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APPLICATION OF ESSENTIAL OILS AS A PRESERVATIVE TO IMPROVE THE SHELF LIFE OF NILE TILAPIA (*Oreochoromisniloticus*)

APLICACIÓN DE ACEITES ESENCIALES COMO CONSERVANTES PARA MEJORAR EL TIEMPO DE VIDA ÚTIL EN TILAPIA NILÓTICA (*Oreochromisniloticus*)

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ABSTRACT

Some essential oils extracted from herbs and spices have antioxidant activity, it can be used to increase the shelf-life of fillets and cuts of different post-mortem meat. The aim of this work was to study the effect of the antioxidant activity of Thyme and rosemary essential oils on Nile tilapia fillets preservation. The effect of essential oils in tilapia shelf-life was evaluated through the determination of the oxidation products by TBARS analysis and their comparison to some samples without essential oil application. The obtained results show that the use of the two essential oils reduces, between 53 and 96.5%, the oxidative processes of tilapia fillets from the ninth day of storage.

Keywords: Tilapia, oxidation, TBARS, essential oils, Shelf life.

RESUMEN

Algunos aceites esenciales extraídos de hierbas aromáticas y especias han presentado actividad antioxidante, aspecto que puede ser usado en el aumento de la vida útil de filetes y cortes post-mortem de diferentes carnes. El objetivo del presente artículo fue estudiar el efecto de la utilización de aceites esenciales de tomillo y romero como conservantes en filetes de tilapia nilótica empleando su actividad antioxidante. Se evaluó el efecto final de los aceites en la vida útil del alimento a través de la determinación de los productos de la oxidación por medio de análisis TBARS y su comparación con muestras a las cuales no se les aplica ningún tipo aceite. Los resultados mostraron que para los dos aceites esenciales empleados, se obtuvieron reducciones para los procesos oxidativos de los filetes de tilapia, entre 53 y 96.5% a partir del noveno día de almacenamiento.

Palabras clave: Tilapia, oxidación, TBARS, aceites esenciales, vida útil.

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INTRODUCTION

Recently, food industry has made many efforts towards Good manufacturing practices (GMP) implementation and food safety, being the latest more and more important in public health day by day (1). Food spoilage is in part due to the enzymes existent in the food which produce chemical reactions transforming biomolecules, altering the product. This chemical reactions include the oxidation reactions, which are chain reactions (reactions which once they start, they continue accelerating up to reaching the total oxidation of the sensible substances) (2, 3). Furthermore, regarding food spoilage, it is estimated that up to 30% of the people from industrialized countries suffer a disease transmitted by food (ETA in Spanish) every year (4). According to OMS, 1.5 millions of children die annually because of the ETA, which is a very alarming number (5). Therefore, it is necessary to reduce or eliminate the pathogenic agents of alimentary transmission by applying new methods or combining the existing ones (6). In Latin America, the largest amount of food involved in foodborne illnesses of known origin are water, (20%) and fish (16%) (7).

Fish is one of the foods which suffer fast decomposition processes, with preservation times on ice between 8 - 17 days and 20 - 40 days for the freshwater fish species of template waters (trout, perch and carp) and the fish from tropical waters (like pacu fish, croaker, shad) respectively (8, 9). Frequently, Tilapia reaches a range of 10 to 27 days when it is conserved on ice. It can generate losses before consumption when compared to the time required for its transportation by maritime ways, representing a huge problem when thinking Tilapia as an exportation product to European and Asian markets (10).

The conditions affecting the most of the properties and product quality are the oxidation reactions and the transformations caused by microorganism present in products (8, 10). However, taking into account the environmental conditions, the concentration of certain ingredients and other factors also play an important role in the decomposition or preservation processes. The reaction products by oxidation affect the generation of free radicals and the reaction speeds which promote the lipids and proteins rupture and generate oxidation compounds. Therefore, the usage of methods or processes which stop or decrease the oxidation reactions would increase the shelf life of the products.

Natural preservatives

One of the existent techniques for the microbial and chemical spoilage control is the addition of synthetic (chemical origin) or natural preservatives. In the last years, there has been a tendency to use and implement natural preservatives that may have a positive effect on human health (11). In natural preservatives, essential oils research has presented great advances in their usage; their compounds are used to reduce microbial and chemical spoilage (12-14).

Essential oils

Western society presents a tendency to "green" or ecological consumption (15), looking for less synthetic additives and more products with a minor impact on the environment. Recently, the World Health Organization has urged a reduction on salt consumption in order to reduce the incidence of cardiovascular diseases (6). By reducing the salt levels on processed foods, it is necessary to use other additives that ensure the preservation of food, hence, is generated a research field in safe foods development that contains natural ingredients displaying no detrimental effect on health. One possibility is the use of essential oils (EO's) as antibacterial additives (16-18), which are aromatic compounds obtained from vegetal material (flowers, shoots, seeds, leaves, twigs, bark, grasses, wood, fruits or roots). About 3000 EO's are well-known, 300 of them are commercially important in flavor and fragrances industries (18). Some methods such as fermentation, enfleurage or extraction are used for their obtaining. However, steam distillation is used the most in commercial production (19).

Some studies have shown that certain EO and some spices have properties or antimicrobial effects (17, 20, 21), antivirals (22), antimicotics (23-26), antioxidants (27, 28), antiparasitic (29, 30) and insecticides properties (14, 31, 32), therefore, the investigation field is broad. The main compounds responsible for the antibacterial properties of the Essential oils are the phenolic compounds (32, 33) such as Timol, Carvacol, acid 1, 8 Cafeic and Transcinnamaldehyde; as well as some of the components with mayor presence on the essential oils of thyme, rosemary, cinnamon and oregano; which are complemented with compounds such as decanal, terpinene, p-cymene, and α-pinen in a less proportion (34-36).

The minor compounds of these oils have shown that they play an important role against bacterial activity in some cases. It can be possibly due to the synergic effect when combined with major compounds as it occurs in sage, some thyme species (35, 37, 38) and oregano (39).

Rosemary (Rosmarinus officinalis L.) and Thyme (Thymus vulgaris) EO's.

In Rosemary case (*Rosmarinus officinalis L.),* extracts have a potent antioxidant activity, and they are widely used in food industry. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which inhibit the chain reactions of free radicals with hydrogen donation (40, 41). Some researchers had reported the effectiveness of the rosemary extracts in delaying the lipidic oxidation on different foods, as in the case of meat fillets where were used 200-1000 oil mg / meat kg (42). Also, rosemary natural extracts have been used as antioxidants in pork sausages (43). Concentrations among 200 and 1000 mg / kg have been used on different food (more than ten types of meats and fishes) (44). In the case of thyme, *(Thymus vulgarism)* its natural antimicrobial properties and antioxidants have been investigated to show its efficiency in improving the foods shelf-life. Thymol and Carvacrol are the most active components on Thyme essential oil, with a wide range of antimicrobial and antioxidant properties (8, 45). Thyme essential oil not only generates an antioxidant effect, but it was proved that it has a medicinal value against diseases such as cough, pharyngitis, emphysema and asthma (16, 19, 46).

The aim of this work is to evaluate the antioxidant effect of the rosemary and thyme essential oils over the oxidation process in fillets of Nile tilapia, by applying different solution concentrations in the fillets immersion.

MATERIALS AND METHODS

Nile tilapia fillets *(Oreochromisniloticus)* were used in this work. They were subjected to different concentrations of essential oils by making vehiculization tests before applying the EO solutions, and it was studied the antioxidant effect at different storage times.

EO´s Vehiculization

Propyleneglycol and tween® (Polyoxyethylene (20) sorbitanmonooleate 99.9 % w/w) were evaluated as emulsifiers of EO´s emulsification in water. These emulsifiers were evaluated separately and mixed at different concentrations. In order to select the proper mix to be applied in the methodology, the permanence of a homogeneous stage of EO solution was evaluated during a time lapse of three hours (express recommendation of the providers). From the obtained results, the combination that presented a homogeneous mix in a water solution 80% v/v; propyleneglycol 20% v/v, tweet® 0.02% v/v was used, which match with provider's recommendations.

Oils application in fillets.

Analyses were performed in 50 g of Nile tilapia fillet, which were kept under refrigeration (2 - 4°C) and kept in contact with the solution of essential oil through 1 day in order to allow a uniform distribution. Afterwards, samples were withdrawn from the solution and stored in refrigeration conditions during experimental period.

Rosemary and thyme Oils were used with three different concentrations (2, 5 and 8% v/v). Additionally, it was prepared a blank test which did not have any essential oil application. Analyses were performed for triplicate for each sampling days (0, 3, 6, 9 and 12 days of refrigerated storage).

To each fillet was applied 10 mL of essential oil dilution during the refrigerated storage in each case, according to the experimental design.

TBARS determinations

The sample for determining the antioxidant effect of tilapia fillets was performed according to the method presented by Chandrika (47). To determine the index TBARS of oxidation, 10 ± 0.1 g of tilapia fillet were weighed. Then, it was macerated with distilled water until it reached its homogeneity. Later, it was completed to a (90 mL) volume with distilled water, and 10 mL of HCl (0.1 N) were added. This mixture was distilled for 10 minutes, collecting the distillate in a 50 mL volumetric balloon. An aliquot of 5 mL of distillate was reacted with 5 mL of Thiobarbituric acid (0.02 M), heated in a water bath at 80 - 90°C during 40 minutes to obtain a TBA-MDA colored complex. The reading was made on a V-530 Jasco spectrophotometer®

model (Jasco Inc., USA) at 532 nm. Final results were expressed based on a standard curve prepared with 1,1,3,3 - tetramethoxypropane (TMP).

Statistical analysis

Data were statistically analyzed by using the Analysis of Variance (ANOVA) and the statistical package STATGRAPHICS® CENTURION XV (Statistical Graphics Corp., USA). A multiple range test was performed by using the Fisher least significant difference procedure (LSD) in a significance of ($p < 0.05$).

RESULTS AND DISCUSSION

Evaluation of rosemary oils implementation

The data obtained from Thiobarbituric acid reaction tests (TBARS), in the application at different concentrations of essential Rosemary´s oils in Nile tilapia fillets, is represented in figure 1.

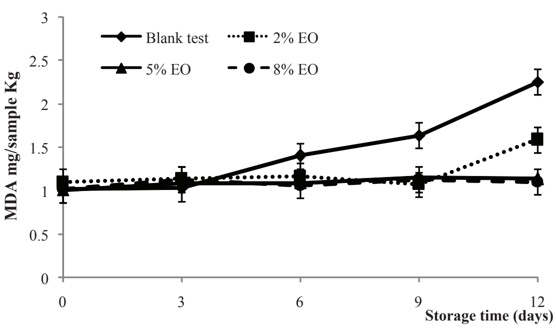

Figure 1. TBARS values (MDA mg/ sample Kg) by Rosemary essential oil application in Nile tilapia at different storage times.

As shown in figure 1, the concentration values of Malonaldehyde (MDA) in the test without any application of essential oils (blank) increased according to the storage time. It implies an increase in the tilapia oxidation.

It is possible to observe how the concentration values of Malonaldehyde (MDA) for fillets, which the application was performed with different concentrations of essential oil, did not display a significant increase (p-value > 0.05), from application (day zero) until day twelve, which shows that in the case of Nile tilapia, Rosemary essential oil has an antioxidant effect, as evidenced by other authors (48 - 51), due to the presence of the compounds: Carnosic, Carnosol, Rosmanol acids, Rosmariquinone and Rosmaridiphenol (40, 41). The values of TBARS oxidation index for the different treatments had statistically significant differences (p-value < 0.05), from ninth day of storage, contrasted with the obtained values for the blank test without any application of essential oils.

In the case of Rosemary essential oil showed in figure 1, there were no statistically significant differences for the TBARS values among the used treatments at concentrations of 2, 5 and 8% except for twelfth day of storage, where TBARS average value for the application at 2% is higher than for the other concentrations.

An effect that is possible to observe in the behavior of Rosemary essential oil is the tendency that it showed during the first three days of storage, where the average values of Malonaldehyde concentration are superior to the blank, without significant differences. This fact may be due to the low levels of fat oxidation in fish (52) and by an interference effect of this type of oils in the determinations.

Evaluation of Thyme oils implementation

Figure 2 shows the values of oxidation expressed as Malonaldehyde concentration for Nile tilapia fillets which were applied Thyme essential oil at different concentrations.

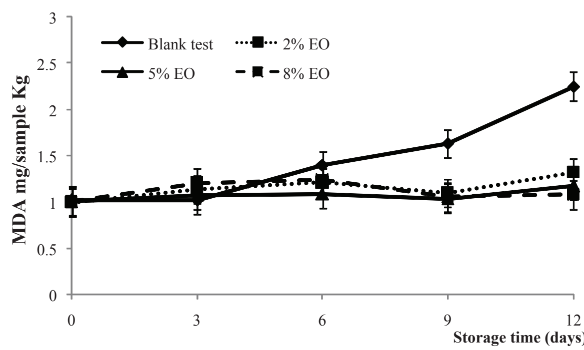

Figure 2. TBARS values (MDA mg/Sample Kg) by the Thyme essential oil application in Nile tilapia at different storage times.

As in the case of Rosemary essential oil application, it can be seen that from third day of storage, oxidation values increased in fillets without the application of essential oil, while fillets with any application of Thyme essential oil did not present any statistically significant differences among the different days of storage for the different concentrations used. It confirmed that the Thyme´s oil and particularly its active compounds, Thymol and Carvacol (26, 39, 53), may pursue an antioxidant effect on the tilapia fillets, as it has been shown in the use of other types of meat. No statistically significant differences were observed in the different concentrations of the Thyme oil used (2, 5 and 8%) (p-value > 0.05) for any of the analyzed days of storage. This implies that Thyme oil and its use in low concentrations may present an antioxidant power similar to the one presented by higher oil concentrations.

The values of TBARS oxidation index obtained for tests at different concentrations of Thyme oil, as well as in the case of Rosemary, presented statistically significant differences (p-value < 0.05) from ninth day of storage compared to the values obtained for the blank test without any application of essential oils. This is because the oxidation processes in fish develop faster from the sixth day of storage.

The reduction level in oxidation processes for the Rosemary and Thyme essential oils application at different concentrations are showed in table 1. This table clearly suggests that, in the early days of storage, oils application did not generate a significant decrease in Tilapia fillets oxidation. However, from ninth day of storage it was observed how the used oils caused a reduction of the oxidation products. By twelfth day of storage the reduction values were between 53.9 and 93.0% for the Rosemary essential oil, and between 75.3 and 94.7% for the Thyme essential oil, compared with the oxidation levels observed in blank tests. This shows a significant improvement in the preservation of the fillets against oxidative processes that occurs during storage.

By using the statistical analysis (ANOVA) it is concluded that when comparing the levels of oxidation of blank against the levels of oxidation of samples, with the application of Rosemary and Thyme oil in concentrations of 2, 5 and 8% v/v, they were significantly different, but they have a similar behavior in their role as antioxidants. This is consistent with other studies that have been conducted on the effects of essential oils to protect meat and meat products against oxidative processes (41, 46, 54, 55).

Table 1. Reduction level values of oxidation in fillets of Nile tilapia.

Day	ROSEMARY 2%	ROSEMARY 5%	ROSEMARY 8%	THYME 2%	THYME 5%	THYME 8%
0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
9	91.0% ± 0.21	78.2% ± 0.38	82.1% ± 0.32	86.6% ± 0.26	96.5% ± 0.17	93.8% ± 0.21
12	53.9% ± 0.18	90.4% ± 0.12	93.0% ± 0.09	75.3% ± 0.18	87.3% ± 0.12	94.7% ± 0.08

CONCLUSIONS

This study showed that Rosemary and Thyme essential oils at low concentrations (2, 5 and 8%) in a solution of water, Propyleneglycol and an emulsifying agent, presents a high effectiveness as antioxidants in the use of Nile Tilapia fillets, at refrigeration temperatures and by placing the fillet immersed in the solution. For longer cooling times in the storage of tilapia fillets, the oxidative processes are diminished by the use of Thyme or Rosemary essential oils.

In general, the reduction of oxidative processes in tilapia fillets by using the two essential oils occurred between 53 and 96.5%. This demonstrates their high effectiveness, even at low concentrations of essential oils.

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