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Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films

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ABSTRACT

The chemical composition of the essential oils obtained by hydrodistillation from fully-formed, dried oregano leaves (*Origanum vulgareOriganum vulgare*) and lavender leaves and flowers (*Lavandula officinalis*) were analyzed by GC/MS. The effectiveness of oregano (OEO) and lavender (LEO) essential oils and a mixture LEO:OEO (50:50) in inhibiting *Escherichia coli* and *Staphylococcus aureus* growth were determined. Both essential oils inhibited the growth of the microorganisms tested, being more sensitive to gram-positive bacteria. OEO yielded the lowest values of minimum inhibitory concentration (MIC_{OEO} = 1600–1800 ppm vs. MIC_{LEO} = 2000 ppm against *E. coli*; MIC_{OEO} = 800–900 ppm vs. MIC_{LEO} = 1000–1200 ppm against *S. aureus*), due to the higher content of phenolic compound, which also provides antioxidant capacity ($IC50_{OEO} = 297 \pm 89$ ppm vs. $IC50_{LEO}$ » 6000 ppm). Mixture results indicated an antagonist antimicrobial effect between OEO and LEO. Gelatin-based films added with OEO or LEO, were prepared by casting (2000–6000 ppm). Mechanical, optical and water vapor barrier properties were determined to observe film functionality. OEO effect on the functional properties of gelatin films was not significant. LEO, in the highest concentration analyzed, promotes a slight change in water vapor permeability of Ge-based films (1.46×10^{-13} to 6.8×10^{-14} Kg.m/Pa.s.m^2), due to its high hydrophobic nature. Oregano containing gelatin films exhibited the highest antimicrobial and antioxidant properties.

1. Introduction

The main driving force for the growth of worldwide food industry is the scope and range of food preservation and shelf life extension technology (Sadaka et al., 2013). Active packaging is gaining increasing attention from researchers and the industry due to its potential to provide quality and safety benefits. Active packaging is a type of packaging that changes its conditions as a way to extend life or enhance safety or sensory properties while maintaining food quality (Vermeiren et al., 1999). In view of the health concerns expressed by consumers and current environmental problems, research is now focusing on the development of sustainable packaging materials based on annually renewable natural biopolymers such as polysaccharides and proteins (Gomez-Estaca et al., 2010). As a multifunctional protein, gelatin is an heteropolymer derived from collagen. Gelatin-based systems are applied in numerous fields, including food, pharmaceuticals, photographic industry, as well as materials intended for food packaging applications (Gómez-Guillén et al., 2007; Martucci and Ruseckaite, 2009; Ahmad et al., 2012; Martucci et al., 2012; Teixeira et al., 2014). Biodegradability, excellent biocompatibility, plasticity, adhesiveness, abundance, and low cost are the main reasons for the wide range of applications of this biopolymer.

The consumer's desire for natural ingredients and for chemical preservative-free foods has increased the popularity of natural antimicrobial agents (Sadaka et al., 2013). In this framework, the addition of essential oils to biopolymer films as natural bacteriostatics could be an interesting election. Essential oils have well-recognized properties, such as antimicrobial (Kulevanova and Panovska, 2001; Gende et al., 2010,b; Teixeira et al., 2013a,b), antibacterial (Canillac and Mourey, 2001; Min and Oh, 2009,b; Teixeira et al., 2013a,b) and antioxidant properties (Burt, 2004; Kačániová et al., 2012; Danh et al., 2012,b; Teixeira et al., 2013a,b). These properties can be attributed to the high content of terpenic compounds (α-pinene, β-pinene, 1,8-cineol, menthol, linalool) or phenolic compounds such as carvacrol, eugenol and thymol (Burt, 2004). It is common knowledge that essential oils are characterized by changes in their chemical composition, depending on the state of

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development of the plant, the part used for the extraction, the geographical location, and the physical and chemical characteristics of the soil and climate (Gende et al., 2010).

The use of gelatin based films with aqueous plant extracts appears to be a promising technology in food packaging materials. These films can reduce surface microbial populations, enhance oxygen barrier, and reduce the use of synthetic packaging materials since gelatin and essential oils are derived from renewable resources (Gómez-Estaca et al., 2009, 2010; Perez-Mateos et al., 2009; Min and Oh, 2009; Ahmad et al., 2012; Teixeira et al., 2014). The inclusion of antimicrobial and/or antioxidant compounds into edible films provides a novel way to improve the safety and shelf life of ready-to-eat foods. Some plant EOs and their components are compatible with the sensory characteristics of fruits and vegetables and have been shown to prevent bacterial growth, as reported by several reviews about this subject (Burt, 2004; Sánchez-González et al., 2011; Eça et al., 2014)

The objectives of this study were to determine the antimicrobial activity of oregano (*Origanum vulgare L.*) and lavender (*Lavandula officinalis L.)* essential oils and their main components against *Escherichia coli* and *Staphylococcus aureus* by a serial dilution method. It was also to investigate the antibacterial efficacy of both oils incorporated into mammalian gelatin-based films using the agar diffusion method. Optical and mechanical properties, water vapor permeability and the ability of the films to provide microorganisms' protection and lipid oxidation were analyzed.

2. Experimental

2.1. Materials

Bovine hide gelatin (Ge) type B was kindly supplied by Rousselot (Argentina), Bloom 150, isoionic point (Ip) 5.3. Buffer Phosphate pH 7 (Ciccarelli, Argentina), Propylene Glycol (PG, molar mass: 76.09 g/mol; HLB: 7.4–9.3) (Bolivar chemicals, Argentina), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich, EEUU), potassium hexacyanoferrate III and trichloroacetic acid (Ciccarelli, Argentina) were analytical grade and used as received.

2.2. Source of Bacteria

Food-borne pathogens were used to assess the antimicrobial proprieties, which includes the gram-negative bacteria *Escherichia coli O157*:*H7* ATCC 32158 (ATCC, American Type Culture Collection) and Gram-positive *Staphylococcus aureus* ATCC 25923. These strains were obtained in Eosin-methylene blue (EMB) and Baird Parker agar respectively. Vegetative cells of each microorganism were streaked on Mueller Hinton agar and incubated at $37 \pm 0.5\,^{\circ}C$ for 24 h. Microbial broth was then suspended in double distilled sterile water. The density of bacteria suspension was adjusted until the visible turbidity was equal to 0.5 Mc Farland standard before testing.

2.3. Isolation and characterization of oregano and lavender essential oils

Falciform, fully-formed oregano leaves (*Origanum vulgare L.)* and lavender leaves and flowers (*Lavandula officinalis L.*) were collected in the geographical area of Mar del Plata (38° 00′ 24.17" S-57° 33′ 55.89" W) during July 2011. Plant specimens were classified and stored in the herbarium of vascular plants (AL 17 and PV 97, Arthropods laboratory, Faculty of Sciences, Universidad Nacional de Mar del Plata). Essential oils were extracted by hydrodistillation using a Clevenger type apparatus according to the method reported elsewhere (Gende et al., 2010) from freshly dried plant material

The quantitative and qualitative analysis of lavender and oregano oils were carried out by gas chromatography (GC) coupled to mass spectrometry (GC/MS). The experiments were performed using an Agilent gas chromatograph (GC) model 7890 A (Agilent, Palo Alto, USA) equipped with an auto-sampler ALS and coupled to an Agilent single quadrupole mass spectrometer (MS) model 5975C (Agilent, Palo Alto, USA). The GC was equipped with an Agilent 5MS column (100 m × 0.25 mm internal diameter and 0.25 μm thickness). Helium was used as carrier (1.5 mL/min) in constant flow mode, with a total GC run time of 30 min. The injector temperature was kept at 280 °C in a split less mode and using an injection volume of 1 μL. The oven temperature was programmed to increase from 50 °C, hold 2 min, increased to 260 at 10 °C/min and then hold for 2 min. The mass spectrometer was operated in electron impact (EI) mode at 70 eV with anion source temperature at 230 °C and quadrupole temperature 190 °C. A scan rate of 0.6 s (cycle time: 0.2 s) was applied, covering *m/z* range from 29 to 500. The identification of EOís components was achieved by matching their mass spectra to that reported in the literature (Adams, 2007). Quantitative data were derived by integration of FID area percentages with no use of collection factors.

2.4. Preparation of control and EO-added gelatin films

Gelatin power (5 g) was dissolved in 100 mL of buffer phosphate (pH 7) at room temperature. After dissolution for 30 min under continuous stirring, propylene glycol (8% v/v of solution) was added as plasticizer and emulsifier agent. An adequate mixing of the plasticizer, lavender essential oil (LEO) or oregano essential oil (OEO) was incorporated to obtain final concentrations between 2000 and 6000 ppm. Control formulation was prepared in the same way, replacing EOs by buffer phosphate. Afterwards mixtures were homogenized at 20000 rpm for 5 min by using a homogenizer (UltraturaxT25 basic, IKA-Werke GMBH & Co., KG Staufen, Germany). Films were obtained by casting and dried at 35 °C in a forced-air oven (Memmert UFE550, Germany) for 20 h until constant weight. Dried film samples were manually peeled off from the mold and conditioned in a laboratory humidity chamber at $25 \pm 2\,^{\circ}C$ and $65 \pm 2\%$ relative humidity (RH) prior to analysis. Resultant films were designated as Ge (control gelatin films), OEO-Ge (oregano essential oil -added gelatin film) and LEO-Ge (lavender essential oil-added gelatin film), respectively.

2.5. Analysis

2.5.1. Thickness

Film thickness was measured using a 0–25 mm manual micrometer, with a resolution of 0.01 mm. The reported values are the average of four readings taken randomly on each film sample.

2.5.2. Optical properties

Color was measured by a CIE L*a*b* system using a LoviBond Colorimeter RT500 (Neu-Isenberg, Germany) with an 8 mm diameter measuring area. Total color difference (ΔE), hue angle (h^*ab) and chroma (C^*ab) were calculated as the average of six samples using the following equations:

$$C*ab = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h*ab = arctg\left(\frac{b*}{a*}\right) \quad (2)$$

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \quad (3)$$

where ΔL^*, Δa^* and Δb^* referred to differences between the white standard (used as the film background) and sample color values.