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Antimicrobial effect of essential oils of *Laurus nobilis* L. and *Rosmarinus officinallis* L. on shelf-life of minced "Maronesa" beef stored under different packaging conditions☆

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ABSTRACT

The aim of this study was to evaluate the effect of essential oils (EOs) of plants naturally occurring in northern Portugal on the spoilage of fresh Maronesa beef burgers stored at 2 and 8 °C under different packaging conditions.

EOs were obtained from dried leaves of laurel (*Laurus Nobilis* L.) and rosemary (*Rosmarinus officinallis* L.) by hydro-distillation using a Clevenger-type apparatus. Analysis of volatile composition of essential oils of rosemary and laurel was achieved by Gas Chromatography–Mass Spectrometry (GC–MS) and Gas Chromatography-Thermal Conductivity Detection (GC-TCD) resulting in the detection of 95.8% and 89.4% of its compounds, respectively.

Fresh beef (*semitendinosus* and *semimembranosus*) of DOP-Maronesa breed (males; n = 4) were obtained from local market and transported to the laboratory. Samples were stored at 2 and 8 °C in two different conditions: aerobiosis (A) and vacuum (V) and analyzed at 0, 1, 2, 3, 5, 7, 10, 14, 21 and 28 days for Lactic acid bacteria (LAB), *Enterobacteriaceae*,*Pseudomonas* spp., Fungi, Total mesophilic (TM) and psychrotrophic (TP), color (L*a*b*) and pH.

Laurel was the most effective EO keeping pH from increasing. Coordinates L* and a* were higher on samples containing laurel EO for both A and V packaging. Laurel also showed better effect in reducing microbiologic counts in samples packed in A at both 2 and 8 °C and packed in V at 8 °C. Rosemary was effective in reducing microbial counts on all V samples stored at 2 °C.

This study allows to conclude that Laurel EO has significant effect in shelf-life, maintaining fresh beef color.

1. Introduction

Meat is a major source of protein of high biological value for humans (Lund, Heinonen, Baron, & Estévez, 2011), being also a source of other important nutrients. It is an ideal substrate for the development of deteriorative and pathogenic microorganisms, therefore it is important to ensure the safety of its consumption (Kodogiannis, Pachidis, & Kontogianni, 2014).

Minced meat has a reduced shelf life when compared to whole meat, since the surface area exposed to the external environment is increased (Limbo, Torri, Sinelli, Franzetti, & Casiraghi, 2010). The best way to improve food safety and shelf life is minimizing contamination and slowing or even inhibiting the growth of deteriorative and pathogenic microorganisms (Sallam & Samejima, 2004).

Deterioration of fresh meat can be subjective since it depends on the culture, economic capacity, level of education and sensory acuity of consumers. Although deterioration is not always apparent, the following aspects are commonly considered as the main criteria for rejection: discoloration, off-odors and off-flavors and slime appearance (Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002).

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There are several factors that can influence shelf-life of meat: temperature, atmospheric oxygen, water activity (a_w), light, endogenous enzymes and microbiological development. All of these factors cause changes in color, odor, flavor and texture. Although the deterioration of meat can be due to processes such as proteolysis, lipolysis and oxidation, microbial growth is the most important factor (Nychas, Marshall, & Sofos, 2007). Microbial loads from 10^7 CFU cm^{-2} are associated with the occurrence of off-odors. Those off-odors can become fruity when the microbial counts rise and become putrid, as a result of amino acid consumption, for microbial counts greater than 10^9 CFU cm^{-2}. When the glucose present in the aqueous phase is used, other substrates are sequentially consumed with the released of odors of ammonia and nitrogenous compounds, such as dimethyl-1 sulfide (Ercolini, Russo, Torrieri, Masi, & Villani, 2006). Aerobic bacteria and Gram negative facultative anaerobic are considered the group with the greatest spoilage potential. Many members of the Enterobacteriaceae family contribute to the meat spoilage. However, in refrigerated meat stored under aerobic conditions, *Pseudomonas*, *Acinetobacter*, *Psychrobacter* and *Moraxella* present high growth rates (Ercolini et al., 2006) and the genus *Pseudomonas* is generally dominant, actively contributing to the deterioration, due to their ability to degrade glucose and amino acids at reduced temperatures (Mohareb et al., 2015). Although *Acinetobacter* would compete with *Pseudomonas* for amino acids and lactic acid, those have little affinity for oxygen, which favors *Pseudomonas* (Ercolini et al., 2006). Even though the dominant spoilage microflora in the fresh meat is generally Gram negative, the initial population can include Gram positive genera such as Lactic Acid Bacteria (LAB) and *Brochothrix thermosphacta* (Mohareb et al., 2015). LAB plays an important role in the spoilage of refrigerated fresh meat and are important competitors of other groups of deteriorative microorganisms. *Brochothrix thermosphacta* is a microorganism that may develop under aerobic and anaerobic conditions, resulting in the release of off-odors (Ellis et al., 2002).

Recently it has been observed a growing interest in the search for natural products with antimicrobial and antioxidant properties in order to replace chemical and synthetic additives currently used in the food industry (Wang, Wu, Zu, & Fu, 2008).

Since ancient times, spices and herbs are used not only for medicinal purposes but also to improve organoleptic characteristics of food (Calo, Crandall, O'Bryan, & Ricke, 2015). EOs can be used as food preservatives to improve food hygiene, reducing the microbiota development and enhancing shelf-life of meat. They are aromatic oily liquids obtained from various organs of plants such as flowers, leaves, seeds, roots, fruits and others (Korifi, Le Dréau, Antinelli, Valls, & Dupuy, 2013). They are secondary metabolites synthesized by herbs as a form of protection from bacteria, virus, fungi, insects, herbivores and the climate. On the other hand they can attract some insects to promote the dispersal of pollen and seeds (Burt, 2004).

The antibacterial, antiviral, anti-parasitic, anti-mycotic, anti-toxicogenic activity and insecticidal properties of some EOs are well known, hence the interest in the study of its applicability in foodstuff preservation (Burt, 2004).

The quality, quantity and chemical composition of the EOs may vary according to the weather and composition of the soil, plant organ from it is extracted, age and stage of the growth cycle (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Thus, in order to obtain EOs of constant composition, plants must be collected under the same conditions.

In vitro physical and chemical studies characterize most of the compounds present in EOs as antioxidants. Depending on the concentration, these can be cytotoxic but are generally non-genotoxic (Bakkali et al., 2008). The characteristics of the food matrix such as lipid composition, proteins, a_w, pH and enzymes can diminish or enhance the effectiveness of the EOs. According to Bajpai, Baek, and Kang (2012), low pH may increase the solubility and stability of the EO increasing the antimicrobial capacity.

According to Fisher and Phillips (2008), the compounds present in the EO penetrate the protein structure of the cell wall causing protein denaturation and destruction of the cell membrane. Thus, the operation of the cellular components, including the core, are reduced by the presence of compounds in the EOs due to changes in cell membrane permeability (Fisher & Phillips, 2008).

Some studies suggest that generally Gram positive bacteria are more sensitive to compounds of the EOs than Gram negative bacteria; this is thought to be related to the Gram negative impermeable outer membrane (Fisher & Phillips, 2008). However, according to Smith-Palmer, Stewart, and Fyfe (2001), over time the EOs ultimately have the same effect on both types.

Rosemary (*Rosmarinus officinalis* L.) is a shrub that grows in all Mediterranean countries. Of the different species (*R. officinalis*, *R. eriocalyx*, *R. lavandulaceus* and *R. laxiflorus*), only *R. officinalis* grows naturally in the Mediterranean (Angioni et al., 2004; Tassou & Nychas, 1995). Besides being used for centuries as a food flavoring, it is also very important in traditional medicine and it is used to combat cramping and relieve the symptoms of diseases of the nervous system (Miresmailli, Bradbury, & Isman, 2006; Wang et al., 2008). *R. officinalis* L. is one of the spices with anti-inflammatory (Steiner et al., 2001) and high antioxidant properties (Bozin, Mimica-Dukic, Samojlik, & Jovin, 2007), attributed to phenolic compounds such as carnosol, carnosic acid, rosmanol, rosmadial, epirosmanol, rosmadiferol and rosmarinic acid. These compounds promote the maintenance of the nervous tissues (Offord, Aescgbach, Loliger, & Pfeifer, 1997). Carnosic acid has anticancer properties, inhibiting the proliferation of abnormal cells (Chan, Ho, & Huang, 1995; Kosaka & Yokoi, 2003). It is effective in controlling various pathogenic and deteriorative microorganisms (Chan et al., 1995).

Laurel (*Laurus Nobilis* L.) is an evergreen shrub or tree native of the southern Mediterranean region (Sellami et al., 2011). Its dried leaves and EO are used in the food industry as a spicy for flavoring and food preservative and are used in folk medicine (Ali-Shtayeh et al.,2000Ali-Shtayeh, Yaniv, & Mahajna, 2000) (Kilic, Hafizoglu, Kollmannsberger, & Nitz, 2004; Ramos et al., 2012). According to Sellami et al. (2011), 1,8-cineole is the major laurel EO component with percentages ranging between 31.4 and 56%. Other compounds were present in appreciable amounts include linalool, *trans*-sabinene hydrate, α-terpinyl-acetate, methyl eugenol, sabinene and eugenol. Benzene compounds present in percentages ranging between 1 and 12%, are responsible for the spicy aroma of the leaves and are extremely important factors determining its sensory quality.

The purpose of this work was to assess the effect of EOs of laurel (*Laurus Nobilis* L.) and rosemary (*Rosmarinus officinallis* L.) naturally occurring in Trás-os-Montes e Alto Douro, Portugal, on the spoilage of fresh Maronesa beef burgers stored at 2 and 8 °C under different packaging conditions.

2. Material and methods

2.1. Extraction of essential oils

For the extraction of EOs, leaves of laurel (*Laurus Nobilis* L.) and rosemary (*Rosmarinus officinallis* L.) were obtained from wild regions in northern Portugal. The material was weighed and dried in an oven at approximately 40 °C until no weight change was found and sealed under vacuum until used. In order to obtain the EOs, the material was submitted to hydro-distillation using a Clevenger-type apparatus for 3 h. In this method, the dried sample is ground and put in a volumetric flask with distilled water (1:10), placed in a heating