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Storage Stability and Antibacterial Activity against *Escherichia coli* O157:H7 of Carvacrol in Edible Apple Films Made by Two Different Casting Methods

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The antimicrobial activities against *Escherichia coli* O157:H7 as well as the stability of carvacrol, the main constituent of oregano oil, were evaluated during the preparation and storage of apple-based edible films made by two different casting methods, continuous casting and batch casting. Antimicrobial assays of films and high-performance liquid chromatography (HPLC) analysis of film extracts following storage up to 49 days at 5 and 25 °C revealed that (a) optimum antimicrobial effects were apparent with carvacrol levels of ~1.0% added to the purees prior to film preparation, (b) carvacrol in the films and film weights remained unchanged over the storage period of up to 7 weeks, and (c) casting methods affected carvacrol concentration, bactericidal activity, physicochemical properties, and colors of the apple films. Carvacrol addition to the purees used to prepare the films reduced water vapor and oxygen permeability of apple films. The results indicate that carvacrol has a dual benefit. It can be used to both impart antimicrobial activities and enhance barrier properties of edible films. The cited observations facilitate relating compositional and physicochemical properties of apple puree films containing volatile plant antimicrobials to their use in foods.

KEYWORDS: Carvacrol; film casting; antibacterial apple films; *E. coli* 0157:H7; HPLC

INTRODUCTION

Outbreaks of food-borne illness cause concern among consumers, the food industry, and regulatory agencies (*1, 2*). The majority of cases associated with fresh produce is caused by *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter jejuni* (*3*). Of special concern is the virulent *E. coli* O157:H7 strain, a foodborne pathogen that has been reported to contaminate various foods, including raw milk (*2*), cheese (*4, 5*), undercooked meat (*6*), and spinach (*7–9*). A need therefore exists to discover new, food-compatible ways to protect foods against this and other pathogens.

Unresolved is the question of how best to use these naturally occurring, food-compatible, and safe compounds to protect foods and feeds against pathogenic bacteria. In principle, this can be accomplished in at least two ways: (a) incorporation of the antimicrobial into the food and (b) protecting the food with an edible antimicrobial film.

As part of this effort, we previously reported that edible-film-forming solutions and films made from apples containing low levels of plant essential oils (oregano, cinnamon, and lemongrass) and their major constituents (carvacrol, cinnamaldehyde, and citral) induced rapid reduction of pathogenic bacteria, such as *E. coli* O157:H7 (*10, 11*).

Films made from fruits or vegetables have the potential to be used commercially to protect food against contamination by pathogenic bacteria on surfaces of food, thus extending the shelf life and safety of meat, fruits, and vegetables (*12*). The major objectives of the present study were (a) to evaluate antimicrobial activities as well as the storage stability and physical properties of carvacrol-containing apple-based films with the aid of high-performance liquid chromatography (HPLC) and physicochemical assays, (b) to optimize antimicrobial effects of the films by determining whether carvacrol contents of films correlate with antimicrobial efficacy, and (c) to evaluate the effect of two different casting methods used to prepare the films from the purees on antimicrobial activities and physical properties of apple 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 apple juice associated with an outbreak (*13, 14*).

Preparation of Apple Films. *Preparation of the Apple Film Forming Solution.* Golden Delicious apple puree (38 °Brix) (Sabroso Co., Medford, OR) was the primary ingredient in all apple-based film-

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Table 1. Effect of Carvacrol Concentration (% w/w) on Water Vapor (WVP) and Oxygen Permeabilities (O_2P) of Apple Puree Edible Films[a,b]

casting method	carvacrol concentration (% w/w)	thickness (mm)	RH inside cup (%)	WVP (g mm kPa^{-1} h^{-1} m^{-2})	O_2P (cm^3 μm m^{-2} d^{-1} kPa^{-1})
batch cast	0	0.152 a	79.0 ab	4.39 ± 0.20 b	83.56 ± 6.50 b
	0.5	0.154 a	79.1 ab	4.32 ± 0.22 b	67.98 ± 1.15 a
	1.0	0.154 a	80.4 b	3.99 ± 0.21 a	71.70 ± 0.86 a
	1.5	0.166 b	77.6 a	5.05 ± 0.27 c	80.40 ± 1.00 b
continuous cast	0	0.144 B	77.7 NS	4.39 ± 0.44 B	74.98 ± 0.84 B
	0.5	0.136 AB	78.2	4.00 ± 0.16 A	63.00 ± 2.28 A
	1.0	0.135 A	78.0	4.02 ± 0.18 A	58.17 ± 0.70 A
	1.5	0.131 A	78.3	3.81 ± 0.17 A	70.27 ± 4.38 B

[a] Data reported are mean values ± standard deviations. Means in column with different letters are significantly different at $p < 0.05$ for each of the casting methods. NS = no significant differences between films. [b] Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP Correction Method (*16*).

Table 2. Effect of Carvacrol Concentration (% w/w) on the Tensile Properties of Apple Puree Edible Films[a]

casting method	carvacrol concentration (% w/w)	tensile strength (MPa)	elongation (%)
batch cast	0	1.45 ± 0.13 a	45.94 ± 2.54 a
	0.5	1.93 ± 0.17 c	45.02 ± 2.90 a
	1.0	1.82 ± 0.11 c	46.51 ± 2.85 a
	1.5	1.60 ± 0.11 b	50.31 ± 1.63 b
continuous cast	0	1.25 ± 0.14 A	38.1 ± 2.4 NS
	0.5	1.85 ± 0.36 B	38.4 ± 4.8
	1.0	1.92 ± 0.24 B	39.5 ± 2.9
	1.5	1.81 ± 0.13 B	38.4 ± 3.5

[a] Same footnote as in **Table 1**.

forming solutions (APFFS). Glycerol (Fisher Scientific, Waukesha, WI) was added as a plasticizing agent. Ascorbic acid (BASF, Mount Olive, NJ) and citric acid (Archer Daniels Midland, Decatur, IL) were used as browning inhibitors. Low methoxyl pectin (Systems BioIndustries, Fair Lawn, NJ) was added to increase film strength and facilitate the release from cast surfaces. Carvacrol, the main antibacterial component of oregano essential oil (from *Origanum vulgare*), was donated by Millenium Chemical Co. (Jacksonville, FL).

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 (*15*). This solution also contained ascorbic and citric acids (2.5 g, 0.25% w/w) and glycerol (30 g, 3% w/w). Carvacrol was then incorporated into the apple puree solutions at the following concentrations: 0 (control), 0.5, 1.0, and 1.5% (w/w). These solutions were homogenized for 3 min at 12 500 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.

Film Casting. Two methods were used to cast films: continuous casting and batch casting. Edible apple films were continuous cast with a Mathis Labcoater unit (Werner Mathis AG, Zürich, Switzerland) by spreading 41 mil (1 mil = 0.001 in.) thickness apple solution on a Mylar sheet conveyor moving at 0.11 m/min. The film was first partially dried by an infrared heater adjusted to 0 and 90% energy emission at the bottom and top, respectively. Next, the convective heating stage was controlled at 132 °C and 1500 m/min air velocity. Batch cast films were produced on the bench. They were made using a 45 mil gap draw down bar to spread the apple solutions on a flat Mylar sheet on 29 × 29 cm^2 glass plate, which was then dried overnight at room temperature (20–25 °C) in a sterile biohood.

Physical Properties of Films. *Water Vapor Permeability (WVP).* The Gravimetric Modified Cup Method (*16*) based on standard method American Society for Testing and Materials (ASTM) E96-80 (*17*) 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, MA). Fan speeds were set to achieve air velocities of 80 m/min to ensure uniform relative humidity throughout the cabinets. 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 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. The steady state of the water vapor transmission rate was achieved within 2 h. Each cup was weighed 8 times at 2 h intervals. Eight replicates of each film were tested. RHs at the film undersides and WVPs were calculated using the WVP Correction Method (*16*). 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 calculated the WVP of the films

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

where WVTR is the water vapor transmission rate and p_{A1} and p_{A2} are the water vapor partial pressure inside and outside the cup, respectively. Units for WVP are g mm kPa^{-1} h^{-1} m^{-2}.

Oxygen Permeability (O_2P). Oxygen permeability of apple films was determined using a Coulox detector at 55% RH through a 100% oxygen differential. An Ox-Tran 2/20 modular system (Modern Controls, Inc., Minneapolis, MN) was used to measure oxygen transmission rates through the films according to standard method ASTM D3985 (*18*). Oxygen transmission rates were determined at 23 °C and 55 ± 1% RH. Each film was placed with the shiny side down 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 2 h waiting period and up to 10 cycles of readings each for 2 h 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. The oxygen permeability was reported in cm^3 μm m^{-2} day^{-1} kPa^{-1} units. Three replicates of each film were evaluated.

Tensile Properties. Standard method ASTM D882-97 (*19*) was used to measure tensile properties of films. Films were cut into strips with a test dimension of 165 × 19 mm according to standard method ASTM D638-02a (*20*). All films were conditioned for 48 h at 23 ± 2 °C and 50% ± 2% RH before testing using a saturated 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 Model 55R4502 Universal Testing Machine (Instron, Canton, MA) 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) versus strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron, Canton, MA). A total of 15 specimens of each film were evaluated.

Table 3. Effect of Carvacrol Concentration (% w/w) on Color Parameters and Whitish Index of Apple Puree Edible Films[a]

casting method	carvacrol concentration (% w/w)	L^*	A^*	b^*	Whitish Index
batch cast	0	86.0 ± 0.6 b	−1.5 ± 0.1 d	16.4 ± 1.4 a	78.4 ± 1.3 c
	0.5	85.7 ± 0.8 ab	−1.8 ± 0.1 c	17.1 ± 1.8 ab	77.6 ± 1.8 bc
	1.0	85.6 ± 0.5 ab	−2.0 ± 0.1 b	19.3 ± 1.7 b	75.8 ± 1.6 b
	1.5	85.1 ± 0.5 a	−2.4 ± 0.1 a	23.0 ± 2.0 c	72.5 ± 1.9 a
continuous cast	0	86.0 ± 0.4 c	−2.3 ± 0.2 A	21.1 ± 2.3 A	74.6 ± 2.0 c
	0.5	84.2 ± 0.7 B	−1.7 ± 0.5 B	25.8 ± 2.9 B	69.6 ± 2.7 B
	1.0	83.3 ± 0.9 AB	−1.1 ± 0.7 c	28.9 ± 2.6 c	66.6 ± 2.7 AB
	1.5	82.6 ± 1.1 A	−0.7 ± 0.5 c	30.8 ± 2.6 c	64.6 ± 2.8 A

[a] Same footnote as in **Table 1**.

Figure 1. HPLC chromatogram of an apple film extract containing an initial 1.5% carvacrol in the apple puree used to prepare the films.

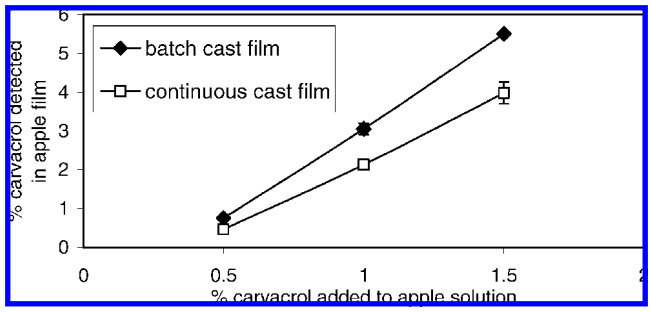

Figure 2. Relationship between the carvacrol content determined by HPLC in apple films made by two casting methods and carvacrol added to the apple-film-forming solutions.

Film Colors. Color of films under a Minolta standard white reflector plate was directly 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 11 films were evaluated for each carvacrol concentration. Three readings were made in each replicate by changing the position of the Chroma Meter over the film. Previously described numerical values of L^*, a^*, and b^* parameters were employed to calculate the Whitish Index as $Wi = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (*21*).

Storage Studies. Continuous and batch cast apple films were shaped into 50 mm diameter discs by cutting with a razor blade around the edge of a watch glass over the film and stored without covering on meshed plastic shelves in Forma Scientific refrigerated incubators (Mallinckrodt, Inc., Marietta, OH) at 5 °C and 24% RH and at 25 °C and 22% RH. The stored films were sampled at day 1 and weekly for 7 weeks. Five film discs were taken out for testing on each sampling time. Stability of carvacrol in apple films was determined by extracting the carvacrol from the films and then analyzing for carvacrol content by HPLC as described below. The antimicrobial activities of the stored films were also determined concurrently as described below.

Preparing Film Discs for Microbiology and HPLC Studies. For microbiology studies, each 50 mm film disk was further 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 until used. The weight and thickness of films used for microbial testing were measured with an analytical balance and a micrometer, respectively.

For HPLC studies, three 50 mm diameter film discs were covered in folded aluminum foil, sealed in plastic bags, and stored under refrigeration until testing.

Antimicrobial Assay of E. coli O157:H7—Overlay Experiment. Frozen cultures of *E. coli* O157:H7 strain RM1484 from our Bacterial Strain Collection were streaked on tryptic soy agar (TSA) and incubated at 37 °C for 24 h. One isolated colony was restreaked on TSA and then incubated at 37 °C for 24 h. One isolated colony was then inoculated into a tube with 5 mL of trypticase soy broth (TSB) and incubated at 37 °C for 24 h with agitation. The microbial broth was serially diluted (10×) in 0.1% peptone containing 0.85% NaCl. Then, 0.1 mL of 10^5 colony-forming units (CFU)/mL of *E. coli* O157:H7 were plated onto each of six MacConkey−sorbitol agar (MSA) plates. The bacteria inoculum was spread evenly throughout each plate and left to dry for 5 min in a biosafety hood.

Each agar plate was divided evenly into four areas and labeled with the different carvacrol concentrations. On the center of each area, one aseptically cut 12 mm diameter edible film disk was deposited over the inoculated agar with the shiny side of the film down. The plates were incubated at 35 °C for 48 h. The inhibition radius around the film disk (colony-free perimeter) was measured with a digital caliper (Neiko Tools, Ontario, CA) in triplicate after 24 and 48 h of incubation, respectively. The inhibition area was then calculated.

HPLC Method for Measurement of Carvacrol in Films. Each film was weighed (ca. 230 mg) and homogenized in an Omni International Homogenizer (Gainesville, VA) in 10 mL of 50% ethanol (prepared from 95% ethanol, ACS/USP grade) for 5 min on low speed with a blade attachment and then for 5 min on high speed with a generator probe. The extract was filtered through a 0.45 μm nylon membrane (Sigma, St. Louis, MO) and injected directly into the HPLC column.

The HPLC system consisted of a Beckman 110B pump, a Thermo Separation Products AS3500 Autosampler (loop size of 100 μL), and a UV 3000HR scanning detector with both deuterium and tungsten lamps. Thermo Separation Products PC1000 System Software controlled the system. The following conditions were used: a Supelco LC-ABZ column was used (250 × 4.6 mm plus a 2 cm precolumn); the particle size of the column packing was 5 μm; the eluent consisted of 50% acetonitrile, 50 mM ammonium phosphate, and 0.05% phosphoric acid

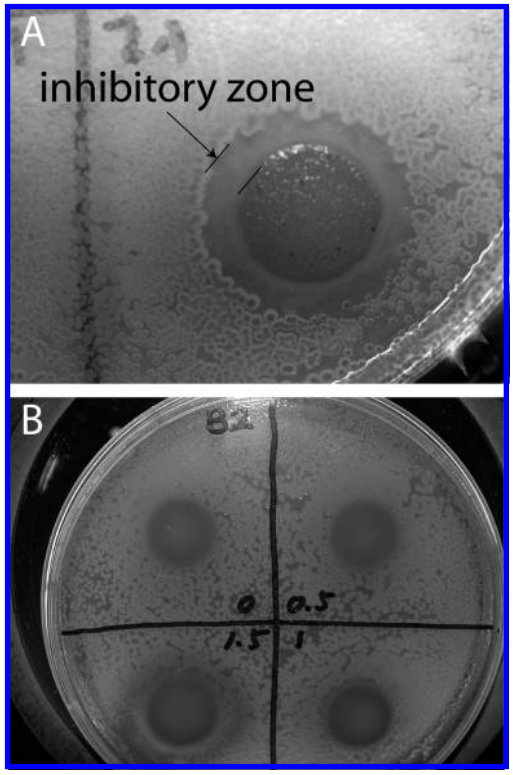

Figure 3. (**A**) Inhibitory zone (antimicrobial effect) of *E. coli* O157:H7 growth on a bacterial plate induced by a carvacrol-containing apple film measured with a digital caliper. (**B**) Inhibitory zones of *E. coli* O157:H7 growth around films with different concentrations of carvacrol (0−1.5%) added to apple puree solutions used to prepare the films.

at pH 3.1. The eluent was degassed once before use. The flow rate of the pump was 1 mL/min, and the sample volume injected was 20 μL. Absorbance was monitored at 200 nm.

Statistical Analysis. Data were analyzed by two- and one-way analysis of variance (ANOVA) using Minitab version 13.31 software (Minitab, Inc., State College, PA). Tukey test was used to determine the difference at a 5% significance level (*22*).

RESULTS AND DISCUSSION

We will first describe effects of added carvacrol on physical−chemical properties of the films followed by a discussion of the influence of carvacrol levels on antimicrobial and storage properties of the films.

Physical−Chemical Properties of Films. *Water Vapor Permeability.* **Table 1** shows that based on two-way ANOVA, WVP was significantly higher ($p < 0.05$) for batch cast films than for those made continuously. Higher casting temperatures used in the Labcoater increased rates of both carvacrol and water evaporations and made the films thinner and denser, probably reducing interstitial spaces for molecular diffusion. Because all of the films were stored in an incubator at constant temperature and humidity, moisture was not measured. The batch cast apple films were less dense than the continuous cast apple films, as evidenced by the following calculated respective average densities ($n = 42$): 1.365 ± 0.009 and 1.424 ± 0.008 g/cm^3 ($p < 0.001$).

Table 1 also shows that WVP was reduced significantly ($p < 0.05$) when carvacrol was added to the continuous cast apple films made in the Labcoater. The addition of carvacrol to apple film solutions at any concentration between 0.5 and 1.5% reduced WVP of films compared to control apple films without carvacrol. Increases in the carvacrol concentration in continuous cast films were accompanied by reduced WVP values. This effect was not evident in batch cast films. The effect may be related to differences in film thicknesses and associated %RH differentials in both sides of the films.

Oxygen Permeability. Oxygen permeability (O_2P) was significantly higher ($p < 0.05$) for batch cast films than for those made continuously (**Table 1**). The higher casting temperatures used in the Labcoater increased carvacrol evaporation and made the films thinner and denser, probably reducing interstitial spaces for oxygen diffusion, thus lowering O_2P. **Table 1** also shows that O_2P was also reduced significantly ($p < 0.05$) when carvacrol was added to the apple films made with both casting methods. Previously, we reported a slight decrease in O_2P of apple films formulated with lemongrass essential oil and its active compound citral (*11*). The previous and present studies thus indicate that the chemical nature of essential oil components influence the barrier properties of edible films. Our results suggest that carvacrol may have a dual benefit. It can be used to both impart antimicrobial activities as well as enhance barrier properties of the films.

Tensile Properties. Tensile strength, elongation, and elastic modulus are parameters that relate mechanical properties of films to their chemical structures (*23*). **Table 2** shows that the addition of carvacrol to apple films increased tensile strength, while the casting methods had no significant effect. Elongation at the break is a measure of the stretch ability of the film prior to breakage (*24*). **Table 2** shows that the percent elongation of batch cast films was significantly higher than that of continuous cast films ($p < 0.05$). It also demonstrates that the concentration of carvacrol added did not affect the percent elongation of continuous cast films. Batch cast films with the highest carvacrol concentration had significantly higher percent elongation. We have no obvious explanation for the differences in the elongation of the films prepared by the two methods.

Film Colors. **Table 3** shows that batch cast films had a significantly ($p < 0.05$) higher Whitish Index and L^* values and significantly lower a^* and b^* values. The observed color changes are presumably due to the browning of the films caused by the higher casting temperatures used in the Labcoater. The Whitish Index, L^* and a^* values increased, whereas the b^* value of the apple films decreased with an increasing carvacrol concentration. The effect of an increased carvacrol concentration on color parameters was more pronounced in films continuously cast in the Labcoater than those prepared in batches under the biohood.

Carvacrol Stability during Storage of Apple Films. Figure 1 shows that, at an absorbance of 200 nm, carvacrol eluted at ~12 min on the HPLC column. **Figure 2** demonstrates that the carvacrol concentration is higher for batch cast than for continuous cast films, presumably because the high casting temperature used in the Labcoater induced faster carvacrol evaporation. The relative concentration of carvacrol in cast films increased by 3.2 and 2.2 times the initial concentration in the apple solution used to cast the films in the batch and continuous casting processes, respectively. Carvacrol has a boiling point of 237.7 °C, and its vapor pressure is 0.0232 mmHg at 25 °C. It is considered to be a volatile molecule.

The structure of carvacrol [2-methyl-5-(1-methylethyl)-phenol] shown in **Figure 1** suggests that it is an antioxidative hydrophilic−hydrophobic phenolic compound. However, the calculated hydrophilic–lipophilic balance (HLB) number for carvacrol of 4.15 by the semiempirical method (*25*) suggests that the compound is predominantly a hydrophobic molecule

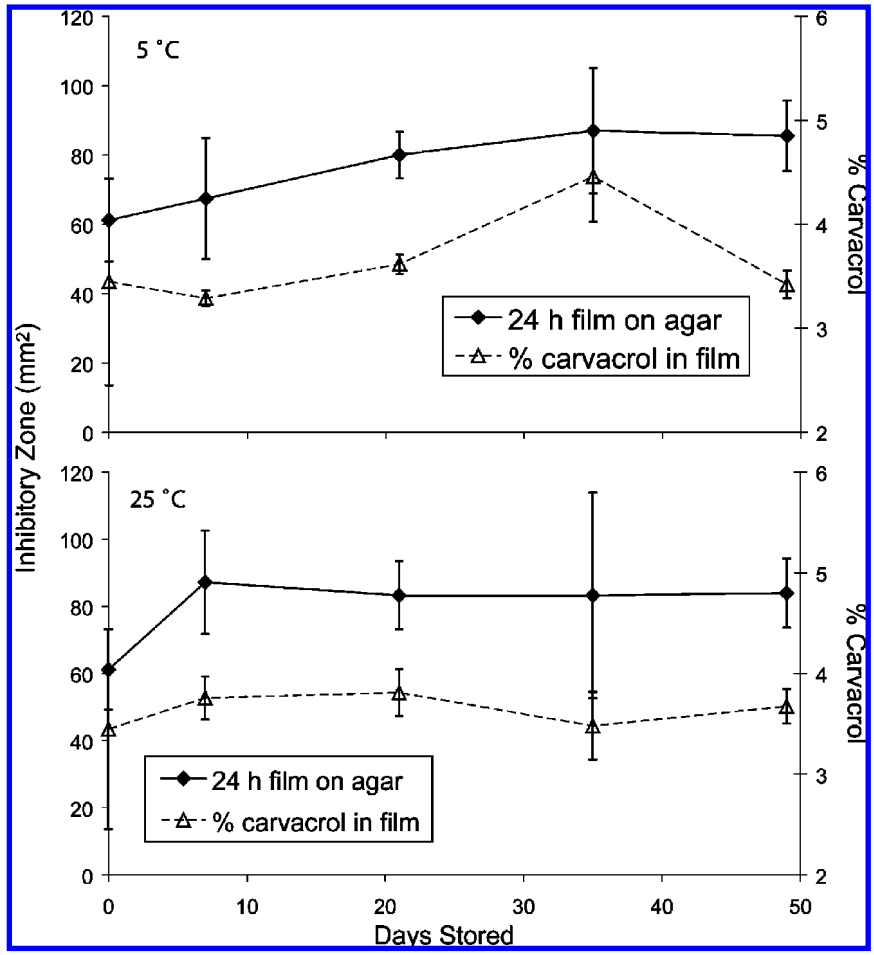

Figure 4. Effect of storage of continuous casting films at 5 and 25 °C during 49 days on the antimicrobial activity (inhibitory zones) and the carvacrol concentration of apple films with added 1.5% carvacrol.

that would dissolve preferentially in oil, stabilize oil-in-water emulsions, and form micelles in water.

Figure 4 shows that the carvacrol concentrations determined by HPLC with 50% ethanol extracts of apple films made by the continuous casting did not significantly change during storage of the apple films up to 7 weeks at 5 and 25 °C. The same pattern was observed with films made by the batch process (results not shown). The films were stored in temperature- and humidity-controlled incubators on open shelves. None of the films showed a significant loss of carvacrol during the 7 week storage period at either temperature. Additional studies (results not shown) indicate that film weights also did not significantly change following storage for up to 7 weeks.

Antimicrobial Activities of Stored Apple Films. *E. coli* O157:H7 generally grew normally on agar plates with films lacking carvacrol incubated at 35 °C for 24 or 48 h. In contrast, no growth was observed on the plates around the 12 mm diameter film discs containing 1.0 or 1.5% carvacrol added to the apple puree slurries (**Figure 3** and **4**). Generally, the extent of inhibition of growth of the bacteria increased as the percent of carvacrol in the films was increased. The inhibition area was also dependent upon the concentration of carvacrol in dried films but independent of the casting methods (data not shown). Because the results of the antimicrobial assays performed at 24 and 48 h were similar, we only report the 24 h data.

Mathematical Analysis of Relationship between Carvacrol Content in Film-Forming Solutions and Activity in Final Films. Using data in **Figure 2**, the following equations were developed to describe the linear relationship of the carvacrol concentration in film-forming solutions and final dried cast films:

$$B = 4.747c - 1.643 \quad (n = 3;\ r^2 = 0.9998) \qquad (2)$$

$$C = 3.516c - 1.322 \quad (n = 3;\ r^2 = 0.9995) \qquad (3)$$

where, B is the percentage of carvacrol in dried batch cast apple films, C is the percentage of carvacrol in dried continuous cast apple films, c is the percentage of carvacrol in apple-film-forming solution, and r^2 is the correlation coefficient.

On the basis of these relationships and the observed antimicrobial effects as a function of the carvacrol concentration in the films, we conclude that the minimum amount of carvacrol to be added in the apple solution to ensure effective antibacterial activity is 1.0 and 0.75% for continuous and batch cast films, respectively. These concentrations of added carvacrol will ensure a minimum effective antimicrobial concentration of 1.95% final carvacrol in the films dried by the two casting methods.

Related Studies with Edible Films. The results of our present study complement and extend reported studies on antimicrobial properties of edible antimicrobial films prepared from other edible food ingredients. These include films made from alginate with essential oils (*26–28*), starch−alginate with lemongrass oil (*29*), cellulosic ethers containing fatty acids and nisin (*30*), hydroxypropylmethylcellulose (HPMC) with nisin (*31*), chitosan with essential oils (*32, 33*), starch−casein with a neem plant extract (*34*), chitosan with nisin (*35*), tapioca starch with potassium sorbate (*36*), chitosan−glucomannan−nisin

blends (*37, 38*), chitosan−starch blends (*39, 40*), fish-skin gelatin with lysozyme (*41*), milk proteins with essential oils (*42*), sodium caseinate with potassium sorbate, sodium lactate, and nisin (*43*), soy protein with nisin and grape seed and green tea extracts (*44*), fermented soybean and corn germ meals without added antimicrobials (*45, 46*), whey protein with *p*-aminobenzoic and sorbic acids (*47–49*), whey protein with lactoferrin, lysozyme, and lactoperoxidase (*50*), whey protein with essential oils (*51*), zein corn protein with lysozyme (*52, 53*), and zein corn protein with nisin (*54*).

It is instructive to compare the results of the present study with results from related published studies. Zivanovic et al. (*33*) found that antimicrobial properties of chitosan films containing anise, basil, coriander, and oregano plant essential oils were similar to those observed with pure oils. Some of these films also inactivated *Listeria monocytogenes* and *E. coli* O157:H7 pathogens on the surface of meat products. Related studies showed that alginate-based films containing garlic oil (*55*), whey protein-based films containing several essential oils (*51*), and partially hydrolyzed sago starch−alginate films containing lemongrass oil (*29*) were also active against foodborne pathogens. The cited studies indicate that the development and application of edible antimicrobial films is currently a very active area of worldwide research. However, unlike the approach used in the present study, previous studies did not evaluate the storage stabilities of the antimicrobial constituents of the films or the fate of antimicrobials in films prepared by different film-casting methods.

In conclusion, the present study reports for the first time on a HPLC method to measure the concentrations of the plant antimicrobial carvacrol in apple-based films prepared by two different casting methods, on the stability of carvacrol during storage of the films and on the relationship between carvacrol levels and antimicrobial activities of the films against a virulent foodborne pathogen. Further studies are needed to demonstrate the potential of the food-compatible edible apple films to reduce the human pathogen burden of foods. To meet this need, we are also evaluating the potential value of antimicrobial-containing apple films for use in foods.

NOTE ADDED AFTER ASAP PUBLICATION

The version published ASAP March 27, 2008 contained incorrect information concerning an author name and Table 2. The current version of April 3, 2008 is correct.

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