Antibacterial Effects of Allspice, Garlic, and Oregano Essential Oils in Tomato Films Determined by Overlay and Vapor-Phase Methods

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ABSTRACT: Physical properties as well as antimicrobial activities against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* of allspice, garlic, and oregano essential oils (EOs) in tomato puree film-forming solutions (TPFFS) formulated into edible films at 0.5% to 3% (w/w) concentrations were investigated in this study. 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 results indicate that the antimicrobial activities against the 3 pathogens were in the following order: oregano oil > allspice oil > garlic oil. *Listeria monocytogenes* was less resistant to EO vapors, while *E. coli* O157:H7 was more resistant to EOs as determined by both overlay and vapor-phase diffusion tests. The presence of plant EO antimicrobials reduced the viscosity of TPFFS at the higher shear rates, but did not affect water vapor permeability of films. EOs increased elongation and darkened the color of films. The results of the present study show that the 3 plant-derived EOs can be used to prepare tomato-based antimicrobial edible films with good physical properties for food applications by both direct contact and indirectly by vapors emanating from the films.

Keywords: edible film, *Escherichia coli* O157:H7, *Listeria monocytogenes*, plant essential oils, *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 losses of volatile aromas and flavors (Kester and Fennema 1986). Chemical and physical properties and applications of edible films and coatings have been extensively reviewed (McHugh and others 1996; Min and others 2005; Bravin and others 2006; Jagannath and others 2006; Serrano 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). Plant 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).

In addition to its flavor properties, tomatoes are reported to possess numerous beneficial nutritional and bioactive components that may also benefit human health. These include the nutrients vitamin A, vitamin C, iron, and potassium; nonnutritive digestible and indigestible dietary fiber; the antioxidative compounds lycopene, β-carotene, and lutein (Frusciante and others 2007; Dorais and others 2008); and the cholesterol lowering (Friedman and others 2000a; Friedman and others 2000b) and immune system enhancing glycoalkaloids tomatine and dehydrotomatine (Morrow and others 2004). Consumption of tomatoes, tomato products, and of isolated bioactive tomato ingredients is reported to be associated with lowered risk of cancer (Friedman and others 2007), heart disease (Willcox and others 2003), diabetes (Bose and Agrawal 2007), and hypertension (Engelhard and others 2006). Edible tomato films containing antimicrobials may have multiple benefits which include protection of food against contamination by pathogenic microorganisms, and nutritional and health benefits associated with the consumption of the previously mentioned tomato ingredients that may be present in the films.

EOs from allspice, garlic, and oregano are compatible with the sensory characteristics of tomato-based films. In addition to desirable antimicrobial and barrier properties, they exhibit antioxidative and other beneficial effects that are reported to be associated with tomatoes (Bakkali and others 2008; Bauermann and others 2008; De Rovira 2008). It was therefore of interest to find out whether previously reported antimicrobial activities of these oils in phosphate buffers against foodborne pathogens (Friedman and others 2002, 2004) would also be active in films prepared from fruits and vegetables. In previous studies we showed that this is indeed the case (Rojas-Graü and others 2006, 2007a, 2007b; Du and others 2008a, 2008b, 2009).

Antimicrobial assays of tomato films indicated that optimum antimicrobial effects occurred with carvacrol (a major constituent of oregano oil) levels of approximately 0.75% added to tomato purees before film preparation. High-performance liquid chromatography (HPLC) analysis of the films indicated that the carvacrol concentrations and bactericidal effect of the films remained unchanged over

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a storage period of up to 98 d at 5 or 25 °C (Du and others 2008a). In a related study, Du and others (2008b) demonstrated that carvacrol in apple films also inhibited the growth of *E. coli* O157:H7, even after storage at 5 or 25 °C for 7 wk.

The biological functions of garlic are mainly due to their high content of volatile compounds, including allicin, diallyl sulfide, diallyl disulfide, and low amounts of nonvolatile water-soluble sulfur compounds. These compounds are responsible for the flavor, and biological properties of garlic (Lanzotti 2006; Corzo-Martinez and others 2007). Allspice is used in bakery products and has antimicrobial, antioxidant, and medicinal properties. Eugenol is the major component of allspice EO and it also contains cineol, phellandrene, caryophyllene, and the antioxidant pimentol (Shylaja and Peter 2004).

Because of the cited potential inherent advantages of tomato based films for human health, the major objectives of the present study were (1) to determine antimicrobial activities of 3 EOs added to tomato puree film-forming solutions (TPFFS) used in the preparation of tomato puree films against *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, and (2) to evaluate the effects of the EOs on physicochemical properties of the films.

Materials and Methods

Source of bacteria

The Food and Drug Administration (FDA) provided the *E. coli* O157:H7 bacteria (our strain designation RM1484; original designation SEA13B88) isolated from tomato 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 tomato films

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

Tomato puree film-forming solution (TPFFS)

Oregano and allspice EOs were obtained from Lhasa Karnak Herb Co. (Berkeley, Calif., U.S.A.). Mexican garlic oil was purchased from Aldrich (Milwaukee, Wis., U.S.A.). Table 1 shows the origin and major components of the 3 EOs provided by the suppliers. The content of the major constituents listed in the table is similar to that reported in the literature (Elnima and others 1983; Kikuzaki and others 1999; Teuscher 2006; Minott and Brown 2007; De Rovira 2008; Parthasarathy and others 2008).

Table 1 — Origin and major components of oregano, allspice, and garlic essential oils evaluated in the present study.

Essential oil	Origin	Major components
Oregano[a]	Hungary	63.4% Carvacrol
Allspice[a]	Jamaica	68.6% Eugenol 4.4% β-Caryophyllene
Garlic[b]	Mexico	30% to 50% Diallyl disulfide 10% to 13% Diallyl trisulfide

[a]Data provided by supplier: Lhasa Karnak Herb Co., Berkeley, Calif., U.S.A.
[b]Data provided by supplier: Sigma-Aldrich Co., Milwaukee, Wis., U.S.A.

Hot break tomato puree (31 °Brix, The Morning Star Packing Co., Los Baños, Calif., U.S.A.) was the primary ingredient in all tomato-based film forming solutions (pastes). High methoxyl pectin 1400 (TIC Gums, Belcamp, Md., U.S.A.) was added to increase film strength and facilitate release from cast surfaces. The tomato paste (30% [w/w]; 300 g of 31 °Brix tomato puree and 700 g of 3% [w/w] pectin solution) was combined in a mixer bowl, and mixed on slow speed for 30 min. The oils were then incorporated into the tomato puree solutions at the following concentrations: 0 (control), 0.5%, 1%, 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 film casting.

Viscosity of tomato 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 diameter, 1.23-mm long) was used to measure the viscosity of the TPFFS at 3 constant shears rates by rotation at 5, 125, and 250 rpm, respectively. For the experiments, 8.5 ± 0.1 g TPFFS was added to the small sample adapter. The testing temperature remained constant at 25 °C during the tests. A total of 5 viscosity readings were made on each TPFFS from 1 to 5 min at constant shear rate and temperature.

Film casting

Tomato films were cast on the bench. They were made using a 45 mil gap draw down bar to spread the TPFFS on a flat Mylar sheet on 29 × 29 cm square glass plate which was then dried overnight at room temperature (20 to 25 °C) in a sterile biohood. Dried films were shaped into 50 mm diameter discs by cutting with a razor blade around the edge of a watch glass over the film, or cut into 12 mm diameter 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) per milliliter of each bacterial culture was plated onto each of the 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 diameter 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 or 48 h of incubation, respectively. The inhibition area was then calculated in mm^2.

For vapor-phase diffusion tests, edible films with different concentration of EOs were aseptically cut into 50 mm diameter discs

and then placed on the lids of TSA plates, which had been previously spread with 0.1 mL 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 set-up 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 or 48 h. The inhibition radius (absence of bacteria) on each TSA plate was measured with a digital caliper. The values obtained were used to calculate inhibition area in mm^2.

Water vapor permeability

Thickness of film, percent relative humidity at the film underside, and water vapor permeability were determined according to the methods described by McHugh and others (1993).

Tensile properties

Tensile strength, elastic modulus, and percent elongation were determined according to the method described by Rojas-Graü and others (2006).

Color of solutions and films

Color of tomato films was done according to the method described by Du and others (2008a). Color of TPFFS was evaluated through clear beakers. A total of 10 TPFFS and tomato films were evaluated for each EO concentration.

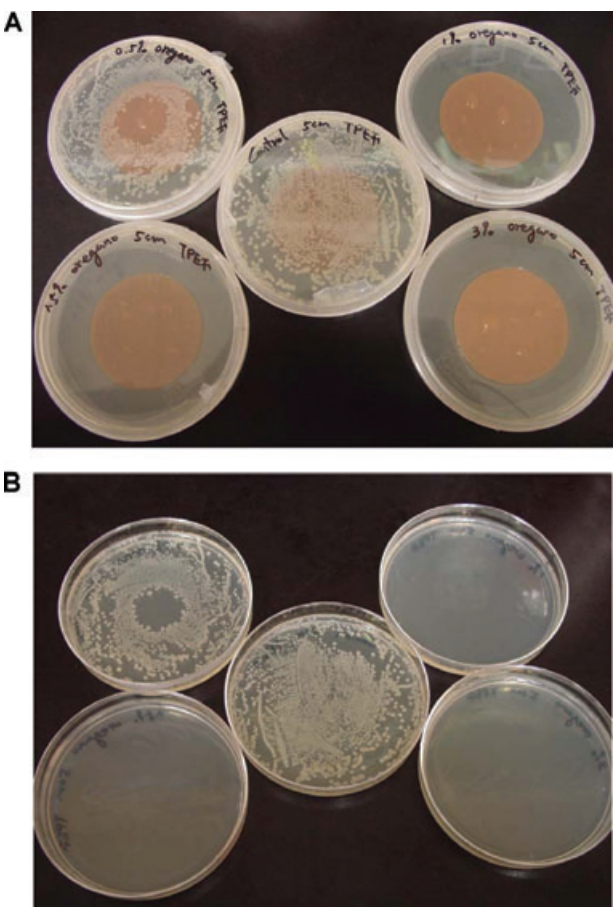

Figure 1 — (A) Vapor-phase test set-up. (B) Vapor-phase inhibitory zone (bacterial colony free spot area) of tomato puree edible films containing 0.5% and 3% oregano oil against *S. enterica*. Petri dish at center in each picture illustrates tomato film without added essential oil (control), showing negative inhibition of bacteria.

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 *t*-tests were used for vapor-phase diffusion tests to determine differences at 5% significance.

Results and Discussion

Antimicrobial activity of EOs in tomato films

In previous studies (Friedman and others 2002, 2004), we evaluated the bactericidal activities of 120 plant essential oils and oil constituents in a pH 7 phosphate in terms of BA_{50} values, defined as the percentage of the botanical compound that kills 50% of the bacteria under the test conditions. The lower the BA_{50} value, the higher the activity.

The 10 most active oils against *E. coli* O157:H7 (BA_{50}, 0.046% to 0.014%) were: oregano, thyme, cinnamon, palmarosa, bay leaf, clove bud, lemon grass, and allspice. The 10 most active oils against *S. enterica* (BA_{50}, 0.045% to 0.14%) were: thyme, oregano, cinnamon, clove bud, allspice, bay leaf, palmarosa, and marjoram. The 10 most active oils against *L. monocytogenes* were (BA_{50}, 0.057% to 0.092%) were: gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, allspice, thyme, and patchouli.

A number of associations were observed from comparisons of the chemical structures of the pure oil compounds and their antimicrobial activities. Both the aldehyde compounds (cinnamaldehyde, citral, citronellal, perillaldehyde, and salicylaldehyde) and phenolic monoterpene compounds (carvacrol, eugenol, and thymol) were highly active. Analysis of selected oils by HPLC showed that bactericidal results are related to the content of the major oil components.

As flavor and taste of oils can vary widely (for example, spicy cinnamon oil, mild oregano oil), specific oils would be more compatible than others for different food categories. The antimicrobial activities under food processing conditions such as baking, cooking, frying, and microwaving are mostly unknown. The most active oils and oil compounds provide candidates for evaluation of bactericidal efficacy after incorporation into edible fruit and vegetable films, including tomato films evaluated in the present study.

The experimental inhibition areas for overlay and vapor-phase diffusion for EOs at 24 or 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 measurement of clear inhibition zones surrounding the film discs in the agar overlay tests and the circular inhibition areas by the vapor-phase tests, respectively. The photographs in Figure 1B illustrate typical inhibitory areas obtained against *S. enterica* by different concentrations of oregano oil in the tomato films.

Tomato film without EOs served as control to determine any possible antimicrobial effect 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 film did not inhibit the bacteria and the area was assigned a zero value.

All tomato film containing EOs inhibited the growth of the 3 pathogens in a concentration-dependent manner. Tomato films formulated with garlic oil were not effective against *E. coli* O157:H7 or *S. enterica*. They were, however, effective against

Table 2— Effect of concentration (% w/w) of 3 plant essential oils in edible tomato films against *E. coli* O157:H7 determined by overlay and vapor-phase diffusion methods.

Film	Essential oil (% w/w)	Overlay test 12 mm diameter disc (113 mm²)		Vapor-phase test 50 mm diameter disc (1964 mm²)	
		Perimetral inhibitory zone (mm²)		Circular inhibitory zone (mm²)	
		24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Oregano oil	0.5	0	0	724 ± 97^{a}	687 ± 153^{a}
	1.0	9.4 ± 3.0^{a}	0.7 ± 1.8^{a}	5913 ± 777^{b}	5319 ± 1807^{b}
	1.5	54.5 ± 14.8^{b}	34.3 ± 10.7^{b}	4859 ± 1303^{b}	4430 ± 1682^{b}
	3.0	416 ± 71^{c}	336 ± 62^{c}	6362^{b}	6362^{b}
Allspice oil	0.5	0	0	0	0
	1.0	0	0	1286 ± 95^{A}	1130 ± 185^{A}
	1.5	4.0 ± 9.8^{A}	0	1764 ± 231^{B}	1696 ± 200^{B}
	3.0	95.8 ± 26.6^{B}	82.9 ± 22.5	1852 ± 26^{B}	1800 ± 61^{B}
Garlic oil	0.5	0	0	0	0
	1.0	0	0	0	0
	1.5	0	0	0	0
	3.0	0	0	0	0

Means in same column for essential oil and control films with different letters are significantly different at $P < 0.05$.

Table 3— Effect of concentration (% w/w) of 3 plant essential oils in edible tomato films against *S. enterica* determined by overlay and vapor-phase diffusion methods.

Film	Essential oil (% w/w)	Overlay test 12 mm diameter disc (113 mm²)		Vapor-phase test 50 mm diameter disc (1964 mm²)	
		Perimetral inhibitory zone (mm²)		Circular inhibitory zone (mm²)	
		24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Oregano oil	0.5	0	0	311 ± 235^{a}	264 ± 240^{a}
	1.0	25.2 ± 6.7^{a}	15.3 ± 8.6^{a}	3276 ± 314^{b}	3178 ± 56^{b}
	1.5	78.1 ± 19.0^{b}	54.0 ± 14.3^{b}	6362^{c}	6362^{c}
	3.0	415 ± 37^{c}	398 ± 48^{c}	6362^{c}	6362^{c}
Allspice oil	0.5	0	0	0	0
	1.0	0	0	832 ± 160^{A}	755 ± 367^{A}
	1.5	8.3 ± 3.3^{A}	6.8 ± 1.2^{A}	1252 ± 63^{B}	1304 ± 84^{B}
	3.0	97.9 ± 14.1^{B}	93.6 ± 16.4^{B}	1630 ± 77^{C}	1576 ± 179^{C}
Garlic oil	0.5	0	0	0	0
	1.0	0	0	0	0
	1.5	0	0	0	0
	3.0	0	0	0	0

Means in same column for essential oil and control films with different letters are significantly different at $P < 0.05$.

Table 4— Effect of concentration (% w/w) of three plant essential oils in edible tomato films against *L. monocytogenes* determined by overlay and vapor-phase diffusion methods.

Film	Essential oil (% w/w)	Overlay test 12 mm diameter disc (113 mm²)		Vapor-phase test 50 mm diameter disc (1964 mm²)	
		Perimetral inhibitory zone (mm²)		Circular inhibitory zone (mm²)	
		24 h	48 h	24 h	48 h
Control	0	0	0	0	0
Oregano oil	0.5	0	0	956 ± 409^{a}	100 ± 173^{a}
	1.0	25.6 ± 5.2^{a}	8.8 ± 2.4a	6362^{b}	6362^{b}
	1.5	132.8 ± 21.2^{b}	54.0 ± 9.7b	6362^{b}	6362^{b}
	3.0	683.9 ± 96.3^{c}	495 ± 101c	6362^{b}	6362^{b}
Allspice oil	0.5	0	0	0	0
	1.0	0	0	315 ± 18^{A}	0
	1.5	0	0	1093 ± 358^{B}	1022 ± 446^{A}
	3.0	95.4 ± 18.1	14.8 ± 11.1	1962 ± 217^{C}	2085 ± 285^{B}
Garlic oil	0.5	24.4 ± 7.3^{x}	0	6362^{NS}	310 ± 282^{x}
	1.0	78.8 ± 12.4^{y}	0	6362	1442 ± 203^{y}
	1.5	79.8 ± 10.3^{y}	0	6362	1834 ± 169^{y}
	3.0	131.4 ± 15.9^{z}	9.5 ± 4.8	6362	2326 ± 153^{z}

Means in same column for essential oil and control films with different letters are significantly different at $P < 0.05$.
NS = not significantly different.

L. monocytogenes at all concentrations levels after 24 h of incubation. Except at the highest concentration, the antibacterial activity against *L. monocytogenes* disappeared after 48 h of incubation. This result agrees with several other studies that have shown that the inhibitory effect of phenolic compounds from plant EOs is more effective on Gram-positive than on Gram-negative bacteria (Beuchat and Golden 1989). Resistance of Gram-negative bacteria is usually associated with the presence of a lipopolysaccharide layer, which might be involved in reducing the sensitivity of these bacteria against plant EOs (Sivarooban and others 2008).

The inhibitory zone by the overlay tests with the 3 pathogenic bacteria was significantly reduced in going from 24 to 48 h. By contrast, the inhibitory zones induced by the vapor-phase tests of films containing oregano and allspice oils were only slightly reduced for the 3 pathogenic bacteria in going from 24 to 48 h incubation times. The vapor-phase test results indicated that the effective levels of 0.5% and 1% oregano and allspice oils added to tomato films were lower than for overlay tests. This observation suggests that volatile components in these 2 EOs diffuse more efficiently through the air gap than through the agar media. The data obtained by both overlay and vapor-phase methods indicate that *L. monocytogenes* was more susceptible to inactivation than was *E. coli* O157:H7.

Table 2 to 4 also show that against *E. coli* O157:H7 and *S. enterica*, oregano and allspice oils in the films were effective at concentrations of 1% and 1.5%, respectively. By contrast, garlic oil in the films was not an effective antibacterial agent against the 2 pathogens, even at a concentration of 3%. However, against *L. monocytogenes*, the lowest concentrations of garlic oil and the highest concentration of allspice oil suppressed the growth of the bacteria. This observation confirms a previous report by Kim and others (2007) that garlic oil is highly effective against different strains of *L. monocytogenes*. Oregano oil in the tomato films consistently inhibited the growth of all 3 pathogenic bacteria. This observation confirms reported related observations by Seydim and Sarikus (2006) on antibacterial properties of oregano and garlic oils in whey protein-based films. The overlay tests show that the relative antibacterial resistance against the 3 EO was in the following order: *E. coli* O157:H7 > *S. enterica* > *L. monocytogenes*. The antibacterial activities of the 3 oils were in the following order: oregano oil > allspice oil > garlic oil.

Oregano oil contains about 63.4% of the active antimicrobial, carvacrol (Table 1). Because oregano oil is used as a salad dressing and has a pleasant taste (McGee 2004), this compound merits use in antimicrobial edible films. As indicated in Table 1, eugenol is the major constituent of allspice oil (Shylaja and Peter 2004). Garlic oil contains different types of sulfide active antimicrobial compounds (Elmina and others 1983).

Viscosity of TPFFS

A decrease in viscosity values of TPFFS by applied constant shear rates during 5 min was indicative of a non-Newtonian thixotropic fluid behavior (Singh and Heldman 1993). The viscosity of TPFFS evaluated in the present study was also reduced by increasing shear rates (Table 5). The TPFFS behaved as a shear thinning, pseudoplastic liquid, typical of fruit purees (Singh and Heldman 1993). The shear thinning behavior (decreasing viscosity with increasing shear rate) could be explained by several phenomena: as the shear rate increased, the asymmetric dispersed molecules tend to align themselves with the shear planes so that frictional resistance is reduced. Higher shear rates would progressively remove the solvated layers, leading to a reduced aggregated size and, hence, lower apparent viscosities through the intermediate shear range (Tung 1978). The addition of 1% allspice to the TPFFS further reduced the viscosity of the solutions at each of the 3 shear rates. This effect was only evident at the highest shear rate with films containing garlic and oregano oils. Arfa and others (2007) also reported that the addition of 20% (w/w) carvacrol did not modify the shear thinning flow behavior of a soy protein isolate solution, but decreased its apparent viscosity.

Water vapor permeability

An important role of edible films is to reduce exchange of water between the wrapped product and environment. The water barrier properties of such films depend on both molecular diffusion coefficient and solubility of water in the matrix (McHugh and others 1994). Water vapor permeability is a measure of the facility with which a material can be penetrated by water vapor (Cagri and others 2001). To compare the Water vapor permeability (WVP) of the tomato films with different EO formulations, it is important to have 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 tomato films with added garlic oil, indicating similar diffusion driving forces. In the present study, WVP properties were not affected by the incorporation of the EOs into the film compared to control tomato films with added oils, presumably because the EOs consist mostly of terpene-like compounds, not lipids. By contrast, Rojas-Graü and others (2006) reported a significant decrease in WVP in apple films formulated with pectin and cinnamon oil.

Table 5 — Effect of concentration (% w/w) of 3 plant essential oils on viscosity of TPFFS at different shear rates at 25 °C.

Films	Essential oil (% w/w)	4.65 s^{-1} shear rate (5 rpm) (cP)	116.25 s^{-1} shear rate (125 rpm) (cP)	232.50 s^{-1} shear rate (250 rpm) (cP)
Control	0	$6,960 \pm 160^{NSDxy}$	$1,589 \pm 28^{NSENS}$	$1,086 \pm 13^{bcCz}$
Oregano oil	0.5	$6,940 \pm 101^{NS}$	$1,539 \pm 33^{NS}$	$1,060 \pm 16^{ab}$
	1.0	$6,940 \pm 137$	$1,535 \pm 39$	$1,048 \pm 15^{a}$
	1.5	$6,940 \pm 77$	$1,581 \pm 38$	$1,092 \pm 19^{bc}$
	3.0	$6,940 \pm 120$	$1,577 \pm 36$	$1,090 \pm 16^{bc}$
Allspice oil	0.5	$6,980 \pm 210^{D}$	$1,549 \pm 13^{D}$	1083 ± 20^{C}
	1.0	$5,140 \pm 40^{A}$	$1,373 \pm 20^{A}$	954 ± 13^{A}
	1.5	$5,480 \pm 103^{B}$	$1,414 \pm 28^{B}$	979 ± 13^{AB}
	3.0	$5,780 \pm 177^{C}$	$1,443 \pm 10^{C}$	1005 ± 15^{B}
Garlic oil	0.5	$7,140 \pm 256^{y}$	$1,539 \pm 33^{NS}$	$1,051 \pm 20^{xy}$
	1.0	$6,760 \pm 103^{x}$	$1,535 \pm 39$	1033 ± 13^{x}
	1.5	$6,780 \pm 40.0^{x}$	$1,581 \pm 38$	1041 ± 11^{xy}
	3.0	$6,800 \pm 113^{x}$	$1,577 \pm 36$	1059 ± 13^{y}

Means in same column for essential oil and control films with different letters are significantly different at $P < 0.05$.
NS = not significantly different.

Tensile properties

Tensile strength indicates the maximum tensile stress that the film can sustain, percent elongation is the maximum change in length of a test specimen before breaking, and elastic modulus is a measure of the stiffness of the film (Srinivasaa and others 2007). Incorporation of EOs in TPFFS caused a significant reduction ($P < 0.05$) in tensile strength of tomato films (Table 7). This effect was more pronounced in films containing oregano oil. Addition of EOs did not affect film elongation. Elastic modulus was only reduced by the addition of oregano oil to tomato films. Zinoviadou and others (2009) also reported that the addition of oregano oil in the sorbitol-plasticized whey protein isolate films resulted in a decrease of elastic modulus with increasing oil concentration. Previously, we found that in general, addition of EOs to apple films resulted in significant reduction of tensile strength and elastic modulus and a higher film elongation (Du and others 2009). Added lipids or EOs are reported to cause decreases in tensile strength and elastic modulus of the films (Pérez-Gago and Krochta 2000; Yang and Paulson 2000; Fang and others 2002; Zivanovic and others 2005; Rojas-Graü and others 2006, 2007b). Lipid addition induces the development of a heterogeneous film structure, featuring discontinuities. The latter may affect the stretching ability of the film based on the characteristics of the added lipids. Since oregano oil is liquid at room temperature, it will be present in the film in the form of oil droplets that can easily be deformed.

Color of solutions and films

The L^* values (a measure of lightness) range from 0 to 100 from dark to light, respectively. The a^* value is a measure of greenness and the b^* value of yellowness (Rojas-Graü and others 2007b). The hue angle and chroma parameters combine the a^* and b^* color parameters. The hue angle value ranges from 0 to 360. A red color is described by hue angle values from 0 to 60. The higher this value the greater the yellowness. Addition of EOs to TPFFS increased the L^*, a^*, b^*, hue angle, and chroma of the tomato solutions (Table 8). The increase was directly related to the EO concentration in the TPFFS solutions. The addition of garlic oil resulted in a higher increase of color parameters than was the case with added oregano and allspice oils (Table 8). Added garlic oil induced a shift of the red tomato color of the solution to a yellow color.

Generally, the L^* and hue angle values of tomato films decreased with increased concentration of EOs, while a^*, b^*, and chroma values increased as the concentration of EOs increased (Table 9).

Table 6—Effect of concentration (% w/w) of 3 plant essential oils on water vapor permeability (WVP) of edible tomato puree films.

Films	Essential oil (% w/w)	Thickness[1] (mm)	%RH inside cup[1,2] (%RH)	WVP[1,2] (g-mm/Kpa-h-m^2)
Control	0	0.108 ± 0.022^{NS}	80.4 ± 1.0^{aANS}	2.77 ± 0.54^{NS}
Oregano oil	0.5	0.097 ± 0.011^{NS}	80.1 ± 1.1^{a}	2.56 ± 0.39^{NS}
	1.0	0.098 ± 0.014	80.9 ± 1.0^{ab}	2.46 ± 0.37
	1.5	0.101 ± 0.011	80.3 ± 0.8^{a}	2.63 ± 0.30
	3.0	0.100 ± 0.012	81.9 ± 0.6^{b}	2.35 ± 0.29
Allspice oil	0.5	0.101 ± 0.017^{NS}	80.3 ± 0.9^{A}	2.60 ± 0.38^{NS}
	1.0	0.099 ± 0.015	80.5 ± 0.6^{A}	2.55 ± 0.43
	1.5	0.100 ± 0.013	80.4 ± 1.0^{A}	2.59 ± 0.29
	3.0	0.101 ± 0.014	81.6 ± 0.8^{B}	2.42 ± 0.34
Garlic oil	0.5	0.098 ± 0.008^{NS}	80.4 ± 0.8^{NS}	2.52 ± 0.22^{NS}
	1.0	0.096 ± 0.011	80.5 ± 0.6	2.45 ± 0.28
	1.5	0.096 ± 0.012	80.7 ± 0.8	2.45 ± 0.37
	3.0	0.099 ± 0.013	80.3 ± 1.4	2.58 ± 0.47

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

Table 7—Effect of concentration (% w/w) of 3 plant essential oils on the tensile properties of edible tomato puree edible films.

Films	Essential oil (% w/w)	Film thickness[1] (mm)	Tensile strength[1] (MPa)	Elongation[1] (%)	Elastic modulus[1] (MPa)
Control	0	0.115 ± 0.006^{aAx}	9.13 ± 1.38^{cBy}	32.2 ± 4.0^{NS}	64.2 ± 14.1^{bNS}
Oregano oil	0.5	0.114 ± 0.007^{a}	8.78 ± 1.35^{bc}	30.3 ± 4.7^{NS}	68.7 ± 16.9^{b}
	1.0	0.118 ± 0.004^{a}	7.91 ± 0.81^{b}	29.8 ± 6.7	57.6 ± 15.7^{ab}
	1.5	0.125 ± 0.005^{b}	8.04 ± 1.0^{b}	29.5 ± 6.4	57.7 ± 14.6^{ab}
	3.0	0.131 ± 0.007^{c}	6.61 ± 0.82^{a}	29.4 ± 6.9	44.8 ± 12.8^{a}
Allspice oil	0.5	0.115 ± 0.006^{A}	8.59 ± 1.34^{B}	31.7 ± 6.7^{NS}	62.3 ± 21.8^{NS}
	1.0	0.117 ± 0.005^{A}	8.14 ± 1.08^{AB}	30.0 ± 6.2	61.0 ± 16.5
	1.5	0.119 ± 0.004^{A}	8.24 ± 1.18^{AB}	30.8 ± 5.7	59.4 ± 14.0
	3.0	0.132 ± 0.006^{B}	7.13 ± 0.87^{A}	30.3 ± 6.1	53.0 ± 14.0
Garlic oil	0.5	0.117 ± 0.005^{xy}	8.98 ± 1.25^{xy}	29.5 ± 4.5^{NS}	68.4 ± 9.6^{NS}
	1.0	0.118 ± 0.006^{xy}	9.16 ± 1.56^{y}	30.8 ± 5.8	67.8 ± 17.6
	1.5	0.118 ± 0.005^{xy}	8.97 ± 1.19^{xy}	32.2 ± 6.3	62.8 ± 17.4
	3.0	0.122 ± 0.006^{y}	7.79 ± 1.13^{x}	31.6 ± 6.9	54.1 ± 15.6

[1] Thickness, tensile strength, elongation, and elastic modulus data are mean values ± standard deviations.
Means in same column for control and essential oil films with different letters are significantly different at $P < 0.05$
NS = not significantly different.

Table 8 — Effect of concentration (% w/w) of 3 plant essential oils on color parameters of tomato puree film forming solutions.[1]

Solutions	Essential oil (% w/w)	L^*	a^*	b^*	Hue angle	Chroma
Control	0	29.6 ± 0.2^{aAu}	24.8 ± 0.6^{aAu}	28.9 ± 0.2^{aAu}	49.3 ± 0.7^{aABx}	38.1 ± 0.4^{aAu}
Oregano oil	0.5	39.4 ± 0.1^{b}	25.1 ± 0.5^{ab}	32.5 ± 0.3^{b}	52.4 ± 0.5^{b}	41.1 ± 0.5^{b}
	1.0	45.7 ± 0.1^{c}	25.7 ± 0.5^{b}	33.4 ± 0.2^{c}	52.4 ± 0.4^{b}	42.1 ± 0.5^{c}
	1.5	49.0 ± 0.2^{d}	25.3 ± 0.5^{ab}	36.4 ± 0.2^{d}	55.1 ± 0.4^{d}	44.3 ± 0.4^{d}
	3.0	53.8 ± 0.1^{e}	27.5 ± 0.6^{c}	37.0 ± 0.4^{e}	53.4 ± 0.3^{c}	46.1 ± 0.7^{e}
Allspice oil	0.5	38.4 ± 0.1^{B}	24.8 ± 0.5^{B}	33.3 ± 0.2^{B}	50.2 ± 0.4^{B}	43.3 ± 0.4^{B}
	1.0	43.0 ± 0.4^{C}	27.9 ± 0.9^{B}	33.5 ± 0.2^{B}	50.2 ± 0.9^{B}	43.6 ± 0.6^{B}
	1.5	45.7 ± 0.1^{D}	28.5 ± 1.1^{B}	33.4 ± 0.3^{B}	49.5 ± 1.1^{AB}	43.9 ± 0.8^{B}
	3.0	49.6 ± 0.2^{E}	30.0 ± 0.8^{C}	34.5 ± 0.5^{C}	48.9 ± 0.4^{A}	45.7 ± 0.9^{C}
Garlic oil	0.5	43.5 ± 0.2^{w}	28.5 ± 1.0^{w}	34.8 ± 0.5^{w}	49.3 ± 0.7^{y}	45.0 ± 1.0^{w}
	1.0	47.3 ± 0.1^{x}	33.0 ± 0.5^{x}	37.9 ± 0.3^{x}	50.7 ± 0.3^{x}	50.2 ± 0.5^{x}
	1.5	48.6 ± 0.2^{y}	35.8 ± 0.5^{y}	41.4 ± 0.3^{y}	49.0 ± 0.2^{x}	54.7 ± 0.6^{y}
	3.0	49.5 ± 0.1^{z}	39.4 ± 0.3^{z}	47.6 ± 0.2^{z}	50.3 ± 0.2^{y}	61.8 ± 0.3^{z}

[1] L^*, a^*, b^*, hue angle and chroma data are mean values ± standard deviations.
Means in same column for control and essential oil films with different letters are significantly different at $P < 0.05$.

Table 9 — Effect of concentration (% w/w) of 3 plant essential oils on color parameters of edible tomato puree films.[1]

Films	Essential oil (% w/w)	L^*	a^*	b^*	Hue angle	Chroma
Control	0	61.3 ± 1.0^{cCy}	26.7 ± 1.0^{aAu}	43.0 ± 0.7^{aAu}	58.2 ± 1.0^{bCz}	50.6 ± 0.8^{aAu}
Oregano oil	0.5	60.2 ± 1.5^{c}	27.4 ± 1.4^{a}	45.6 ± 1.2^{c}	59.0 ± 1.8^{b}	53.2 ± 0.9^{b}
	1.0	56.6 ± 1.0b	30.4 ± 1.1^{b}	44.4 ± 0.5^{b}	55.6 ± 1.0^{a}	53.8 ± 0.6^{b}
	1.5	56.6 ± 0.6^{b}	30.1 ± 0.5^{b}	47.8 ± 0.7^{d}	57.8 ± 0.8^{b}	56.4 ± 0.3^{c}
	3.0	55.2 ± 0.7^{a}	33.6 ± 0.6^{c}	48.8 ± 0.5^{d}	55.5 ± 0.7^{a}	59.2 ± 0.3^{d}
Allspice oil	0.5	60.6 ± 0.7^{C}	28.9 ± 0.7^{B}	45.3 ± 0.7^{C}	57.5 ± 0.8^{C}	53.7 ± 0.6^{B}
	1.0	55.7 ± 1.7^{B}	34.5 ± 1.6^{C}	44.5 ± 0.7^{B}	52.2 ± 1.7^{B}	56.3 ± 0.5^{C}
	1.5	55.1 ± 1.0^{AB}	35.5 ± 1.0^{C}	44.4 ± 0.5^{B}	51.3 ± 0.9^{AB}	56.8 ± 0.6^{C}
	3.0	54.4 ± 0.6^{A}	37.8 ± 0.5^{D}	45.8 ± 0.3^{C}	50.4 ± 0.5^{A}	59.3 ± 0.2^{D}
Garlic oil	0.5	57.2 ± 3.0^{x}	30.2 ± 1.4^{w}	46.5 ± 0.8^{w}	57.0 ± 1.6^{z}	55.4 ± 0.5^{w}
	1.0	55.8 ± 1.5^{x}	36.2 ± 1.5^{x}	49.5 ± 1.1^{x}	53.8 ± 1.7^{y}	61.3 ± 0.3^{x}
	1.5	55.5 ± 0.9^{x}	39.3 ± 0.8^{y}	52.9 ± 1.0^{y}	53.4 ± 1.1^{y}	65.9 ± 0.3^{y}
	3.0	55.5 ± 0.6^{x}	43.3 ± 0.4^{z}	54.5 ± 0.6^{z}	51.5 ± 0.5^{x}	69.6 ± 0.4^{z}

[1] L^*, a^*, b^*, hue angle and chroma data are mean values ± standard deviations.
Means in same column for control and essential oil films with different letters are significantly different at $P < 0.05$.

Color of the film may influence consumer acceptability of a product (Kunte and others 1997). Rhim and others (2000) reported that the addition of various compounds that structurally bind with the film-forming solutions changed the native color of the soy protein film. Sivarooban and others (2008) also reported that the incorporation of 1% grape seed extract into soy protein isolate films significantly ($P < 0.05$) influenced the L^*, a^*, and b^* values. Sensory studies need to be conducted for evaluating consumer acceptability of this color change in tomato film.

Conclusions

The antimicrobial activity of oregano oil was greater than the activities of allspice and garlic oils in tomato puree edible films against *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*. The added oils did not adversely affect water vapor permeability and effects on tensile properties were also minor. The antimicrobial data obtained with the vapor diffused from the tomato puree films can serve as a guide for selection of appropriate levels of volatile plant essential oils and their active constituents for incorporation into antimicrobial edible films. Incorporating essential oils into edible films provides novel ways to enhance, by direct and indirect contact, the microbial safety and shelf life of foods. The edible tomato films containing essential oils have the potential to provide multiple benefits to consumers.

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