *Resources*: J. M. Lopes, I. P. Doihara and C. S. Teixeira. *Methodology*: J. M. Lopes. *Writing (original draft): A. Pretto. Writing (proofreading & editing): A. Pretto, J. M. Lopes, I. P. Doihara and C. S. Teixeira.*

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