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ORIGINAL PAPER

Two Different Processes to Obtain Antimicrobial Packaging Containing Natural Oils

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Abstract In this study, antimicrobial packaging materials were developed by incorporating known concentrations (*w*/*w*) of essentials oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) into low-density polyethylene (LDPE), suitable for use as food packaging, via two different methods: ionizing treatment and directly by extrusion. The mechanical, barrier, and antimicrobial properties of the packaging were evaluated against the following foodborne pathogens: *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli O157:H7*. The results demonstrate that films developed by extrusion incorporating 4% (*w*/*w*) of essential oils had a higher inhibitory effect than those obtained using the ionizing treatment. The packaging developed by extrusion containing 1% (*w*/*w*) showed a positive inhibitory effect, while those obtained by the ionizing treatment had no inhibitory effect against any of the test microorganisms. The incorporation of essential oils on the LDPE films generated a plasticizer effect, whereas the ones obtained by means of ionizing treatment did significantly affect the barrier properties of the films. The results of this study showed that plant-derived essential oils could be incorporated in active films for food packaging.

Keywords Antimicrobial packaging · Essential oils · Low-density polyethylene

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Introduction

Food packaging has been traditionally defined as a passive barrier that delays environment effects on food products (Brody 2002; López-Rubio et al. 2004). However, trends in current research involve the development of packaging materials that can positively interact with the environment and food, playing an active role in preservation. To date, active packaging is a novel food biopreservation technique for extending the shelf life of food products (Barros Velazquez 2011). Antimicrobial packaging, an innovative concept, can be defined as a kind of active packaging in which the package, the product, and the environment interact to reduce, inhibit, or retard the growth rate of microorganisms (Suppakul et al. 2008). In these technologies, researchers are developing food packaging materials such as synthetic films (Suppakul et al. 2006, 2008; López et al. 2007) and edible films (Natrajan and Sheldon 2000a; Ouattara et al. 2000; Jagannath et al. 2006; Kim et al. 2006; Seydim and Sarikus 2006; Rojas-Grau et al. 2006; Campos et al. 2010) with antimicrobial properties. Polymers are effective vehicles for the active substance (López-Rubio et al. 2004), giving the possibility to incorporate different antimicrobial additives. A first example is low-density polyethylene (LDPE), which may incorporate imazalil (Weng and Hotchkiss 1992; Vartiainen et al. 2003), organic acids (Dobias et al. 2000), nisin (Scannell et al. 2000; Natrajan and Sheldon 2000b), hexamethylenetetramine (Devlieghere et al. 2000a), or food preservatives (Devlieghere et al. 2000b). A second example is polyethylene (PE), which may be coated with an antimicrobial peptide (Miltz et al. 2006). A third example is hydroxypropylmethylcellulose, which may incorporate nisin by cross-linking (Sebti et al. 2003).

Although many studies have demonstrated the antimicrobial effect of essential oils and their active compounds against a broad spectrum of pathogenic bacteria in food (Burt and Reinders 2003; Di Pascua et al. 2005; Carson et al. 2006), there are very few publications that discuss their incorporation as additives in polymeric plastic films (Ha et al. 2001; Suppakul et al. 2006, 2008; López et al. 2007). Given that the US Food and Drug Administration categorizes natural extracts such as essential oils (EOs) and their constituents as generally recognized as safe, packaging manufacturers and demanding consumers consider the incorporation of these natural extracts in plastic films an appealing way of avoiding microbial food spoilage (Nerín et al. 2008). The current study aims to develop antimicrobial LDPE films using two incorporation methods, to determine the antimicrobial activity of these films against selected pathogenic microorganism and to assess the effect of incorporation of oregano and thyme essential oils on mechanical and barrier properties in the active films.

Materials and Methods

Materials

Essential oils of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) were used as the antimicrobial agents. The EOs were obtained from Primavera Life (Sulzberg, Germany) and were selected for their high antimicrobial activity and wide availability. The polymer used in these studies was LDPE (PEMEX 20020X, Monterrey, Mexico); attractive characteristics of this polymer include ease of processing, chemically inertness, and low cost.

Development of Antimicrobial Films

The EOs were incorporated into polyolefin films using two processing methods that would not compromise the antimicrobial potency of the oils: an ionizing treatment and extrusion.

Method 1—Ionizing Treatment

In this method, the EOs were incorporated by deposition on the surface of the film after surface modification using an ionizing treatment. Initially, LDPE films were produced using a single screw extruder (Kilion Extruders Inc, Verona, NJ, USA) with an L/D ratio screw of 24:1 and an operating speed of 30 rpm. The temperature profile from the first barrel zone to the die was 140/160/190/190 °C. The film surface was ionized by electronic radiation using the Laboratory Corona Treater model BD-20AC (Electro-Technic Products, Inc., IL, USA). The wire electrode was passed back and forth approximately 2.5 cm above each film surface for 2 min at high frequency of 4–5 MHz. After the film surface was ionized by electronic radiation, the films were characterized with a sessile drop contact angle measurement in order to determine the change in surface hydrophilicity Then, samples of the treated films (5×5 cm) were taken randomly from an area of 1 m^2 and a volume of each essential oil was placed on each sample. The samples were placed in an incubator (30±2 °C) to evaporate the solvents. LDPE films incorporating 1% and 4% (*w*/*w*) of EOs and control films were produced without EO.

Method 2—Extrusion

The EOs were preblended with polymer resin LDPE into a master batch (Brabender Instruments, Inc., South Hackensack, NJ, USA). Two hundred fifty grams of resin was added to the mixer with 40 ml of EO at 110 °C and 50 rpm for 30 min. The preblended oil resin was then ground in a knife mill to produce 2-mm fragments and finally incorporated with virgin resin pellets in a single screw extruder (Kilion Extruders Inc.) with an L/D ratio screw of 24:1 and an operating speed of 30 rpm. The temperature profile from the fed zone to the die was 120/155/175/175 °C. The extrusion and master batch temperatures of the LDPE (120–190 °C) were changed to protect the active oil compounds from oxidation based on the thermal study of EOs (DTA, data not shown). LDPE films incorporating 1% and 4% (*w*/*w*) of EOs and control films without EOs were produced.

The antimicrobial films developed with both methods were immediately wrapped in aluminum foil to minimize the loss of the antimicrobial agent by evaporation and were then stored at room temperature for up to 1 week prior to testing. An average of 10 measurements were taken at different points on the film sample using a digital micrometer (Digimatic Outside Micrometer, Mitutoyo, Japan) to measure the thickness of the sample.

Contact Angle Measurements

After the film surface was ionized by electronic radiation (method 1, mentioned above), the films were characterized with a sessile drop contact angle measurement in order to determine the change in surface hydrophilicity. Contact angle measurements were obtained using a goniometer (Ramé-Hart, Mountain Lakes, NJ, USA). For these measurements, water, a standard solution for a 32° contact angle, thyme EO, and oregano EO were used as test solutions. A drop of test solution (5 µl) from a microsyringe (Hamilton-Bonaduz, GR, Switzerland) was placed on the surface of the modified films. The sample stage and CCD camera position were adjusted so that the image of the drop was clearly obtained on the computer monitor, and the image was captured 5 s after depositing the drop. The angle formed between the liquid drop and the film was recorded as the contact angle. Reported contact angle values are an average of five measurements.

Determination of Antimicrobial Effect of Active Films

The antimicrobial activity of active films was determined quantitatively using the agar plate diffusion method. The zone of inhibition on solid media was used to determine the antimicrobial effects of active films against *Escherichia coli 0157:H7*, *Salmonella typhimurium*, and *Listeria monocytogenes*. From each bacterial dispersion (10^6 CFU/ml), 100 μl was spread onto an agar plate medium. Then, each antimicrobial film (2×2 cm) was placed over the surface of the agar plate medium. The plates were incubated at 37 °C for 24 h in an appropriate incubation chamber. The antimicrobial activity was observed as a zone of inhibition of the targeted microorganisms around the active film, and the diameter of the zone was measured with a digital micrometer (Digimatic Outside Micrometer, Mitutoyo). All tests were performed in triplicate. LDPE films without essential oils were included as controls.

Tensile Properties of the Films

A standard method D882-02 (ASTM 2002) was used to measure the tensile properties of the films. Films were cut into strips with a test dimension of 169×19 mm according to the standard method D638-08 (ASTM 2008). All films were conditioned for 48 h at 23±2 °C and 50±2% RH before testing. The strips were mounted and clamped with pneumatic grips on a universal testing machine (United Calibration Corp. and United Testing Systems, Inc., California, USA) with a 100-N load cell. The initial gauge length was set to 100 mm, and films were stretched using a crosshead speed of 10 mm/min. The parameters of tensile strength and elongation at break were determined. Measurements were performed on five replicates.

Oxygen Transmission Rate of the Films

Measurements were performed with an Ox-Tran 2/21 Oxygen Transmission Rate System (Mocon, Modern Controls Inc., Minneapolis, MN, USA) according to the standard method D3985-05 (ASTM 2005). Oxygen transmission rates (OTR) were determined at 23 °C and 0% RH.

Water Vapor Transmission Rate of the Films

A Permatran-W 3/31 Permeation Analysis System (Mocon, Modern Controls Inc.) was used to the measure water vapor transmission rate (WVTR) according to the standard method F-1249-06 (ASTM 2006). Tests were carried out at 37.8 °C temperature and 100% RH.

Statistical Analysis

Data points were presented as the mean of the measured values. The data were subjected to an analysis of variance and the Turkey test at the 0.05 level of significance (Xlstat 2009).

Results and Discussion

The activation of LDPE films with thyme and oregano EOs was performed using two different methods. LDPE films with thicknesses of 23±3 μm were prepared using the ionizing treatment and LDPE films with thickness of 27.5±3.5 μm using the extrusion method. To incorporate and protect the essential oils, the standard extrusion conditions of LDPE films (120–190 °C) were modified. Reductions of 20 °C in the first barrel zone and 15 °C in the other zones were obtained without compromising the structural and mechanical integrity of the film.

Contact Angle Measurements of Ionized Films

The measurements of contact angle were studied in order to test the effectiveness of ionizing radiation and their impact on the surface properties of LDPE film. The ionizing treatment altered the PE surface from hydrophobic to relatively hydrophilic by introducing mainly oxygen (Yamamoto et al. 2011). The contact angle between liquid–solid interfaces is a good indicator of adsorption because it increases the capacity of the liquid to spread, thereby decreasing the contact angle (Rubin 2004). Therefore, liquids with a lower contact angle will have a greater capacity to spread and penetrate a solid surface.

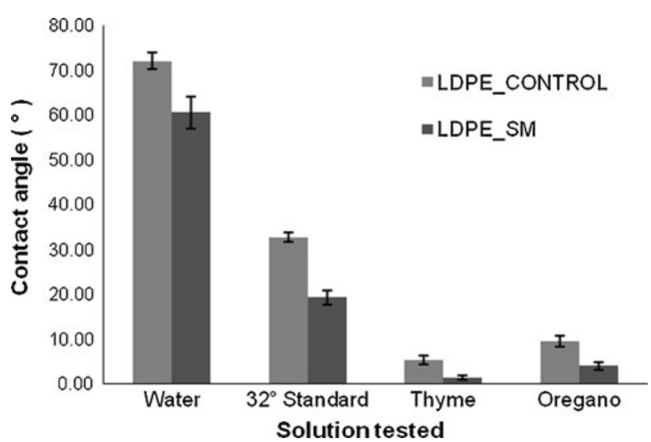

Fig. 1 Contact angle of surface-modified LDPE films by means of ionizing method. *SM* surface modified

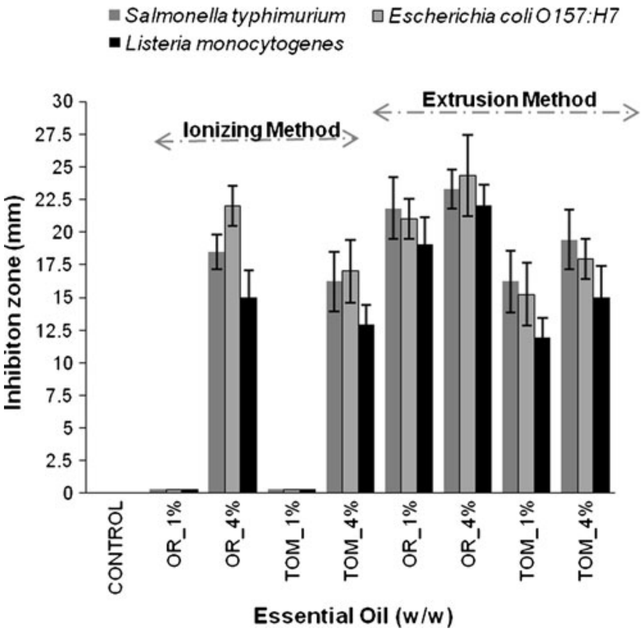

Fig. 2 Antimicrobial activity of LDPE films incorporating thyme and oregano essential oils against *E. coli* 0157:H7, *S. typhimurium*, and *L. monocytogenes*

The measured contact angles for each solution tested are illustrated in Fig. 1. All tested solutions showed a reduction in contact angle. Water, which initially had the highest contact angle due to the hydrophobic nature of polyolefin films, showed a 15.97% reduction of its contact angle. The contact angle of a standard solution for a 32° contact angle showed a reduction of 41.22%. Initially, the oregano and thyme EOs showed significantly lower contact angles compared to water and the standard solution due to the polyolefin's high affinity for fatty substances. However, the contact angles of the oils also showed the largest percentage reductions. Reductions of 76.19% and 57.89% were observed in the contact angles of oregano and thyme EOs, respectively. The results showed that the ionization treatment modified the film surface and increased the surface energy, resulting in a chemically active surface that increased the essential oil's effectiveness and ease of adhesion.

Antimicrobial Activity Assay of Active Films

Figure 2 shows the antimicrobial activity of LDPE active films against *E. coli 0157:H7*, *S. typhimurium*, and *L. monocytogenes*. As expected, in all cases, the films without essential oils did not show antimicrobial activity against any microorganisms. The ionization and extrusion methods both yielded positive results, although the resulting antimicrobial activity was significantly different in each case.

LDPE active films developed using the ionizing method incorporating 4% (*w*/*w*) of EOs were effective against all tested microorganisms (Fig. 2). However, the ionized films incorporating 1% (*w*/*w*) of EOs showed no antimicrobial activity. This effect may be due to the lower concentration of oil, which does not allow effective adhesion of the oil to the film surface. Although the ionization method showed no activity at low concentrations, it would be worthwhile to explore ways of improving it (varying power, time, and electrode type). On the other hand, the inhibition tests were conducted with microorganisms at concentrations well above those that are normally involved in food deterioration.

The LDPE active films developed using the extrusion method incorporating 1% and 4% (*w*/*w*) of thyme and oregano

Fig. 3 Representative picture of inhibitory zones of LDPE films incorporated with 4% (*w*/*w*) oregano essential oil compared to control against *E. coli* 0157:H7, *S. typhimurium*, and *L. monocytogenes*

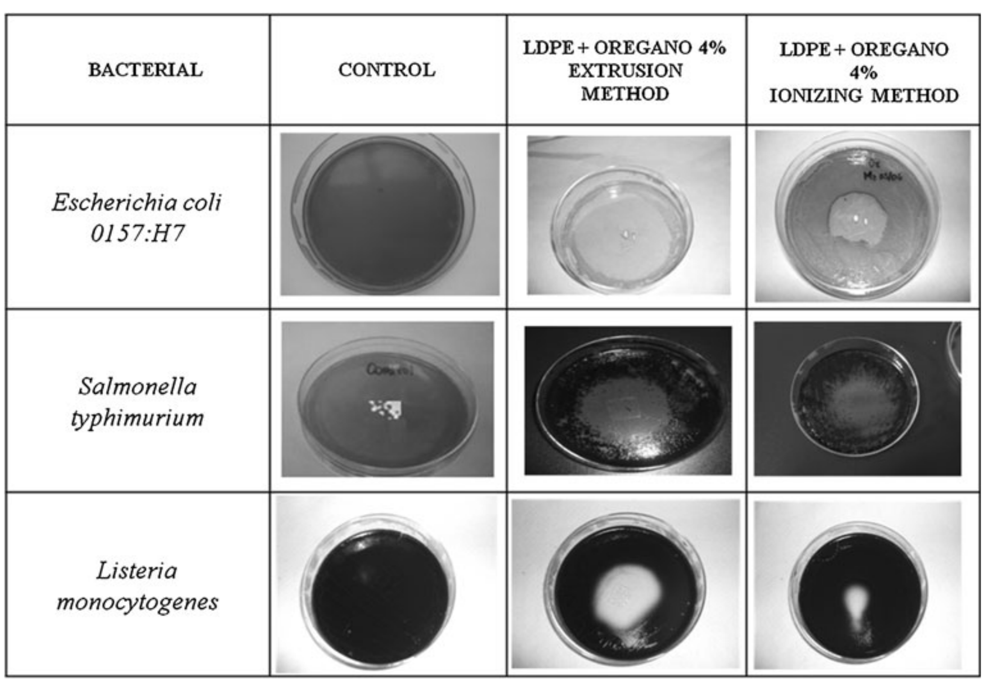

Table 1 Effect of the incorporation of essential oils on mechanical properties of LDPE films

Incorporation method	Type of polymer	Properties		
		Thickness (μm)	Tensile strength (kg/mm^2)	Elongation at break (%)
–	LDPE (control)	0.023±0.003a	1.319±0.107a	171±22b
Ionizing method	LDPE + 1% oregano	0.024±0.003a	1.227±0.121a	178±28ab
	LDPE + 4% oregano	0.022±0.004a	1.357±0.121a	174±12ab
	LDPE + 1% thyme	0.022±0.003a	1.322±0.129a	180±15ab
	LDPE + 4% thyme	0.025±0.002a	1.180±0.124a	172±25ab
Extrusion method	LDPE + 1% oregano	0.023±0.002a	1.282±0.082a	180±17ab
	LDPE + 4% oregano	0.024±0.002a	0.832±0.085b	221±11a
	LDPE + 1% Thyme	0.023±0.002a	1.202±0.124a	177±21ab
	LDPE + 4% thyme	0.023±0.002a	0.791±0.063b	225±21a

Thickness (N=10), tensile strength (N=5) and elongation (N=5) data are mean values±standard deviation. Means in same column with different lowercase letters are significantly different ($p<0.05$)

LDPE low-density polyethylene

EOs showed antimicrobial activity against all tested microorganism (Fig. 2). Other authors also reported the effectiveness of LDPE films incorporating essential oils (propolis, clove, basil, oregano, and cinnamon) at concentrations from 0.5% to 5% (*w/w*) (Hong et al. 2000; López et al. 2007; Suppakul et al. 2008) with different activation procedures than those presented in this study. These results demonstrate the powerful utility of LDPE films as antimicrobial packaging materials when they are formulated with EOs.

LDPE active films developed by extrusion showed a significantly higher inhibition than films developed using the ionizing method. The results suggest that the extrusion method allowed a better incorporation of the active compounds on the polymer. Figure 3 shows pictures representing the zones of inhibition of antimicrobial films incorporated with oregano EO against tested microorganisms.

There were also differences in the antimicrobial activity as a function of the EO incorporated; the films containing oregano oil were found to be more effective than those containing thyme oil, as illustrated in Fig. 2. The interactions of EOs have crucial effects on the antimicrobial activity of active films. Ting and Deibel (1991), Smith-Palmer et al. (1998), Hammer et al. (1999), and Burt and Reinders (2003) all observed strong antibacterial properties of oregano essential oil when directly applied against *E. coli O157:H7*, *L. monocytogenes*, *S. typhimurium*, *Staphylococcus aureus*, *Candida albicans*, *Aeromonas sobria*, and *Pseudomonas aeruginosa*. These authors found that the inhibitory effect of oregano was due to the high concentration of phenolic compounds such as carvacrol and thyme. Carvacrol and thyme (the major components of oregano and thyme EOs) are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt 2004).

Effect of Incorporation of EOs on the Mechanical and Barrier Properties of LDPE Plastic Films

Mechanical Properties of Antimicrobial LDPE Films

Tensile strength, elongation, and thickness were measured in order to study the effect of EO incorporation on the

Table 2 Effect of the incorporation of essential oils on the barrier properties of LDPE films

Incorporation method	Type of polymer	Properties		
		Thickness (μm)	WVTR (g/m^2/day)	OTR (ml/m^2/day)
	LDPE (control)	0.023±0.003	13.8±0.3a	6,658.7±66.5b
Ionizing method	LDPE + 1% oregano	0.024±0.003	13.6±0.3a	6,791.7±39.6b
	LDPE + 4% oregano	0.022±0.004	11.8±0.7b	6,213.6±14c
Extrusion method	LDPE + 1% oregano	0.023±0.002	10.2±0.4b	5,070.3±5.5c
	LDPE + 4% oregano	0.024±0.002	13.2±1.1ab	7,857.9±4.5a

Data are expressed as mean±standard deviation (N=4).Means in same column with different lowercase letters are significantly different ($p<0.05$)

LDPE low-density polyethylene, *WVTR* water vapor transmission rate, *OTR* oxygen transmission rate mechanical properties of films. Mechanical properties of films with and without EOs are shown in Table 1. No significant differences were observed between the thicknesses of films with and without EOs. Incorporating any of the EOs in the plastic films developed using the ionizing method did not affect the mechanical properties of films. However, the active films developed using the extrusion method showed a significant reduction in tensile strength and an increase in elongation. These results suggest that incorporating EOs in LDPE films using the extrusion method produces a plasticizing effect on the films. This effect manifested as a 9.28% reduction in the tensile strength and a 7.75% increase in the percentage elongation of the films. Similar results were reported by Dobias et al. (2000). They found statistically significant differences between the mechanical properties of films without antimicrobial agents and those with different agents at concentrations of 0.5% (*w*/*w*) and 1% (*w*/*w*), and found that the tensile and sealing strengths were lower in all samples.

Barrier Properties of Antimicrobial LDPE-Based Films

Only the LDPE films incorporated with oregano essential oil were evaluated, because these active films exhibited the greatest antimicrobial effect using both methods of incorporation. Table 2 shows OTR and WVTR values of the active films. In the ionized films, incorporating 1% *w*/*w* of oregano EO had no effect on the WVTR and OTR properties. However, a slight decrease in these properties was observed after incorporating 4% *w*/*w* oregano EO. In the extrusion method, incorporating oregano EO significantly affected the WVTR and OTR properties of the films. The films incorporating 1% (*w*/*w*) of oregano EO showed a reduction in the OTR and WVTR; and those with 4% (*w*/*w*) showed an unexpected increase in the OTR property. These results suggest that in the extrusion method, the oregano EO (1% *w*/*w*) was solubilized and could migrate to the amorphous region of the polymeric structure due to the nature of the oils. However, the amorphous region was saturated after the addition of 4% (*w*/*w*) of oregano EO, and the addition of further oregano EO interfered with the polymer–polymer interactions, resulting in the increased in the OTR property of the LDPE films.

Conclusions

The direct addition of oregano and thyme EOs into LDPE polymer matrices using two simple methods was possible. The antimicrobial films developed showed antimicrobial activity against food pathogens such as *S. typhimurium*, *L. monocytogenes*, and *E. coli O157:H7*. Antimicrobial films incorporating 4% (*w*/*w*) of the EOs developed using the extrusion method showed a greater inhibitory effect than those obtained using the ionizing method. The incorporation of essential oils into LDPE films changed some characteristics of the packaging material slightly, such as the mechanical and barrier properties. Incorporating EOs using the extrusion method significantly affected the mechanical and barrier properties of the films, while the incorporation using the ionizing method significantly affected only the barrier properties.

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