Effects of Allspice, Cinnamon, and Clove Bud Essential Oils in Edible Apple Films on Physical Properties and Antimicrobial Activities

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ABSTRACT: Essential oils (EOs) derived from plants are rich sources of volatile terpenoids and phenolic compounds. Such compounds have the potential to inactivate pathogenic bacteria on contact and in the vapor phase. Edible films made from fruits or vegetables containing EOs can be used commercially to protect food against contamination by pathogenic bacteria. EOs from cinnamon, allspice, and clove bud plants are compatible with the sensory characteristics of apple-based edible films. These films could extend product shelf life and reduce risk of pathogen growth on food surfaces. This study evaluated physical properties (water vapor permeability, color, tensile properties) and antimicrobial activities against *Escherichia coli* O157:H7, *Salmonella enterica,* and *Listeria monocytogenes* of allspice, cinnamon, and clove bud oils in apple puree film-forming solutions formulated into edible films at 0.5% to 3% (w/w) concentrations. Antimicrobial activities were determined by 2 independent methods: overlay of the film on top of the bacteria and vapor phase diffusion of the antimicrobial from the film to the bacteria. The antimicrobial activities against the 3 pathogens were in the following order: cinnamon oil > clove bud oil > allspice oil. The antimicrobial films were more effective against *L. monocytogenes* than against the *S. enterica.* The oils reduced the viscosity of the apple solutions and increased elongation and darkened the colors of the films. They did not affect water vapor permeability. The results show that apple-based films with allspice, cinnamon, or clove bud oils were active against 3 foodborne pathogens by both direct contact with the bacteria and indirectly by vapors emanating from the films.

Keywords: allspice, cinnamon, clove bud, edible film, *Escherichia coli* O157:H7, essential oils, *Listeria monocytogenes, Salmonella enterica*

Introduction

Edible films can improve shelf life and food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation, and production of volatile aromas and flavors (Kester and Fennema 1986). For reviews, see Min and others (2005), Serrano and others (2006), Bravin and others (2006), and Jagannath and others (2006). The use of edible films and coatings for food products, including fresh and minimally processed fruits and vegetables, is of interest because films can serve as carriers for a wide range of beneficial food additives, including plant-derived, safe antimicrobials (Pranoto and others 2005). Increased interest in antimicrobial films is the result of increased consumption of contaminated fresh-cut produce. Such consumption has resulted in occasional outbreaks of illness associated with contaminated fruits and vegetables (Brackett 1999; Thunberg and others 2002). For example, the presence of *Escherichia coli* O157:H7 on fruit surfaces may adversely affect the microbial safety of fresh and fresh-cut fruit (Del Rosario and Beuchat 1995; Thunberg and others 2002).

Essential oils (EOs) and oil compounds have been previously evaluated for their ability to protect food against pathogenic bacteria contaminating apple juice (Friedman and others 2004) and other foods (Burt 2004). McHugh and others (1996) developed the 1st edible films made from fruit purees. They found that apple-based edible films are excellent oxygen but not very good moisture barriers. Because addition of EOs and texturing agents such as pectin and alginate may improve the barrier properties of fruit-based films (Mancini and McHugh 2000), novel films can be developed by combining fruit purees with various gelling agents. For example, Rojas-Graü and others (2006, 2007a, 2007b) studied the antimicrobial, mechanical and barrier properties of apple films and coatings formulated with pectin or alginate, as well as application on sliced apples, incorporating EOs and their active antimicrobial compounds. Recently, Du and others (2008b) demonstrated that carvacrol, the main ingredient of oregano oil, in apple films inhibited the growth of *E. coli* O157:H7, even after storage at 5 or 25 °C for 7 wk.

EOs from cinnamon, allspice, and clove bud are compatible with the sensory characteristics of apple-based edible films, while also incorporating functional properties related to potential antibacterial activities and improved barrier and mechanical film behavior. It was of interest to find out whether antimicrobial activities of these EOs in phosphate buffers against foodborne pathogens evaluated by Friedman and others (2002, 2004) would also be active in films prepared from fruits and vegetables (Rojas-Graü and others 2006, 2007a, 2007b; Du and others 2008a, 2008b). The evaluation of antimicrobial effectiveness of EOs in films can be done by different methods depending on relevant applications. The overlay (Janssen and others 1986; Rojas-Graü and others 2006) method can be related to direct contact of the films on food surfaces and

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the vapor phase method (López and others 2007) can be related to inactivation of pathogens from a distance without direct contact of the film with the contaminated food. The objectives of this study were therefore (1) to determine antimicrobial activities of allspice, cinnamon, and clove bud oils added to apple puree film forming solutions used in the preparation of apple puree films against *E. coli* O157:H7, *Salmonella enterica,* and *Listeria monocytogenes*, and (2) to evaluate the effects of the EOs on physicochemical properties of the films.

Materials and Methods

Source of bacteria

The sources of the bacteria used in the present study have been previously described (Friedman and others 2002). Briefly, the Food and Drug Administration (FDA) provided the *E. coli* O157:H7 bacteria (our strain designation RM1484; original designation SEA13B88) isolated from apple juice associated with an outbreak. *S. enterica* serovar Hadar (our strain designation RM1309; original designation strain MH136) was isolated from ground turkey and obtained from the Produce Safety and Microbiology unit at Western Regional Research Center. *L. monocytogenes* was obtained from Univ. of California, Berkeley (our strain designation RM2199; original designation strain F2379) isolated from cheese associated with an outbreak.

Preparation of apple films

The methods we used to prepare the films were adapted from previous studies (Du and others 2008b). These are briefly summarized below.

Apple puree film-forming solution (APFFS)

Cinnamon oil, allspice oil, and clove bud oil were obtained from Lhasa Karnak Herb Co. (Berkeley, Calif., U.S.A.). Golden Delicious apple puree (38 °Brix) (Sabroso Co., Medford, Oreg., U.S.A.) was the primary ingredient in all apple-based film-forming solutions (APFFS). Glycerol (Fisher Scientific, Waukesha, Wis., U.S.A.) was added as a plasticizing agent. Ascorbic acid (BASF, Mount Olive, N.J., U.S.A.) and citric acid (Archer Daniels Midland, Decatur, Ill., U.S.A.) were utilized as browning inhibitors. Low methoxyl pectin (Systems BioIndustries, Fair Lawn, N.J., U.S.A.) was added to increase film strength and facilitate release from cast surfaces. APFFS (26%, w/w; 260 g of 38 °Brix apple puree plus 705 g of 3% [w/w] pectin solution) was prepared according to the method of McHugh and Senesi (2000). This solution also contained ascorbic and citric acids (2.5 g, 0.25% [w/w]) and glycerol (30 g, 3% [w/w]). Cinnamon oil, allspice oil, and clove bud oil were then incorporated into the apple puree solutions at the following concentrations: 0% (control), 0.5%, 1.0%, 1.5%, and 3% (w/w). These solutions were homogenized for 3 min at 12500 rpm using a Polytron 3000 homogenizer (Kinematica, Littau, Switzerland). Each homogenate was degassed under vacuum for 15 min and then used for casting the films.

Viscosity of apple film forming solutions

Viscosity studies were determined in a Brookfield Digital Rheometer model DV−III+ with a TC-500 Refrigerated Bath/Circulator using a model 107 Programmable Temperature Controller running Rheocalc for Windows (Brookfield Engineering Laboratories Inc., Middleboro, Mass., U.S.A.). A small sample adapter along with spindle SC4–21 (0.66 mm dia, 1.23 mm long) was used to measure the viscosity of the APFFS at 3 constant shears rates by rotation at 5, 125, and 250 rpm, respectively. For the experiments, 8.5 ± 0.1 g APFFS was added to the small sample adapter. The testing temperature remained constant at 25 °C during the tests. Five viscosity readings were made on each APFFS from 1 to 5 min at constant shear rate and temperature.

Film casting

Apple films were cast on the bench. They were made using a 45 mil gap draw down bar to spread the APFFS on a flat Mylar sheet on 29 × 29 cm square glass plate which was then immediately moved into a sterile biohood and dried overnight at room temperature (20 to 25 °C). Dried films were shaped into 50-mm-dia discs by cutting with a sterilized razor blade around the edge of a watch glass over the film, or cut into 12-mm-dia discs using a sterilized cork borer. The film discs were stored on layers of aluminum foil in sealed, sterilized glass containers or zip plastic bags until used. The weight and thickness of films used for microbial testing were measured with an analytical balance and a micrometer, respectively.

Antimicrobial assay of pathogenic bacteria

Frozen cultures of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* were streaked on Trypticase Soy Agar (TSA) and then incubated at 37 °C for 24 h. One isolated colony was re-streaked on TSA and then incubated at 37 °C for 24 h. This was followed by inoculating 1 isolated colony into a tube with 5 mL Trypticase Soy Broth (TSB) and incubating at 37 °C for 24 h with agitation. The microbial broth was then serially diluted (10×) in 0.1% peptone water.

For overlay diffusion tests, 0.1 mL of 10^5 colony forming units (CFU)/mL of each bacterial culture was plated onto each of 6 TSA plates. The inoculum was spread evenly throughout each plate and then let to dry for 5 min in a biosafety hood. Each agar plate was divided evenly into 2 to 4 areas and labeled with the different EO concentrations. On the center of each area, 1 aseptically cut 12-mm-dia edible film disc was deposited over the inoculated agar with the film's shiny side down. The plates were incubated at 35 °C for 48 h. The inhibition radius around the film disc (colony-free perimeter) was measured with a digital caliper (Neiko Tools, Ontario, Calif., U.S.A.) in triplicate after 24 and 48 h of incubation, respectively. The inhibition area was then calculated.

For vapor phase diffusion tests, edible films with different concentration of EOs were aseptically cut into 50-mm-dia discs and then placed on the lids of TSA plates, which had been previously spread with 0.1 mL of 10^5 CFU/mL of each bacterial inoculum. The inoculated TSA plate was inverted with dish on the top of each lid containing antimicrobial film. Parafilm was used to tightly seal the edge of each TSA plate. Figure 1A shows the setup used for vapor phase tests. All sealed and inverted plates were incubated at 35 °C. The growth of each pathogen on the TSA plates was checked after incubation for 24 and 48 h. The inhibition radius (absence of bacteria) on each TSA plate was measured with a digital caliper (Neiko Tools). The values obtained were used to calculate inhibition area.

Film thickness

Film thickness was measured with a micrometer IP 65 (Mitutoyo Manufacturing, Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in) at 5 random positions around the film. The mean value was used to calculate water vapor permeability (WVP) and tensile strength.

Water vapor permeability of films

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh and others 1993) was used to determine WVP. A cabinet with a variable speed fan was used to test film WVP. Cabinet temperature of 25 ± 1 °C was maintained in a Forma Scientific reach-in incubator (Thermo Electron Corp., Waltham, Mass., U.S.A.). Fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity throughout the cabinets. Cabinets were

pre-equilibrated to 0% room humidity (RH) using anhydrous calcium sulfate (W.A. Hammond Drierite, Xenia, Ohio, U.S.A.). Circular test cups made from polymethylmethacrylate (Plexiglas^{TM}) were used. A film was sealed to the cup base with a ring containing a 19.6 cm^2 opening using 4 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 in the bottom of the test cups to expose the film to a high percentage RH inside the test cups. Average stagnant air gap heights between the water surface and the film were measured. Test cups holding 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 8 times at 2 h intervals. Eight replicates of each apple film were tested. Relative humidities at the film undersides and WVPs were calculated using the WVP correction method (McHugh and others 1993).

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

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

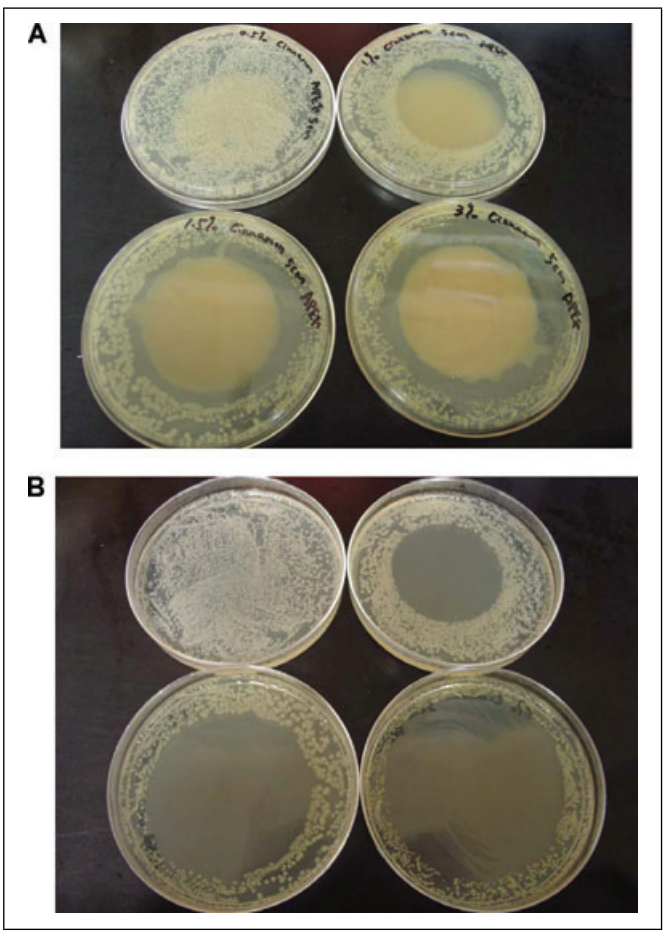

Figure 1—(A) Vapor phase test setup. (B) Vapor phase inhibitory zone (bacterial colony free spot area) of apple puree edible films containing 0.5% to 3.0% cinnamon oil against *S. enterica*. Top left in each picture illustrates apple film with 0.5% cinnamon oil, showing no inhibition of bacteria, similar to control films without added EOs.

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

Tensile properties of films

Standard method D882-97 (ASTM 1997) was used to measure tensile properties of apple films. Films were cut into strips with a test dimension of 165 × 19 mm according to standard method D638-02a (ASTM 2002). All films were conditioned for 48 h at 23 ± 2 °C and 50 ± 2% RH before testing using a saturated salt solution of magnesium nitrate (Fisher Scientific, Fair Lawn, N.J., U.S.A.). The ends of the equilibrated strips were mounted and clamped with pneumatic grips on an Instron Model 55R4502 Universal Testing Machine (Instron, Canton, Mass., U.S.A.) with a 100 N load cell. The initial gauge length was set to 100 mm and 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) compared with strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron). Fifteen specimens of each film were evaluated.

Color of apple puree film forming solutions and films

Color of APFFS through clear beakers and apple films under a Minolta standard white reflector plate was measured with a Minolta Chroma Meter (Model CR-400, Minolta, Inc., Tokyo, Japan). The color was measured using the CIE L^*, a^*, and b^* coordinates. Illuminant D65 and 10° observer angle were used. The instrument was calibrated using a Minolta standard white reflector plate. A total of 10 apple solutions and apple films were evaluated for each EO concentration. Three readings were made in each replicate by changing the position of the Chroma Meter over the solution and film. Previously described experimental values of L^*, a^*, and b^* parameters were employed to calculate the Whitish Index as $Wi = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Avena-Bustillos and others 1994).

Statistical analysis

Data were analyzed by one-way and two-way analysis of variance (ANOVA) using Minitab version 13.31 software (Minitab Inc., State College, Pa., U.S.A.). Tukey test was used to determine the difference at 5% significance level. Paired Student's t-tests were used for vapor phase diffusion tests to determine differences at 5% significance.

Results and Discussion

The main desired characteristics of an ideal edible film would be low WVP and high mechanical strength. The physicochemical properties of edible films (color, tensile strength, water vapor, and oxygen permeability) relate to coating enhancement of mechanical integrity of foods, inhibition of moisture loss and oxidative rancidity, and final-product appearance (Debeaufort and others 1998). A complete analysis of both antimicrobial and physicochemical properties is important for predicting the behavior of antimicrobial edible films in food system (McHugh and Krochta 1994; Cagri and others 2001).

Composition of allspice, clove bud, and cinnamon oils

Table 1 shows the origin and major components of the 3 oils provided by the supplier. The content of the major constituents listed in the table is similar to that reported in the literature (Kikuzaki and others 1999; Teuscher 2006; Minott and Brown 2007; De Rovira 2008; Parthasarathy and others 2008).

Antimicrobial activity of essential oils in apple films

The experimental inhibition areas for overlay and vapor phase diffusion induced by the oil-containing films at 2 time periods, 24 and 48 h, against *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* are shown in Table 2, 3, and 4, respectively. The listed inhibitory activities were estimated from area measurements of clear inhibition zones surrounding the film discs in the agar overlay tests, and the circular inhibition spots by the vapor phase tests (Figure 1B), respectively. Figure 1 shows typical inhibitory zones induced by different concentrations of the oil-containing films.

Apple film without oils served as controls to determine possible antimicrobial effects of the film without additives. The control film did not inhibit the growth of the 3 pathogenic bacteria. If a circular spot in the vapor phase test or surrounding clear zone in the overlay test was not present, it was assumed that the oil was not inhibitory and the area was assigned a zero value, as illustrated in Figure 1A and 1B for apple films with 0.5% cinnamon oil. All apple films containing oils inhibited the growth of the 3 pathogens in a concentration-dependent manner. The inhibitory zones produced by the overlay tests with the 3 pathogenic bacteria were larger when tested after 24 h than those observed after 48 h. We have no apparent explanation for this effect.

By contrast, the inhibitory zones induced by the vapor phase tests were largely unchanged for *E. coli* O157:H7 and *L. monocytogenes*. However, they were significantly smaller for *S. enterica* than those observed with the other 2 pathogens. The overlay tests show that the relative antibacterial resistance against the 3 EOs was in the following order: *E. coli* O157:H7 < *L. monocytogenes* < *S. enterica*. The antibacterial activities of the 3 oils were in the following order: cinnamon > allspice > clove bud.

Table 2 to 4 also show that against *E. coli* O157:H7 and *S. enterica*, cinnamon oil in the film was effective at 1%; allspice, at 1.5%; and clove bud oil, at 3%. Lower concentrations of allspice and clove bud oils in the films (1% and 1.5%, respectively) suppressed the growth of *L. monocytogenes*.

Most of the existing methods for testing the antimicrobial activities of substances require direct contact between the active agent

Table 1 — Origin and major components of allspice, cinnamon, and clove bud essential oils evaluated in the present study.[a]

Essential oil	Origin	Major components
Allspice	Jamaica	68.60% eugenol 4.40% β-caryophyllene
Cinnamon	Sri Lanka	70.42% cinnamaldehyde 2.45% eugenol
Clove bud	Madagascar	81.59% eugenol 11.53% eugenol acetate 5.12% β-caryophyllene 0.56% α-humulene

[a]Data provided by supplier: Lhasa Karnak Herb Co., Berkeley, Calif., U.S.A.

Table 3 — Effect of concentration (% w/w) of 3 EOs in edible apple films against *S. enterica* determined by overlay direct contact and vapor phase diffusion methods.

		Overlay test 12-mm-dia disc (113 mm^2)		Vapor phase test 50-mm-dia disc (1964 mm^2)	
		Perimetral inhibitory zone (mm^2)		Circular inhibitory zone (mm^2)	
Film	EO (% w/w)	24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Cinnamon oil	0.5	0	0	0	0
	1.0	10.8 ± 5.6	12.1 ± 4.6	1959	1755
	1.5	43.1 ± 7.2	28.8 ± 6.3	2920	2882
	3.0	240.1 ± 41.8	205.7 ± 36.3	3709	3675
Allspice oil	0.5	0	0	0	0
	1.0	0	0	353	154
	1.5	8.2 ± 3.2	0	965	932
	3.0	37.2 ± 10.1	27.6 ± 10.0	1820	1700
Clove bud oil	0.5	0	0	0	0
	1.0	0	0	0	0
	1.5	0	0	1089	884
	3.0	26.1 ± 4.5	18.1 ± 6.8	1873	1293

Table 2 — Effect of concentration (% w/w) of 3 EOs in edible apple films against *E. coli* O157:H7 determined by overlay direct contact and vapor phase diffusion methods.

		Overlay test 12-mm-dia disc (113 mm^2)		Vapor phase test 50-mm-dia disc (1964 mm^2)	
		Perimetral inhibitory zone (mm^2)		Circular inhibitory zone (mm^2)	
Film	EO (% w/w)	24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Cinnamon oil	0.5	0	0	0	0
	1.0	9.2 ± 1.7	5.4 ± 6.4	3402	3118
	1.5	74.8 ± 22.7	38.0 ± 16.2	3444	3352
	3.0	490.4 ± 28.5	435.2 ± 34.3	4976	4782
Allspice oil	0.5	0	0	0	0
	1.0	0	0	1026	783
	1.5	10.2 ± 11.6	0	1607	1719
	3.0	61.4 ± 10.5	43.6 ± 14.3	1931	2115
Clove bud oil	0.5	0	0	0	0
	1.0	0	0	1081	827
	1.5	0	0	1311	1468
	3.0	100.1 ± 14.7	78.7 ± 12.7	1374	1615

Table 4 — Effect of concentration (% w/w) of 3 EOs in edible apple films against *L. monocytogenes* determined by overlay direct contact and vapor phase diffusion methods.

		Overlay test 12-mm-dia disc (113 mm^2)		Vapor phase test 50-mm-dia disc (1964 mm^2)	
		Perimetral inhibitory zone (mm^2)		Circular inhibitory zone (mm^2)	
Film	EO (% w/w)	24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Cinnamon oil	0.5	0	0	0	0
	1.0	1.6 ± 1.6	0	4263	4208
	1.5	45.2 ± 21.9	2.6 ± 4.4	6362	6362
	3.0	450.4 ± 32.9	122.2 ± 78.1	6362	6362
Allspice oil	0.5	0	0	0	0
	1.0	3.6 ± 3.2	0	1267	538
	1.5	15.4 ± 2.3	0.9 ± 2.3	1442	1270
	3.0	71.7 ± 19.6	33.2 ± 12.0	1978	2286
Clove bud oil	0.5	0	0	0	0
	1.0	0	0	515	0
	1.5	10.5 ± 5.9	5.5 ± 4.4	1243	1228
	3.0	54.0 ± 8.9	27.7 ± 2.6	1807	1992

and the microorganism (that is, food), and thus are not relevant to many commercial products in which there is little or no direct contact between the food and packaging material (Rodríguez and others 2007). Vapor phase tests, which are not direct contact assays, can be used to assess the protection provided by the antimicrobial volatile materials under no direct contact conditions.

One advantage of EOs is their bioactivity in the vapor phase, a characteristic that makes them useful as possible fumigants for stored commodity protection. Volatile compounds from plants usually have a relatively high vapor pressure and are capable of approaching an organism through the liquid and the gas phase (Fries 1973). Therefore, overlay test (direct contact) and vapor phase test (not direct contact) were used in this study to compare antimicrobial activity of EOs in the apple films.

Cinnamon oil contains about 85% of the active antimicrobial, cinnamaldehyde (Friedman and others 2004). Because cinnamon oil is present in numerous commercial foods (Friedman and others 2000), has a pleasant taste, and is generally recognized as safe (GRAS) listed (Adams and others 2004), the compound merits use in antimicrobial edible films. Eugenol is the major constituent of allspice (Kikuzaki and others 1999) and clove bud (Guan and others 2007). The 2 oils and eugenol were previously found to be active *in vitro* against *S. enterica* and *L. monocytogenes* (Friedman and others 2002).

We found no reports in the literature on films containing allspice and clove bud oils, which were evaluated in the present study. With respect to cinnamon oil, López and others (2007) reported that very high concentrations (up to 10%) of the oil in nonedible polymeric films were needed to inactivate pathogenic bacteria. By contrast, films made from apples evaluated in the present study containing 3% cinnamon oil or less were found to be effective against the pathogens.

Viscosity of apple puree film-forming solutions

The decrease in viscosity values of the apple solutions by applied constant shear rates during 5 min is indicative of a non-Newtonian thixotropic fluid behavior (Singh and Heldman 1993). Viscosities evaluated in the present study were reduced by increasing shear rates (Table 5). The apple solutions behaved as a shear thinning, pseudoplastic liquid, typical of fruit purees (Singh and Heldman 1993). The addition of 1% to 1.5% oils to the apple solution further reduced viscosities. This effect was more pronounced by cinnamon and allspice oils than by clove bud oil.

Water vapor permeability

WVP is a measure of the ability of a material to be penetrated by water vapors (Cagri and others 2001). To compare the permeabilities of the apple films containing different EO formulations, it is important to expose the films to the same percent relative humidity (RH) differential as the driving force for water diffusion. Table 6 shows nonsignificant differences in %RH at the film underside for the apple films, indicating similar diffusion driving forces.

Table 5— Effect of concentration (% w/w) of 3 EOs on viscosity of apple puree film-forming solutions at different shear rates at 25 °C.

Films	EO (% w/w)	4.65 per second shear rate (5 rpm) (cP)	116.25 per second shear rate (125 rpm) (cP)	232.50 per second shear rate (250 rpm) (cP)
Control	0	$5,940.0 \pm 40.0^{cCz}$	$1,437.4 \pm 28.9^{bBz}$	988.2 ± 12.7^{bcBy}
Allspice oil	0.5	$5,660.0 \pm 136.6^{bc}$	$1,405.0 \pm 24.0^{ab}$	969.6 ± 11.3^{ab}
	1.0	$5,140.0 \pm 40.0^{a}$	$1,372.8 \pm 19.6^{a}$	953.6 ± 13.4^{a}
	1.5	$5,480.0 \pm 103.3^{b}$	$1,413.8 \pm 28.4^{ab}$	979.2 ± 13.4^{b}
	3.0	$5,780.0 \pm 177.4^{c}$	$1,442.6 \pm 9.7^{b}$	$1,004.8 \pm 15.3^{c}$
Cinnamon oil	0.5	$5,600.0 \pm 65.3^{B}$	$1,374.7 \pm 26.4^{A}$	948.8 ± 13.4^{A}
	1.0	$5,360.0 \pm 172.8^{A}$	$1,367.7 \pm 22.0^{A}$	947.2 ± 13.2^{A}
	1.5	$5,260.0 \pm 40.0^{A}$	$1,355.5 \pm 11.4^{A}$	946.9 ± 13.4^{A}
	3.0	$5,440.0 \pm 65.3^{AB}$	$1,420.2 \pm 23.4^{B}$	984.0 ± 13.4^{B}
Clove bud oil	0.5	$5,340.0 \pm 76.6^{xy}$	$1,367.0 \pm 15.2^{xy}$	947.8 ± 13.9^{x}
	1.0	$5,200.0 \pm 113.1^{x}$	$1,358.1 \pm 20.2^{x}$	944.0 ± 14.0^{x}
	1.5	$5,480.0 \pm 80.0^{y}$	$1,389.4 \pm 8.6^{xy}$	969.3 ± 12.4^{y}
	3.0	$5,200.0 \pm 65.3^{x}$	$1,397.8 \pm 24.7^{y}$	971.5 ± 12.4^{y}

[a,B,x] Means in same column for EO and control films with different letters or numbers are significantly different at $P < 0.05$.

Table 6— Effect of concentration (% w/w) of 3 EOs on water vapor permeability (WVP) of edible apple puree films.

Films	EO (% w/w)	Thickness[C] (mm)	%RH inside cup[C,D] (%RH)	WVP[C,D] (g-mm/kPa-h-m^2)
Control	0	0.126 ± 0.015^{NS}	78.5 ± 1.5^{NS}	$3.62 \pm 0.34^{ab,AB,xy}$
Allspice oil	0.5	0.130 ± 0.017^{NS}	78.3 ± 1.2^{NS}	3.80 ± 0.44^{b}
	1.0	0.133 ± 0.014	78.4 ± 1.1	3.85 ± 0.28^{b}
	1.5	0.123 ± 0.011	79.1 ± 1.3	3.43 ± 0.19^{a}
	3.0	0.126 ± 0.008	79.2 ± 1.2	3.51 ± 0.23^{a}
Cinnamon oil	0.5	0.127 ± 0.010^{NS}	79.3 ± 1.4^{NS}	3.50 ± 0.24^{A}
	1.0	0.127 ± 0.012	77.6 ± 1.4	3.86 ± 0.28^{B}
	1.5	0.121 ± 0.004	78.0 ± 1.4	3.62 ± 0.17^{AB}
	3.0	0.137 ± 0.007	79.0 ± 1.2	3.83 ± 0.12^{B}
Clove bud oil	0.5	0.124 ± 0.007^{NS}	79.0 ± 1.0^{NS}	3.49 ± 0.17^{x}
	1.0	0.127 ± 0.008	79.0 ± 1.3	3.57 ± 0.18^{xy}
	1.5	0.129 ± 0.003	78.0 ± 1.3	3.83 ± 0.14^{y}
	3.0	0.130 ± 0.005	78.0 ± 1.1	3.73 ± 0.15^{xy}

[C] Thickness and RH data are mean values. WVP data are mean values ± standard deviations.
[D] Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP correction method (McHugh and others 1993).
[NS] = not significantly different.
[a,B,x] Means in same column for EO and control films with different letters or numbers are significantly different at $P < 0.05$.

In the present study, WVP properties were not affected by the incorporation of the oils into the film compared to control apple films with added oils, presumably because the oils consist mostly of terpene-like compounds, not lipids. However, a slight decrease in WVP was observed with 1.5% to 3% (w/w) clove bud oil (Table 6). Rojas-Graü and others (2006) reported a significant decrease in permeability through apple films formulated with pectin and cinnamon oil.

Tensile properties

Addition of EOs to the apple solution caused a significant reduction ($P < 0.05$) in tensile strength and elastic modulus of the resulting apple films (Table 7). This effect was more pronounced in films containing clove bud oil, which displayed lower tensile strength and elastic modulus values than films with allspice and cinnamon oil. The tensile strength and elastic modulus of apple film without added oils were significantly greater (3.5 and 5.2 MPa, respectively) than most of the films containing the oils (Table 7). The addition of EOs resulted in higher film elongations (Table 7). The percent elongation of apple film without oils (48.6%) increased in all films to a maximum value of 54% with 3% added cinnamon oil (Table 7). Similar effects on tensile properties of apple films were reported by Rojas-Graü and others (2006, 2007a, 2007b) (Du and others 2008b).

Colors of solutions and films

The color parameter L^* provides a measure of lightness. Its dark to light values range from 0 to 100. A positive a^* value is a measure of redness, and a negative value of greenness. A positive b^* value is a measure of yellowness, and a negative value of blueness. The whitish index combines the 3 experimental color parameters. Their values range from 0 to 100. The higher the combined value the greater the whiteness. Addition of the oils to the apple solution increased their L^*, b^* values, as well as the whitish index (Table 8). The increase was directly related to the concentration of the oils. The a^* value decreased for all 3 oils. The oil-containing solutions were less green compared to the original apple solution without added oils. Cinnamon oil in the film induced higher increases in the L^* values and the whitish index than was the case with added allspice and clove bud oils (Table 8). The concentration-dependent increases of the whitish indices of the apple solutions may be related to higher light scattering associated with the formation of large numbers of emulsion droplets.

The color parameters of apple films with different concentrations of oils are listed in Table 9. The L^*, a^*, and b^* values of apple films formulated with the 3 oils indicate a light yellowish–greenish color, similar to the results reported by Rojas-Graü and others (2007b). The L^* and a^* values of apple films decreased with concentration of allspice and cinnamon, but not with clove bud oil. The b^* values increased with concentration of all 3 oils. Increasing oil concentrations resulted in reduced whitish index values of all apple films (Table 9). Lower values for L^* and whitish index indicate darker films.

Conclusions

The antimicrobial activity of cinnamon oil was significantly greater than the activities of allspice and clove bud oils in apple puree edible films against *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*. Incorporation of the oils into the films did not adversely affect WVP. Effects on tensile properties were also minor.

The antimicrobial data obtained with vapors diffused from the apple films can serve as a guide for selection of appropriate levels of volatile EOs and their active constituents for incorporation into

Table 7 — Effect of concentration (% w/w) of 3 EOs on the tensile properties of edible apple puree films.

Films	EO (% w/w)	Film thickness (mm)	Tensile strength[d] (MPa)	Elongation[d] (%)	Elastic modulus[d] (MPa)
Control	0	0.143 ± 0.005^{aAx}	3.47 ± 0.28^{cBCz}	48.6 ± 3.4^{aAx}	5.16 ± 0.60^{bBz}
Allspice oil	0.5	0.144 ± 0.009^{a}	3.63 ± 0.12^{bc}	52.3 ± 2.2^{b}	4.80 ± 0.64^{b}
	1.0	0.155 ± 0.010^{b}	3.30 ± 0.23^{b}	51.8 ± 3.3^{b}	4.51 ± 0.64^{b}
	1.5	0.152 ± 0.011^{b}	3.31 ± 0.21^{b}	52.6 ± 1.7^{b}	4.57 ± 0.77^{b}
	3.0	0.167 ± 0.005^{c}	2.98 ± 0.20^{a}	53.5 ± 2.0^{b}	3.78 ± 0.79^{a}
Cinnamon oil	0.5	0.144 ± 0.004^{A}	3.61 ± 0.21^{C}	51.5 ± 2.5^{B}	4.95 ± 0.86^{B}
	1.0	0.154 ± 0.006^{B}	3.51 ± 0.18^{BC}	52.5 ± 2.0^{BC}	4.76 ± 0.84^{B}
	1.5	0.154 ± 0.009^{B}	3.36 ± 0.18^{B}	52.6 ± 1.9^{BC}	4.30 ± 0.43^{AB}
	3.0	0.169 ± 0.009^{C}	3.05 ± 0.13^{A}	54.0 ± 1.5^{C}	4.03 ± 0.35^{A}
Clove bud oil	0.5	0.147 ± 0.005^{x}	3.33 ± 0.24^{yz}	49.7 ± 3.5^{x}	4.61 ± 0.32^{y}
	1.0	0.147 ± 0.010^{x}	3.17 ± 0.23^{y}	50.8 ± 2.6^{xy}	4.25 ± 0.36^{xy}
	1.5	0.146 ± 0.003^{x}	3.19 ± 0.14^{y}	50.5 ± 2.5^{xy}	4.16 ± 0.22^{x}
	3.0	0.158 ± 0.005^{y}	2.85 ± 0.13^{x}	52.7 ± 1.8^{y}	3.66 ± 0.38^{w}

[d]Thickness, tensile strength, elongation, and elastic modulus data are mean values ± standard deviations.
[a,A,w] Means in same column for control and EO films with different letters or numbers are significantly different at $P < 0.05$.

Table 8 — Effect of concentration (% w/w) of 3 EOs on color properties of apple puree film-forming solutions.

Solutions	EO (% w/w)	L^*	a^*	b^*	Whitish index
Control	0	39.66 ± 0.52^{aAu}	-3.97 ± 0.07^{aAu}	10.58 ± 1.03^{aAx}	38.60 ± 0.41^{aAu}
Allspice oil	0.5	59.75 ± 0.57^{b}	-3.65 ± 0.12^{b}	15.24 ± 0.93^{b}	56.80 ± 0.25^{b}
	1.0	70.07 ± 0.62^{c}	-2.85 ± 0.12^{c}	16.58 ± 0.79^{c}	65.66 ± 0.18^{c}
	1.5	73.94 ± 0.69^{d}	-2.44 ± 0.17^{d}	16.76 ± 0.80^{c}	68.91 ± 0.19^{d}
	3.0	79.60 ± 0.71^{e}	-1.70 ± 0.28^{e}	16.59 ± 0.96^{c}	73.62 ± 0.07^{e}
Cinnamon oil	0.5	65.98 ± 0.41^{B}	-3.39 ± 0.10^{B}	17.54 ± 0.49^{B}	61.57 ± 0.17^{B}
	1.0	76.42 ± 0.52^{C}	-2.53 ± 0.21^{C}	18.31 ± 0.67^{B}	70.03 ± 0.07^{C}
	1.5	80.86 ± 0.41^{D}	-2.02 ± 0.27^{D}	17.34 ± 0.62^{B}	74.08 ± 0.13^{D}
	3.0	85.21 ± 0.27^{E}	-1.30 ± 0.23^{E}	18.15 ± 0.38^{B}	76.55 ± 0.15^{E}
Clove bud oil	0.5	59.13 ± 0.44^{w}	-3.72 ± 0.10^{w}	15.38 ± 0.64^{y}	56.17 ± 0.23^{w}
	1.0	69.14 ± 0.40^{x}	-2.99 ± 0.07^{x}	16.41 ± 0.62^{z}	64.92 ± 0.09^{x}
	1.5	74.28 ± 0.38^{y}	-2.42 ± 0.13^{y}	17.20 ± 0.62^{z}	68.96 ± 0.15^{y}
	3.0	79.28 ± 0.33^{z}	-1.83 ± 0.08^{z}	16.95 ± 0.37^{z}	73.17 ± 0.28^{z}

[a,A,u] Means in same column for control and EO films with different letters or numbers are significantly different at $P < 0.05$.

Table 9 — Effect of concentration (% w/w) of 3 EOs on color parameters of edible apple puree films.

Films	EO (% w/w)	L^*	a^*	b^*	Whitish index
Control	0	86.08 ± 0.40^{abD}	-1.35 ± 0.38^{bB}	17.9 ± 2.5^{aAx}	77.3 ± 2.1^{cDz}
Allspice oil	0.5	86.21 ± 0.53^{b}	-1.74 ± 0.28^{a}	19.8 ± 2.3^{a}	75.8 ± 2.1^{c}
	1.0	85.75 ± 0.45^{ab}	-1.29 ± 0.28^{b}	24.4 ± 2.2^{b}	71.7 ± 2.0^{b}
	1.5	85.94 ± 0.48^{ab}	-1.16 ± 0.22^{b}	25.8 ± 2.0^{bc}	70.6 ± 1.9^{b}
	3.0	85.51 ± 0.51^{a}	-1.04 ± 0.25^{b}	28.3 ± 1.1^{c}	68.2 ± 1.2^{a}
Cinnamon oil	0.5	85.18 ± 0.22^{C}	-1.89 ± 0.13^{A}	30.9 ± 0.9^{B}	65.7 ± 0.9^{C}
	1.0	84.02 ± 0.49^{B}	-1.66 ± 0.32^{AB}	36.7 ± 1.3^{C}	60.0 ± 1.3^{B}
	1.5	83.45 ± 0.79^{AB}	-1.14 ± 0.55^{B}	39.8 ± 1.4^{D}	56.9 ± 1.6^{A}
	3.0	82.97 ± 0.37^{A}	-0.37 ± 0.24^{C}	38.8 ± 0.5^{D}	57.6 ± 0.5^{A}
Clove bud oil	0.5	86.06 ± 0.26^{NS}	-1.12 ± 0.08^{NS}	23.7 ± 1.08^{y}	72.5 ± 1.0^{y}
	1.0	85.90 ± 0.15	-1.28 ± 0.07	25.1 ± 0.65^{yz}	71.2 ± 0.6^{xy}
	1.5	86.07 ± 0.44	-1.29 ± 0.25	26.1 ± 1.72^{z}	70.4 ± 1.6^{x}
	3.0	86.01 ± 0.45	-1.36 ± 0.20	27.3 ± 1.5^{z}	69.3 ± 1.5^{x}

[a.A.x] Means in same column for control and EO films with different letters or numbers are significantly different at $P < 0.05$.
[NS] = not significantly different.

antimicrobial edible films. Edible fruit and vegetable films containing plant-derived volatile EOs provide new ways to enhance microbial safety and shelf life of foods by direct and/or indirect contacts of the antimicrobials in the films with the food. Moreover, in addition to antimicrobial effects, the evaluated essential oils are also reported to exhibit antioxidative and beneficial sensory properties (Bakkali and others 2008; Bauermann and others 2008; De Rovira 2008). The edible apple films containing essential oils, therefore, have the potential to provide multiple benefits to the consumers.

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