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Mechanical, Barrier, and Antimicrobial Properties of Apple Puree Edible Films Containing Plant Essential Oils

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Edible films, as carriers of antimicrobial compounds, constitute an approach for incorporating plant essential oils (EOs) onto fresh-cut fruit surfaces. The effect against *Escherichia coli* O157:H7 of oregano, cinnamon, and lemongrass oils in apple puree film-forming solution (APFFS) and in an edible film made from the apple puree solution (APEF) was investigated along with the mechanical and physical properties of the films. Bactericidal activities of APFFS, expressed as BA_{50} values (BA_{50} values are defined as the percentage of antimicrobial that killed 50% of the bacteria under the test conditions) ranged from 0.019% for oregano oil to 0.094% for cinnamon oil. Oregano oil in the apple puree and in the film was highly effective against *E. coli* O157:H7. The data show that (a) the order of antimicrobial activities was oregano oil > lemongrass oil > cinnamon oil and (b) addition of the essential oils into film-forming solution decreased water vapor permeability and increased oxygen permeability, but did not significantly alter the tensile properties of the films. These results show that plant-derived essential oils can be used to prepare apple-based antimicrobial edible films for various food applications.

KEYWORDS: Apple puree film; plant essential oils; physicochemical properties; antimicrobial activity; *Escherichia coli* O157:H7

INTRODUCTION

Epidemiological studies indicate that *Escherichia coli* serotypes are responsible for about 100,000 outbreaks of foodborne illness in the United States each year, resulting in about 110 fatalities, with the O157:H7 serotype accounting for the greatest proportion of cases (*1*, *2*). These data suggest the need to protect the food against contamination as well as the consumer against infection by foodborne pathogenic bacteria.

The increase in consumption of fresh-cut produce has resulted in frequent outbreaks of illness associated with raw fruits and vegetables (*3*, *4*). During minimal processing, spoilage and pathogenic microorganisms can gain access to the nutrients inside fruits and multiply (*5*−*7*). The presence of *E. coli* O157:H7 on the surface of fruits may adversely affect the safety of fresh and fresh-cut fruit. Controlling the numbers and the growth of pathogenic bacteria is a challenging problem for the food-processing industry (*8*). The use of edible films and coatings for a wide range of food products, including fresh and minimally processed vegetables and fruits, has received increasing interest because films can serve as carriers for a wide range of food additives, including antimicrobials (*9*). Incorporating antimicrobial compounds into edible films or coatings provides a novel way to enhance the safety and shelf life of ready-to-eat foods (*10*). Essential oils (EOs) have been extensively evaluated for their abilities to protect food against pathogenic bacteria contaminating apple juice (*15*) and other foods (*11*). They are also used as flavoring agents in baked goods, sweets, ice cream, beverages, and chewing gum (*26*) and are designated as Generally Regarded as Safe (GRAS) (*11*). EOs are regarded as alternatives to chemical preservatives, and their use in foods meets the safety demands of consumers for mildly processed natural products, as reviewed by Burt (*11*). The antimicrobial activity of EOs is associated with the terpenoid and phenolic components of the oils (*11*).

To assess the antimicrobial effectiveness of natural compounds and plant extracts, we previously evaluated the bactericidal activities of about 200 plant essential oils, oil compounds, phenolic compounds, and flavonoids against major foodborne pathogenic bacteria including antibiotic-resistant bacteria (*12*−*16*). Several of these compounds were previously also found to be active in apple juice (*15*).

The physicochemical properties of edible films (color, tensile strength, water vapor, and oxygen permeability) relate to coating enhancement of the mechanical integrity of foods, inhibition of moisture loss and oxidative rancidity, and final-product

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appearance (*17*). Combined analysis of antimicrobial and physicochemical properties is crucial for predicting the behavior of antimicrobial edible films (*10*, *18*). No prior research has been reported on the antimicrobial effects against *E. coli* O157:H7 of essential oil containing apple puree edible films. The objectives of this study were (a) to determine antimicrobial activities against the *E. coli* O157:H7 of apple puree film-forming solutions used in the preparation of films, and of apple puree films, both of which contain select essential oils and oil compounds, and (b) to evaluate the effects of added natural antimicrobials on changes in the physicochemical properties of the films.

MATERIALS AND METHODS

Test Compounds. Golden Delicious apple puree (38 °Brix) (Sabroso Co., Medford, OR) was the primary ingredient in all apple-based film-forming solutions (APFFS) and edible films (APEF). Glycerol was added as a plasticizing agent (Fisher Scientific, Waukesha, WI). Ascorbic acid (BASF, Mount Olive, NJ) and citric acid (Archer Daniels Midland, Decatur, IL) were utilized as browning inhibitors. High-methoxyl pectin (Systems BioIndustries, Fair Lawn, NJ) was added to films to assist in film release from the cast surface. Oregano oil (from *Origanum vulgare*), lemongrass oil (from *Cymbopogon citratus*), and cinnamon oil (from *Cinnamomum cassia*) were the EOs tested and were obtained from Lhasa Kamash Herb Co. (Berkeley, CA).

Preparation of APFFS. APFFS (26% w/w) (260 g of 38 °Brix apple puree plus 700 g of 3% w/w pectin solution) was prepared according to the method of McHugh and Senesi (*19*). This solution also contained 5 g of ascorbic and citric acids (0.5% w/w) and 30 g of glycerol (3% w/w). Pectin was added to increase film strength. Natural antimicrobial essential oils (oregano, lemongrass, and cinnamon) were then incorporated into APFFS at the following concentrations: 0 (control), 0.05, 0.075, 0.1, and 0.5% (w/w). These solutions were homogenized for 3 min at 12,500 rpm using a Polytron 3000 homogenizer (Kinematica, Littau, Switzerland) and then used for bactericidal studies and casting the films.

Preparation of APEF. Apple puree edible film-forming solutions were prepared as described previously. Vacuum was then applied to remove bubbles. Films were then cast on 29 × 29 cm square plates and dried at ambient conditions for ~24 h. Dried films were cut and peeled from the casting surface. These samples were used for determinations of physicochemical and antimicrobial properties of the films.

Test Buffers. Phosphate-buffered saline (PBS, pH 7.0) was prepared by mixing dibasic sodium phosphate (100 mM) and monobasic sodium phosphate (100 mM) at a 2:1 ratio, diluting by half with H_2O, and adding NaCl (150 mM). For lower pH buffers (saline solutions), 2 mM citric acid−150 mM NaCl was adjusted to pH 3.3−3.7 with 1 N HCl.

Bactericidal Assays of APFFS. The source of *E. coli* O157:H7 used in this study is given in ref *12*. To facilitate pipetting, the 26% APFFS solution was further diluted by half with pH 3.3 saline solution (v/v). This APFFS sample was used to prepare suspensions of APFFS for the assay. Oregano oil (10 μL) was added to 9.99 mL of diluted APFFS. Lemongrass oil or cinnamon oil (50 μL) was added to 9.95 mL of diluted APFFS in 50 mL tubes. The tubes were warmed in a microwave oven for 10 s and then shaken to form uniform suspensions. The contents of the tubes were then diluted as follows: saline solution (500 μL) was added to five sterile 1.9 mL tubes. Serial dilutions were made starting with 1 mL of each original test solution, using 500 μL for each transfer for a total of five dilutions. Microtiter plates with 96 wells (Nalge, Rochester, NY) were prepared with saline pH 3.3 negative controls (100 μL each in 6 wells) and three test substances with five dilutions plus the test solution (100 μL of each dilution per well, 6 wells). Each of these 24 wells were sampled at three time intervals: 3, 30, and 60 min at 21 °C. Bactericidal assays were then carried out in duplicate using previously described procedures (*12*, *14*).

Bactericidal Activities (BA_{50} Values). Bactericidal activity, defined as the percentage of test compound that kills 50% of the bacteria under the test conditions, was determined as follows. Each compound was tested at a series of dilutions. The control pH 3.3 saline diluent was matched with the pH of APFFS. The colony-forming unit (CFU) values from all experiments were transferred to a Microsoft Excel 8.0 spreadsheet. The number of CFUs from each dilution was matched with the average control value to determine the percentage of bacteria killed per well. Each of the dose−response profiles (percent of test compound versus percent of bactericidal activity) was examined graphically, and the BA_{50} values were estimated by linear regression. The lower the BA_{50} value, the higher the antimicrobial activity.

Antimicrobial Activity of APEF. Disk inhibition zone assays were performed as a qualitative test for antimicrobial activity of the films. APEFs with and without EOs (control) were aseptically cut into 12 mm diameter disks and then placed on MacConkey−Sorbitol agar (Biokar Diagnostics, Beauvais, France) plates for *E. coli* O157:H7, which had been previously spread with 0.1 mL of inoculum containing 10^5 CFUs/mL. Plates were incubated at 37 °C for 48 h. The thickness (millimeters) of the inhibition zone around the film disk (colony-free perimeter) was measured with a millimeter scale, and the growth below the film disks (the contact area of edible film with agar surface) was visually examined. Tests were done in duplicate.

Film Thickness. Film thicknesses were measured with a micrometer IP 65 (Mitutoyo Manufacturing, Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in.) at five random positions around the film. The mean value was used to calculate water vapor permeability (WVP), oxygen permeability (O_2P), and tensile strength.

WVP of Films. The gravimetric Modified Cup method based on ASTM E96-92 (*20*) was used to determine WVP. A cabinet (Thermo Electron Corp., Waltham, MA) with a variable-speed fan was used to test film WVP at 25 ± 1 °C. Fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity throughout the cabinets. The cabinets were pre-equilibrated to 0% relative humidity (RH) using anhydrous calcium sulfate (W. A. Hammond Drierite, Xenia, OH). Circular test cups made from polymethylmethacrylate (Plexiglas) were used. A film was sealed to the cup base with a ring containing a 19.6 cm^2 opening using four screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed at the bottom of the test cups to expose the film to a high percentage of RH inside the test cups. The average stagnant air gap heights between the water surface and the film were measured. Test cups, holding the films, were then inserted into the pre-equilibrated 0% RH desiccator cabinets. Steady state of water vapor transmission rate was achieved within 2 h. Each cup was weighed eight times at 2 h intervals. Eight replicates of each film were tested. Relative humidities at the film undersides and WVPs were calculated using the WVP correction method (*20*).

The WVP of the films was calculated by multiplying the steady-state water vapor transmission rate by the average film thickness, determined as described above, and dividing by the water vapor partial pressure difference across the films

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times \mathrm{thickness}}{p_{A1} - p_{A2}} \quad (1)$$

where WVTR = water vapor transmission rate and p_{A1} and p_{A2} = partial pressure of water vapor inside and outside the cup, respectively.

O_2P of Films. An Ox-Tran 2/20 ML modular system (Modern Controls Inc., Minneapolis, MN) was utilized to measure oxygen transmission rates through the films (standard method D3985) (*21*). Oxygen transmission rates were determined at 23 °C and 50 ± 1% RH. Each film was placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into the test cell and exposed to 98% N_2 + 2% H_2 flow on one side and pure oxygen flow on the other. The system was programmed to have a 10 h waiting period to allow the films to achieve equilibrium. Oxygen permeability was calculated by dividing the O_2 transmission rate by the difference in O_2 partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness measured at five random places. Four replicates of each film were evaluated.

Tensile Properties of Films. Standard method D882-97 (*22*) was used to measure the tensile properties of films. Films were cut into strips with a test dimension of 165 mm × 19 mm (standard method

Table 1. Bactericidal Activities (BA_{50} Values) of Essential Oils against *E. coli* O157:H7 in Apple Puree Film Forming Solution (APFFS)[a] Incubated for 3, 30, and 60 min at 21 °C

essential oil (% w/w) in 50% APFFS	BA_{50} value for *E. coli* O157:H7[b] 3 min	30 min	60 min
oregano, 0.1%	0.034 ± 0.01	0.024 ± 0.007	0.019 ± 0.004
cinnamon, 0.5%	>0.34[c]	0.12	0.094 ± 0.04
lemongrass, 0.5%	0.28 ± 0.03	0.078 ± 0.02	0.059 ± 0.005

[a] APFFS is 50% apple puree film formula suspension in saline pH 3.7 buffer. [b] Average values and standard deviations of two replicates of BA_{50} values. [c] > signifies that <50% of bacteria were killed at the highest dose used.

D638-02a) (*23*). Before testing, all of the films were conditioned for 48 h at 23 ± 2 °C and 50 ± 2% RH using a saturated salt solution of magnesium nitrate (Fisher Scientific, Fair Lawn, NJ). The ends of the equilibrated strips were mounted and clamped with pneumatic grips on an Instron Universal Testing Machine (model 55R4502, Instron, Canton, MA) with a 100 N load cell. The initial gauge length was set to 100 mm, and the films were stretched using a crosshead speed of 7.5 mm/min. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron, Canton, MA). Fifteen specimens of each type of film were evaluated.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) using Minitab (version 13.31) software (Minitab Inc., State College, PA). Tukey's test was used to determine the differences at 5% significance level (*24*).

RESULTS AND DISCUSSION

Antimicrobial Activity of Plant EOs in APFFS. Table 1 lists the experimental BA_{50} values for EOs at three time periods, 3, 30, and 60 min. All compounds inhibited the growth of *E. coli* O157:H7. APFFS in saline pH 3.3, without EOs and containing ascorbic acid and citric acid as antibrowning agents, was not effective against the pathogen.

Oregano oil at a concentration of 0.1% in APFFS was effective at 3 min with a BA_{50} value of 0.034 (0.034% of oregano oil inhibited 50% of the *E. coli* O157:H7 after 3 min). The activity at 30 and 60 min was slightly greater (BA_{50} = 0.024 and 0.019%, respectively) than the activity at 3 min (**Table 1**). Thus, oregano oil appears to be a highly potent antimicrobial against *E. coli* O157:H7. In contrast, cinnamon oil at a concentration of 0.5% in APFFS was effective at only 30 and 60 min with BA_{50} values of 0.12 and 0.094%, respectively. These results indicate that cinnamon oil at a 5-fold greater concentration was less effective against *E. coli* O157:H7 than oregano oil.

Table 1 also shows that the activity of lemongrass oil against *E. coli* O157:H7 at a concentration of 0.5% in APFFS is similar to that of cinnamon oil. The BA_{50} value of 0.059 at 60 min means that 0.059% of lemongrass oil inhibited 50% of the bacteria under the test conditions. Compared to oregano oil, it took about a 5 times higher concentration of cinnamon or lemongrass oils to achieve the same activity against *E. coli* O157:H7.

It is also instructive to compare the activities of the same compounds in the APFFS to activities previously observed in "cloudy" apple juice (*15*). The comparison against *E. coli* shows that (a) the BA_{50} values at 60 min and 21 °C for oregano oil, cinnamon oil, and lemongrass oil (0.019, 0.094, and 0.059%, respectively) in apple puree were similar to those in apple juice following incubation at 37 °C for 60 min and (b) although *E. coli* O157:H7 resists inactivation in acidic pH (*25*), the low pH of both apple juice (~3.7) and the apple puree (~3.3) does not seem to contribute significantly to the antimicrobial effects of the test substances.

Table 2. Antibacterial Activity of Essential Oils Incorporated into Apple Puree Edible Films against *E. coli* O157:H7

essential oil	concn (% w/w)	*E. coli* O157:H7 inhibitory zone[a] (mm)	inhibitory effect[b]
oregano	0 (control)	0	–
	0.05	<1	+
	0.075	1.2	+
	0.1	1.4	+
cinnamon	0 (control)	0	–
	0.05	0	+
	0.075	<1	+
	0.1	<1	+
	0.5	1.1	+
lemongrass	0 (control)	0	–
	0.05	0	–
	0.075	0	–
	0.1	<1	+
	0.5	1.2	+

[a] Values are measurements of thickness (mm) of inhibitory zone (colony-free perimeter). [b] +, inhibitory effect; –, no inhibitory effect.

Antibacterial Activity of Plant EOs in APEF. Antibacterial activities of APEF with EOs are shown in **Table 2**. The inhibitory activities were based on the measurement of clear inhibition zones surrounding film disks. If surrounding clear zones were not present, it was assumed that the compound was not inhibitory and the diameter was assigned as zero. APEF without EOs served as control to determine any possible antimicrobial effect of the normal film. The control film did not inhibit *E. coli* O157:H7.

As with APFFS, in APEFs, oregano oil was once again the most effective compound against *E. coli* O157:H7. The inhibitory zone increased with increasing concentration of oregano oil in the films (**Table 2**). **Figure 1** shows the inhibitory effect of APEF against *E. coli* O157:H7 with 0.1% oregano oil and without the oil. The figure shows that the film without the oil had no effect on the bacteria. In contrast, a film containing 0.1% oregano oil completely killed all of the surrounding bacteria. Previously, it was suggested that diffusion of antimicrobials from a film disk depends on the size, shape, polarity of the diffusing molecule, chemical structure of the film, and degree of molecular cross-linking (*10*). We do not know whether these factors also govern antimicrobial properties of fruit and vegetable films.

In contrast, inhibition of *E. coli* O157:H7 by cinnamon oil and lemongrass oil at concentrations of <0.5% in the films was lower than those observed with oregano oil (**Table 2**). However, the inhibitory effect increased when the concentration of both EOs (cinnamon and lemongrass) was increased to 0.5%. Compared to oregano oil, it took about a 5 times higher concentration of cinnamon or lemongrass oils to achieve the same activity against *E. coli* O157:H7. As cinnamon oil is added to numerous commercial foods (*27*), for its pleasant flavor, and is GRAS-listed (*28*), the compound merits use in antimicrobial edible films.

WVP. McHugh (*29*) developed the first edible films made from fruit purees, characterizing their permeability properties. Because apple-based edible films were not very good moisture barriers, addition of lipids was necessary to improve the water barrier properties. In this study, it was observed that all of the WVP values decreased when the fraction of the hydrophobic

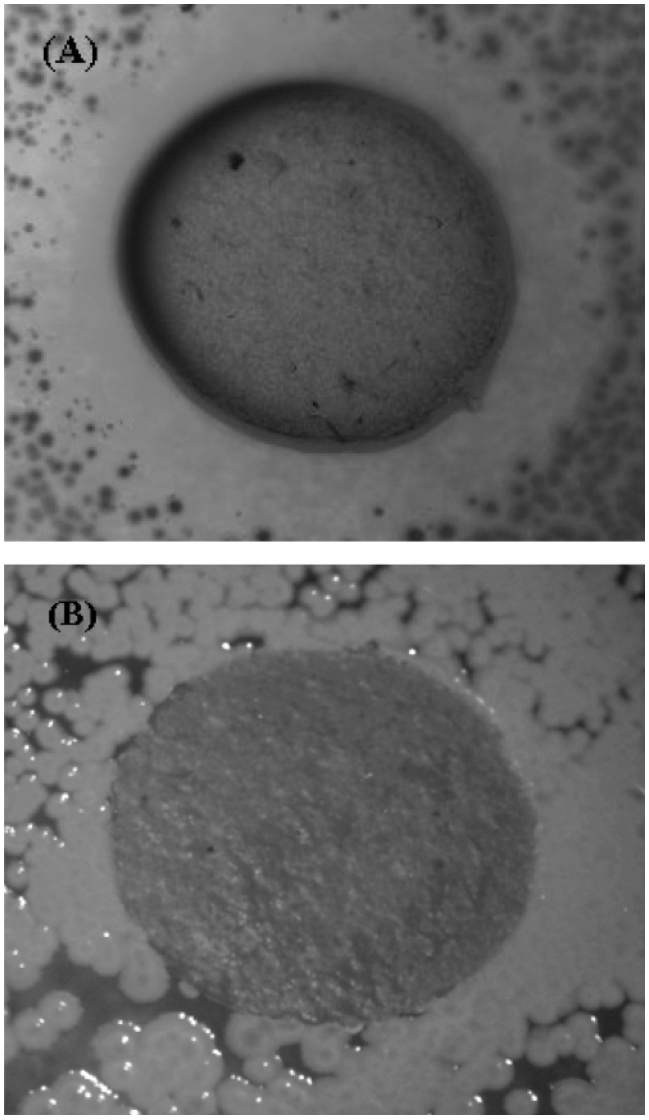

Figure 1. Inhibitory zone (*E. coli* O157:H7 colony-free perimeter) of apple puree edible films containing 0.1% oregano oil (**A**) compared to control without oregano oil (**B**).

compounds (EOs) increased. This effect was more prominent with oregano oil. At the maximum concentration (0.1% w/w), oregano oil induced a significant ($p < 0.05$) decrease in film WVP (**Table 3**).

Water vapor transfer generally occurs through the hydrophilic portion of the film and therefore depends on the hydrophilic−hydrophobic ratio of the film constituents (*30*). Because each hydrophobic substance has unique physicochemical properties, films based on lipids have variable behavior against moisture transfer. In this study, the behavior observed in films containing different EOs might be due to differences in the hydrophobicity−hydrophilicity parts of each molecule (*31*).

Our results suggest that oregano oil may have a dual benefit. It can be used both to impart antimicrobial activities and to enhance barrier properties of the films.

O_2P. APEF is a good oxygen barrier, exhibiting values of 22.64 ± 1.28 cm^3-μm/m^2-d-kPa. This result agrees with earlier observations (*29*). The O_2P values increased with increasing amounts of EOs in the films. Hence, a significant difference ($p < 0.05$) was observed with oregano oil (0.1% w/w), reaching values of 38.12 ± 0.80 cm^3-μm/m^2-d-kPa compared to the control value of 22.64 ± 1.28 cm^3-μm/m^2-d-kPa (**Table 3**).

Table 3. Effect of Concentration of Essential Oils on Water Vapor Permeability (WVP) and Oxygen Permeability (O_2P) Properties of Apple Puree Edible Films

essential oil	essential oil concn (% w/w)	thickness[a] (mm)	RH inside cup[a,b] (%)	WVP[a,b] (g-mm/kPa-h-m^2)	O_2P[a] (cm^3-μm/m^2-d-kPa)
control	0	0.153ab	62.6ab	7.04 ± 0.63b	22.64 ± 1.28a
oregano	0.05	0.142ab	60.3a	7.06 ± 0.18b	32.82 ± 0.79c
	0.075	0.152ab	63.7b	6.67 ± 0.57ab	33.72 ± 0.80c
	0.1	0.135a	62.8ab	6.17 ± 0.56a	38.12 ± 0.80d
lemongrass	0.05	0.140ab	61.7ab	6.71 ± 0.33ab	28.92 ± 1.57bc
	0.075	0.148ab	63.4ab	6.59 ± 0.58ab	26.83 ± 0.84b
	0.1	0.150ab	62.5ab	6.92 ± 0.29ab	34.24 ± 1.55cd
	0.5	0.150ab	63.7b	6.62 ± 0.86ab	30.25 ± 0.78bc
cinnamon	0.05	0.155b	61.4ab	7.48 ± 0.42b	30.52 ± 0.79c
	0.075	0.145ab	62.0ab	6.83 ± 0.41ab	28.21 ± 0.63bc
	0.1	0.143ab	62.2ab	6.74 ± 0.83ab	31.72 ± 0.99c
	0.5	0.152ab	63.3ab	6.82 ± 0.85ab	32.08 ± 0.50c

[a] Thickness and RH data are mean values. WVP and O_2P data are mean values ± standard deviations. Means in same column with different letters are significantly different ($p < 0.05$). [b] Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP correction nethod (*16*).

Table 4. Effect of Concentration of Essential Oils on the Tensile Properties of Apple Puree Edible Films

essential oil	essential oil concn (% w/w)	tensile strength[a] (MPa)	elongation[a] (%)	elastic modulus[a] (MPa)
control	0	0.64 ± 0.017b	25.4 ± 2.1ab	5.06 ± 0.54ab
oregano	0.05	0.61 ± 0.04b	27.4 ± 2.2b	5.47 ± 0.64b
	0.075	0.61 ± 0.03b	27.4 ± 3.7b	4.41 ± 0.40ab
	0.1	0.62 ± 0.02b	26.5 ± 2.0b	4.73 ± 0.32ab
lemongrass	0.05	0.65 ± 0.03b	25.8 ± 1.0ab	5.18 ± 0.33ab
	0.075	0.57 ± 0.06ab	24.2 ± 2.6ab	5.58 ± 1.03b
	0.1	0.54 ± 0.02a	23.5 ± 1.5ab	4.01 ± 0.70a
	0.5	0.63 ± 0.02b	24.8 ± 1.9ab	4.49 ± 0.53a
cinnamon	0.05	0.72 ± 0.05c	25.2 ± 2.6ab	7.60 ± 0.59c
	0.075	0.79 ± 0.09c	26.1 ± 2.2b	7.00 ± 1.03c
	0.1	0.75 ± 0.05c	25.3 ± 2.6ab	5.65 ± 0.37b
	0.5	0.61 ± 0.04ab	22.6 ± 1.8a	4.04 ± 0.39a

[a] Tensile strength, elongation, and elastic modulus data are mean values ± standard deviations. Means in same column with different letters are significantly different at $p < 0.05$.

Nonpolar materials such as lipids act as excellent moisture barriers, but are less effective gas barriers. McHugh and Krochta (*18*) indicated that films containing lipids exhibit relatively poor oxygen barrier properties. The chemical nature of oil plays a major role in the barrier properties of edible films.

Tensile Properties. Tensile strength expresses the maximum stress developed in a film during tensile testing (*32*). The tensile properties of the APEF with and without EOs are summarized in **Table 4**.

Lemongrass oil and cinnamon oil had different effects on the tensile strength of the films. Compared to the control films without added EOs, increasing the concentration of lemongrass oil from 0.05 to 0.1% w/w caused a significant reduction ($p < 0.05$) in tensile strength. In contrast, addition of the same amount of cinnamon oil increased the tensile strength (**Table 4**). The difference between the two films may due to difference in polarities of the added compounds. High concentrations (0.5% w/w) of these oils added tensile strength to control films. These results agree with those obtained by Pranoto (*9*) for physical and antibacterial properties of an alginate edible film containing garlic oil.

Elongation at break is a measure of the film's stretchability prior to breakage (*33*). Generally, the presence of EOs did not significantly change the elongation of the films. However, at a level >0.5% (w/w), cinnamon oil significantly reduced ($p < 0.05$) the elongation at break value (**Table 4**).

No significant differences were observed in the elastic modulus between films with and without oregano or lemongrass oils ($p < 0.05$). However, a significant increase in the elastic modulus of the film was noted at a concentration of 0.075% cinnamon oil (**Table 4**). In related studies, Zivanovic (*34*) noted a decrease in tensile strength and an increase in elongation of chitosan films enriched with essential oils. Begin and Van Calsteren (*35*) noted that an increased molecular weight of the counterion resulted in thicker and more elastic but weaker chitosan films.

In conclusion, the antimicrobial activity of oregano oil in apple puree edible films and film-forming solutions against *E. coli* O157:H7 was significantly greater than the activities of cinnamon oil and lemongrass oil. There was no adverse effect of additives on water vapor permeability properties. The antimicrobial films showed good oxygen barrier properties. The tensile strength of the films containing certain levels of antimicrobials did not differ significantly from control films without added antimicrobials. The antimicrobial activity data obtained with the APFFS (BA_{50} values) can serve as a guide for the selection of appropriate levels of plant compounds for incorporation into antimicrobial edible films. Our ultimate goal is to develop commercially viable technologies for the manufacture of fruit- and vegetable-based films incorporating antimicrobial phytochemicals in an efficient manner for protection of *E. coli* O157H7 contaminated foods such as fresh-cut salad mixes. Future research could be conducted to evaluate the sensory aspects of using these natural essential oil compounds in edible films and coatings, as well as to characterize their stability.

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LITERATURE CITED

(1) Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M.; Tauxe, R. V. Food-related illness and death in the United States. *Emerg. Infect. Dis.* **1999**, *5*, 607−625.

(2) Smith, J. L.; Fratamico, P. M. Diarrhea-inducing *Escherichia coli*. In *Foodborne Pathogens—Microbiology and Molecular Biology*; Fratamico, P. M., Bhunia, A. K., Smith, J. L., Eds.; Caister Academic Press: Norfolk, U.K., 2005; Chapter 18, pp 357−382.

(3) Brackett, R. E. Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biol. Technol.* **1999**, *15*, 305−311.

(4) Thunberg, R. L.; Tran, T. T.; Bennett, R. W.; Matthews, R. N.; Belay, N. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot.* **2002**, *65*, 677−682.

(5) Del Rosario, B. A.; Beuchat, L. R. Survival and growth of enterohemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. *J. Food Prot.* **1995**, *58*, 105−107.

(6) Dingman, D. W. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. *Appl. Environ. Microbiol.* **2000**, *66*, 1077−1083.

(7) Thunberg, R. L.; Tran, T. T.; Bennett, R. W.; Matthews, R. N.; Belay, N. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot.* **2002**, *65*, 677−682.

(8) Burt, S. A.; Reinders, R. D. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* **2003**, *36*, 162−167.

(9) Pranoto, Y.; Salokhe, V. M.; Rakshit, S. K. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. *Food Res. Int.* **2005**, *38*, 267−272.

(10) Cagri, A.; Ustunol, Z.; Ryser, E. T. Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing *p*-aminobenzoic or sorbic acids. *J. Food Sci.* **2001**, *66*, 865−870.

(11) Burt, S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **2004**, *94*, 223−253.

(12) Friedman, M.; Henika, P. R.; Mandrell, R. E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **2002**, *65*, 1545−1560.

(13) Friedman, M.; Henika, P. R.; Mandrell, R. E. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **2003**, *66*, 1811−1821.

(14) Friedman, M.; Buick, R.; Elliott, C. T. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. *J. Food Prot.* **2004**, *67*, 1774−1778.

(15) Friedman, M.; Henika, P. R.; Levin, C. E.; Mandrell, R. E. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. *J. Agric. Food Chem.* **2004**, *52*, 6042−6048.

(16) Friedman, M.; Henika, P. R.; Levin, C. E.; Mandrell, R. E.; Kozukue, N. Antimicrobial activities of tea catechins and theaflavins and tea extracts against *Bacillus cereus*. *J. Food Prot.* **2006**, *69*, 354−361.

(17) Debeaufort, F.; Quezada-Gallo, J. A; Voilley, A. Edible films and coatings: tomorrow's packagings: a review. *Crit. Rev. Food Sci.* **1998**, *38*, 299−313.

(18) McHugh, T.; Krochta, J. M. Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *J. Agric. Food Chem.* **1994**, *42*, 841−845.

(19) McHugh, T. H.; Senesi, E. Apple wraps: a novel method to improve the quality and extend the shelf life of fresh-cut apples. *J. Food Sci.* **2000**, *65*, 480−485.

(20) McHugh, T. H.; Avena-Bustillos, R.; Krochta, J. M. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *J. Food Sci.* **1993**, *58*, 899−903.

(21) ASTM. Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. D3985-95. In *Annual Book of American Standard Testing Methods*; ASTM: Philadelphia, PA, 1995.

(22) ASTM. Standard test method for tensile properties of thin plastic sheeting. D882-97. In *Annual Book of American Standard Testing Methods*; ASTM: Philadelphia, PA, 1997.

(23) ASTM. Standard test method for tensile properties of plastic. D638-02.a. In *Annual Book of American Standard Testing Methods*; ASTM: Philadelphia, PA, 2002.

(24) SAS. *OnLine Doc*; SAS Institute, Inc.: Cary, NC, 1999.

(25) Roering, A. M.; Luchansky, J. B.; Ihnot, A. M.; Ansay, S. E.; Kaspar, C. W.; Ingham, S. C. Comparative survival of *Salmonella typhimurium* DT 104, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. *Int. J. Food Microbiol.* **1999**, *46*, 263−269.

(26) Fenaroli, G. *Fenaroli's Handbook of Flavor Ingredients*, 3rd ed.; CRC Press: Boca Raton, FL, 1995; 990 pp.

(27) Friedman, M.; Kozukue, N.; Harden, L. A. Cinnamaldehyde content in foods determined by gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 5702−5709.
(28) Adams, T. B.; Cohen, S. M.; Doull, J.; Feron, V. J.; Goodman, J. I.; Marnett, L. J.; Munro, I. C.; Portoghese, P. S.; Smith, R. L.; Waddell, W. J.; Wagner, B. M. The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food Chem. Toxicol.* **2004**, *42*, 157−185.
(29) McHugh, T. H.; Huxsoll, C. C.; Krochta, J. M. Permeability properties of fruit puree edible films. *J. Food Sci.* **1996**, *61*, 88−91.
(30) Hernandez, E. Edible coatings for lipids and resins. In *Edible Coatings and Films To Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M.O., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 279−304.
(31) Roberts, J.; Caserio, M. C. *Basic Principles of Organic Chemistry*; Benjamin: New York, 1965.
(32) Gennadios, A.; Brandenburg, A. H.; Park, J. W.; Weller, C. L.; Testin, R. F. Water vapor permeability of wheat gluten and soy protein isolate films. *Ind. Crops Prod.* **1994**, *2*, 189−195.
(33) Krochta, J. M.; De Mulder-Johnston, C. Edible and biodegradable polymer films: challenges and opportunities. *Food Technol.* **1997**, *51*, 61−72.
(34) Zivanovic, S.; Chi, S.; Draughon, A. F. Antimicrobial activity of chitosan films enriched with essential oils. *J. Food Sci.* **2005**, *70*, M45−M51.
(35) Begin, A.; Van Calsteren, M. R. Antimicrobial films produced from chitosan. *Int. J. Biol. Macromol.* **1999**, *26*, 63−67.

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