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Physicochemical Properties of Alginate/Polycaprolactone-Based Films Containing Essential Oils

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Oregano, savory, and cinnamon essential oils (EOs) 1% (w/v) were separately incorporated as natural antioxidant agents in alginate/polycaprolactone-based films. Films were then treated in 2 or 20% (w/v) $CaCl_2$ solutions in order to generate insoluble films. The mechanical properties and the insoluble matter of films were determined. Intermolecular interactions between film components and alginate cross-linkage were characterized by Fourier transform infrared (FTIR) spectroscopy. Surface morphology of the polymer membrane was determined by scanning electron microscopy (SEM) analysis. Antiradical properties of films were also evaluated following a modified colorimetric method using the *N,N*-diethyl-*p*-phenylenediamine (DPPD) discoloration test. Results showed that films treatment with $CaCl_2$ (20%) solution increased the percentage of insoluble matter in films but did not enhance their mechanical properties. FTIR analysis showed higher interactions in films treated in $CaCl_2$ solutions despite the concentration used (2 vs 20%). The SEM observations of films showed a smoother surface with a higher density when films were treated in $CaCl_2$ (20%) solution. The DPPD test showed that the oregano-based films had the highest antiradical properties.

KEYWORDS: Bioactive packaging; alginate; polycaprolactone; cross-linking; essential oil; solubility; tensile properties; FTIR; SEM; antiradical activity

INTRODUCTION

During the past two decades, a wide variety of packaging materials have been developed to interact with food (*1*). This innovative concept, termed as "active packaging", is based on a mode of interaction between the package, the product, and the environment to extend shelf life or enhance safety or sensory properties, while maintaining the nutritional quality of the product (*2*). Edible or biodegradable films constitute an appropriate way to prolong the shelf life of foods and to increase their quality without contributing to environmental pollution (*3*). These materials not only act as selective barriers for moisture, gas, and solute migration but also as food additive carriers (*4*). Hence, various kinds of compounds such as antioxidants (*5*), antimicrobial agents (*6*), colorings, flavors, and spices (*7*) can now be incorporated into packaging materials to improve their functionality and give them novel or extra functions (*2*, *7*).

Essential oils (EOs) extracted from herbs and their components have many applications in medicine, in fragrance, and also in food flavoring and preservation (*8*). Oxidative deterioration of food components can result in alterations of organoleptic characteristics, e.g., taste and aroma, in the food products, making them unacceptable to the consumer. However, recent studies on the antioxidant activity of spices have also been reported (*9*). In this context, oregano, savory, and cinnamon EO-based films have been reported to prevent lipid oxidation when added in various food systems (*10*).

Alginate, a polysaccharide extracted from brown seaweed, possesses good film-forming properties, and the inclusion of polyols such as glycerol or poly(ethylene glycol) can improve their flexibility (*11*). Also, alginate-based films are impervious to oils and fats but are poor moisture barriers and are water-soluble (*12*). In addition, they are good oxygen barriers (*13*), can improve the flavor and the texture of foods, and can delay lipid oxidation (*11*). An important property of alginate resides in its ionotropic gelation induced by multivalent cations that cross-link carboxylate groups in the uronate blocks to give an insoluble gel (*14*). Poly(ϵ-caprolactone) (PCL) is a semicrystalline biodegradable polyester, resulting from a chemical synthesis from crude oil, and is also used to produce biodegradable films. PCL-based films have good water resistance, low melting point, low glass transition temperature, and good processability (*15*). It has been shown that PCL exhibited desirable characteristics as a diffusion-controlled delivery system, including biodegradability, biocompatibility, commercial availability, and affordability (*16*).

Milk protein-based films containing EOs were previously developed in our laboratory to improve the shelf life and to control the growth of pathogenic bacteria on beef muscle slices (*17*). The addition of emulsifying agents in the film formulation

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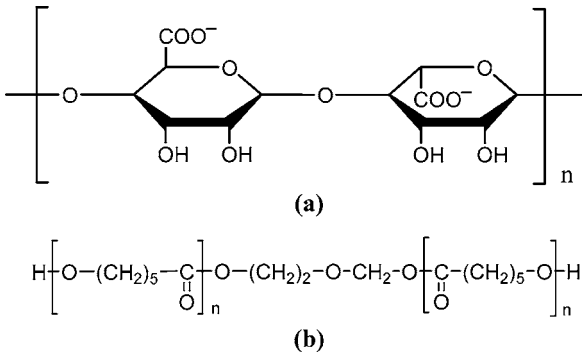

Figure 1. Chemical structure of alginate (**a**) and poly(ϵ-caprolactone) diol (PCL) (**b**).

resulted in a more stable emulsion when EOs were encapsulated. Therefore, these films allowed progressive release of bioactive compounds and showed constant availability during 7 days of storage. However, the films were not water-resistant during storage and showed poor mechanical properties. Other studies also showed that oregano, savory, and cinnamon EOs had the most effective antimicrobial properties (*18*). Consequently, a new formulation based on alginate and PCL was developed to obtain hydrophobic films containing oregano, savory, and cinnamon EOs. The availability of the encapsulated EOs in films and their release properties have already been determined by Oussalah et al. (*19*) during storage when applied on meat products. The amount of EOs in films was 30.71, 35.91, and 45.48 mg/(g of film) for oregano-, savory-, and cinnamon-based films, respectively.

The aim of the present study was to evaluate the effects of treatment in 2 or 20% (w/v) $CaCl_2$ solution on the solubility, the mechanical properties, and the microstructure of alginate/PCL-based films. The effect of EOs on the antiradical properties of films was also evaluated. Molecular interactions of components present in films were also examined by Fourier transform infrared (FTIR) spectroscopy, and the observations were correlated with the mechanical properties of films.

MATERIALS AND METHODS

Film Preparation. Bioactive films were prepared according to a method developed in our laboratory. A preheated aqueous solution (45 °C) containing 3% alginate (from brown algae, low viscosity; Sigma-Aldrich Canada Ltd., Oakville, ON, Canada), 1% poly(ϵ-caprolactone) diol (PCL; M_w, 1250; Sigma-Aldrich), and 2% glycerol (Laboratoire Mat, Beauport, QC, Canada) was sterilized at 121 °C for 20 min. **Figure 1** shows the chemical structures of alginate (a) and PCL (b). Spanish oregano (*Corydothymus capitatus*), winter savory (*Satureja montana*), and Chinese cinnamon (*Cinnamomum cassia*) essential oils were used as bioactive agents and were purchased from Cedarome Canada Inc. (Brossard, QC, Canada). For each type of film (oregano-, savory-, and cinnamon-based films), 1% of the respective EO was added in the film-forming solution. The solution was then homogenized using a Ultra-Turrax TP18/1059 homogenizer (Janke & Kunkel, Staufen, Germany) at 45 °C and 25 000 rpm for 1 min. Films were then cast by applying 5 mL of the film-forming solution onto Petri dishes (50 × 9 mm; VWR International, Ville Mont-Royal, QC, Canada) and allowed to dry for 24 h, at room temperature and 40−50% relative humidity (RH). Dried water-soluble films were peeled and treated by immersion in $CaCl_2$ solutions. Two concentrations of $CaCl_2$ solution (2 and 20%) were used for insolubilization treatment of films, to compare respectively the effect of mild and strong insolubilization processes upon the physicochemical properties of films. The time of the insolubilization process was also evaluated. Film immersion was done for 1, 10, 20, or 30 min. These treatments allowed formation of insoluble films by ionotropic gelation between alginate and calcium ions. Films were then washed in distilled water and dried at room temperature and 40−50% RH for 30 min, before testing.

Determination of Insoluble Matter. The percentage of insoluble matter in films was determined following the method of Le Tien et al. (*20*). The average dry weight of the films was determined by drying them in an oven at 90 °C until constant weight was achieved (72 h). Each type of film was immersed into distilled water with mild stirring for 24 h at room temperature. Residual films were then recovered by filtration (Whatman no. 1; Whatman Inc., Florham Park, NJ) and dried as previously described. On the basis of dry matter, differences between the initial weight and the weight after treatment in water were calculated. Results were expressed as the recovery yield (RY, insoluble matter), which was calculated using the equation

$$\mathrm{RY}\ (\%) = (m_f/m_i) \times 100$$

where m_i is the initial weight of the film and m_f is the weight of the recovered film after treatment in water.

Mechanical Properties of Films. *Film Thickness.* Film thickness was measured using a Mitutoyo digimatic Indicator (Mitutoyo MFG, Tokyo, Japan) at five random positions around the film, by slowly reducing the micrometer gap until the first indication of contact.

Puncture Strength and Puncture Deformation. Puncture strength (PS) and puncture deformation (PD) measurements were carried out using a Stevens-LFRA texture analyzer (model TA-1000; Texture Technologies Corp., Scarsdale, NY), as described by Letendre et al. (*21*). Film samples were equilibrated in a desiccator containing a saturated NaBr solution ensuring 56% RH at room temperature (21 °C) for at least 24 h. Films were then fixed between two perforated Plexiglass plates (3.2 cm diameter), and the holder was held tightly with two screws. A cylindrical probe (2 mm diameter) was moved perpendicularly to the film surface at a constant speed (1 mm·s^{-1}) until it passed through the film. Strength and deformation values at the puncture point were used to calculate the hardness and deformation capacity of the film. In order to avoid any variation related to the film thickness, the PS values were divided by the thickness of the films. PS was calculated using the equation

$$\mathrm{PS}\ (\mathrm{N{\cdot}mm^{-1}}) = (9.81F)/x$$

where F is the recorded force value (g), x is the film thickness (μm), and 9.81 m·s^{-2} is the gravitational acceleration.

PD was calculated from the PS curve, using the equation

$$\mathrm{PD}\ (\mathrm{mm}) = d/8.33$$

where d is the distance (mm) recorded between the time of first contact probe/film and the time of puncture point and 8.33 is a corrective factor related to the fixed parameters of the texture analyzer.

Viscoelasticity. Viscoelastic properties were evaluated using relaxation curves. The same puncture test procedure as described before was used, but the probe was stopped at 3 mm and maintained for 1 min. The Young's modulus (Y) was calculated using the equation

$$Y_{(1\ \mathrm{min})} = (F_i - F_f)/F_i$$

where F_i is the initial recorded value (g) and F_f the second value measured after 1 min of relaxation. A low viscoelasticity coefficient ($Y \rightarrow 0$) indicates high film elasticity, whereas a high coefficient ($Y \rightarrow 1$) indicates high film plasticity, implying a more rigid and easily distorted material.

FTIR Spectroscopy. FTIR spectra of EO-free films and their components were recorded using a Spectrum One spectrophotometer (Perkin-Elmer, Woodbridge, ON, Canada) equipped with an attenuated total reflectance (ATR) device for solids analysis and a high linearity lithium tantalate (HLLT) detector. Spectra were analyzed using the Spectrum software (version 3.02.01). Films were stored at room temperature for 72 h in a desiccator containing a saturated NaBr solution ensuring 56% RH. Films were then placed onto a zinc selenide crystal, and the analysis was performed within the spectral region of 650−4000 cm^{-1} with 128 scans recorded at a 4 cm^{-1} resolution. After attenuation of total reflectance and correction of the baseline, spectra

were normalized with a limit ordinate of 1.5 absorbance units. Resulting FTIR spectra were compared in order to evaluate the effect of each compound addition (alginate, PCL, glycerol) and ionic cross-linking degree on the intensity and shift of IR bands related to molecular interactions.

Scanning Electron Microscopy Analysis. Film samples (5 × 5 mm) were deposited on a brass hold and sputtered with gold (coating thickness, 150−180 Å) in a Polaron PS3 sputter coater (Soquelec Ltd, Mississauga, ON, Canada). Scanning electron microscopy (SEM) photographs were taken with a Hitachi S-4300SE/N scanning electron microscope (Hitachi Canada Ltd., Mississauga, ON, Canada) at two required magnifications (500× and 5000×) at room temperature. The working distance was maintained between 15.4 and 16.4 mm, and the acceleration voltage used was 5 kV, with the electron beam directed to the surface at a 45° angle and a secondary electron imaging (SEI) detector.

Antiradical Properties. Antiradical properties of films were evaluated following the procedure of Caillet et al. (*22*) using *N*,*N*-diethyl-*p*-phenylenediamine (DPPD) reagent. A piece of film (25−30 mg) was placed in an electrolytic cell (platinum electrodes) containing 3 mL of 0.15 M NaCl and submitted to electrolysis for 1 min (10 mA direct current (DC), 400 V). After electrolysis, an aliquot of 200 μL of the electrolyte was added to 2 mL of DPPD solution (25 mg/mL; Sigma-Aldrich). The generated reactive oxygen species (ROS) such as superoxide anions ($\bullet O_2^-$), singlet oxygen ($^1O_2$), hydroxy radicals ($\bullet$OH), and their byproducts such as hydrogen peroxide (H_2O_2) and hypochlorite ion (OCl^-) react instantly with DPPD, producing a red coloration that can be measured at 515 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). The antiradical activity of films is equivalent to their capacity to inhibit the accumulation of oxidative species and, consequently, the red coloration. The colorimetric reaction was calibrated using the nonelectrolyzed NaCl solution (no oxidative species, ascribed to 100% scavenging) and the electrolyzed NaCl solution (0% scavenging, in the absence of any antioxidants). The scavenging percentage was calculated using the equation

$$\text{scavenging (\%)} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$$

where A_{control} is the absorbance of electrolyzed solution in the absence of sample. The degree of discoloration indicated the scavenging potential of the bioactive films. This colorimetric method was standardized with FCC/USP ascorbic acid (Laboratoires Denis Giroux Inc., Saint-Hyacinthe, QC, Canada). Thus, the antiradical activity of films was estimated from a calibration curve ($R^2 = 0.9752$; relative standard deviation < 6.4%) by plotting known solutions of USP ascorbic acid (0.6, 1.2, 1.8, 2.4, and 3.0 USP units) against % scavenging and results were expressed in USP unit/(g of film). One USP (United States Pharamacopeia) unit represents the free radical-scavenging activity of 0.05 mg of the USP ascorbic acid reference standard.

Statistical Analysis. Experiments for the determination of insoluble matter and mechanical property measurements of films were carried out using a 3 × 3 × 4 factorial design [3 replicates, 3 treatments (control, $CaCl_2$ (2%), $CaCl_2$ (20%)), 4 types of films (EO-free films (control) and oregano-, savory-, and cinnamon-based films)]. Free radical-scavenging capacity analysis was performed using a 3 × 4 factorial design [3 replicates, 4 types of films (EO-free films (control) and oregano-, savory-, and cinnamon-based films)]. For each measurement, three samples in each replicate were tested. Analysis of variance and Duncan's multiple-range tests were used to perform statistical analysis on all results, using SPSS Base 10.0 software (Stat-Packets statistical analysis software, SPSS Inc., Chicago, IL). Differences between means were considered to be significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Preliminary Results. Preliminary tests were conducted in order to determine the concentration of calcium (2 vs 20%) and the time of immersion (1, 5, 10, and 20 min) to obtain an insoluble film with a low (2% $CaCl_2$) and high (20% $CaCl_2$) cross-linking degree. Films without immersion treatment had good puncture strength (74.46 N·mm^{-1}) but were completely soluble in water. Films treated with $CaCl_2$ (2%) for 1 min were insoluble but had a poor puncture strength (18.35 N·mm^{-1}). Films treated with $CaCl_2$ (20%) for 20 min were insoluble with a good puncture strength (79.69 N·mm^{-1}). Consequently, on the basis of these preliminary tests, films with a high puncture strength (high cross-linking degree due to optimal treatment in 20% $CaCl_2$ for 20 min) were compared to films with a low puncture strength (low cross-linking degree due to treatment in 2% $CaCl_2$ for 1 min) in order to evaluate the effect of $CaCl_2$ immersion on the rheological properties of films relative to their structural modification after treatment.

Table 1. Effect of Film Formulation and Treatment with $CaCl_2$ on the Recovery Yield of Films (Fraction of Insoluble Film Matter)

type of film	treatment[a]	recovery yield[b,c] (%)
control	0	0.00 ± 0.00 a, A
	1	85.06 ± 2.01 b, B
	2	86.25 ± 1.32 b, B
oregano	0	0.00 ± 0.00 a, A
	1	62.86 ± 1.34 b, A
	2	81.17 ± 5.72 c, AB
savory	0	0.00 ± 0.00 a, A
	1	81.21 ± 3.03 b, B
	2	84.66 ± 4.08 b, B
cinnamon	0	0.00 ± 0.00 a, A
	1	64.00 ± 3.42 b, A
	2	74.34 ± 3.76 c, A

[a] Treatment 0 corresponds to untreated films, treatment 1 to films immersed in a $CaCl_2$ solution (2%) for 1 min, and treatment 2 to films immersed in a $CaCl_2$ solution (20%) for 20 min [b] For the same type of film (control, oregano, savory, or cinnamon), means followed by the same lower case letter between treatments are not significantly different ($p > 0.05$). [c] For the same treatment, (0, 1, 2), means followed by the same upper case letter between types of film are not significantly different ($p > 0.05$).

Otherwise, the use of PCL was necessary to stabilize the emulsion and to form a homogeneous film after addition of EOs. Indeed, the film formulation without PCL (alginate/glycerol only) did not form homogeneous films after incorporation of EOs (visible diphasic system). It is well-known that the polarity and structure of hydrophobic substances play an important role in the emulsion stability and the repartition of these hydrophobic substances into the polymeric support (*23*).

Determination of Insoluble Matter. The insolubility of the films after treatment with $CaCl_2$ is shown in **Table 1**. Results showed that films without immersion were entirely soluble in water (RY = 0%) notwithstanding the EO added in the film. When the control film and oregano- and savory-based films were treated with $CaCl_2$ (20%), a RY value of 86, 81, and 85% was, respectively, observed, as compared to a RY of 74% obtained with cinnamon-based films ($p \leq 0.05$). It is also interesting to note that the RY value of cinnamon-based films was significantly lower ($p \leq 0.05$) than EO-free films despite the treatment with $CaCl_2$ used (2 vs 20%). These results suggest that the presence of cinnamon EO in the films treated with $CaCl_2$ (20%) increased their solubility.

The immersion treatment in $CaCl_2$ increased the RY of all films significantly ($p \leq 0.05$). Furthermore, a significant increase of RY ($p \leq 0.05$) was observed in oregano- and cinnamon-based films by increasing the $CaCl_2$ concentration from 2 to 20%. The RY values after immersion treatment with $CaCl_2$ (2%) were 63 and 64% for oregano- and cinnamon-based films, respectively, as compared to 81 and 74% for the same respective films after immersion in $CaCl_2$ (20%). However, the increase of the $CaCl_2$ concentration did not have any significant effect ($p > 0.05$) on the RY values in EO-free and savory-based films.

Table 2. Effect of Film Formulation and Treatment with $CaCl_2$ on Mechanical Properties of Films

type of film	treatment[a]	thickness[b,c] (μm)	puncture strength[b,c] (N.mm^{-}1)	puncture deformation[b,c] (mm)	viscoelasticity coefficient[b,c] (%)
control	0	105.87 ± 4.63 a, A	74.46 ± 7.89 b, B	8.71 ± 0.29 c, B	41.38 ± 0.19 a, B
	1	105.50 ± 3.05 a, A	18.35 ± 1.32 a, C	7.05 ± 0.61 b, B	45.39 ± 0.42 b, B
	2	104.08 ± 6.35 a, A	79.69 ± 3.47 b, D	6.12 ± 0.33 a, A	48.99 ± 0.33 c, AB
oregano	0	128.72 ± 2.91 a, B	62.77 ± 3.53 a, C	8.96 ± 0.17 b, B	37.15 ± 0.15 a, A
	1	126.38 ± 5.04 a, B	9.88 ± 0.32 a, A	6.03 ± 0.48 a, A	38.55 ± 1.50 a, A
	2	126.17 ± 5.95 a, B	57.65 ± 4.18 b, B	6.19 ± 0.28 a, A	51.48 ± 1.46 b, B
savory	0	101.20 ± 2.67 a, A	57.89 ± 6.71 b, A	7.91 ± 0.32 b, A	36.85 ± 1.53 a, A
	1	102.11 ± 5.78 a, A	11.41 ± 0.96 a, B	6.09 ± 0.48 a, A	47.19 ± 1.95 b, B
	2	100.43 ± 6.71 a, A	68.11 ± 9.33 c, C	5.84 ± 0.48 a, A	51.98 ± 2.48 c, B
cinnamon	0	125.93 ± 4.35 a, B	60.35 ± 1.64 c, A	8.81 ± 0.27 c, B	44.29 ± 2.48 ab, B
	1	123.92 ± 5.08 a, B	10.21 ± 0.79 a, AB	6.93 ± 0.62 b, B	40.43 ± 2.29 a, A
	2	126.93 ± 4.60 a, B	49.29 ± 5.11 b, A	6.11 ± 0.56 a, A	47.74 ± 3.44 b, A

[a] Treatment 0 corresponds to untreated films, treatment 1 to films immersed in a $CaCl_2$ solution (2%) for 1 min, and treatment 2 to films immersed in a $CaCl_2$ solution (20%) for 20 min. [b] For the same type of film (control, oregano, savory, or cinnamon), means followed by the same lower case letter between treatments are not significantly different ($p > 0.05$). [c] For the same treatment, (0, 1, 2), means followed by the same upper case letter between types of film are not significantly different ($p > 0.05$).

After immersion in $CaCl_2$ (2%), the RY values were respectively 85 and 81% for EO-free and savory-based films as compared to 86 and 85% after immersion in $CaCl_2$ (20%) for the same respective films. These results suggest that a $CaCl_2$ concentration of 20% was necessary to optimize the RY of oregano- and cinnamon-based films and only a concentration of 2% of $CaCl_2$ was necessary to optimize the RY of EO-free and savory-based films. The highest RY values were obtained for the control film and oregano- and savory-based films after immersion in $CaCl_2$ (20%), showing respective values of 86, 81, and 85% respectively), whereas cinnamon-based films showed the lowest RY (64 and 74%) notwithstanding the concentration of $CaCl_2$ used.

Chemical analysis of EOs provided by the manufacturer had shown that the content of phenols and terpene hydrocarbons in oregano EO was respectively 84 and 13% (w/w) as compared to 56 and 43% in savory EO. The chemical analysis of cinnamon EO showed essentially the presence of aldehydes (91%). Hence, the higher solubility of cinnamon films could be explained by the fact that *trans*-cinnamaldehyde and its derivatives, which are the main components of cinnamon EO, are more polar than phenols and monoterpene hydrocarbons contained in oregano and savory EOs, implying a better affinity for water (*24*). Furthermore, a significant concentration of terpene hydrocarbons in savory EO (43%) as compared to oregano EO (13%) could explain why savory-based films are less soluble than oregano-based ones after a treatment with $CaCl_2$ (2%). Consequently, it seems that the composition of the EOs used has an effect on the film solubility. According to Oussalah et al. (*17*, *19*), the polarity, the structural configuration, and the functional groups of EO components could have an influence on the EO emulsion stability after immobilization into the polymeric network. Indeed, it has been assessed that hydrogen, hydrophobic, and ionic interactions are contributory factors in emulsion formation and stability (*25*). Dispersion of hydrophobic substances can also modify significantly their interactions with film-forming agents (*23*). In agreement with Parris et al. (*26*), savory EO particles containing relatively hydrophobic substances would be out of phase with the hydrophilic polymer matrix and would disperse non-homogeneously therein. This implies greater ionic and hydrogen bonding between polymer chains, leading to less soluble gels. In opposition, a homogeneous dispersion of oregano and cinnamon EOs into polymeric network could increase the spacing between macromolecule chains, which would reduce ionic and hydrogen bonding between the chains, and give more soluble films.

Mechanical Properties. *Film Thickness.* The thickness of films treated with $CaCl_2$ (2 and 20%) are presented in **Table 2**. Depending on the formulation and the immersion treatment in $CaCl_2$, the average film thickness was from 100 to 130 μm (standard deviation $\leq$ 6.71 μm). For each formulation, the immersion treatment in $CaCl_2$ had no significant effect ($p > 0.05$) on the film thickness. The thickness of oregano- and cinnamon-based films was from 123.9 to 128.7 μm. These values are significantly higher ($p \leq 0.05$) than the thickness obtained for the control and savory-based films (from 100.4 to 105.9 μm). These variations may be explained by the difference in the chemical composition of EOs and by their interactions with the polymeric matrix.

Puncture Strength. PS values obtained for films are presented in **Table 2**. Results showed that the incorporation of EOs in films had detrimental effects on the PS values. Indeed, PS values obtained for EO-based films were significantly lower ($p \leq 0.05$) than EO-free films despite the treatment used. When films were treated with $CaCl_2$, PS values obtained for 2% $CaCl_2$-treated films were in the decreasing order control > savory > cinnamon > oregano and PS values obtained for 20% $CaCl_2$-treated films were in the decreasing order control > savory > oregano > cinnamon. These results follow the same trend as results obtained for RY of films since cinnamon- and oregano-based films were the most soluble ones compared to control and savory-based films which were the least soluble ones notwithstanding the treatment used (2 or 20% $CaCl_2$).

The effect of treatment with $CaCl_2$ on PS values also showed that films treated with $CaCl_2$ (2%) had significantly ($p \leq 0.05$) lower PS values compared to untreated ones and films treated with $CaCl_2$ (20%). Moreover, compared to untreated films, the treatment of films with $CaCl_2$ (20%) improved the PS value of savory-based films significantly ($p \leq 0.05$) by 20% (from 57.89 to 68.11 N·mm^{-1}). However, a significant decrease ($p \leq 0.05$) of the PS value was observed in oregano- and cinnamon-based films by 8 and 18%, respectively (from 62.77 to 57.65 N·mm^{-1} for oregano-based films and from to 60.35 to 49.29 N·mm^{-1} for cinnamon-based films; **Table 2**). No significant effect ($p > 0.05$) of the treatment with $CaCl_2$ was observed in EO-free films. This phenomenon does not correlate with results obtained for solubility of films since RY values of films treated with $CaCl_2$ were significantly ($p \leq 0.05$) higher than RY values of untreated films (RY = 63−86% for films with or without EOs treated in 2 and 20% $CaCl_2$ as compared to RY = 0% for untreated films with or without EOs; **Table 1**). As reported by Pranoto et al. (*27*), the presence of EO particles in the internal structure of films probably interferes with ionic and polymer−polymer interactions, thus providing films with lower PS values.

Puncture Deformation. The PD values of films are presented in **Table 2**. For all films treated with $CaCl_2$ (20%), the results showed no significant difference ($p > 0.05$) between control films and EO-based films, showing PD values varying from 5.84 to 6.19 mm, suggesting that EO addition did not affect the PD of films when treated with $CaCl_2$ (20%).

For all types of films, the effect of treatment with $CaCl_2$ on PD values showed that films treated with $CaCl_2$ (2 or 20%) had significantly ($p \leq 0.05$) lower PD values as compared to untreated films. For films not treated with $CaCl_2$, the PD values observed for EO-free film and oregano-, savory-, and cinnamon-based films were respectively 8.7, 9.0, 7.9, and 8.8 mm. A significant decrease ($p \leq 0.05$) was observed after treatment with $CaCl_2$ (2%), showing PD values of 7.1, 6.0, 6.1, and 6.9 mm, respectively. Results also showed a significant decrease of PD values ($p \leq 0.05$) in control and cinnamon-based films when the $CaCl_2$ concentration increased from 2 to 20% (from 7.1 to 6.1 mm for control films and from 6.9 to 6.1 mm for cinnamon-based films), whereas no significant effect of the $CaCl_2$ concentration from 2 to 20% on PD values was observed ($p > 0.05$) in oregano- and savory-based films. This reduction of PD values in all films (EO-free and EO-based films) after treatment with $CaCl_2$ could be explained by the formation of new ionic intermolecular bridges ($Ca^{2+}\cdots COO^-$). Indeed, ionic interactions can retain strongly two macromolecular chains of alginate and, consequently, decrease the films' tensile characteristics, as compared to untreated films that were characterized by weaker bonds (hydrogen bonds, hydrophobic interactions). Similar observations were reported by Le Tien et al. (*20*) for protein and polysaccharide-based films.

Viscoelasticity. The viscoelasticity of films are presented in **Table 2**. A low viscoelasticity coefficient (*Y*) means that the material is highly elastic, whereas a high coefficient indicates that the material is more rigid and easily distorted. The results showed that when films were treated with $CaCl_2$ (20%), the addition of EOs did not alter the viscoelasticity of films ($p > 0.05$). Moreover, the results showed that cinnamon-based films treated with $CaCl_2$ (20%) were significantly ($p \leq 0.05$) more viscoelastic than oregano- and savory-based films. A *Y* coefficient of 47.74% was observed for cinnamon-based films, as compared to 51.48 and 51.98% for oregano- and savory-based films, respectively (**Table 2**). The viscoelasticity results obtained for cinnamon-based films treated with $CaCl_2$ (20%) are in agreement with PS results since cinnamon-based films had a significantly lower PS value than oregano- and savory-based films (49.29, 57.65, and 68.11 $N\cdot mm^{-1}$ respectively; $p \leq 0.05$). This phenomenon could be explained by the significant concentration of aldehydes in cinnamon-based films, as compared to oregano- and savory-based films where the content in phenols and monoterpenes (more hydrophobic components) is significant. These results suggest different interactions with polymer network when a tensile stress occurs. Similar behaviors were reported by Ressouany et al. (*28*) and Sabato et al. (*29*). Their study showed a loss of film rigidity and a gain in elasticity after addition of a more water-soluble plasticizer in their film formulations.

The treatment with $CaCl_2$ (2%) decreased the viscoelasticity of EO-free and savory-based films significantly ($p \leq 0.05$) by 10 and 28%, respectively. However, this treatment did not affect the viscoelasticity of oregano- and cinnamon-based films significantly ($p > 0.05$). The treatment with $CaCl_2$ (20%) decreased the viscoelasticity of EO-free, oregano- and savory-based films significantly ($p \leq 0.05$) by 18, 39, and 41%, respectively. However, cinnamon-based films were not affected significantly ($p > 0.05$) by this treatment (**Table 2**). Also, a significant decrease ($p \leq 0.05$) of the viscoelasticity values was observed by increasing the $CaCl_2$ concentration from 2 to 20% in all types of films. These observations suggest that an increase of $CaCl_2$ concentration increased the polymeric network density and therefore decreased the elasticity of films. It is important to note that a correlation was observed between the viscoelasticity and the PD values of films. Indeed, for each film formulation, the elasticity of films tends to decrease with the PD after treatment with $CaCl_2$. In addition, an increase from 2 to 20% of the $CaCl_2$ concentration increased the PS of films and decreased their viscoelasticity significantly ($p \leq 0.05$) and tends to decrease their PD values, which signifies that the increase of $CaCl_2$ concentration improved the resistance of films and reduced their elongation and elasticity. Le Tien et al. (*20*) and Lee et al. (*30*) observed similar relations between cross-linking degree, puncture strength, puncture deformation, and viscoelasticity of protein-based films.

FTIR Spectroscopy. Figure 2 shows the FTIR spectra of pure PCL (a), alginate−glycerol film (b), and PCL−alginate−glycerol film (c). The absorption peaks can be mainly assigned to the following IR vibrations: O−H stretching (3000−3600 cm^{-1}), overlapping symmetric and asymmetric C−H stretching (2850−2950 cm^{-1}), C═O lactones stretching (1720 cm^{-1}), asymmetric COO^- stretching (1600 cm^{-1}), overlapping symmetric COO^- stretching and O−H bending (1410 cm^{-1}), C−O lactones stretching (1180 cm^{-1}), and C−O−C stretching (1030 cm^{-1}). It is important to note that the characteristic sharp peaks of PCL at 2850−2950, 1720, and 1180 cm^{-1} (**Figure 2a**) were not altered in the presence of alginate matrix (**Figure 2c**), thereby indicating the absence of covalent bonds between PCL and alginate (*31*).

Figure 3 shows the FTIR spectra of untreated PCL-alginate-glycerol films (control, a), treated with $CaCl_2$ (2%) (b), and treated with $CaCl_2$ (20%) (c). The comparison of spectra a−c allowed characterization of the insolubilization effect of calcium addition, via $Ca^{2+}\cdots COO^-$ bonds, by the variation of intensity and shift in the absorption bands assigned to alginate and PCL as $CaCl_2$ concentration increases. Indeed, an increase of peak intensity related to chemical functions of PCL (hydrophobic compound) and a decrease of peak intensity related to chemical functions of alginate and glycerol (hydrophilic compounds) were observed with an increase of $CaCl_2$ concentration, successively for spectra a−c. For alginate bands, results showed a decrease of peak intensity related to O−H stretching and bending and COO^- and C−O−C stretchings as the $CaCl_2$ concentration increases (*32*). In contrast, for PCL bands, an increase of peak intensity was observed for C−H stretching and C═O lactone stretching and bending with an increase of $CaCl_2$ concentration. The decrease of the O−H peak intensity can be explained by higher intermolecular associations involving C(2)OH···O═C(6) and C(3)OH···O═C(6) hydrogen bonds resulting from cross-linking (*33*). The increase of C−H stretching peaks at 2865 and 2940 cm^{-1} are attributable to the alkyl groups of PCL (*34*). These results showed that, after dehydration of the polymeric network by addition of $CaCl_2$, the variation of vibrational intensities in IR bands is intimately associated with polar interactions between film components and unbounded or "free" water retained in films (*20*). As described by Wong et al. (*35*), interactions between alginate and Ca^{2+} is mainly characterized by a decrease in the wavenumber of the carbonyl peak from 1600 to 1590 cm^{-1} and a reduction in the peak intensities associated with the free carboxylate groups of alginate (1600 and 1410 cm^{-1}) and the hydroxyl groups of alginate. This

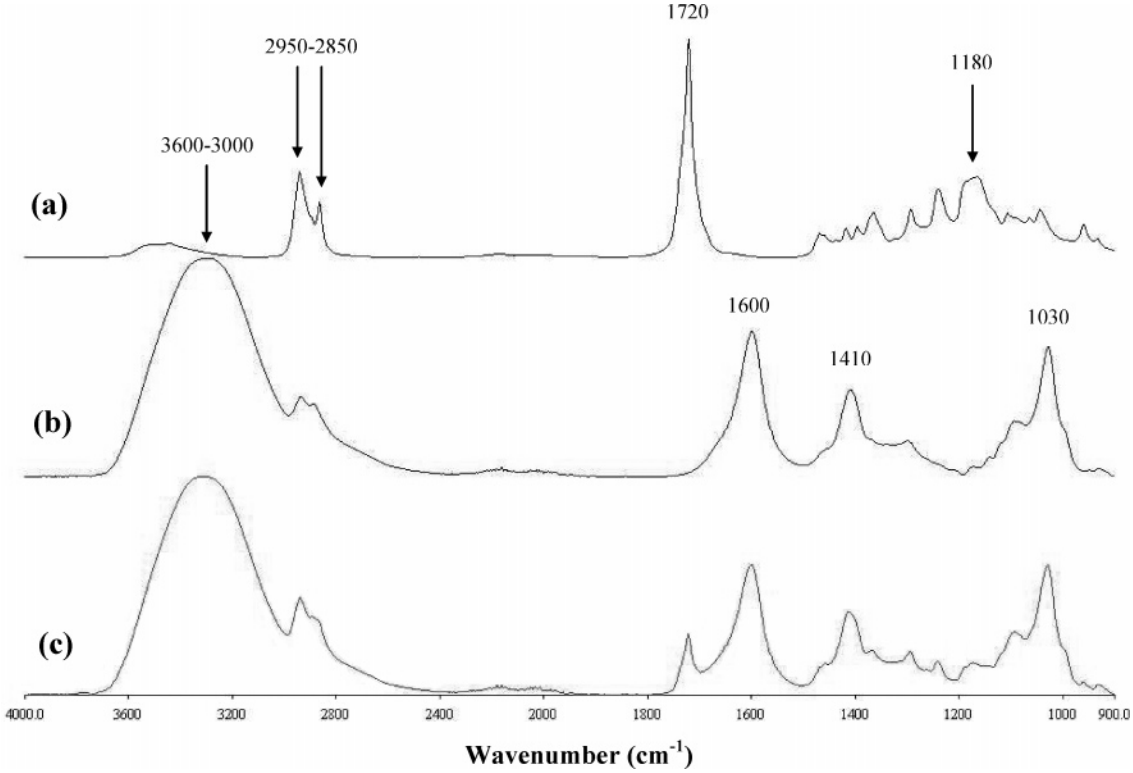

Figure 2. FTIR spectra of pure PCL (**a**), alginate-glycerol film (**b**), and poly(ϵ-caprolactone)/alginate/glycerol film (**c**).

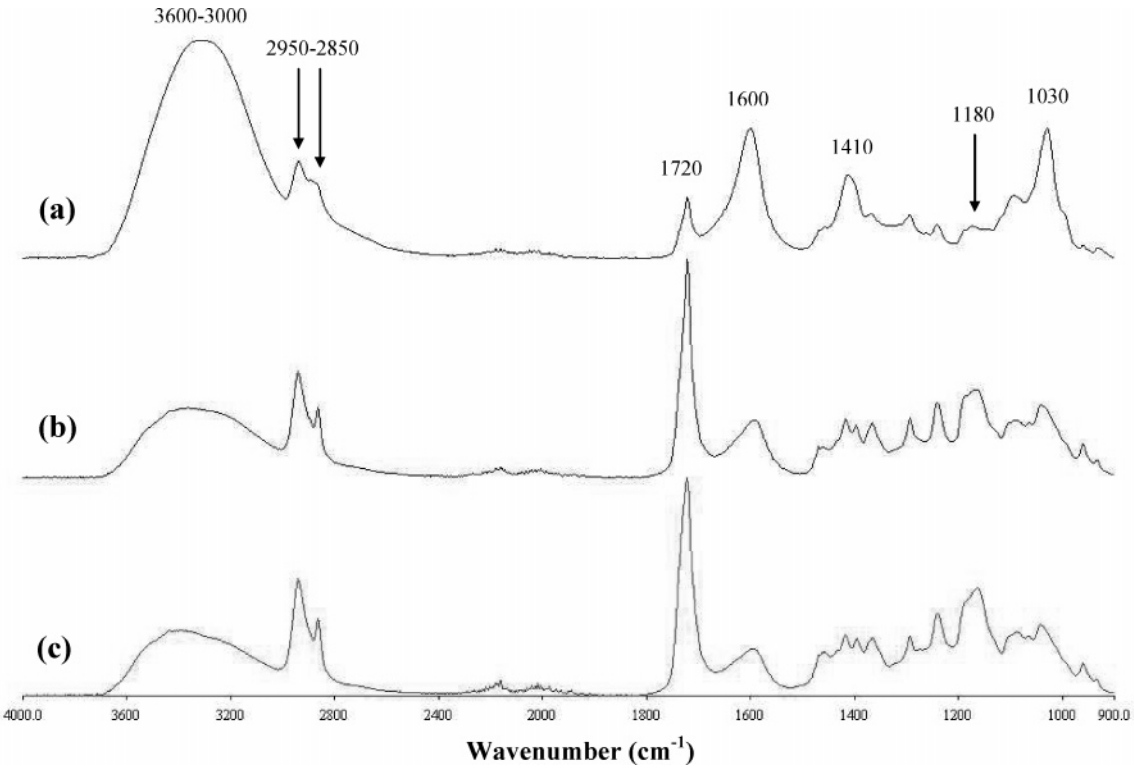

Figure 3. FTIR spectra of untreated (**a**), 2% $CaCl_2$-treated (**b**), and 20% $CaCl_2$-treated (**c**) poly(ϵ-caprolactone)/alginate/glycerol films.

indicates that ionotropic gelation increased the extent of polymer−polymer interactions via hydroxyl and carbonyl moieties, which was expected to enhance film insolubility (*11*, *12*, *35*). Wang et al. (*12*) observed a similar evolution and showed that chitosan−alginate coacervates presented IR bands with higher intensities as films were kept in wetter conditions. Consequently, a significant reduction of O−H stretching band intensity can be related to a reduction of polymer−water interactions via alginate cross-linking that increases polymer intermolecular hydrogen bonding and consequently reduces its susceptibility to hydration. A similar explanation was postulated by Le Tien et al. (*20*), relative to the affinity of cellulose-based films for water.

SEM Analysis. SEM photographs of untreated EO-free films (control), films treated with $CaCl_2$ (2%), and films treated with $CaCl_2$ (20%) are presented in **Figure 4**. Two magnifications

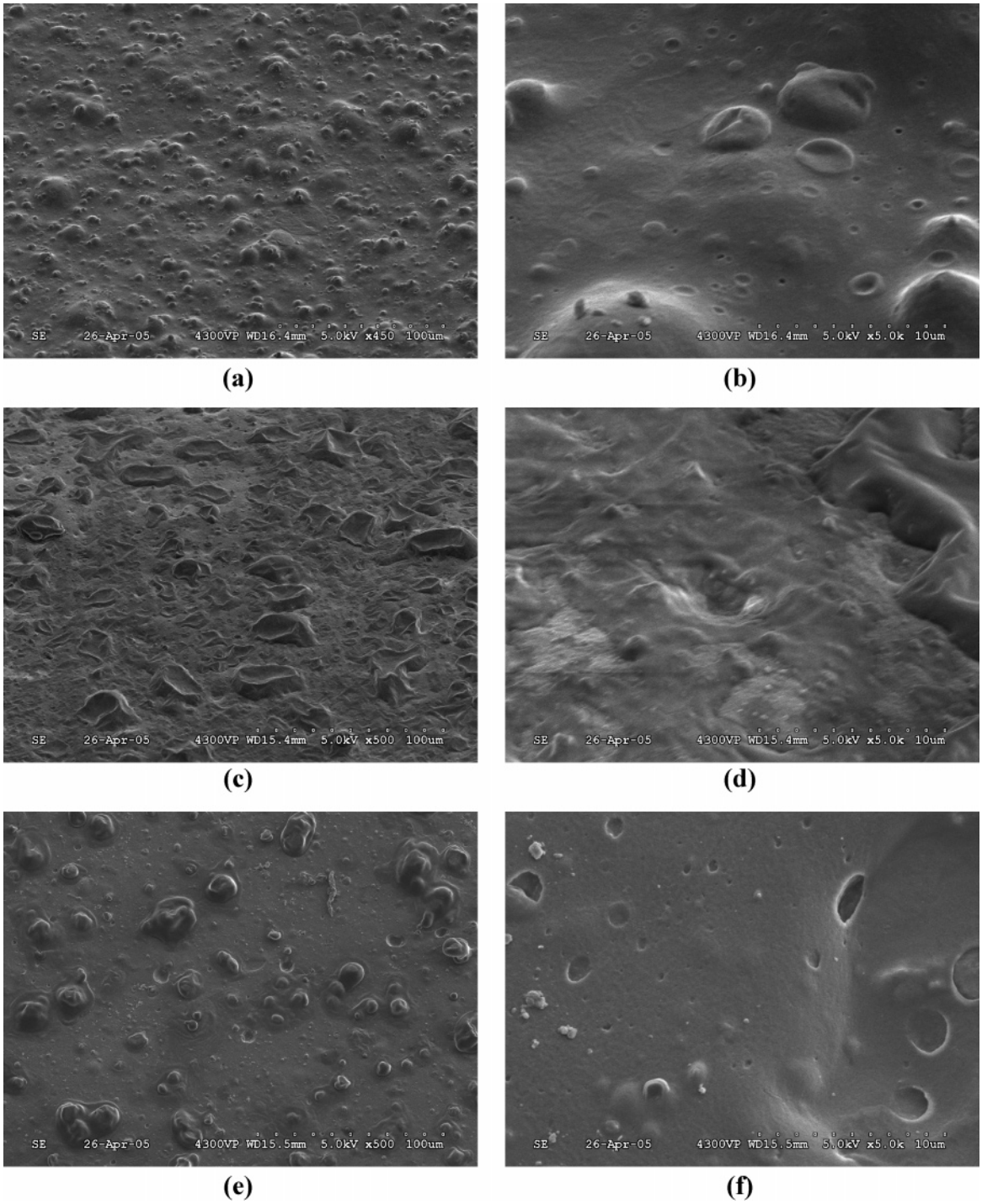

Figure 4. Surface morphology of alginate/PCL-based films. Untreated EO-free films (control): (**a**) magnification 500×; (**b**) magnification 5000×. EO-free films treated with in $CaCl_2$ (2%): (**c**) magnification 500×; (**d**) magnification 5000×. EO-free films treated in $CaCl_2$ (20%): (**e**) magnification 500×; (**f**) magnification 5000×.

(500× and 5000×) were used in order to evaluate the microscopic appearance of films following the cross-linking of alginate. Parts **a** and **b** of **Figure 4** show SEM photographs of the surface of untreated films (500× and 5000×). These photos suggest a smooth undulating surface of the polymeric matrix that exposes slight granulations dispersed homogeneously. The untreated films were the most visually transparent films. Parts **c** and **d** of **Figure 4** show photographs of the surface of films treated with $CaCl_2$ (2%). It is obvious that the surface smoothness is significantly affected after treatment with calcium, showing a heterogeneous and amorphous granular dispersion. The surface of these films appears to be rougher because they contain parts of the polymer blend both cross-linked and solubilized, implying a disorganized state and a low adhesion between cross-linked alginate and PCL. Parts **e** and **f** of **Figure 4** present photographs of the film surface treated with $CaCl_2$ (20%). The different physical appearance of these films is characterized by a more condensed and more regular smooth surface, with less interspersed granulations, as compared to untreated films and films treated with $CaCl_2$ (2%). It is important to note the correlation between the photographs and the PS results since the heterogeneous and uncoordinated surface of films treated with $CaCl_2$ (2%) is in agreement with the lower PS of these films, as compared to untreated films and films

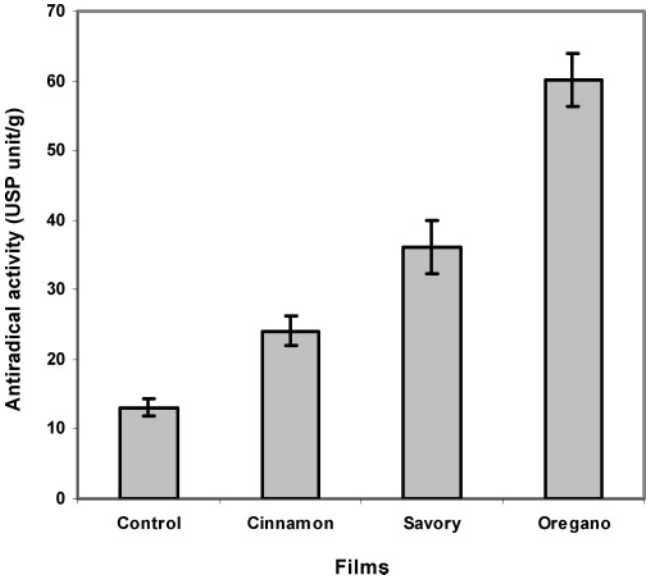

Figure 5. Antiradical activity of films as affected by incorporation of EOs.

treated with $CaCl_2$ (20%). Furthermore, the evaluation of physicochemical properties of EO-free films showed a significant ($p \leq 0.05$) decrease of solubility, deformability, and elasticity of these films by increasing the $CaCl_2$ concentration, which is directly related to an increase of cross-linking density (*23*). These observations confirm the competition that occurs between the kinetics of film hydration and that of cross-linking, as previously supported for PS results.

Antiradical Properties. The antiradical properties of water-soluble films are presented in **Figure 5**. The antiradical properties of films decreased significantly ($p \leq 0.05$) in the order oregano > savory > cinnamon > control, showing respective values of 60.1, 36.1, 24.1, and 13.1 USP units/g. Hence, incorporation of EOs increased the antiradical properties of the control films significantly ($p \leq 0.05$). As a result, it can be assessed that oregano-based films presented a high antiradical activity (3.0 times higher than ascorbic acid) and savory-based films exhibited a lower antiradical activity (1.8 times higher than ascorbic acid) whereas cinnamon films were the least antiradical films (only 1.2 times higher than ascorbic acid).

According to Škerget et al. (*36*), the effect of natural antioxidants on lipid molecules is influenced by the system (composition of the oil/emulsion), the hydrophobicity/hydrophilicity ratio (related to the polarity of components), and the total number and location of hydroxyl groups or aromatic rings. EOs are complex mixtures constituted by numerous components with different polarities, and this complexity often makes it difficult to explain the activity pattern (*9*). Many reports on the antioxidant potentials of the EOs refer to concepts such as synergism, antagonism, and additivity (*37*). It should be noted that EOs are stabilized in an oil-in-water emulsion system, the active surface being oriented to the oil−water interface (*38*). However, the complex interfacial affinities between air−oil and oil−water interfaces involved in the film matrix could influence the evaluation of the activity of films containing EOs, as compared to that of EOs alone (*39*).

Radonic and Milos (*40*) observed that antiradical properties of EOs are related to their chemical composition in free volatile compounds. The high antiradical activity of oregano and savory films may be attributed primarily to the high content of phenolic components present in EOs used in this study (*37*). Indeed, the chemical composition of the EOs of oregano and savory provided by the manufacturer indicates that they are rich in carvacrol and thymol (80.0% of total content for oregano and 48.4% for savory), followed by their biosynthetic precursors *p*-cymene and γ-terpinene (7.0% of total content for oregano and 20.3% for savory). The free radical-scavenging ability of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (*41*). Botsoglou et al. (*42*) reported that carvacrol and thymol, the two main phenols that constitute the major fraction of oregano and savory EOs, have antioxidant ability. Therefore, the high antiradical activity of these EOs can be mainly related to the high content of these components. They also observed that other minor constituents such as the two monoterpenic hydrocarbons *p*-cymene and γ-terpinene may contribute to this activity (*43*). Moreover, it has been established that inhibition of oxidation by the EOs was highly dependent on the content of both carvacrol and thymol (*44*). Consequently, these facts could explain the observation that oregano-based films possess higher antiradical properties than savory-based films. Many studies have also reported the strong antioxidant effect of oregano EOs on the process of oxidation in different food systems (*45*). Although cinnamaldehyde, the primary constituent of cinnamon EO, has long been recognized for its antimicrobial properties (*46*), some authors have recently reported that it also possessed a significant antioxidant activity in several food models (*47*). However, studies on selected spices showed the most effective compounds generally appear to be those with a phenolic structure, such as eugenol, carvacrol, or thymol (*10*, *40*), which could explain the weak antiradical activity of cinnamon-based films as compared to oregano- and savory-based films.

In summary, homogenization of film-forming solution was achieved to stabilize the dispersion of EOs into the matrix and therefore to improve the appearance of films. However, the incorporation of EOs in alginate/PCL produced films that were less resistant to puncture but without decreasing their deformation to puncture and their viscoelasticity despite the cross-linking treatment used. Hence, this suggests that EOs can act as plasticizers in addition to their bioactive role. In order to improve their physicochemical properties, cross-linking of films by $CaCl_2$ (20%) decreased their solubility, their puncture deformation, and their viscoelasticity. However, in the presence of savory EO, only the puncture strength was enhanced by treatment with $CaCl_2$ (20%). Also, the addition of EOs in films increased their radical-scavenging properties significantly. Oregano-based films exhibited the most effective antiradical properties. Therefore, the encapsulation of oregano EO in film formulations could be further explored in food applications to prevent lipid oxidation in food systems.

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