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Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil

Yue Zhang, Qiumin Ma, Faith Critzer, P. Michael Davidson, Qixin Zhong*

Department of Food Science and Technology, University of Tennessee, Knoxville, United States

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ABSTRACT

Edible antimicrobial films with essential oils are potential intervention strategies to improve microbiological safety of foods. The aim of this work was to study the effects of incorporating soybean oil (SBO) on the physical and antibacterial properties of alginate films prepared with 1% or 2% cinnamon bark oil (CBO). The incorporation of SBO improved the microstructure homogeneity and transparency, reduced tensile strength, elongation at break, water solubility, and total solids content but had no obvious effects on the water vapor permeability of films. Films prepared with 1% CBO had a low residual CBO after film formation and storage. For 2% CBO treatments, the addition of SBO reduced the loss of CBO during storage of films and improved the antimicrobial activity against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica*. The results suggest that SBO can be used to improve certain physical characteristics and the antibacterial efficacy of edible films containing essential oils.

1. Introduction

Edible films and coatings are potential strategies to improve the safety and quality of perishable food products (Zhou, Xu, & Liu, 2010). Coatings improve the quality of citrus and apples by controlling weight loss and moisture during storage (Valencia-Chamorro, Palou, del Río, & Pérez-Gago, 2011). Edible coatings applied on fresh, frozen, and processed meat, poultry, and seafood products can reduce moisture loss, prevent lipid oxidation and discoloration, and generally enhance product appearance and quality (Gennadios, Hanna, & Kurth, 1997). Edible films and coatings can also serve as carriers of food additives such as antimicrobials, antioxidants, colorants, flavors, nutrients and spices (Gennadios et al., 1997; Pranoto, Salokhe, & Rakshit, 2005). With the potential for reducing contamination on food surfaces by spoilage and pathogenic microorganisms, coatings and films incorporated with antimicrobial agents have received much attention (Coma, Sebti, Pardon, Deschamps, & Pichavant, 2001).

Essential oils (EOs) distilled from plants or plant parts such as cinnamon bark oil (CBO) have excellent antimicrobial activity against a broad spectrum of pathogenic and spoilage microorganisms (Burt, 2004). The antimicrobial properties of EOs and their generally-recognized-as-safe (GRAS) regulatory status have attracted much interest for incorporation in edible films and many studies have shown the effectiveness of these antimicrobial films to control microbial growth (Valencia-Chamorro et al., 2011). Polysaccharides, proteins, and lipids have been studied as film matrices for incorporation of EOs for potential use as edible antimicrobial films to improve food safety and quality (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011).

Alginates are extracted from brown seaweed. Several studies have shown that sodium alginate films containing EOs demonstrate significant inhibition against different pathogenic bacteria (Benavides, Villalobos-Carvajal, & Reyes, 2012; Pranoto et al., 2005). Films prepared with cinnamon oil showed a higher antibacterial activity against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* than those prepared with clove bud and allspice oils (Du et al., 2009) Cinnamic aldehyde, the major component in CBO also has excellent fungitoxicity (Kishore, Mishra, & Chansouria, 1993). Therefore, edible films with CBO have great potential to inhibit pathogenic and spoilage microorganisms. However, EOs including CBO are volatile and these studies also showed that a large percentage of the EOs were lost during film preparation. Hence, a high concentration of EOs was needed to prepare films to ensure their antibacterial efficacy on fruits throughout the duration of the shelf-life (Benavides et al., 2012;

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* Corresponding author. Department of Food Science and Technology, The University of Tennessee, 2510 River Drive, Knoxville, TN 37996, United States. Tel.: +1 865 974 6196; fax: +1 865 974 7332.

E-mail address: qzhong@utk.edu (Q. Zhong).

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Sánchez-González, González-Martínez, Chiralt, & Cháfer, 2010). Multivalent metal cations, particularly calcium ions, can form ionic crosslinks in alginates to improve physical and mechanical properties of the fabricated polymeric materials (Benavides et al., 2012). Because of its hydrophilic nature, crosslinking alginate with calcium can improve water barrier properties, mechanical resistance, and rigidity of films (Rhim, 2004) and control the release of encapsulated compounds (Badwan, Abumalooh, Sallam, Abukalaf, & Jawan, 1985). Furthermore, soybean oil (SBO) was shown to lower the volatility of EOs (Kim, Wu, Kubota, & Kobayashi, 1995). Physically, because SBO is more non-polar than ambient air and interior aqueous food matrices, incorporation of SBO can decrease the polarity of alginate films to favor the retention of CBO during film preparation and storage. The enhanced retention of CBO in films may also reduce the diffusion into food matrices to cause undesirable flavor notes (Dhall, 2013).

Therefore, the aim of this study was to prepare alginate films with CBO and SBO at different mass ratios and characterize the physical, mechanical, and antimicrobial properties of the films. Physical properties studied were color, water vapor permeability (WVP), water solubility, total solids content, and the retention of CBO in films during storage. The antimicrobial properties of these films were evaluated against *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*.

2. Materials and methods

2.1. Materials

Sodium alginate and CBO (containing 55–78% (v/v) cinnamic aldehyde) were purchased from Sigma–Aldrich Corp. (St. Louis, MO). SBO (containing 50–54% (v/v) linoleic acid) was procured from MP Biomedicals, LLC (Santa Ana, CA). Other chemicals were obtained from either Sigma–Aldrich or Fisher Scientific (Pittsburgh, PA).

2.2. Preparation of films

Alginate (2% w/v) stock solutions were prepared by dissolving 8 g of sodium alginate in 400 mL of sterile distilled water at 70 °C. After stirring for 30 min for complete dissolution of alginate, glycerol was added at 0.3 g/g alginate as a plasticizer while $CaCl_2$ (0.05 g/g alginate) was added to strengthen the film. CBO was mixed with polysorbate 80 (Tween 80) at different mass ratios on a magnetic stir plate for 30 min and incorporated into alginate solutions. SBO was then added into the mixture at different concentrations. Distilled water was added to make up the film-forming mixture to a final alginate concentration of 1% w/v. The final film-forming mixtures contained 1% w/v sodium alginate, 0.5% w/v Tween 80, 0, 0.5 or 1.0% w/v SBO, and 1.0 or 2.0% w/v CBO. The mixtures were stirred for 30 min on a magnetic stir plate before casting 40 mL of each of the mixtures on 17.8 cm × 17.8 cm glass plates. After drying at room temperature (21 °C) for 40 h, films were peeled and stored in desiccators maintained at 0% relative humidity (RH) until use (Tong, Xiao, & Lim, 2008). Films used in WVP tests were conditioned for at least 24 h at 58% RH maintained by a saturated sodium bromide solution in desiccators at 21 °C.

2.3. Hydrodynamic diameters (D_h) of film-forming mixtures

The D_h and polydispersity index (PDI) of film-forming mixtures were measured using dynamic light scattering instrument (Delsa Nano C particle size/zeta potential analyzer, Beckman Coulter, Fullerton, CA) with a scattering angle of 165°. Mixtures were diluted to a final CBO content of 0.03%, and measurements were done in triplicate at 21 °C for each sample.

2.4. Physical properties of films

2.4.1. Thickness and color measurements

The thickness of films was measured using a digital microcaliper with 0.001 mm precision (Mitutoyo Corp., Kawasaki, Japan). Measurements were taken at five different locations of each film. Two film replicates were measured.

The color of films was measured using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were performed by placing the film sample over the standard yellow plate ($L^* = 64.50$, $a^* = 38.80$, $b^* = 62.00$). Color differences (ΔE) were also calculated using the following equation:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \tag{1}$$

where L^*, a^*, b^* are the color parameter values of the yellow plate, and L, a, b are the values of film samples.

2.4.2. Total solids content

Film samples (~0.1 g) were weighed and dried at 105 °C in an oven until a constant mass was reached (Norajit, Kim, & Ryu, 2010). The total solids content was determined as the percentage of residual mass after drying with respect to the initial film mass.

2.4.3. Water vapor permeability (WVP)

The WVP of films was measured based on the modified ASTM method E96-95 as described previously (McHugh, Avena-Bustillos, & Krochta, 1993). Each film sample was sealed on a permeation test cup with a circular opening of 12.6 cm^2. The bottom of cups was filled with 5 g distilled water that corresponded to 100% RH and 2.487×10^3 Pa vapor pressure at 21 °C. The cups holding films were then placed in a desiccator containing saturated sodium bromide solution to maintain 58% RH. The mass of the permeation test cell was determined at intervals of 1.5 h for 9 h. A linear regression analysis of mass loss vs. time was performed, and the slope was determined as the water vapor transmission rate (WVTR). The WVP (g/m h Pa) was then calculated as Eq. (2).

$$\mathrm{WVP} = (\mathrm{WVTR}\ L)/\Delta p \tag{2}$$

where L is the thickness of a film (m), and Δp is the water vapor pressure difference between two sides of the film (1.05×10^3 Pa).

2.4.4. Water solubility

The water solubility of films was determined using the method of Norajit et al. (2010). The initial dry matter of circular film discs (~28 cm^2) was first determined by drying film discs at 105 °C in an oven until a constant mass was obtained. The films were then immersed in 40 mL distilled water and stirred at 250 rpm on a magnetic stir plate for 24 h at 21 °C. The samples were then filtered through pre-weighed Whatman No.1 filter paper. Filter papers containing any insolubilized film material were dried at 105 °C for 24 h and the final dry matter of film discs was determined after subtraction of the filter paper mass. The film solubility (%) was calculated using the following equation:

$$\begin{aligned}\mathrm{Solubility}(\%) = {} & (\mathrm{Initial\ dry\ matter} - \mathrm{Final\ dry\ matter}) \\ & \times /\mathrm{Initial\ dry\ matter} \times 100\%\end{aligned} \tag{3}$$

2.5. Mechanical properties of films

Tensile strength (*TS*) and elongation at break (*E*) were determined using a texture analyzer in the tensile mode (TA.XTplus Texture Analyzer, Texture Technologies Corp., Scarsdale, NY) according to ASTM standard method D882 as described previously (Benavides et al., 2012). Films were cut into 10 cm × 1 cm strips. Strips held at an initial gap of 8 cm were tested at a speed of 1 mm/s. *TS* was calculated by dividing the maximum force by the cross-section area of each film (thickness × width), and the *E* was calculated as the percentage of extension at break with respect to the original strip length.

2.6. Scanning electron microscopy (SEM)

The alginate films were placed on a double-sided adhesive tape mounted on a stainless steel stub and then imaged using a LEO 1525 SEM microscope (SEM/FIB Zeiss Auriga, Oberkochen, Germany) at a voltage of 1.00 kV.

2.7. Quantification of CBO in alginate films

CBO content in alginate films was determined after storage in a desiccator at ambient temperature (21 °C) and 0% RH for 0–14 days. Films were cut into 15 mm diameter discs and weighed. Extraction of CBO was performed by immersing the discs in 5 mL ethanol (Areias, Valentão, Andrade, Ferreres, & Seabra, 2000). After 2 h mixing, the samples were centrifuged at 6000 *g* for 2 min (Minispin plus, Eppendorff, Hamburg, Germany). The extraction procedures were repeated three times. The collected supernatants then were combined and diluted in ethanol to measure the absorbance at 280 nm (ThermoScientific UV/Vis spectrophotometer, Waltham, MA). CBO concentration was determined based on a standard curve established using standard solutions with various concentrations of CBO dissolved in ethanol. Three independent film replicates were measured to estimate the residual content of CBO (Eq. (4)) and entrapment efficiency (Eq. (5)).

$$\text{Residual CBO mass in a film (g)} = \frac{\textit{Area of film}}{\textit{Area of disc}} \times m_{\mathrm{CBO}} \tag{4}$$

$$\text{Entrapment efficiency}(\%) = \frac{\textit{Residual CBO mass in a film}(\mathrm{g})}{\textit{Theoretical } 100\% \textit{ CBO mass in a film } (\mathrm{g})} \times 100\% \tag{5}$$

where m_{CBO} is the mass of CBO in each tested film disc, and the theoretical 100% CBO mass is the amount of CBO (0.4 and 0.8 g for 1% and 2% CBO formulations, respectively) used to prepare a 17.8 cm × 17.8 cm film.

2.8. Antimicrobial properties of films

Bacterial strains were obtained from the culture collection of the Department of Food Science and Technology at the University of Tennessee (Knoxville, TN). A cocktail was prepared from 5 strains each of *E. coli* O157:H7 (H1730, F4546, K3995, K4492 and 932) and *L. monocytogenes* (LM1, LM2, 310, Scott A, and V7) or 5 serovars of *S. enterica* (Agona, Montevideo, Gaminara, Michigan, and Saint Paul). Each test strain or serovar stored in glycerol at −20 °C was transferred twice to tryptic soy broth (TSB) at 37 °C for *E. coli* O157:H7 and *S. enterica* or 32 °C for *L. monocytogenes* and incubated for 24 h. The 5 test strains or serovars were combined to yield a cocktail containing equal proportions of each test strain/serovar and diluted to ~10^6 CFU/mL as the working culture.

The agar diffusion method was used to determine the antimicrobial activity of the films (Pranoto et al., 2005). The films stored for 7 days were cut into 15 mm diameter discs that were placed on tryptic soy agar (TSA) plates, which had been previously spread with 0.1 mL of the working culture (~10^5 CFU). The plates were incubated at 37 °C for *E. coli* O157:H7 and *S. enterica* or 32 °C for *L. monocytogenes* for 24 or 48 h. The growth of bacteria under the film discs was visually examined. The diameter of the growth inhibition zones including the discs was measured using a ruler with a millimeter resolution (Nguyen, Gidley, & Dykes, 2008). The measurements were replicated four times with two discs from each of two independent film replicates.

2.9. Statistical analysis

All experiments were carried out at least in duplicate. All results were reported as means ± standard deviations. Statistical analyses were performed using the SPSS 16.0 statistical analysis system (SPSS Inc., Chicago, IL). The one-way analysis of variance of means was performed at a significance level (*P*) of 0.05 using the Duncan's test method.

3. Results and discussion

3.1. Appearance and thickness of films

All alginate films were visually homogeneous with no bubbles and were easily removed from the cast plates. The thickness of films varied between 0.021 and 0.045 mm (Table 1). The film thickness increased with the increasing amounts of CBO and SBO used in film preparation, which resulted from the increased content of non-solvent matters in the film forming mixture (Benavides et al., 2012).

As shown in Table 1, the incorporation of CBO affected the ΔE of the films that was 10.01 and 31.27 for the treatments prepared with 1% and 2% CBO, respectively, and was significantly greater than that of the control film (3.61). The increase in ΔE was mainly attributed to the decrease in yellowness (positive *b* value). The *L* values were slightly higher (64.96 and 68.80 for films prepared with 1% and 2% CBO, respectively) than that of the control (62.98), showing that the incorporation of CBO decreased the transparency of alginate film (Fig. 1) which was similar to that found by (Li, Zivanovic, Davidson, & Kit, 2010). When SBO was also incorporated in films with CBO, the ΔE values were significantly reduced (Table 1), especially for the sample prepared with 1% CBO and 1% SBO that had ΔE value close to the control film. The improvement in transparency of films after incorporating SBO (Fig. 1) may expand the application of coatings/films on food products.

3.2. Surface microstructure of films

The surface microstructure of alginate films studied by SEM is presented in Fig. 2. The control alginate film had a smooth and homogeneous surface, indicating the formation of an ordered matrix that resulted in transparent films (Fig. 1A). After incorporating CBO, the films showed a heterogeneous surface that corresponded to the decreased transparency of films (Fig. 1B–C). The arrangements of alginate molecules and the aggregation of oil droplets during film formation (Ahmad, Benjakul, Prodpran, & Agustini, 2012; Shojaee-Aliabadi et al., 2013) can result in either concave or convex irregularities of the surface of films (Atarés, Bonilla, & Chiralt, 2010). The microstructure is further impacted by the evaporation of CBO and the subsequent impact on oil droplet structures during drying (Atarés et al., 2010). Generally speaking,

Table 1
Thickness and color of alginate films with cinnamon bark oil (CBO) and soybean oil (SBO).[a]

Additive	Thickness (mm)	*L* (Lightness)	*a* (Redness)	*b* (Yellowness)	*ΔE*
None (control)	0.021 ± 0.004^{D}	62.98 ± 0.61^{D}	38.66 ± 0.44^{A}	58.90 ± 1.71^{A}	3.61 ± 1.32^{E}
1% CBO	0.029 ± 0.004^{C}	64.96 ± 0.14^{C}	36.59 ± 0.61^{B}	52.71 ± 2.11^{A}	10.01 ± 2.20^{D}
1% CBO, 0.5% SBO	0.034 ± 0.003^{BC}	65.42 ± 0.57^{C}	37.52 ± 0.41^{AB}	56.45 ± 1.97^{A}	5.77 ± 2.09^{DE}
1% CBO, 1% SBO	0.034 ± 0.004^{BC}	65.08 ± 1.41^{C}	37.33 ± 1.71^{AB}	60.32 ± 1.43^{A}	3.00 ± 2.38^{E}
2% CBO	0.035 ± 0.004^{B}	68.80 ± 0.43^{A}	29.28 ± 0.45^{D}	32.53 ± 0.89^{C}	31.27 ± 0.90^{A}
2% CBO, 0.5% SBO	0.037 ± 0.005^{B}	66.73 ± 0.40^{B}	32.76 ± 0.82^{C}	39.2 ± 2.53^{B}	23.70 ± 2.65^{B}
2% CBO, 1% SBO	0.045 ± 0.002^{A}	66.49 ± 0.62^{B}	34.10 ± 1.45^{C}	43.57 ± 4.62^{B}	17.25 ± 4.78^{C}

[a] Numbers are mean ± standard deviation (n = 10 for thickness, 6 for color). Mean values with different uppercase superscript letters in the same column are significantly different ($P < 0.05$).

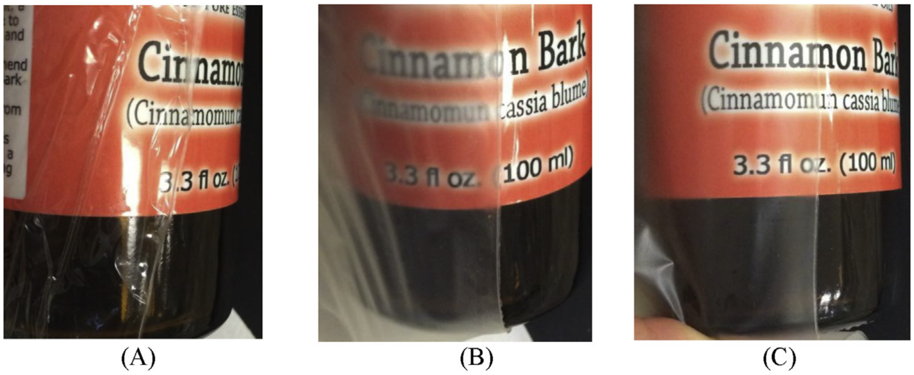

Fig. 1. Transparency of alginate films prepared (A) without and with (B) 2% CBO or (C) 2% CBO and 0.5% SBO.

films with a higher CBO content showed more heterogeneous surfaces (Fig. 2B–D vs. Fig. 2E–G) that likely resulted from a larger population of oil droplets after drying. For films containing SBO, a higher content of SBO, i.e., a lower CBO:SBO mass ratio, reduced the heterogeneity of surface microstructure. For example, the film with a CBO:SBO mass ratio of 1:1 (Fig. 2D) was more homogenous than that of 2:1 (Fig. 2C).

The surface microstructure of films generally agreed with the changes of D_h and PDI of film-forming mixtures in that a mixture with smaller D_h and PDI corresponded to a more homogeneous surface (Fig. 2 and Table 2). It is expected that bigger droplets and a wider distribution of droplet size in the film forming mixture can result in more heterogeneous film microstructures. Increases in emulsion droplet dimension due to mechanisms such as Ostwald ripening and coalescence are also expected during drying to form films. Ostwald ripening results from the polarity of EOs but can be eliminated by incorporation of nonpolar lipids (Lim et al., 2011). For the treatment without SBO, the larger starting droplets (Table 2) and possible droplet growth during film formation may have caused the more heterogeneous microstructures of films (Fig. 2B, E). The film-forming mixture with a higher amount of SBO had smaller D_h (Table 2) and, together with the reduced possibility of Ostwald ripening, resulted in a more homogenous film (Fig. 2). The decrease in droplet dimension after incorporation of SBO can be attributed to the decreased polarity of the oil phase and the increased compatibility of oil phase with Tween 80 (Salmieri & Lacroix, 2006; Ma & Zhong, 2015).

3.3. Physical properties of films

The water solubility, WVP and total solids content of films are summarized in Table 3. Water solubility reflects the water resistance and the biodegradability of films when used as packaging materials (Berger, Ludwig, & Wielich, 1953; Maizura, Fazilah, Norziah, & Karim, 2007). The control alginate film showed a very high solubility in water (~99.5%) due to the hydrophilic property of alginate molecules that enabled the dissolution of alginate film (Berger et al., 1953; Maizura et al., 2007). Incorporation of CBO in films reduced the water solubility of films that was further reduced following incorporation of SBO. This may have resulted from the overall increase in film hydrophobicity (Ojagh, Rezaei, Razavi, & Hosseini, 2010) and decrease of water sorption (Abdollahi, Rezaei, & Farzi, 2012). For films with SBO, the higher solubility of films prepared with 2% CBO than those of 1% CBO can be explained by the fact that CBO is much more polar than SBO and the larger amount of CBO can increase the spacing between SBO chains to form smaller droplets (Salmieri & Lacroix, 2006), which increases the loss of film mass after swelling and filtration. Water solubility determines if films can be washed off by consumers before consumption and also the structural stability of films when applied on moist foods (Rhim & Wang, 2013).

The incorporation of CBO increased the WVP of films (Table 3). This agreed with the increased irregularity of film structures (Fig. 2) that can lower mass transfer resistance for water molecules (Norajit et al., 2010). As for the total solids content, films prepared with 1% CBO had a higher total solids content than the control film, while the opposite trend was observed for films prepared with 2% CBO. The increased WVP after incorporation of CBO in films can facilitate the moisture evaporation during drying to form films and therefore increase the total solids content. Conversely, since CBO is easily evaporated at 105 °C, the loss of CBO during drying to determine total solids content may cause the under-estimation of total solids content of films. These two opposite impacts of CBO may have resulted in the opposite trend for films prepared with 1% and 2% CBO. Since WVP is proportional to the thickness of films (Eq. (2)), the impact of SBO on WVP was not observed (Table 3), which agrees with a previous study by (Shaw, Monahan, O'Riordan, & O'Sullivan, 2002). Conversely, SBO decreased the water solubility and increased total solids content of films, and the impacts were greater for the 1% CBO treatments with a higher overall concentration of

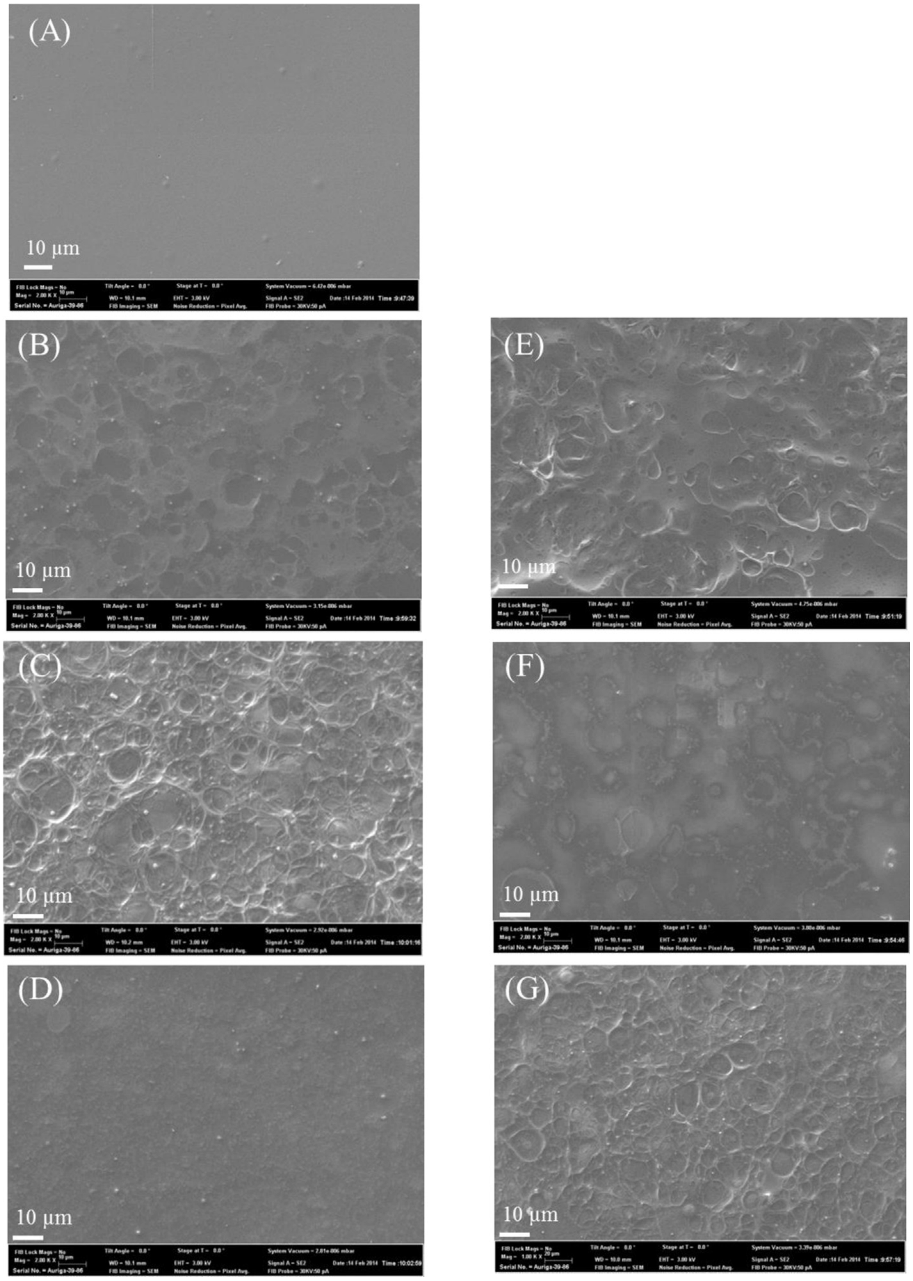

Fig. 2. SEM micrographs showing the surface structure of (A) the control alginate film and after addition of (B) 1% CBO, (C) 1% CBO and 0.5% SBO, (D) 1% CBO and 1% SBO, (E) 2% CBO, (F) 2% CBO and 0.5% SBO, or (G) 2% CBO and 1% SBO in the film-forming mixture.

SBO (Table 3). This could mainly be attributed to the increased hydrophobicity of films by SBO.

3.4. Mechanical properties of films

TS and *E* are important mechanical properties of films and have been used to study structures of films (Tara Habig McHugh & Krochta, 1994). The influence of CBO and SBO on mechanical properties of alginate films is also presented in Table 3. The incorporation of CBO resulted in stronger and more elastic films, as indicated by larger values of *TS* and *E*. Such an improvement was previously interpreted by the strengthened interactions between biopolymers in film matrix (Ojagh et al., 2010). After addition of SBO, the *TS* and *E* of films with CBO significantly reduced. This likely resulted from the increased content of droplets containing non-

Table 2
Hydrodynamic diameters of film forming mixtures containing 1% w/v alginate, cinnamon bark oil (CBO) and soybean oil (SBO) at different mass ratios.[a]

Additive	Hydrodynamic diameter (nm)	Polydispersity index
1% CBO	1429.4 ± 43.0^{B}	0.521 ± 0.011^{B}
1% CBO, 0.5%SBO	523.3 ± 112.3^{C}	0.228 ± 0.035^{C}
1% CBO, 1% SBO	495.3 ± 98.5^{C}	0.225 ± 0.038^{C}
2% CBO	2082.7 ± 712.8^{A}	0.785 ± 0.276^{A}
2% CBO, 0.5% SBO	1180.5 ± 309.4^{B}	0.356 ± 0.051 ^{BC}
2% CBO, 1% SBO	888.0 ± 221.5 ^{BC}	0.295 ± 0.065^{C}

[a] Numbers are mean ± standard deviation (n = 3). Mean values with different uppercase superscript letters in the same column are significantly different ($P < 0.05$).

Table 3
Mechanical and physical properties of alginate films incorporated with cinnamon bark oil (CBO) and soybean oil (SBO).[a]

Additive	*TS* (MPa)	*E* (%)	WVP ($\times 10^{-7}$ g/m h Pa)	Total solids content (%)	Water solubility (%)
None (control)	6.51 ± 1.04^{C}	11.99 ± 2.39^{D}	4.36 ± 0.34^{D}	77.51 ± 1.40^{C}	99.50 ± 0.50^{A}
1% CBO	16.03 ± 3.14^{A}	36.32 ± 3.38^{B}	5.08 ± 0.12^{C}	81.25 ± 0.35^{B}	97.40 ± 0.51^{BC}
1% CBO, 0.5% SBO	9.67 ± 0.58^{B}	27.63 ± 1.79^{C}	5.44 ± 0.14^{BC}	82.74 ± 1.04^{AB}	91.76 ± 0.26^{D}
1% CBO, 1% SBO	6.67 ± 1.92^{BC}	16.11 ± 0.97^{D}	5.50 ± 0.20^{BC}	84.46 ± 0.34^{A}	90.68 ± 0.71^{D}
2% CBO	15.94 ± 1.08^{A}	45.97 ± 8.82^{A}	6.38 ± 0.05^{AB}	72.57 ± 1.12^{D}	97.65 ± 0.46^{B}
2% CBO, 0.5% SBO	9.68 ± 1.36^{B}	26.04 ± 1.59^{C}	5.86 ± 0.13^{B}	72.77 ± 0.95^{D}	96.39 ± 1.40^{BC}
2% CBO, 1% SBO	7.31 ± 0.88^{BC}	25.13 ± 1.28^{C}	6.88 ± 0.21^{A}	74.21 ± 1.29^{D}	96.04 ± 0.27^{C}

[a] Numbers are mean ± standard deviation (n = ≥5 for *TS* and *E*, n = 3 for other measurements). Mean values with different uppercase superscript letters in the same column are significantly different ($P < 0.05$).

polar SBO that have different structures from film matrix (Yang & Paulson, 2000). The negative effect of long chain fatty acids on the mechanical properties of films was also observed by others (Park, Weller, Vergano, & Testin, 1993; Yang & Paulson, 2000). The decreased *TS* and *E* after incorporation of SBO indicate the decreased deformability of films such as the increased chance of fracture and reduced stretchability (Rhim & Wang, 2013).

3.5. The CBO mass in alginate films during storage

The mass of CBO in freshly prepared films and during storage in a desiccator at 21 °C and 0% RH is presented in Table 4. For films prepared with 1% CBO, the film without SBO had the highest retention of CBO after film preparation and following storage. The increased concentration of SBO significantly decreased the content of CBO in the fresh films. All films prepared with 1% CBO showed no significant ($P > 0.05$) change of CBO content during storage (Table 4). For films prepared with 2% CBO, the overall CBO content was significantly higher than those prepared with 1% CBO (Table 4), and the difference was more than 2 folds in fresh films. The CBO content in the treatment prepared with 2% CBO without SBO decreased significantly from 0.503 g to 0.236 g after 3-day storage and was similar ($P > 0.05$) to the treatment prepared with 1% CBO after 7 days. The loss of CBO during storage was likely the result of the volatility of CBO. After addition of SBO in 2% CBO treatments, the CBO content was reduced in the fresh films but was retained to a significantly higher extent after storage when compared to the treatment without SBO ($P < 0.05$).

The effect of SBO addition on the retention of CBO in fresh films and during the subsequent storage may be explained by two competing factors. Firstly, Tween® 80 is the only surfactant used at a constant amount in the film forming mixture to initially emulsify CBO. After blending SBO, the amount of Tween® 80 associated with CBO likely is reduced because of increased miscibility between the oleate fraction of Tween® 80 and long-chain fatty acids of SBO (Takagi & Rahman, 1996). The reduced attraction between Tween® 80 and CBO after addition of SBO resulted in the greater extent of CBO evaporation during film formation and storage. Secondly, once CBO is associated with SBO, the volatility of CBO can be lowered. The first factor may have been dominant during film formation where CBO molecules have to diffuse through the wet mixture to evaporate, corresponding to the observation that a higher SBO content reduced the CBO content to a greater extent in fresh films (Table 4). After removal of most water to form films, the competition of oils for Tween 80 becomes insignificant, and CBO in films is retained to a greater extent by a larger amount of SBO during storage.

3.6. Antimicrobial properties of films

The antimicrobial activity, as defined as diameter of clear zones surrounding film discs of CBO-loaded alginate films, against cocktails of two Gram-negative and one Gram-positive pathogenic bacteria is shown in Table 5. The control alginate film did not show any inhibition of the three bacteria. The inhibition zones of films with CBO against *L. monocytogenes* were larger than the other bacteria after 24 h incubation, suggesting that *L. monocytogenes* was more sensitive to CBO. The observation was in accordance with previous studies showing lower bacteriostatic and bactericidal concentrations against *L. monocytogenes* than *E. coli* O157:H7 and *S. enterica* (Friedman, Henika, & Mandrell, 2002; Smith-Palmer, Stewart, & Fyfe, 1998). For all three bacteria, the inhibition zones generally decreased after further incubation for 48 h, possibly due to the evaporation of CBO during incubation or adaptive tolerance of the microorganisms. The reduction was more significant for *L. monocytogenes* (with no measurable zone area) than the other two bacteria, showing a significant recovery of *L. monocytogenes* that may be due to a more rapid adaptation by *L. monocytogenes* (Lundén, Autio, Markkula, Hellström, & Korkeala, 2003). Overall, the CBO-loaded film discs were more effective against the two Gram-negative bacteria than *L. monocytogenes* after 48 h. Less inhibition of *L. monocytogenes* than *E. coli* O157:H7 or *S. enterica* was also observed for cinnamon oil in another study (Smith-Palmer et al., 1998).

Antimicrobial activity generally agreed with the CBO content in films after storage for 7 days in desiccators. For films with CBO only, there is no significant increase in the inhibition zone for1% vs. 2% CBO treatments. This agreed with the similar content of CBO in

Table 4
Content of cinnamon bark oil (CBO) in entire alginate films prepared with CBO and soybean oil (SBO), after storage in a desiccator at 21 °C.[a]

Additive[b]	Content of CBO (g) after storage			
	Day 0	Day 3	Day 7	Day 14
1% CBO	0.194 ± 0.025^{GH}	0.171 ± 0.016^{HI}	0.164 ± 0.034^{HIJ}	0.169 ± 0.042^{HI}
1% CBO, 0.5% SBO	0.103 ± 0.015^{IJK}	0.102 ± 0.017^{IJK}	0.104 ± 0.007^{IJK}	0.091 ± 0.005^{IJK}
1% CBO, 1% SBO	0.075 ± 0.009^{K}	0.085 ± 0.010^{JK}	0.066 ± 0.003^{K}	0.058 ± 0.002^{K}
2% CBO	0.503 ± 0.065^{A}	0.236 ± 0.022^{FGH}	0.187 ± 0.016^{GH}	0.165 ± 0.009^{HIJ}
2% CBO, 0.5% SBO	0.423 ± 0.036^{BC}	0.446 ± 0.007^{AB}	0.288 ± 0.119^{DEF}	0.253 ± 0.075^{EFG}
2% CBO, 1% SBO	0.327 ± 0.033^{DE}	0.360 ± 0.041^{CD}	0.240 ± 0.033^{FGH}	0.211 ± 0.021^{FGH}

[a] Numbers are mean ± standard deviation (n = 3). Mean values with different uppercase superscript letters are significantly different ($P < 0$ 0.05).
[b] The overall CBO content in film-forming mixtures containing 1% and 2% CBO was 0.40 and 0.80 g, respectively.

Table 5
Inhibition zone diameters of alginate film discs (diameter = 15 mm) prepared with cinnamon bark oil (CBO) and soybean oil (SBO) on tryptic soy agar against five-strain cocktails of pathogenic bacteria after 24 and 48 h at 37 or 32 °C.

Bacteria	Additive	Zone diameter (mm)[a,b]	
		24 h	48 h
Listeria monocytogenes	None (control)	–	–
	1% CBO	19.7 ± 5.5^{C}	+
	1% CBO, 0.5% SBO	17.3 ± 2.3^{C}	+
	1% CBO, 1% SBO	+	+
	2% CBO	22.6 ± 5.6^{C}	+
	2% CBO, 0.5%SBO	31.3 ± 5.0^{B}	16.5 ± 0.5^{C}
	2% CBO, 1% SBO	44.3 ± 4.3^{A}	$19.7 \pm^{C}$
Salmonella enterica	None (control)	–	–
	1% CBO	17.2 ± 1.3^{DEF}	16.5 ± 0.6^{EF}
	1% CBO, 0.5% SBO	16.0 ± 0.0^{F}	16.0 ± 1.0^{F}
	1% CBO, 1% SBO	+	+
	2% CBO	20.0 ± 2.9^{BCDE}	18.6 ± 1.8^{CDEF}
	2% CBO, 0.5% SBO	23.4 ± 4.2^{AB}	20.4 ± 2.9^{BCD}
	2% CBO,1% SBO	25.8 ± 1.8^{A}	22.0 ± 1.4^{BC}
E. coli O157:H7	None (control)	–	–
	1% CBO	18.0 ± 2.0^{CDE}	15.5 ± 0.7^{E}
	1% CBO, 0.5% SBO	16.0 ± 0.0^{DE}	16.0 ± 0.0^{DE}
	1% CBO, 1% SBO	+	+
	2% CBO	19.8 ± 1.3^{BC}	16.5 ± 0.7^{DE}
	2% CBO, 0.5% SBO	31.0 ± 2.5^{A}	19.2 ± 1.3^{BCD}
	2% CBO, 1% SBO	33.2 ± 2.2^{A}	21.3 ± 0.6^{B}

[a] Numbers are mean ± standard deviation (n = 4). Mean values with different uppercase superscript letters in the same bacteria treatments are significantly different ($P < 0.05$).

[b] (–): no inhibition; (+): inhibition was observed underneath the discs but the inhibition zone area around the discs was negligible.

these two films (Table 4). For films prepared with 1% CBO, the increased SBO content decreased the overall CBO content and correspondingly the inhibition zone. For films prepared with 2% CBO, the significantly larger inhibition zones of the treatments with SBO than that without SBO were also in agreement with the greater quantities of CBO in films (Table 4).

4. Conclusions

Research presented in this study demonstrates that CBO and SBO can be successfully incorporated in alginate films by simple mixing. The incorporation of CBO decreased alginate film transparency, increased the *TS* and *E*, and increased the WVP. The addition of SBO to alginate films with CBO increased the transparency and total solids content, but decreased *TS*, *E* and water solubility. SBO showed no significant effect on the WVP. Films prepared with 2% CBO showed the effectiveness against both Gram-positive and Gram-negative bacterial pathogens and incorporation of SBO reduced the evaporation of CBO during storage of films thus maintaining antimicrobial activity. Therefore, alginate films with CBO and SBO may be used to improve the microbiological safety and potentially the quality of perishable solid food products.

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