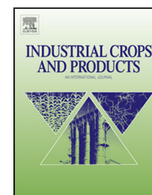
Industrial Crops and Products 71 (2015) 205–213

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop



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

J.F. Martucci[a,*], L.B. Gende[b], L.M. Neira[a], R.A. Ruseckaite[a]

[a] Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Av. Juan B. Justo 4302, 7600 Mar del Plata, Buenos Aires, Argentina
[b] Research Center in Social Bees (Arthropods Laboratory). CONICET- Natural and Exact Sciences Faculty, Mar del Plata University, Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 4 November 2014
Received in revised form 24 March 2015
Accepted 26 March 2015

Keywords:
Active films
Essential oils
Gelatin
Antimicrobial
Antioxidant

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.
© 2015 Elsevier B.V. All rights reserved.

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

* Corresponding author. Tel.: +542234816600x249.
E-mail address: jmartucci@fi.mdp.edu.ar (J.F. Martucci).

http://dx.doi.org/10.1016/j.indcrop.2015.03.079
0926-6690/© 2015 Elsevier B.V. All rights reserved.

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 ± 0.5 °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 ± 2 °C and 65 ± 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:

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

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

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

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

2.5.3. Scanning electronic microscopy (SEM)

Fracture surfaces were observed on a Phillips 505 microscope (Eindhoven, The Netherlands) at 10 kV. All specimens were sputter-coated with gold.

2.5.4. Water uptake

Water uptake test was performed gravimetrically. Samples were placed in pre- weighed aluminum cups and dried at 45 °C in a vacuum oven (Gallenkamp, UK) up to constant weight (m0). Specimens were then kept at 25 ± 2 °C in a humidity chamber at 65 ± 2% RH following the procedure described in ASTM E104-95. Samples were removed at specific time intervals and weighed with a precision of ±0.0001 g until reaching constant weight (mf). The water uptake at equilibrium (WU_{eq}) was calculated as the weight gain according to:

$$WU_{eq}(\%) = 100\frac{(mf - m_0)}{m_0} \quad (4)$$

This experiment was carried out on four specimens of each sample to ensure results reproducibility.

2.5.5. Mechanical properties

Tensile strength at breaking (TS) and percentage of elongation (ϵ%) were measured according to the ASTM 638 94 D standard using an Instron 4467 Universal Testing Machine (Buckinghamshire, England) with a 5 kN load cell and at a crosshead speed of 10 mm/min. Samples were stored at 65 ± 2% RH and 25 ± 2 °C prior to measurements. Reported results were obtained from at least 10 samples for each type of film.

2.5.6. Water vapor permeability (WVP)

Water vapour permeability (WVP) was performed gravimetrically at 25 °C, using the ASTM E96-95 desiccant method. All specimens were equilibrated at 65 ± 2% RH at 25 ± 2 °C for 48 h. Afterwards, test films were fixed onto opening cells containing silica gel (0%RH) and the cells were placed in an controlled humidity chamber at 65 ± 2% RH and 25 ± 2 °C. The air gap inside the cell was ~1.2 cm and the film area exposed for water vapor transmission was 13.8 cm^2. The cells were weighed hourly over a 10 h period. WVP was calculated from the following equation:

$$WVP\left(\text{kg ms}^{-1}\ \text{Pa}^{-1}\text{m}^{-2}\right) = \frac{w}{At\Delta P}e \quad (5)$$

where w is the weight gain of the cup (Kg) at time t (s); e is the film thickness (m); A is the exposed area of the film (m^2); ΔP is the vapor pressure difference across the film (Pa). All measurements were made in triplicate.

2.5.7. 7 In vitro antioxidant assay

2.5.7.1. Ferric reducing antioxidant power (FRAP) assay. FRAP assay was carried out according to the method described by Oyaizu (1986). Basically, 1 mL of the essential oils (2000–6000 ppm) was mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and 2.5 mL of potassium hexacyanoferrate III ($K_3Fe(CN)_6$, 1% (w/v)). The mixture was incubated at 50 °C for 20 min. Afterwards 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added to the mixture, which was then centrifuged at 5000 rpm for 10 min (Sartorius type 4-15, Germany). A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of a 0.1% (w/v) $FeCl_3$ solution. Absorbance was measured at 700 nm in a UV–visible spectrophotometer (Agilent 8453, China). The increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid (AA) was used as reference material and the final results were expressed as ppm of ascorbic acid equivalents (AAE). All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

2.5.7.2. Determination of DPPH radical scavenging activity. DPPH radical scavenging activity (RSA) was determined on the basis of the method by Yen and Hsieh (1995) with slight modifications. A volume of 400 μL of each essential oil solution (0–6000 ppm) was mixed with 2 mL of a 0.06 mmol/L solution of DPPH in methanol. Mixtures were shaken vigorously and allowed to stand in the dark for 30 min at room temperature. The absorbance of the resulting solutions was monitored at 517 nm using an UV–visible spectrophotometer (Agilent 8453, China). The control was prepared in the same manner except that methanol was used instead of the sample. DPPH radical scavenging activity (RSA) was calculated according to the following equation (Yen and Hsieh, 1995):

$$RSA(\%) = \left(1 - \frac{A_{517\text{sample}}}{A_{517\text{control}}}\right)100 \quad (6)$$

where $A_{517\text{sample}}$ and $A_{517\text{control}}$ correspond to the absorbances at 517 nm of the radical (DPPH•) in the absence and presence of antioxidant respectively. IC50 is defined as the efficient concentration required to decrease the initial DPPH radical concentration by 50%.

In the case of films, samples were immersed in liquid nitrogen and subsequently crushed and grounded with a pestle. A precise amount of crushed film (0.4 g) was mixed with 4 mL of methanol in a caped tube, stirred vigorously and allowed to stand overnight (about 12 h). Afterwards, tubes were centrifuged at 5000 rpm for 10 min (Sartorius type 4-15, Germany) and the supernatant was recovered and reserved for DPPH radical scavenging activity and reducing power as outlined above for EOs. In the case of reducing power, final results were expressed per g of film.

2.5.8. In vitro antibacterial assay of essential oils and films

2.5.8.1. Determination of Minimum Inhibitory Concentration (MIC). A broth microdilution method was used to determine MIC (Gende et al., 2010). Appropriate amounts of essential oil were mixed in water and emulsified with 8% (v/v) propylene glycol, thus obtaining a mother solution (MS). For broth microdilution, 100 μL of Muller Hilton medium was placed in each of the 96-well microtiter plates and then diluted with MS to obtain serial dilutions. Microbial biomass suspension of *E. coli* and *S. aureus* (approximately 10^5–10^6 CFU/mL) was individually added to each serial dilution, yielding final value concentrations ranging from 12.5 to 2000 ppm. All microtiter plates (with positive and negative controls) were incubated at 35 ± 0.5 °C for 48 h in order to determine MIC values. Experiments were conducted in triplicate. Chloramphenicol (30 μg) was used as a reference standard.

2.5.8.2. Antimicrobial activity of films by disc diffusion method. Antibacterial activity on films was assessed using the agar diffusion method described by Pereda et al. (2011). Films were aseptically cut into a 10 mm diameter disc using a circular knife and then placed on agar plates, previously seeded with 100 μL of inoculum containing approximately 10^5–10^6 CFU/mL of each tested bacteria. The plates were then incubated at 37 °C for 24 h. The diameter of the growth inhibition zone surrounding the film discs was accurately measured with a manual caliper from the center of the film (Mitutoyo, Japan). Each assay was performed by triplicate on two separate experimental runs.

2.5.9. Statistical analysis

Experimental data were statistically analyzed by one-way analysis of variance (ANOVA) using the OriginPro 8 software and Tukey's test for comparison of means at a 5% of significance level.

Table 1
Main compounds, expressed as percentage of chromatographic area of *L. officinalis* and *O. vulgare* essential oils.

Components(%)			
Lavander (Lavandula officinalis)		Oregano (Origanum vulgare)	
Linalool	53.50	Carvacrol	26.70
Camphor	8.40	p-Cimene	15.20
Terpinen-4-ol	7.60	γ−Terpinene	15.10
1,8- cineol	6.80	Terpinene	7.50
Borneol	4.70	α− Pinene	5.60
Linalyl acetate	4.20	Iso borneol	3.80
Lavandulyl acetate	0.80	Terpinolene	3.40
Hexilacetate	0.60	β- Myrcene	3.40
1-octen-3-ol	0.55	α− Thujene	3.40
3- octanone	0.40	α- Terpineol	2.30
Myrcene	0.30	Methyl carvacrol	2.20
		Caryophyllene	1.40
		Sabinene	1.40
		Endo borneol	1.20
		Thymol	1.10
		Terpinen-4-ol	1.10
		β- Phellandrene	0.60
		Camphene	0.40
		1,8- cineol	0.30
Total	87.85	Total	96.1

3. Results and discussion

3.1. Characterization of oregano and lavender essential oils

Active components in oregano and lavender essential oils identified by GC/MS are summarized in Table 1. The quantitative analysis of lavender essential oil revealed a prevalence of linalool (53.50%), followed by camphor (8.40%), terpinen-4-ol (7.60%) and 1.8-cineol (6.80%). The results of the chemical profile of LEO compare favorably with those of earlier studies (Inouye et al., 2001; Dahn et al., 2012 Teixeira et al., 2013a). The prevailing compounds of oregano essential oil were aromatic monoterpenes: carvacrol (26.70%) and thymol (1.10%) and aliphatic monoterpenes being *p*-cymene (15.20%), γ- terpinene (15.10%) and terpinene (7.50%) the major constituents of this group. Plants that produce carvacrol and thymol almost always have relatively high amounts of *p*-cymene and γ-terpinene, which are biosynthetic precursors for both phenolic monoterpenes (Poulose and Croteau, 1978). Differences in essential oil compounds may be influenced by geographical variables, time of plant harvesting, preparation process extraction method and quantification conditions (Burt, 2004; Gende et al., 2010). Several studies on *O. vulgare ssp.* cultivated in different regions worldwide have centered their attention on the variability of chemical composition. Different percentages of thymol and carvacrol have been reported as majority compounds in this vegetal specie (D'Antuono et al., 2000; Oussalah et al., 2004; Gómez-Estaca et al., 2009).

EOs antibacterial efficiency was quantified by determining the minimum inhibitory concentration (MIC). Oregano and lavender essential oils, together with their major components, c.a. carvacrol and linalool, were evaluated against *E. coli* and *S. aureus.* MIC values are summarized in Table 2. It is noteworthy that greater inhibition of oils and pure compounds was observed against *S. aureus*. This finding is in line with early studies focused on the action of whole EOs against food spoilage organisms and food-borne pathogens (Inouye et al., 2001; Burt, 2004) and it is thought to arise as a result of the differences in their cell membrane structure and the hydrophobic character of essential oils and their components (Vaara, 1992; Inouye et al., 2001 Inouye et al., 2001). Gram negative bacteria possess an outer membrane surrounding the cell wall composed by hydrophilic polysaccharides which restricts diffusion of hydrophobic compounds such as OEs and their main constituents (Inouye et al., 2001). Gram-positive bacteria, in turn, lack the rigid outer membrane being more susceptible to the action of EOs.

The effectiveness of pure constituents of OEO and LEO against the studied bacteria was statistically lower than that of EOs (Table 2) indicating that minor ingredients are critical to the activity, as previously observed by others (Kulevanova and Panovska, 2001; Canillac and Mourey, 2001; Gende et al., 2010; Danh et al., 2012; Teixeira et al., 2013a). Furthermore, both pathogens were less susceptible to the action of lavender oil and to its major component, linalool (Table 2), suggesting that phenolic compounds seem to govern the antibacterial ability of Eos (Burt, 2004; Tassou et al., 2000). Similar trend was previously observed for thyme and basil essential oils (and their major compounds: thymol, estragol, carvacrol, linalool, and *p*-cymene) being carvacrol the one exerting the strongest antibacterial activity against *E. coli* followed by thymol. Estragol and linalool, in turn, exhibited limited antibacterial activity (Bagamboula et al., 2004).

The potential antagonistic or synergic effect among components of both essentials oils was experimentally analyzed on mixtures of LEO:OEO 50:50 The obtained results (Table 2) reflected that the tested microorganisms were less susceptible to the action of the mixture, resulting in a lower antimicrobial effect than the sum of the single effects produced by each essential oil individually, thereby indicating a possible antagonistic effect between their components (Canillac and Mourey, 2001; Sadaka et al., 2013).

The antioxidant capacity of EOs was revealed through their reducing power and DPPH radical scavenging efficiency, respectively. In Fig. 1 a, the FRAP value points reflect a ten-fold higher reducing power of OEO than that of lavender oil, in accordance with the presence of electron donor chemicals such as carvacrol and thymol, which can react with free radicals and turn them into more stable products, and so terminate radical chain reactions (Burt, 2004). Moreover, OEO exhibited a strong dose-dependent reducing power rising from 11 ppm of AA for 200 ppm up to 114 ppm of AA for 3000 ppm. Higher OEO concentrations did not induce significant changes in the reducing power, indicating a saturation level of around 3000 ppm. By contrast, no significant variations were evidenced for LEO activity which exhibited a lag phase of up to 2000 ppm followed by a minor increment of up to 17 ppm for a concentration of 6000 ppm. These results are in agreement with OEO composition constituted by phenolic compounds (Table 1). The phenolic compounds are free radical acceptors that delay or inhibit the autoxidation initiation step or interrupt the autoxidation propagation step (Kačániová et al., 2012; Eça et al., 2014). Fig. 1 b clearly shows that the radical scavenging activity of OEO is significantly higher as compared to that of LEO for the same concentrations. As it can be observed in Fig. 1 b, OEO free radical scavenging effect also exhibited a dose-dependent increase having a 74% RSA for 6000 ppm, in line with the results reported by Kačániová et al. (2012) for the same concentration of oregano essential oil. LEO did not show significant activity for all concentrations tested, i.e., from 2000 to 6000 ppm. The weakest activity of LEO could be associated with the absence of phenolic compounds in its composition.

Table 2
Antimicrobial activity of essential oils against *E. coli* and *S. aureus strains.*

Minimum inhibitory concentration (MIC)	*E. coli*	*S. aureus*
Lavender EO (LEO)	2000	1000–1200
Oregano EO (OEO)	1600-1800	800–900
LEO:OEO 50:50	>2000	1600–1800
Thymol	1000	800–900
Carvacrol	600	500–600
Linalool	2000	2000
Chloramphenicol	8	2

Notes: Data are MIC (μg/mL) range values. The antimicrobial activity was determined by triplicate analyses for oil and strains.

Table 3
Thickness and color parameters of obtained films.

	Thickness (mm)	l*	a*	b*	C*ab	h*ab	ΔE*
Ge	0.11 ± 0.01^{ax}	89.07 ± 0.16^{ax}	0.07 ± 0.07^{a}	2.22 ± 0.51^{a}	2.23 ± 0.51^{ax}	1.54 ± 0.07^{a}	10.81 ± 0.53^{ax}
OEO2000-Ge	0.10 ± 0.01^{a}	88.95 ± 0.15^{a}	0.23 ± 0.09^{a}	2.02 ± 0.60^{a}	2.03 ± 0.60^{a}	1.46 ± 0.09^{a}	10.67 ± 0.71^{a}
OEO3000-Ge	0.13 ± 0.03^{a}	89.01 ± 0.21^{a}	0.23 ± 0.11^{a}	1.49 ± 1.09^{a}	1.50 ± 1.09^{a}	1.42 ± 0.11^{a}	10.25 ± 0.99^{a}
OEO4000-Ge	0.11 ± 0.02^{a}	89.12 ± 0.40^{a}	0.17 ± 0.12^{a}	2.17 ± 1.02^{a}	2.18 ± 1.02^{a}	1.49 ± 0.12^{a}	10.73 ± 1.08^{a}
OEO5000-Ge	0.11 ± 0.02^{a}	88.96 ± 0.36^{a}	-0.23 ± 0.12^{b}	5.12 ± 1.11^{b}	5.13 ± 1.11^{b}	-1.52 ± 0.12^{b}	13.56 ± 1.01^{b}
OEO6000-Ge	0.13 ± 0.02^{a}	88.89 ± 0.17^{a}	-0.24 ± 0.06^{b}	4.82 ± 0.53^{b}	4.82 ± 0.53^{b}	-1.52 ± 0.06^{b}	13.35 ± 0.52^{b}
LEO2000-Ge	0.07 ± 0.02^{y}	89.00 ± 0.28^{x}	-0.29 ± 0.21^{x}	4.96 ± 0.87^{x}	2.68 ± 0.87^{x}	-1.23 ± 0.10^{x}	12.55 ± 1.28^{x}
LEO3000-Ge	0.09 ± 0.02^{xy}	88.96 ± 0.68^{x}	-0.44 ± 0.20^{x}	5.18 ± 1.19^{xy}	5.20 ± 2.19^{y}	-1.49 ± 0.08^{y}	13.71 ± 1.09^{xy}
LEO4000-Ge	0.09 ± 0.02^{xy}	88.18 ± 0.60^{x}	-0.45 ± 0.38^{x}	5.86 ± 1.25^{xy}	3.89 ± 1.25^{xy}	-1.45 ± 0.11^{y}	12.71 ± 1.39^{xy}
LEO5000-Ge	0.11 ± 0.03^{xy}	88.52 ± 0.49^{x}	-0.60 ± 0.23^{x}	5.74 ± 1.75^{xy}	5.77 ± 1.75^{yz}	-1.47 ± 0.13^{y}	14.38 ± 1.83^{y}
LEO6000-Ge	0.11 ± 0.02^{x}	88.02 ± 0.35^{x}	-0.56 ± 0.31^{x}	7.23 ± 1.30^{y}	7.25 ± 3.30^{z}	-1.49 ± 0.11^{y}	15.94 ± 1.38^{y}

* Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Tukey test.

High radical scavenging activity and reducing power has been observed in the main components of oregano essential oil (carvacrol, thymol, γ-terpinene and *p*-cymene) (Aeschbach et al., 1994; Oussalah et al., 2004). However, other compounds could contribute to the antioxidant properties of oregano oil.

The antioxidant capacity of the LEO:OEO 50:50 mixture was analyzed by DPPH radical scavenging activity and Ferric reducing power assay. The results obtained indicated a reduction in the antioxidant capacity in relation to the content of oregano essential oil. The lower antioxidant activity of the mixture denoted a possible antagonistic effect between their components as previously observed in the antimicrobial activity. Taking into account the results reported, EOs mixture was not considered for films formulation.

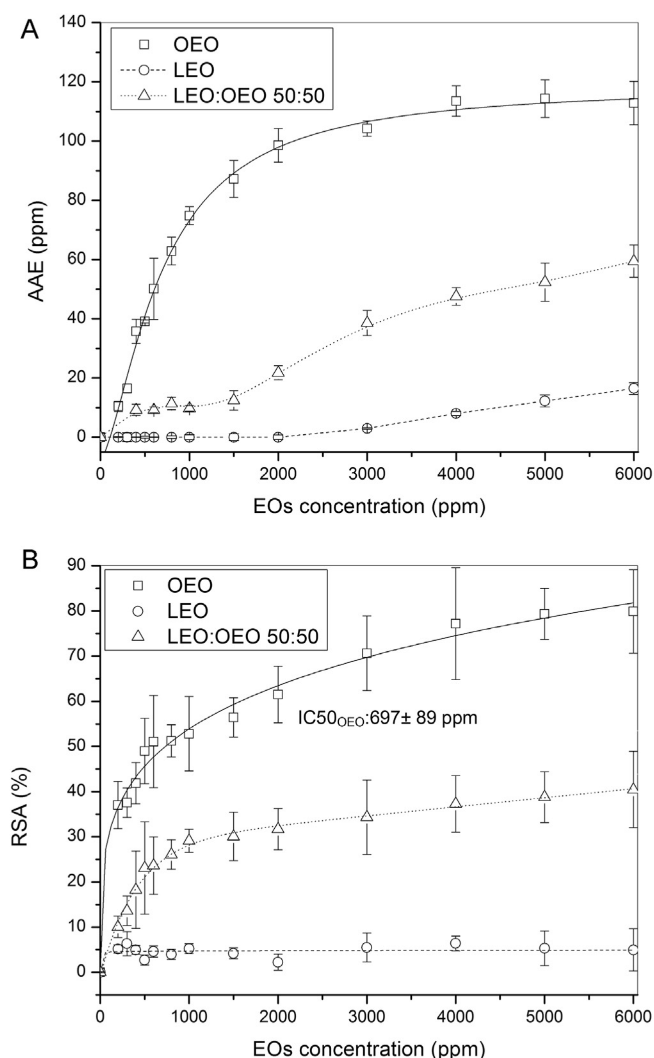


Fig. 1. (a) Reducing Power and (b) DPPH radical scavenging activity (RSA) of oregano and lavender essential oils. Bars represent the standard deviation from triplicate determinations.

3.2. Properties of EO-added gelatin films

3.2.1. Optical properties

Gelatin films added with oregano or lavender essential oils, on an individual basis, were prepared by casting using propylene glycol as plasticizer and emulsifier. The film optical properties are relevant since they play a major role in the appearance of the coated product. In order to compare the differences between control films and those prepared with the essential oils, Table 3 reports the color parameters of the films obtained. Visually; Ge films were quite transparent, though they turned yellowish when the essential oil was added. As far as OEO differences are concerned, they were not significant ($P \geq 0.05$) up to 3000 ppm of OEO (Table 3), which is consistent with the findings reported by Min and Oh (2009) for cat fish gelatin coatings containing oregano essential oil and Teixeira et al. (2014) for fish gelatin films. On the other hand, LEO-Ge films presented higher C*ab and ΔE* values and lower h*ab. ΔE* LEO-Ge values were higher than OEO-Ge ones due to the increase in absorption caused by the interaction between the components. In LEO-Ge films, C*ab values increased with the amount of lavender oil added, indicating an increase in yellowish coloration. These results suggested that the incorporation of essential oil influenced film color, though changes depended on the type of essential oil. Ahmad et al. (2012) showed that the incorporation of lemongrass oil in fish skin gelatin films increased its total color difference, yet there was no difference in the color parameters of the films incorporated with bergamot oil. The considerable effects of the incorporation of cinnamon oil on the color of caseinate-based edible films were reported by Atarés et al. (2010a). However, no marked effect on the color parameters of those films was obtained when ginger oil was added (Atarés et al., 2010a). It was suggested that essential oil also influenced the color of gelatin film, depending on the type and concentrations of the essential oil incorporated.

3.2.2. Films morphology

The cross-section of Ge control film was compact and smooth without pores or cracks (Fig 2a) in agreement with previous reports (Martucci and Ruseckaite, 2009; Tongnuanchan et al., 2012). With the addition of essential oils, the cross-section of films became slightly rougher as compared to the control (Fig 2b–e); however, no oil droplets were observed in the film thickness even at the highest concentration tested. This result indicates that the film-forming dispersion was a stable so that no collapse of emulsion

Table 4
Mechanical properties, water vapor permeability (WVP) and water uptake (WUeq) of Ge films with and without essential oils.

	Mechanical properties		WVP* 10^{13}	WUeq
	ϵ (%)	TS (MPa)	(Kg.m/Pa.s.m^2)	(%)
Ge	10.8 ± 3.0^{ax}	17.7 ± 2.8^{ax}	1.46 ± 0.13^{ax}	26.0 ± 6.8^{ax}
OEO2000-Ge	8.3 ± 3.7^{a}	14.0 ± 1.6^{ab}	1.21 ± 0.45^{a}	23.8 ± 3.7^{a}
OEO3000-Ge	9.2 ± 3.4^{a}	13.9 ± 1.8^{bc}	0.88 ± 0.01^{a}	20.2 ± 1.9^{a}
OEO4000-Ge	9.7 ± 2.6^{a}	12.2 ± 2.8^{bc}	1.10 ± 0.20^{a}	21.0 ± 2.1^{a}
OEO5000-Ge	10.1 ± 2.2^{a}	8.9 ± 3.3^{c}	0.81 ± 0.03^{a}	19.5 ± 1.7^{a}
OEO6000-Ge	10.0 ± 1.9^{a}	11.3 ± 3.2^{bc}	0.84 ± 0.01^{a}	18.1 ± 2.3^{a}
LEO2000-Ge	4.3 ± 0.8^{y}	8.8 ± 2.2^{yz}	1.27 ± 0.07^{x}	20.3 ± 3.1^{x}
LEO3000-Ge	7.6 ± 3.0^{xy}	15.4 ± 2.1^{xw}	0.97 ± 0.10^{y}	19.6 ± 2.3^{x}
LEO4000-Ge	6.3 ± 2.0^{y}	12.1 ± 1.9^{zw}	1.05 ± 0.03^{y}	17.8 ± 1.6^{xy}
LEO5000-Ge	4.6 ± 0.3^{y}	11.7 ± 2.4^{zw}	1.02 ± 0.04^{y}	17.5 ± 1.4^{y}
LEO6000-Ge	7.3 ± 2.0^{xy}	12.8 ± 2.8^{zw}	0.68 ± 0.02^{w}	14.8 ± 1.0^{z}

* Any two means in the same column followed by the same letter are not significantly different ($P > 0.05$) based on the Turkey test.

occurred during dehydration (Hosseini et al., 2009; Atarés et al., 2010b; Tongnuanchan et al., 2012).

3.2.3. Water uptake

Water uptake at the equilibrium of gelatin films incorporated with EOs is summarized in Table 4. Gelatin films exhibited the highest water uptake (i.e., 26.0 ± 6.8%), in accordance to its hydrophilic nature (Martucci and Ruseckaite, 2009; Martucci et al., 2012; Kavoosi et al., 2013). The incorporation of LEO into gelatin matrix reduced the water absorption capacity (14.8 ± 1.0% for 6000 ppm). It is hypothesized that non polar EOs interact favorably with hydrophobic domains in gelatin inducing conformational changes in gelatin chains that cannot promote protein-water interaction (Djagny et al., 2001; Kavoosi et al., 2013). Nevertheless, no significant differences were noted in OEO incorporated films ($P \geq 0.05$). The different behavior could be due to the differences in the hydrophobic nature of the oils used, which resulted in a different ability to attract water to the film network.

3.2.4. Water vapor permeability

WVP values of LEO-Ge films were slightly decreased when lavender concentrations remained above 2000 ppm (Table 4, $P < 0.05$). Nevertheless, in OEO-Ge films, no significant differences (Table 4, $P \geq 0.05$) were found among samples with different contents of oregano oil phase. Lavender and oregano oil incorporation affected WVP of gelatin film in a different way, due to the hydrophobic nature of essential oils, which affects the hydrophilic/hydrophobic balance of the film (Ojagh et al., 2010; Teixeira et al., 2014) based on the water uptake capacity. Similar results were obtained by Ahmad et al. (2012) in fish skin

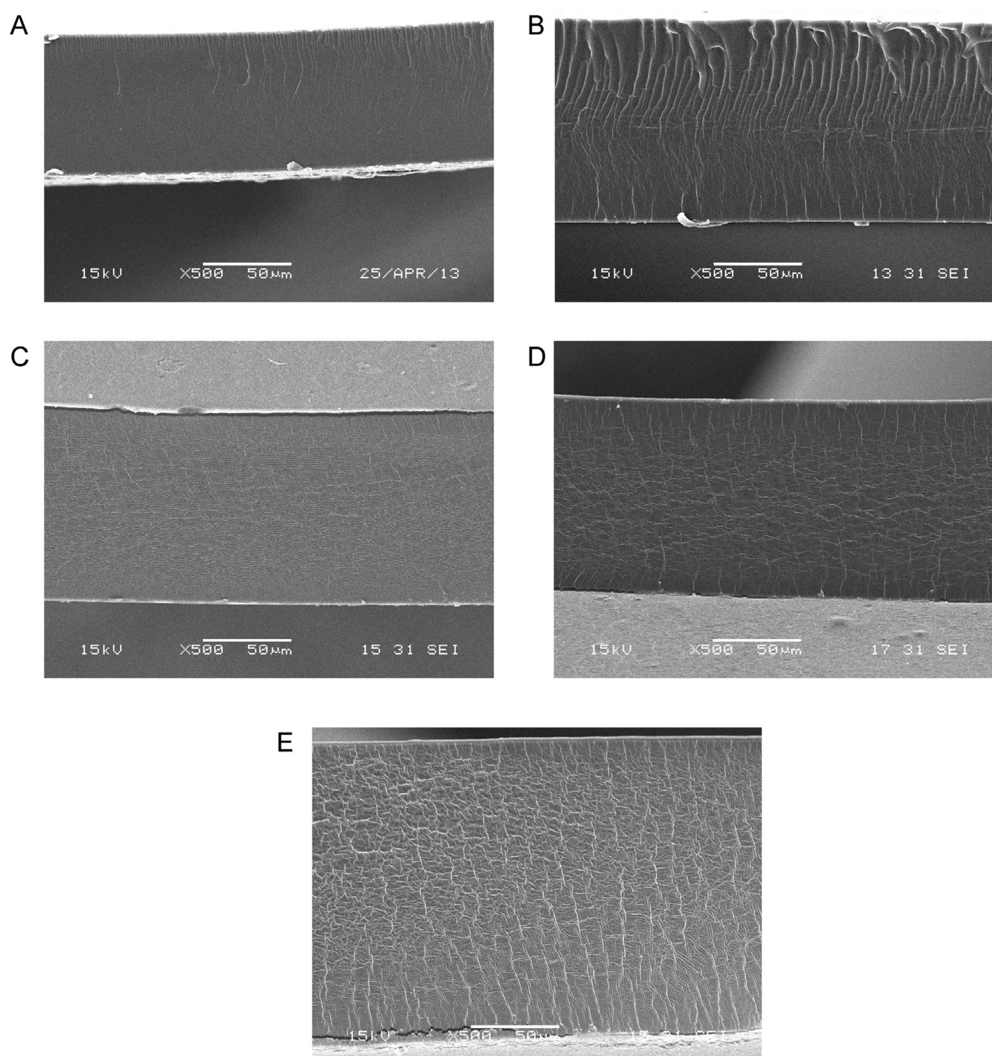


Fig. 2. Scanning electron microscopic images of cross sections of Ge (a); LEO3000-Ge (b); LEO6000-Ge (c); OEO3000-Ge (d) and OEO6000-Ge (e) films.

Table 5
Antimicrobial activity measured as inhibition zone expressed as millimeter (mm) and antioxidant activity measured as DPPH radical scavenging activity (RSA,%) and Ferric reducing power (expressed as ppm of ascorbic acid equivalent, AAEppm/g film) of Ge-based edible films with and without essential oils.

EO(ppm)	Antimicrobial activity[a]		Antioxidant activity			
	E. coli (mm)	*S. aureus* (mm)	RSA (%)		AAE (ppm/g film)	
	OEO-Ge	LEO-Ge	OEO-Ge	LEO-Ge	OEO-Ge	LEO-Ge
0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.1 ± 0.1[a]	0.0 ± 0.0[a]	0.3 ± 0.2[a]	0.1 ± 1.0[a]
2000	9.3 ± 1.0[b]	10.8 ± 0.8[b]	12.7 ± 0.2[b]	1.5 ± 0.6[b]	97 ± 18[b]	0.1 ± 2.4[a]
3000	11.7 ± 0.4[c]	11.3 ± 1.0[bc]	17.5 ± 3.1[c]	3.1 ± 1.1[bc]	129 ± 6[bc]	0.8 ± 2.3[a]
4000	13.7 ± 0.5[d]	10.6 ± 1.5[bc]	36 ± 10[d]	5.4 ± 2.8[c]	135 ± 6[c]	0.3 ± 2.0[a]
5000	14.1 ± 0.4[d]	13.0 ± 0.8[c]	49 ± 5[d]	6.9 ± 3.5[c]	189 ± 2[d]	0.7 ± 2.2[a]
6000	18.0 ± 0.8[e]	15.0 ± 0.5[d]	60 ± 4[e]	8.6 ± 4.4[c]	241 ± 8[e]	26.0 ± 6.6[b]

Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Turkey test.
[a] Standard antibiotic of chloramphenicol (30 μg/disc) was used as reference or positive control. Clearing zones around the discs were between 21 and 23 mm in diameter for both microorganisms.

gelatin films enriched with bergamot and lemongrass essential oils. Bergamot oil caused an increased in WVP, while lemongrass caused a decrease in WVP of the resulting films. Teixeira et al. (2014) showed a significant reduction in WVP values of fish gelatin films by incorporating clove essential oil, while no statistical differences were detected in films containing garlic or oregano essential oils. WVP also decreased significantly with an increase in the concentration of OEO in chitosan-cassava starch, yet this behavior was observed in films with concentrations greater than 0.5% of OEO (Pelissari et al., 2009). Nevertheless, Atarés et al. (2010a) found no significant differences in the WVP of sodium caseinate films (SC) incorporated with cinnamon or ginger essential oils owing to the small amount of essential oil used. In short, types and amount of essential oil affected not only the optical properties but also the water vapor permeability of the films obtained.

3.2.5. Mechanical properties

The oil type significantly affected both TS (resistance to elongation) and ϵ% (capacity for stretching) of the films obtained. The addition of LEO in gelatin film caused a significant decrease in TS and ϵ% if compared to the control ($P < 0.05$) (Table 4). A decrease in the breaking force was previously recorded in gelatin films incorporated with antioxidant extracts (Tongnuanchan et al., 2012; Kavoosi et al., 2013; Li et al., 2014). The addition of EOs possibly resulted in the lowered interaction between gelatin monomers, and probably hindered the polymer chain-to-chain interactions and, consequently, led to a decrease in TS. Other authors assigned this behavior to the presence of the structural discontinuities provoked by oil incorporation. The reduction in the stretching capacity has also been reported by other authors in protein-oil added films (Atarés et al., 2010b; Li et al., 2014).

The incorporation of OEO resulted in a significant decrease of TS ($P < 0.05$), though no significant effect ($P > 0.05$) was observed on deformation at break. A possible explanation therefor lies in the alteration of the plasticizer/gelatin ratio in the EOs added films. Water exerted a plasticizing effect on protein films (Martucci and Ruseckaite, 2009). As expressed above, the water uptake of OEO-Ge film had no significant difference with respect to Ge control film but was higher than LEO-Ge films (Table 4). Other authors reported no difference in the ϵ% and a reduction in TS in biopolymer films incorporated with antioxidant extracts (Sanchez-González et al., 2009; Atarés et al., 2010b; Kavoosi et al., 2013; Wu et al., 2014). Mechanical properties were also affected by the type and content of the essential oil.

3.2.6. Antimicrobial activity

Gelatin films with varying amounts of EOs were tested against *E. coli* and *S. aureus*. To minimize the incorporation of flavor to the films, the oils concentrations incorporated into the FFSs were equal to or above the MIC values of the oils against microorganisms. The inhibitory effect of gelatin films incorporated with 0–6000 ppm of oregano and lavender essential oil against *E. coli* and *S. aureus* is illustrated in Table 5. The results indicate that both microorganisms exhibited sensitivity to all the active films. Ge-EOs films showed an increase in the halo of inhibition with EOs increment. The halos obtained were slightly higher for OEO-Ge films which are in concordance with the greatest antimicrobial effect of carvacrol compared to linalool (the main oil components) (Burt, 2004; Bagamboula et al., 2004). The present study showed that EOs were effective against tested bacteria at concentrations less than or equal to 6000 ppm, which is in accordance with most of the reported works on the antimicrobial effectiveness of protein films containing essential oils, in most cases at concentrations varying between 1 to 5% w/V (Min and Oh, 2009; Gomez-Estaca et al., 2009, 2010, 2010 Ahmad et al., 2012).

Inhibition zones obtained for *S. aureus* yielded lower values than those of *E. coli* despite the lower MIC value. This could be explained by the evaporation of volatile compounds involved in the antimicrobial activity against *S. aureus* during films forming conditions and/or by the gelatin matrix hindering the migration of EO components into the agar medium (Gomez-Estaca et al., 2009Gomez-Estaca et al., 2009 Hosseini et al., 2009; Pereda et al., 2011; Teixeira et al., 2014). Nonetheless, based on the values of the vapor pressure and boiling points of the major components of the essential oils used (Table 6) and the processing temperature (35 °C during drying operations), it was assumed that the content of the active agents in the final films was almost invariable and the essential oil components were retained by the protein matrix.

Table 6
Boiling point (°C) and vapor pressure (mmHg) of the main components of oregano and lavender essential oils.

	Boiling point[a] (°C)	Vapor pressure[b] (mmHg)
Linalool	199	0.17 at 25 °C
Camphor	204	0.65 at 25 °C
Terpinen-4-ol	212	0.40 at 20 °C
1,8-cineol	176	1.90 at 25 °C
carvacrol	236	0.03 at 25 °C
Thymol	232	1.00 at 64 °C
p-cymene	177	3.70 at 38 °C
γ−Terpinene	182	0.70 at 20 °C
Terpinene	174	0.80 at 20 °C
α-Pinene	156	4.75 at 25 °C

[a] THE MERCK INDEX, 1996.- Encyclopedia of chemicals, drugs, and biologicals. 12th Ed.- Merck Research Laboratories. Division of Merck & CO Inc., Whitehouse Station, NJ, USA.
[b] Daubert, T.E., R.P. Danner. Physical and thermodynamic properties of pure chemicals data compilation. Washington, D.C.: Taylor and Francis, 1989.

3.2.7. Antioxidative capacity

The DPPH radical-scavenging activity and reducing power values of gelatin based films are displayed in Table 5. The results are in concordance with the antioxidant capacity of each essential oil. The control film had no DPPH radical scavenging and FRAP activities in accordance with other works on gelatin (Tongnuanchan et al., 2012; Li et al., 2014).

The addition of EOs significantly increased ($P < 0.05$) DPPH radical scavenging activity (Table 5). The results suggested that the addition of 6000 ppm of OEO made the film more active against DPPH radical. Regarding the gelatin-based film mixed with LEO, the DPPH radical scavenging capacity could be negligible compared to OEO films. The differences observed between both essential oils was previously explained by the higher concentration of phenolic compounds present in oregano essential oil (Pelissari et al., 2009; Kačániová et al., 2012; Teixeira et al., 2013b; Li et al., 2014). Along these lines, the films mixed with OEO had higher reducing power as compared to the control and to LEO-Ge films (Table 5). In comparison with EOs (Fig. 1 a and b), the films presented lower DPPH and FRAP values in the same concentration, probably owing to the interactions between gelatin matrix and EO components. The interaction between antioxidant and gelatin molecules in proteins films has been already suggested by different authors (Gomez-Estaca et al., 2009; Pereda et al., 2011; Teixeira et al., 2014).

4. Conclusion

Oregano and lavender essential oils exhibited good antimicrobial properties against *E. coli* and *S. aureus* in concentrations above 2000 ppm. Yet oregano proved to be better due to its phenolic compounds content which also provided antioxidant capacity. Oregano and lavender essential oils incorporated into gelatin films at low content (2000-6000 ppm) led to an increase in their antimicrobial and antioxidant efficacy with little effect on water vapor permeability and mechanical properties of the resultant films. Ge-based films can be used in a wide range of food products, not being harmful for packaging food material. Oregano-based films exhibited the most effective antimicrobial and antioxidant properties. Due to its particularly intense color, the incorporation of OEO in concentrations above 4000 ppm considerably increased the yellowish coloration of gelatin films. OEO4000-Ge film could be a good alternative to natural food preservatives as a microbial growth inhibitor and/or oxidizer. Advances in active packaging materials based on renewable sources, such us, gelatin and essential oils will open new lines of work for the development of improved ecofriendly materials.

Acknowledgments

This research was financed by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, grant number PIP 112-201101-00637) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, grant number PICT 2010-1791) of Argentina. Authors would like to thank Dr. A. Folabella (Biology Department, FCEyN, UNMDP) and Dr. C. Studdert (IIIB, FCEyN, UNMDP) for bacterial strains, to A. Coppola and M. Zorzabal for oregano plant material and Rouselot (Argentina) for kindly supply gelatin material. Special thanks to Ing. Juan P. Espinosa for kindly perform GC/MS experiments.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Cromatography/Mass Spectroscopy, 4th ed. Allured Publishing Corporation, Carol Stream, Illinois.

Ahmad, M., Benjakul, S., Prodpran, T., Agustini, T.W., 2012. Physico–mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloids 28 (1), 189–199.

Aeschbach, R., Loliger, J., Scott, B.C., Murcia, A., Butler, J., Halliwell, B., Aruoma, O.I., 1994. Antioxidant actions thymol, carvacrol, 6-gingerol, zingerone and hydroxy tyrosol. Food Chem. Toxicol. 32, 31–36.

Atarés, L., Bonilla, J., Chiralt, A., 2010a. Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 100, 678–687.

Atarés, L., De Jesús, C., Talens, P., Chiralt, A., 2010b. Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. J. Food Eng. 99 (3), 384–391.

Bagamboula, C.F., Uyttendaele, M., Debevere, J., 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigellasonnei and S. flex. Food Microbiol. 21, 33–42.

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods e a review. Int. J. Food Microbiol. 94, 223–253.

Canillac, N., Mourey, A., 2001. Antibacterial activity of the essential oil of Piceaexcelsa on Listeria Staphylococcus aureus and coliform bacteria. Food Microbiol. 18, 261–268.

D'Antuono, L.F., Galetti, G.C., Bocchini, P., 2000. Variability of essentials oil contents and composition of Origanum vulgare L. populations from North Mediterranean Area (Liguria Region, Northern Italy). Ann. Bot. 86, 471–478.

Danh, L.T., Triet, N.D.A., Han, L.T.N., Zhao, J., Mammucari, R., Foster, N., 2012. Antioxidant activity, yield and chemical composition of lavender essential oil extracted by supercritical CO_2. J. Supercrit. Fluids 70, 27–34.

Djagny, K.B., Wang, Z., Xu, S., 2001. Conformational changes and some functional characteristics of gelatin esterified with fatty acid. J. Agric. Food Chem. 49, 2987–2991.

Eça, K.S., Sartori, T., Menegalli, F.C., 2014. Films and edible coatings containing antioxidants – a review. Braz. J. Food Technol., http://dx.doi.org/10.1590/bjft.2014.017.

Gende, L.B., Maggi, M., van Baren, C., di Leo Lira, A., Bandoni, A., Fritz, R., Eguaras, M., 2010. Antimicrobial and miticide activities of *Eucalyptus globulus* essential oils obtained from different Argentine regions. Spanish J. Agric. Res. 8 (3), 642–650.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M.C., Alemán, A., Montero, P., 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 112, 18–25.

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M.E., Gómez-Guillén, M.C., Montero, P., 2010. Biodegradable gelatine-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. 27, 889–896.

Gómez-Guillén, M.C., Ihl, M., Bifani, V., Silva, A., Montero, P., 2007. Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (UgnimolinaeTurcz). Food Hydrocolloids 21 (7), 1133–1143.

Hosseini, M.H., Razavi, S.H., Mousavi, M.A., 2009. Antimicrobial physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. J. Food Process. Preserv. 33 (6), 727–743.

Inouye, S., Takizawa, T., Yamaguchi, H., 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J. Antimicrob. Chemother. 47, 565–573.

Kačániová, M., Vukovič, N., Hleba, L., Bobková, A., Pavelková, A., Rovná, K., Arpášová, H., 2012. Antimicrobial and antiradicals activity of OriganumVulgare L. and Thymus Vulgaris essential oils. J. Microbiol. Biotechnol. Food Sci. 2 (1), 263–271.

Kavoosi, G., Dadfar, S.M.M., Purfard, A.M., 2013. Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing. J. Food Sci. 78 (2), E244–E250.

Kulevanova, S., Panovska, T.K., 2001. Antioxidant activity of essential oils of different wild Thymus L. species. Bull. Chem. Technol. Maced. 20 (1), 61–66.

Li, J.-H., Miao, J., Wu, J.-L., Chen, S.-F., Zhang, Q.-Q., 2014. Preparation and characterization of active gelatin-based films incorporated with natural antioxidants. Food Hydrocolloids 37, 166–173.

Martucci, J.F., Ruseckaite, R.A., 2009. Tensile properties, barrier properties and biodegradation in soil of compression – molded gelatin-starch dialdehyde films. J. Appl. Polym. Sci. 112, 2166–2178.

Martucci, J.F., Accareddu, A.M.E., Ruseckaite, R.A., 2012. Preparation and characterization of plasticized gelatin films cross-linked with low concentrations of glutaraldehyde. J. Mater. Sci. 47 (7), 3282–3292.

Min, B.J., Oh, J.-H., 2009. Antimicrobial activity of catfish gelatin coating containing origanum (*Thymus capitatus*) oil against gram-negative pathogenic bacteria. J. Food Sci. 74 (4), 143–148.

Ojagh, S.M., Rezaei, M., Razavi, S.H., Hosseini, S.M.H., 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem. 120 (1), 193–198.

Oussalah, M., Caillet, S., Ri, S.S., Saucier, L., Lacroix, M., 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. J. Agric. Food Chem. 52 (18), 5598–5605.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44, 307–331.

Pelissari, F.M., Grossmann, M.V.E., Yamashita, F., Pineda, E.A.G., 2009. Antimicrobial mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. J. Agric. Food Chem. 57 (16), 7499–7504.

Pereda, M., Ponce, A.G., Marcovich, N.E., Ruseckaite, R.A., Martucci, J.F., 2011. Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. Food Hydrocolloids 25, 1372–1381.

Perez-Mateos, M., Montero, P., Gómez-Guillén, M.C., 2009. Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. Food Hydrocolloids 23, 53–61.
Poulose, A.J., Croteau, R., 1978. Gamma-Terpinene synthetase: a key enzyme in the biosynthesis of aromatic monoterpenes. Arch. Biochem. Biophys. 191 (1), 400–411.
Sadaka, F., Nguimjeu, C., Brachais, C.-H., Vroman, I., Tighzert, L., Couvercelle, J.-P., 2013. Review on antimicrobial packaging containing essential oils and their active biomolecules. Innovative Food Sci. Emerg. Technol. 20, 350–355.
Sanchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., Cháfer, M., 2009. Characterization of edible films based on hydroxypropylmethylcellulose and tea tree essential oil. Food Hydrocolloids 23 (8), 2102–2109.
Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., Cháfer, M., 2011. Use of essential oils in bioactive edible coatings: a review. Food Eng. Rev. 3, 1-1610.1007/s12393-010-9031-3.
Tassou, C., Koutsoumanis, K., Nychas, G.J.E., 2000. Inhibition of Salmonella enteritidis and Staphylococcus aureus in nutrient broth by mint essential oil. Food Res. Int. 33, 273–280.
Teixeira, B., Marques, A., Ramos, C., Neng, N.R., Nogueira, J.M.F., Saraiva, J.A., Nunes, M.L., 2013a. Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind. Crops Prod. 43, 587–595.
Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M., Saraiva, J.A., Nunes, M.L., 2013b. Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil. J. Sci. Food Agric. 93 (11), 2707–2714.
Teixeira, B., Marques, A., Pires, C., Ramos, C., Batista, I., Saraiva, J.A., Nunes, M.L., 2014. Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: physical, antioxidant and antibacterial properties. LWT-Food Sci. Technol. 59, 533–539.
Tongnuanchan, P., Benjakul, S., Prodpran, T., 2012. Properties and antioxidant activity of fish skin gelatin film incorporated with citrus essential oils. Food Chem. 134, 1571–1579.
Vermeiren, L., Devlieghere, F., Van Beest, M., De Kruijf, N., Debevere, J., 1999. Developments in the active packaging of foods. Trends Food Sci. Technol. 10 (3), 77–86.
Wu, J., Ge, S., Liu, H., Wang, S., Chen, S., Wang, J.H., Li, J.H., Zhang, Q., 2014. Properties and antimicrobial activity of silver carp (Hypophthalmichthys molitrix) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packag. Shelf Life 2 (1), 7–16.
Yen, G.C., Hsieh, P.P., 1995. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. J. Sci. Food Agric. 67, 415–420.