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Physical, mechanical, and antimicrobial properties of chitosan films with microemulsions of cinnamon bark oil and soybean oil

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ABSTRACT

Antimicrobial films prepared with essential oil emulsions often have a high degree of opacity. This may be prevented using transparent microemulsions. The objective of this study was to characterize physical, mechanical, and antimicrobial properties of films prepared from mixtures with 1% w/w chitosan solution and microemulsions containing 1:0, 2:1, and 4:1 mass ratios of cinnamon bark oil (CBO) (1, 2 and 3% w/w) and soybean oil. Changes in solvent polarity after mixing chitosan solution and microemulsions increased droplet dimension from <30 nm of microemulsions to >88 nm of the film–forming mixtures and induced different extents of coalescence after film formation. Despite these physical changes, films prepared from microemulsions were transparent and had low opacity. The incorporation of microemulsions increased the thickness and water vapor permeability of films and significantly reduced the moisture content and swelling ratio. The retention of CBO was improved for films prepared from microemulsions with 2 and 3% CBO immediately following film formation and after ambient storage, when compared to control films prepared with emulsions with less Tween 80. Large zones of inhibition against foodborne pathogens were observed for film discs prepared with 2 and 3% CBO. These characteristics show the potential of using microemulsions as an easy approach to incorporate EOs in biopolymer antimicrobial films to improve microbiological safety and film transparency.

1. Introduction

Fresh produce is perishable and is sometimes associated with outbreaks of foodborne illnesses due to contamination by microbial pathogens (CDC, 2012a, 2012b). Many strategies to improve the microbiological safety and quality of fresh produce have been studied, including films or coatings with and without antimicrobials. Polysaccharides (Perdones, Vargas, Atarés, & Chiralt, 2014; Zivanovic, Chi, & Draughon, 2005), proteins (Gómez-Estaca, Montero, & Gómez-Guillén, 2014; Moditsi, Lazaridou, Moschakis, & Biliaderis, 2014) and lipids (Arcan & Yemenicioğlu, 2013) have been utilized as edible film–forming or coating materials. Chitosan, a copolymer consisting of β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units, is an excellent film–forming material derived from chitin by N-deacetylation (Domard & Domard, 2001; Elsabee & Abdou, 2013). Chitosan films have good mechanical properties and a selective permeability (higher permeability to CO_2 and lower to O_2) (Despond, Espuche, & Domard, 2001; Elsabee et al., 2013). In addition, chitosan itself has antibacterial and antifungal activity (Kim, Thomas, Lee, & Park, 2003; Tsai, Su, Chen, & Pan, 2002). Thus, coating with chitosan may be a good strategy to improve the microbiological safety and quality of fresh produce (Sangsuwan, Rattanapanone, & Rachtanapun, 2008).

Various antimicrobials have been incorporated into films and plant essential oils (EOs) are frequently studied due to their broad antimicrobial activity (Chen, Zhang, & Zhong, 2014; Ma, Davidson, & Zhong, 2013). Incorporating EOs into chitosan films has been shown to enhance antimicrobial activity and lower water vapor permeability (WVP) (Ojagh, Rezaei, Razavi, & Hosseini, 2010; Pereda, Amica, & Marcovich, 2012; Zivanovic et al., 2005). However, adding EOs into chitosan films can also increase the opacity thus affecting the appearance of products (Hosseini, Razavi, & Mousavi, 2009; Pereda et al., 2012). This largely results from the low-water solubility of EOs that form particulate structures in the film to scatter visible light. In addition, the volatile nature of EOs

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causes their significant loss during film formation and storage (Chi, Zivanovic, & Penfield, 2006). Much work is still needed to improve the properties of antimicrobial films/coatings with EOs.

Colloidal systems are used to improve various functional properties of oil and water mixtures. Microemulsions are thermodynamically stable isotropic mixtures of water, oil, surfactants, and co-surfactants (Danielsson & Lindman, 1981). Microemulsions are transparent because their droplets are from 1 to 100 nm, typically 10–50 nm (Moulik & Paul, 1998; Slomkowski et al., 2011). The interfacial tension in microemulsions is very low, which enables their easy preparation without using high mechanical energy as in conventional emulsification (Klossek, Marcus, Touraud, & Kunz, 2014). Therefore, transparent films may be obtained by incorporating EO microemulsions in a biopolymer matrix. Furthermore, many fresh produce products, such as cantaloupes, have irregular and rough surfaces and entrapment of bacteria in the cavities on the produce surface can reduce or eliminate the effectiveness of antimicrobial films and coatings. This was demonstrated in a study on the influence of surface roughness of fresh produce on the adhesion rate of *Escherichia coli* O157:H7 (Wang, Feng, Liang, Luo, & Malyarchuk, 2009). A positive linear correlation was found between adhesion rate of the bacterium and surface roughness but a negative correlation existed between the surface roughness and inactivation efficacy by acidified electrolyzed water and peroxyacetic acid. In another study, the improved inactivation of *E. coli* O157:H7 on spinach leaves was reported after adding the surfactant, sucrose monolaurate, to a sodium hypochlorite wash solution (Xiao et al., 2011). This study illustrated that surfactants can lower the solid/liquid interfacial tension to facilitate access of antimicrobials to bacteria which are protected by the heterogeneous structures of fresh produce. Because surfactants are a part of microemulsions, coatings prepared from EO microemulsions may have the potential to enhance antimicrobial activity. Additionally, microemulsions can be formulated to dissolve long-chain triacylglycerols such as soybean oil (SBO) that may change evaporation properties of volatile compounds (Kim, Wu, Kubota, & Kobayashi, 1995) and film properties.

In a previous study (Ma & Zhong, 2015), we formulated microemulsions with an oil phase consisting of various mass ratios of cinnamon bark oil (CBO) and SBO using polysorbate 80 (Tween™ 80) as the surfactant and an equal mass of water and propylene glycol (PG) as the polar phase. The objective of the present work was to characterize physical, mechanical, and antimicrobial properties of films cast from mixtures of chitosan solution and the microemulsions formulated with various mass ratios of CBO and SBO.

2. Materials and methods

2.1. Materials

Low molecular weight chitosan (75–85% deacetylated) and CBO were purchased from Sigma–Aldrich Corp. (St. Louis, MO). PG, SBO, glycerol, acetic acid, tryptic soy broth (TSB) and Tween™ 80 were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA).

2.2. Preparation of microemulsions

Microemulsions were prepared by simple mixing as previously described (Ma & Zhong, 2015). Microemulsions contained Tween™ 80, a polar phase with equal mass of PG and water, and an oil phase comprised of CBO and SBO at mass ratios of 1:0, 2:1, or 4:1 (abbreviated as microemulsion 1:0, 2:1, and 4:1, hereafter). Specific compositions of microemulsions are listed in Table 1. Tween™ 80, the oil phase and the polar phase were added together and mixed by hand shaking until a transparent appearance with no further visible changes.

2.3. Film preparation

A chitosan stock solution was prepared at 2% w/w in 1% w/w acetic acid solution. The impurities were removed by filtering the solution through a microcloth (Calbiochem–Novabiochem Corp., San Diego, CA). Microemulsions were mixed with the 2% w/w chitosan stock solution, glycerol (at 20% mass of chitosan), and deionized water to final CBO concentrations of 1, 2, and 3% w/w. The final concentrations of chitosan and acetic acid were 1% and 0.5% w/w, respectively. Films were prepared by casting 30 g of the mixtures on 17.8 cm × 17.8 cm glass plates and drying at ambient conditions (21 °C) for 24 h. Unless stated otherwise, the dried films were peeled and conditioned for about 48 h at room temperature in a desiccator with 57% relative humidity (RH) controlled by a saturated sodium bromide solution prior to physical characterizations. Films without microemulsions were prepared at the same chitosan concentration as a control.

2.4. Hydrodynamic diameters (D_h) of film–forming mixtures

The D_h of fresh film–forming mixtures was measured using dynamic light scattering. A Delsa Nano analyzer (Beckman Coulter, Atlanta, GA) with a scattering angle of 165° was used. Measurements were done in triplicate for each sample.

2.5. Viscosity of film–forming mixtures

The viscosities of film–forming mixtures were measured using an AR2000 rheometer (TA Instruments, Inc., New Castle, DE). About 20 mL of each sample was loaded into a bob–cup geometry with the outer diameter of bob and the inner diameter of cup being 28 and 30 mm, respectively. Shear rate ramps were conducted at a shear rate range of 0.1–100 s^{-1} at 25 °C, and each sample was sheared for 1 min. Each test was repeated at least once.

2.6. Physical and mechanical properties of films

2.6.1. Thickness

The thickness of films was measured using a digital microcaliper (Mitutoyo Corp., Kawasaki, Japan) with 0.001 mm precision. A total of 12 points were measured for each film. Means from two film replicates were reported.

2.6.2. Color and opacity

The color and opacity of films were measured using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The color of films was measured for lightness (L) and chromaticity parameters a (red–green) and b (yellow–blue) in the Hunter Lab scale. Color measurements were performed over the standard white tile. Opacity was measured over the standard white tile and black glass. For each treatment, two film replicates were measured, each tested in triplicate.

2.6.3. Moisture content and swelling ratio

To determine the moisture content (Eq. (1)) and swelling ratio (Eq. (2)) of films, films were cut into 2 × 2 cm squares and weighed (w_1). Moisture contents of films were determined by drying the films at 60 °C for 24 h and weighing after cooling to room temperature in a desiccator filled with anhydrous calcium chloride (w_2). Swelling ratio was measured by immersing the film squares into deionized water for 24 h. Wet samples were tapped with filter paper to remove free water, followed by weighing the wet films

Table 1
Composition of microemulsions used for preparation of chitosan films.

Sample	Tween™ 80 (%w/w)	CBO (%w/w)	SBO (%w/w)	Water-PG (1:1, w:w) (%w/w)
Microemulsion 1:0	40	20	0	40
Microemulsion 2:1	40	20	10	30
Microemulsion 4:1	40	20	5	35

(w_3). Three film replicates prepared from each formulation were tested.

$$\text{Moisture}(\%) = 100\% \times (w_1 - w_2)/w_1 \tag{1}$$

$$\text{Swelling}(\%) = 100\% \times (w_3 - w_1)/w_1 \tag{2}$$

2.6.4. Water vapor permeability (WVP)

The WVP of films was determined by measuring mass changes of Fisher/Payne permeability cups (Fisher Scientific, Pittsburgh, PA) during incubation at room temperature (21 °C). Cups were filled with 5.0 g deionized water, sealed with films, and placed in a desiccator with 57% RH controlled by a saturated sodium bromide solution. The cup mass was measured hourly for 8 h to the nearest 0.0001 g. Water vapor permeation ratio (WVPR) was calculated based on the mass loss (m), time (t), and effective film area (A) as eq. (3), while WVP was determined using Eq. (4) (Pelissari, Grossmann, Yamashita, & Pineda, 2009). Measurements were performed using triplicate films for each formulation.

$$WVPR = \frac{m}{t \times A} \tag{3}$$

$$WVP = \frac{WVPR \times \text{Film thickness}}{sp \times (RH_1 - RH_2)} \tag{4}$$

where sp is the water vapor saturation pressure at the assay temperature (Pa), RH_1 and RH_2 are the relative humidity inside (100%) and outside (57%) the cup, respectively. The difference of water vapor partial pressure between the water surface and the film underside under the studied conditions was confirmed to be negligible based on the derivations of Gennadios, Weller, and Gooding (1994), due to the small gap (0.7 cm) between the two surfaces.

2.6.5. Tensile strength and elongation

Tensile strength and elongation at break were determined using a TA.XTplus Texture Analyzer in the tensile mode (Texture Technologies Corp., Scarsdale, NY). Films were cut into 10 cm × 1 cm strips, the initial gap was 8 cm, and the test speed was 1 mm/s. Tensile strength (Pa) was calculated by dividing the maximum force by the cross-section area of each film, and the elongation at break was calculated as the percentage of extension at break with respect to the original strip length (Pranoto, Salokhe, & Rakshit, 2005).

2.6.6. Scanning electron microscopy

A LEO 1525 surface scanning electron microscope (LEO Electron Microscopy, Oberkochen, Germany) was used to observe the surface morphology of films. Films stored for 1 day at 57% RH were mounted on the specimen holders and imaged at a voltage of 1.00 kV without gold coating.

2.7. Residual content of cinnamon bark oil in films during ambient storage

The loss of CBO from films during ambient storage (21 °C) was quantified. After storage for up to 7 days, films were cut into 2 × 2 cm squares and placed in 20 mL capped vials with 10 mL hexane (Chi et al., 2006). After extraction by stirring overnight at room temperature, the mixture was centrifuged at 11,337 × g for 5 min (Minispin plus, Eppendorff, Hamburg, Germany) and the supernatant was analyzed for absorbance at 280 nm (model Evolution 201, Thermo Scientific, Waltham, MA). The CBO concentration was then determined based on a standard curve constructed from standard CBO solutions in hexane (Pan, Chen, Davidson, & Zhong, 2014). To compare the loss of CBO in films prepared from microemulsions (with 2–6% Tween™ 80) versus conventional emulsions, a set of chitosan films was prepared with 0.5% Tween™ 80 (Chi et al., 2006; Wang et al., 2011; Zivanovic et al., 2005) and 1–3% CBO as controls. The loss of CBO with respect to film–forming mixtures was calculated using Eq. (5) based on three film replicates.

$$Loss(\%) = \frac{C - C_t}{C} \times 100\% \tag{5}$$

where C and C_t are the respective amount of CBO in the film–forming mixtures and after storage of films for t days.

2.8. Target bacteria preparation

Five strain bacterial cocktails were used in the microbiological studies. Each bacterial cocktail consisted of equal populations of 5 test strains of *E. coli* O157:H7, including H1730, F4546, K3995, 658, and 932, and 5 serovars of *Salmonella enterica*, including *S.* Agona, *S.* Montevideo, *S.* Gaminara, *S.* Michigan, and *S.* Saint Paul. For *Listeria monocytogenes*, strains LM1, LM2, 310, Scott A, and V7 were mixed. Before mixing, each strain/serovar was cultured in TSB for 24 h at 32 °C (*L. monocytogenes*) or 37 °C (*S. enterica* and *E. coli* O157:H7) and transferred at least 2 times prior to use. 2 mL of culture from each strain/serovar were mixed to yield the cocktail and diluted to about 10^6 CFU/mL in TSB before testing.

2.9. Antimicrobial properties of films

Antimicrobial properties of the films were evaluated using a disk diffusion assay (Zivanovic et al., 2005). Films were cut into circular discs with a diameter of 10 mm. 200 μL of each cocktail with 10^6 CFU/mL bacteria was spread uniformly on the surface of tryptic soy agar (TSA) or TSA supplemented with yeast extract (TSAYE, for *L. monocytogenes*) plates. Two discs of each film were placed on each plate and incubated for 24 h at 32 °C (*L. monocytogenes*) or 37 °C (*E. coli* O157:H7 and *S. enterica*). Inhibition zone diameters (mm) were then measured. Two films from the same formulation were tested for two discs each (n = 4).

2.10. Statistical analysis

Experiment data were subjected to variance analysis and the Tukey's test using SPSS 20 (IBM, Armonk, NY) at a 5% significance level.

3. Results and discussion

3.1. Viscosity and hydrodynamic diameters (D_h) of film–forming mixtures

The D_h of film–forming mixtures was measured shortly before film casting. As presented in Table 2, at each CBO level, D_h followed an order of microemulsion 2:1 < 4:1 < 1:0, which suggested that an increase in the amount of SBO reduced D_h. The trend agreed with results from microemulsion only (Ma & Zhong, 2015), which is expected because film–forming mixtures had a same

Table 2
Hydrodynamic diameters of mixtures containing 1% w/w chitosan and microemulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w and three CBO:soybean oil (SBO) mass ratios.[a]

Overall CBO concentration	CBO:SBO mass ratio	Hydrodynamic diameter (nm)
1% w/w	1:0	257.1 ± 8.8^{b}
	2:1	191.5 ± 6.9^{de}
	4:1	219.7 ± 24.8^{c}
2% w/w	1:0	273.4 ± 6.7^{ab}
	2:1	115.7 ± 3.1^{f}
	4:1	206.4 ± 5.7^{cd}
3% w/w	1:0	293.3 ± 9.9^{a}
	2:1	88.3 ± 5.5^{g}
	4:1	178.5 ± 3.1^{e}

[a] Numbers are mean ± standard deviation (n = 6). Different superscript letters indicate mean values differ significantly ($p < 0.05$).

concentration of chitosan. The smaller D_h at a higher SBO content is likely because the decreased polarity of the oil phase after mixing CBO with SBO enhances the compatibility with Tween™ 80 (Ma & Zhong, 2015). The D_h of film–forming mixtures was mostly greater than 100 nm and greater than <30 nm of the microemulsion alone (Ma & Zhong, 2015). The D_h being greater than 10–50 nm typically expected in microemulsions (Slomkowski et al., 2011) indicates the film–forming mixtures were no longer microemulsions. This is because microemulsions are formed at a specific combination of thermodynamic conditions but the solvent in the film–forming mixture is no longer being equal masses of PG and water (as in microemulsions before mixing chitosan solution. Nevertheless, microemulsions provide a convenient approach in preparing oil/water/surfactant mixtures to prepare transparent films as presented in the following section. For description simplicity, the term “microemulsions” is still used to present treatments in the rest of this paper.

The rheograms of film–forming mixtures are shown in Fig. 1 and are a function of both chitosan and microemulsion components in the complex mixture (Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2010b). The film–forming mixtures were overall shear thinning fluids, which is expected for biopolymer solutions and deformable droplets (McClements, 2005). As the CBO content increased from 1% to 3%, the viscosity increased significantly, as a result of the increased content of microemulsion components that are more viscous than water. The impacts of CBO:SBO mass ratios on viscosity showed different trends at three CBO levels, indicating different roles of chitosan and microemulsion components (Sánchez-González et al., 2010b). At 1% CBO (Fig. 1A), no statistical difference was observed for the three samples. This may be due to the dominance of chitosan on mixture viscosity. The impacts of microemulsion became significant at the two higher CBO levels. At 2% CBO (Fig. 1B), the mixture with a higher amount of SBO had a higher viscosity at low shear rates, and no difference was observed at high shear rates. As shown in Table 2, mixtures with a higher SBO content had smaller droplets and an overall larger amount of oil phase at the same CBO content, which results in a larger population of smaller oil droplets and eventually an increased effective volume fraction of droplets and viscosity of mixture at low shear rates (McClements, 2005). The deformability of oil droplets likely makes it difficult to differentiate these mixtures at high shear rates under the conditions studied. At 3% CBO (Fig. 1C), the viscosity in the entire shear rate range overall followed the decreasing order for mixtures with microemulsion 1:0 > 4:1 > 2:1, which agrees with the decreasing content of PG (Table 1). At high shear rates, the impacts of chitosan and oil droplets on viscosity are reduced, and the observations in Fig. 1C likely are caused by differences in solvent composition. Because PG is more viscous than water (data not shown), its impact on the mixture viscosity becomes significant for treatments with 3% CBO but not two lower CBO concentrations.

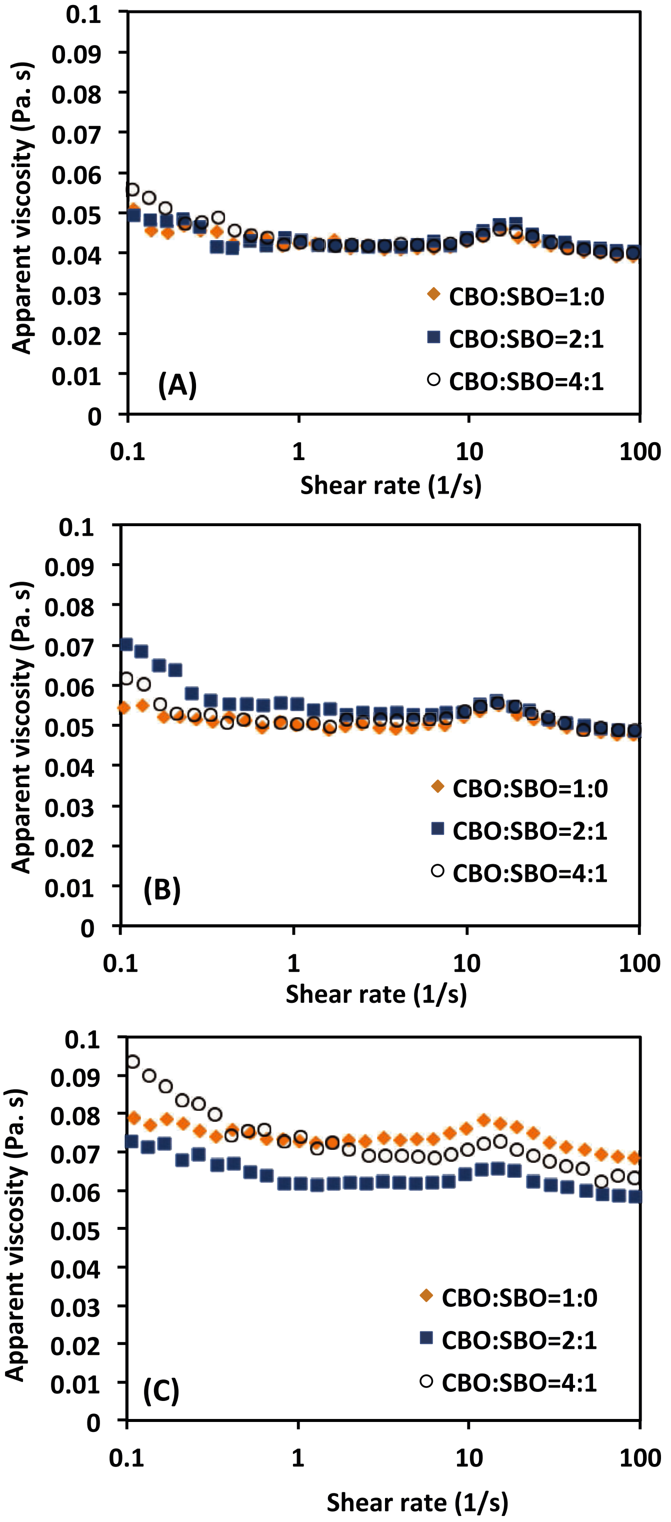

Fig. 1. Rheograms of mixtures containing 1% w/w chitosan and microemulsions at an overall cinnamon bark oil (CBO) concentration of 1% (A), 2% (B), 3% w/w (C) and three CBO:soybean oil (SBO) mass ratios at 25 °C.

Table 3
Thickness and color of films prepared from mixtures containing 1% w/w chitosan and microemulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w and three CBO:soybean oil (SBO) mass ratios.[a]

Overall CBO concentration	CBO:SBO mass ratio	Thickness (mm)	*L*	*a*	*b*
0% (chitosan only)		0.012 ± 0.001^{g}	89.14 ± 0.23^{a}	-1.80 ± 0.06^{a}	1.42 ± 0.12^{e}
1% w/w	1:0	0.040 ± 0.002^{f}	89.43 ± 0.59^{a}	-2.20 ± 0.13^{ab}	2.70 ± 0.30^{cd}
	2:1	0.043 ± 0.004^{f}	89.48 ± 0.39^{a}	-2.38 ± 0.11^{bc}	2.87 ± 0.24^{cd}
	4:1	0.040 ± 0.003^{f}	89.18 ± 0.47^{a}	-2.18 ± 0.18^{ab}	2.57 ± 0.42^{d}
2% w/w	1:0	0.063 ± 0.007^{e}	89.42 ± 0.64^{a}	-2.53 ± 0.07^{bc}	3.62 ± 0.21^{bc}
	2:1	0.069 ± 0.003^{d}	89.31 ± 0.40^{a}	-2.88 ± 0.24^{cd}	4.45 ± 0.44^{b}
	4:1	0.066 ± 0.004^{de}	89.17 ± 0.17^{a}	-2.52 ± 0.13^{bc}	3.54 ± 0.3^{bc}
3% w/w	1:0	0.089 ± 0.009^{c}	89.41 ± 0.42^{a}	-3.31 ± 0.52^{d}	5.70 ± 0.87^{a}
	2:1	0.106 ± 0.009^{a}	89.25 ± 0.35^{a}	-3.37 ± 0.40^{d}	6.30 ± 0.78^{a}
	4:1	0.097 ± 0.007^{b}	89.49 ± 0.55^{a}	-3.34 ± 0.41^{d}	6.18 ± 0.67^{a}

[a] Numbers are mean ± standard deviation (n = 24 for thickness, 6 for color). Different superscript letters indicate mean values in the same column differ significantly ($p < 0.05$).

3.2. Physical properties of films

The thickness of films is shown in Table 3. Control chitosan films were significantly thinner (0.012 mm) than films with microemulsions ($p < 0.05$). Generally, with the increasing content of microemulsion, the thickness of films significantly increased, agreeing with a previous study in which films were prepared from the mixtures with 0.5–1.5% EOs, 0.1% v/v Tween 80, and 1% w/v chitosan homogenized at 7000 rpm for 2 min (Hosseini et al., 2009). For films prepared with 1% w/w CBO, no significant differences in thickness were found with different CBO:SBO mass ratios. For treatments prepared with 2% w/w CBO, a significant difference in film thickness was only observed for films prepared with microemulsion 2:1 (0.069 mm) and 1:0 (0.063 mm). The thickness of films prepared with 3% w/w CBO varied significantly with microemulsion composition, following the order of microemulsion 2:1 (0.106 mm) > 4:1 (0.097 mm) > 1:0 (0.089 mm). Therefore, the impacts of non-volatile SBO on film thickness became significant in films with 3% w/w CBO.

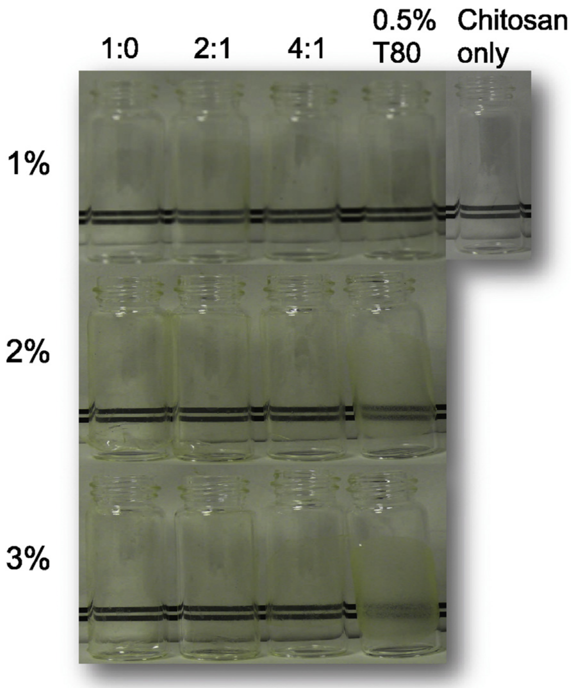

Fig. 2. Appearance of films prepared from mixtures containing 1% w/w chitosan with and without microemulsions or emulsions. Microemulsions were formulated with cinnamon bark oil (CBO): soybean oil (SBO) mass ratios of 1:0, 2:1 and 4:1 and overall CBO concentrations of 1, 2, and 3% w/w. Emulsions consisted of 0.5% w/w Tween™ 80 and 1, 2 or 3% w/w CBO.

No significant differences in lightness (around 89 in *L* values) were found among the samples (Table 3). Generally, as the CBO content in the film–forming mixture increased from 1% to 3%, the positive *b* value (yellowness) of films increased, from 1.40–2.80 to 3.60–6.30, which was due to the increased amount of Tween™ 80 and oil.

Most films prepared with microemulsions were visually transparent in the present study (Fig. 2) and had low opacity (Table 4). An exception was observed in films prepared with microemulsion 2:1 and 1% CBO, which had the highest opacity and were slightly translucent. The low CBO content and a relatively high SBO content of this treatment (Table 1) may have caused a higher extent of coalescence during film preparation. In contrast, control films with 0.5% Tween™ 80 at 2 and 3% CBO levels had much higher opacity (Table 4) and were translucent or opaque (Fig. 2).

The moisture contents of films are listed in Table 5. The control chitosan film had a significantly ($p < 0.05$) higher moisture content (~22%) than films with 1% CBO. For treatments with microemulsions, the measured moisture content was significantly higher ($p < 0.05$) for films without SBO than those containing SBO. Because CBO is volatile, moisture content of films with 2 and 3% CBO were significantly higher than that of films with 1% CBO and thus the moisture content of the films was over-estimated. Nevertheless, the data in Table 5 suggest that microemulsions reduce the retention of moisture during drying and SBO reduces the evaporation of CBO

Table 4
Opacity of films prepared from mixtures containing 1% w/w chitosan with and without microemulsions or emulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w. Mass ratios of CBO and soybean oil in microemulsions were 1:0, 2:1 or 4:1.[a]

Overall CBO concentration	CBO:SBO mass ratio	Opacity (%)
0% (chitosan only)		0.04 ± 0.01^{e}
1% w/w	1:0	0.05 ± 0.01^{e}
	2:1	0.95 ± 0.17^{c}
	4:1	0.08 ± 0.02^{e}
2% w/w	1:0	0.08 ± 0.02^{e}
	2:1	0.30 ± 0.05^{d}
	4:1	0.04 ± 0.01^{e}
3% w/w	1:0	0.03 ± 0.01^{e}
	2:1	0.16 ± 0.01^{de}
	4:1	0.05 ± 0.01^{e}
0.5% Tween™ 80 + 1% CBO		0.07 ± 0.02^{e}
0.5% Tween™ 80 + 2% CBO		1.41 ± 0.21^{b}
0.5% Tween™ 80 + 3% CBO		6.38 ± 2.33^{a}

[a] Numbers are mean ± standard deviation (n = 6). Different superscript letters indicate mean values in the same column differ significantly ($p < 0.05$).

Table 5
Moisture content, swelling ratio, and water vapor permeability (WVP) of films prepared from mixtures containing 1% w/w chitosan with and without microemulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w and three CBO:soybean oil (SBO) mass ratios.[a]

Overall CBO concentration	CBO:SBO mass ratio	Moisture content (%)	Swelling ratio (%)	WVP ($\times 10^{-10}$ g/Pa m s)
0% (chitosan only)		22.44 ± 1.40^{bc}	871.01 ± 53.29^{a}	0.965 ± 0.003^{e}
1% w/w	1:0	18.63 ± 0.76^{e}	8.98 ± 3.44^{g}	2.777 ± 0.108^{d}
	2:1	15.41 ± 0.34^{f}	19.74 ± 1.29^{ef}	2.882 ± 0.026^{d}
	4:1	14.17 ± 0.65^{f}	15.15 ± 2.06^{fg}	2.540 ± 0.056^{d}
2% w/w	1:0	24.31 ± 0.29^{ab}	50.23 ± 2.27^{c}	4.456 ± 0.020^{c}
	2:1	19.61 ± 1.62^{de}	50.72 ± 3.50^{c}	4.683 ± 0.101^{c}
	4:1	21.17 ± 0.42^{cde}	58.07 ± 2.00^{b}	4.440 ± 0.118^{c}
3% w/w	1:0	26.46 ± 0.95^{a}	35.70 ± 0.46^{d}	6.319 ± 0.326^{b}
	2:1	20.83 ± 1.23^{cde}	23.88 ± 1.47^{e}	7.663 ± 0.133^{a}
	4:1	21.81 ± 0.79^{bcd}	31.61 ± 1.62^{d}	6.669 ± 0.062^{b}

[a] Numbers are mean ± standard deviation (n = 3). Different superscript letters indicate mean values in the same column differ significantly ($p < 0.05$).

during drying. The former likely resulted from the reduced water binding capacity of films with a higher content of hydrophobic SBO and CBO (Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2010a). The latter agrees with the previous study showing a reduction of compound volatility by SBO (Kim et al., 1995).

The control chitosan film showed the highest swelling ratio (>800%). Among films with microemulsions, swelling ratios of films prepared with 2% w/w CBO (>50%) were significantly higher than

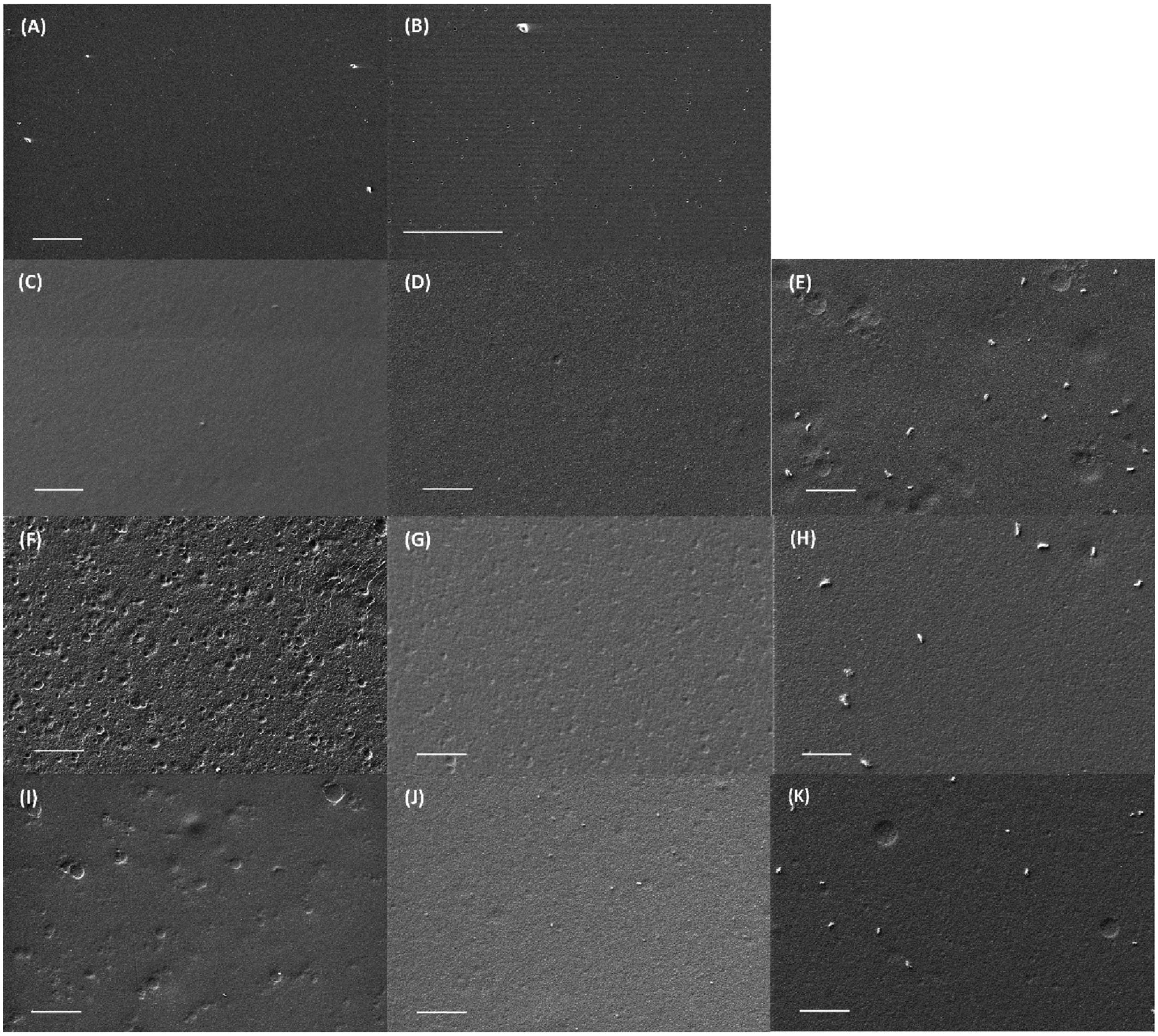

Fig. 3. Scanning electron micrograph images showing surfaces of films prepared from mixtures containing 1% w/w chitosan and microemulsions formulated with cinnamon bark oil (CBO): soybean oil mass ratios of 1:0 (C–E), 2:1 (F–H) and 4:1 (I–K) and overall CBO concentrations of 1, 2, and 3% w/w (left, middle, and right in each row). Control chitosan film is compared in A and B at two magnifications. Bar = 10 μm.

those prepared with 3% w/w CBO (~23–35%), followed by films prepared with 1% w/w CBO (~9–19%). The higher swelling ratio of films prepared with 2% w/w CBO may be due to the intermediate content of Tween™ 80 and lipophilic CBO and SBO. For the 3% w/w CBO treatments, although the increased Tween™ 80 content could increase the water absorption of films due to the water affinity of Tween™ 80, the increased contents of lipophilic CBO and SBO likely increased the film hydrophobicity and therefore reduced film swelling.

The WVP of films (Table 5) had the same trend as the thickness of films (Table 3) and was significantly higher at a larger amount of CBO/microemulsion. The increased WVP with increasing CBO/microemulsion content in the film can be attributed to the increased content of hydrophilic Tween™ 80 (Ziani, Oses, Coma, & Maté, 2008) and PG (Park, Weller, Vergano, & Testin, 1993; Parris & Coffin, 1997), since the Tween™ 80 and PG increase was greater than the increased content of oil (CBO and SBO). The positive correlation between the thickness of films and WVP is expected from Eq. (4), as reported in previous studies (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007; Longares, Monahan, O'riordan, & O'sullivan, 2004). This was the most apparent for films prepared with 3% w/w CBO that showed significant differences in film thickness and WVP for treatments with three different CBO:SBO mass rations, with the microemulsion 2:1 treatment showing the highest thickness (Table 3) and WVP (Table 5). In the present study, films prepared with microemulsion 2:1 had smaller oil droplets than treatments with other CBO:SBO mass ratios (Table 2), which may additionally reduce the mass transfer resistance for water molecules and therefore increase the WVP.

3.3. *Morphology of film surface*

Surface SEM images of films are shown in Fig. 3. The surface of control chitosan film was smooth (Fig. 3A and B). Films with microemulsion 1:0 had smooth surfaces for the 1 and 2% w/w CBO treatments (Fig. 3C and D), while particulates and large pores were observed for the 3% w/w CBO treatments (Fig. 3E). For microemulsions 2:1 and 4:1 treatments, films prepared with 1 and 2% w/w CBO showed many small pores (<~1 μm), while those prepared with 3% w/w CBO also had particulates but fewer large pores than the microemulsion 1:0 treatment. The particulate structures probably resulted from microemulsion components after drying, while pore structures may have resulted from evaporation of CBO during drying.

Because all films were prepared with the same amount of chitosan, different surface morphology of films likely resulted from structure changes of microemulsion components during drying. As presented previously, mixing chitosan solution and microemulsions changes the polarity of solvent, and the film–forming mixtures are not microemulsions anymore. The new oil/water/surfactant mixtures then can experience coalescence and Ostwald ripening as expected in most EO emulsions, resulting in increases of droplet dimension, but the structures of these mixtures are not as well controlled as emulsions prepared with homogenization at defined conditions. The continued changes of solvent composition during drying film–forming mixture can further destabilize oil droplets. The evaporation of water and some CBO during drying can additionally cause the formation of pores in Fig. 2 that are much larger than the D_h of droplets (Table 2). These complicated phenomena make it difficult to clearly correlate formulations with film morphology and correlate film microstructures with mechanical properties in the next section. As a result, no attempt was made to quantify overall pore size and porosity of films, as well as dissecting films to study internal structures.

Nevertheless, several observations can be noted based on surface morphology of films in Fig. 3. For films prepared with microemulsion 1:0, the amount of Tween™ 80 in the mixture with 1 and 2% CBO may have been sufficient to maintain the nanoscale structure after drying, resulting in smooth surface structures (Fig. 3C and D). At 3% CBO (Fig. 3E), the increased droplet concentration may increase the possibility of droplet coalescence during drying and cause the formation of large pores. For films containing SBO, treatments with a CBO:SBO mass ratio of 4:1 have overall less homogeneous surfaces than those at a ratio of 2:1 (Fig. 3F–H vs. I–K), which can result from the coalescence of larger droplets (Table 2) during drying. The increasing homogeneity of film surface structure prepared at a higher CBO level agrees with the reduced droplet dimension in the film–forming mixtures (Table 2). The increased viscosity at a higher CBO level (Fig. 1) also can reduce the coalescence (Thoroddsen, Takehara, & Etoh, 2005) of droplets and increase matrix homogeneity after drying.

3.4. *Mechanical properties of films*

The tensile strength and elongation% at break of films are presented in Table 6. The control chitosan film had the highest tensile strength (627 MPa) and lowest elongation% at break (4.2%). When the amount of CBO in the film-forming mixture increased from 1 to 3% w/w CBO, the tensile strength of films significantly decreased from ~71–121 MPa to ~27–36 MPa, while elongation% at break increased from 13.2–23.7% to 32.7–42.8%. No significant differences in tensile strength were found for films prepared with 2 and 3% w/w CBO, regardless of SBO content. At a higher content of CBO, the population of oil droplets is higher, which can potentially increase the porosity of films after drying. The increased film porosity, together with the greater number of residual oil droplets in film, decreases the homogeneity of chitosan matrix structure to lower the tensile strength and increase elongation% at break (Bonilla, Atarés, Vargas, & Chiralt, 2012; Hosseini et al., 2009).

3.5. *Residual content of cinnamon bark oil in films during ambient storage*

EOs are volatile and can evaporate during film formation and subsequent storage. In a previous study, incorporating Tween™ 20 in chitosan films improved the retention of oregano oil (Chi et al., 2006). SBO was also found to reduce the volatility of garlic volatile compounds (Kim et al., 1995). In the present study, the residual content of CBO in films with microemulsions after formation and during storage under ambient conditions was compared to control chitosan films prepared with conventional emulsions with 0.5% Tween™ 80 and 2% or 3% CBO (Chi et al., 2006; Wang et al., 2011; Zivanovic et al., 2005). For fresh films (day 0) prepared with 2% CBO, about 50% of CBO in the film–forming mixtures was lost in films prepared with microemulsions, compared to a 70% loss of CBO in the control film (Fig. 4A). Similar results were observed for treatments prepared with 3% CBO, showing about 30% loss of CBO in fresh films prepared with microemulsions and about 50% loss in the control film (Fig. 4B). During ambient storage, films prepared with 3% CBO in the microemulsion form had lower losses of CBO than the control emulsion treatment ($p < 0.05$) after 6-day storage (Fig. 4B). The overall trend was similar for films prepared with 2% CBO (Fig. 4A) but the difference between microemulsion treatments and the control emulsion films was less than those prepared with 3% CBO (Fig. 4B). The results confirmed the effectiveness of microemulsion components in reducing the loss of EOs during drying (film formation). At the same concentration of CBO, no significant difference in CBO retention was found among microemulsion films with and without SBO. When CBO evaporates from films, there are overall two mass transfer steps: diffusion from film interior to film surface and then from film surface to ambient air

Table 6
Mechanical properties of films prepared from mixtures containing 1% w/w chitosan with and without microemulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w and three CBO:soybean oil (SBO) mass ratios.[a]

Overall BO concentration	CBO:SBO mass ratio	Tensile strength (MPa)	Elongation at break (%)
0% (chitosan only)		627.5 ± 20.5^{a}	4.2 ± 0.7^{g}
1% w/w	1:0	121.3 ± 7.8^{b}	23.7 ± 4.1^{de}
	2:1	71.2 ± 9.0^{cd}	13.2 ± 3.9^{fg}
	4:1	94.8 ± 8.9^{bc}	15.9 ± 3.2^{ef}
2% w/w	1:0	42.2 ± 11.6^{de}	24.5 ± 9.9^{de}
	2:1	56.0 ± 2.8^{de}	36.7 ± 1.3^{abc}
	4:1	44.4 ± 5.9^{de}	30.2 ± 3.3^{cd}
3% w/w	1:0	36.3 ± 4.6^{e}	42.8 ± 5.5^{a}
	2:1	32.4 ± 3.5^{e}	41.1 ± 3.9^{ab}
	4:1	27.4 ± 6.4^{e}	32.7 ± 9.1^{bcd}

[a] Numbers are mean ± standard deviation (n = 3). Different superscript letters indicate mean values in the same column differ significantly ($p < 0.05$).

(Boland, Buhr, Giannouli, & van Ruth, 2004). The mass transfer rate from the film surface to air may be dominant, because of the convection and the larger concentration gradient than that in the film interior, as quantified in a previous study for mass partition coefficients between a volatile oil and ambient air or a film matrix (Kurek, Moundanga, Favier, Galić, & Debeaufort, 2013). As a result, the hydrophobic interaction between SBO and CBO in films, which impacts the interior mass transfer, becomes insignificant on the overall retention of CBO.

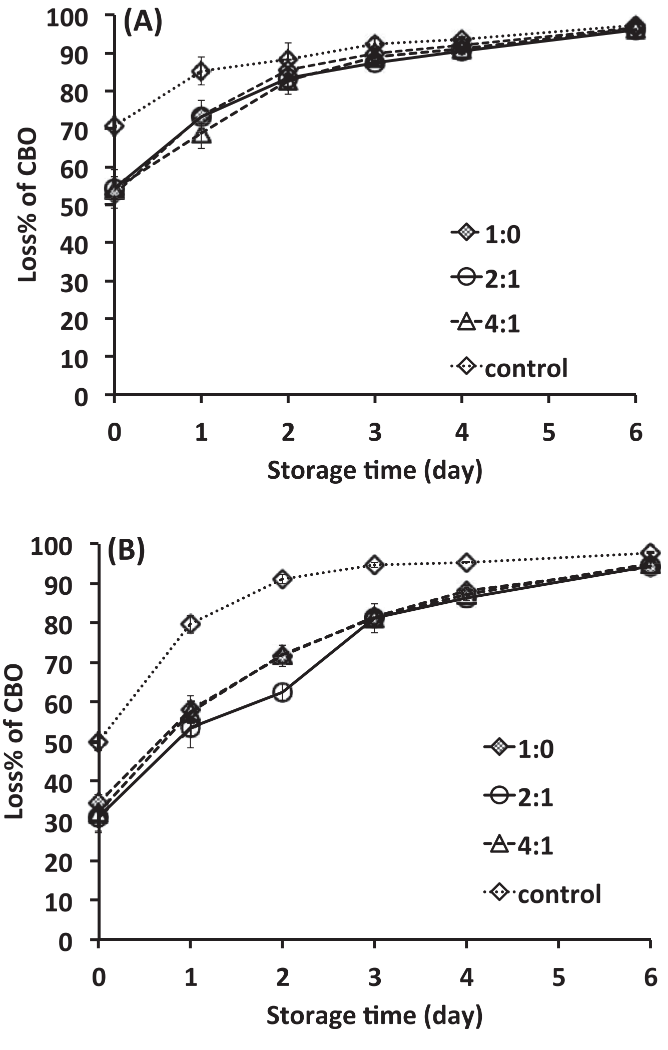

Fig. 4. Loss% of cinnamon bark oil (CBO), with respect to the CBO mass in film-forming mixture, from films during ambient storage (21 °C, n = 3). Films were prepared with microemulsions with CBO:soybean oil (SBO) mass ratios of 1:0, 2:1 and 4:1 at an overall CBO level of (A) 2% and (B) 3%. Data at day 0 are measured for fresh films after drying film–forming mixtures at ambient conditions for 24 h. The control films were prepared with conventional emulsions with 0.5% Tween™ 80 and same CBO concentrations.

3.6. Antimicrobial properties of films

Inhibition zone diameters determined in the disc diffusion method, with an example shown in Fig. 5, are summarized in Table 7. The test was conducted for films conditioned in desiccators for 1 day. Inhibition zone diameters of films prepared with 3% w/w CBO were significantly larger than those with 2% w/w CBO, while those prepared without and with 1% w/w CBO did not show inhibition. The results were in agreement with the greater quantities of CBO in films prepared with a higher CBO level (Fig. 4 and Table 7). The films prepared with 3% CBO had 1.87–1.97 mg CBO/cm^2 film, which was more than twice of about 0.90 mg CBO/cm^2 film in treatments prepared with 2% CBO. There was only ca. 0.10 mg CBO/cm^2 film detected in films prepared with 1% CBO. The maximum local concentration of CBO in the agar underneath films discs prepared with 2 and 3% CBO, estimated by dividing the CBO content in film discs by the mass of TSA/TSAYE underneath without diffusing to nearby media, is about 2600 and 6000 ppm, respectively. These concentrations are significantly higher than the minimum inhibitory concentration (MIC) of CBO, which was previously shown to be a maximum of ca. 250 ppm against the same three foodborne pathogens (Mith et al., 2014). In contrast, films prepared with 1% CBO had a residual CBO content below the MIC that agreed with the lack of any inhibition zone (Table 7).

For the discs prepared with 3% w/w CBO, the inhibition zone diameters were significantly different among bacteria and followed the decreasing order of *L. monocytogenes* (22.0–23.5 mm) > *S. enterica* (18.3–18.5 mm) > *E. coli* O157: H7 (15.3–16.8 mm). In a previous study, *L. monocytogenes* was also inhibited to a greater extent than either *E. coli* O157:H7 or *S.* Typhimurium by trans-cinnamaldehyde, the major component of CBO (Mith et al., 2014).

4. Conclusions

Transparent chitosan films were obtained after incorporating microemulsions with different mass ratios of CBO:SBO. SBO was observed to reduce the loss of CBO when films were tested for moisture content during vacuum drying. However, SBO did not show significant impact on the residual content of CBO after film formation and incubation of films at ambient conditions, which may be due to the dominant mass transfer between film surface and ambient environment. Films with microemulsions were thicker and had higher WVP than the control film, which may be due to increased amounts of hydrophilic Tween™ 80 and PG. Tensile strength of films decreased as the amount of

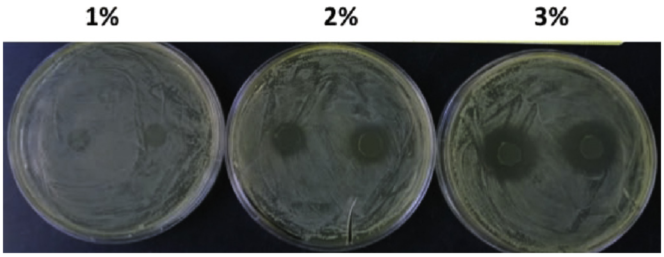

Fig. 5. Representative photograph showing inhibition of *Listeria monocytogenes* around film discs on TSAYE plates. Films were prepared from mixtures containing 1% w/w chitosan and microemulsions at an overall cinnamon bark oil concentration of 1, 2, or 3% w/w without soybean oil.

Table 7
Inhibition zone diameters (including the 10 mm diameter of discs) of film discs prepared from mixtures containing 1% w/w chitosan with and without microemulsions at an overall cinnamon bark oil (CBO) concentration of 1–3% w/w and three CBO:soybean oil (SBO) mass ratios.[a]

Overall CBO concentration	CBO:SBO mass ratio	Mg CBO/cm^2 film	Inhibition zone diameter (mm)		
			E. coli O157:H7	*S. enterica*	*L. monocytogenes*
0% (chitosan only)		0.00	+[b]	+	+
1%	1:0	0.06	+	+	+
	2:1	0.10	+	+	+
	4:1	0.10	+	+	+
2%	1:0	0.90	11.8 ± 0.5^{g}	12.0 ± 0.8^{g}	14.6 ± 0.8^{ef}
	2:1	0.87	11.5 ± 0.6^{g}	11.8 ± 0.5^{g}	13.3 ± 0.9^{fg}
	4:1	0.87	11.6 ± 0.5^{g}	11.4 ± 0.5^{g}	13.0 ± 0.8^{fg}
3%	1:0	1.87	15.3 ± 0.5^{ef}	18.5 ± 1.1^{cd}	22.0 ± 0.0^{ab}
	2:1	1.97	16.8 ± 1.0^{de}	20.0 ± 1.6^{bc}	23.5 ± 2.1^{a}
	4:1	1.94	15.3 ± 0.5^{ef}	18.3 ± 0.9^{cd}	23.5 ± 1.7^{a}

[a] Numbers are mean ± standard deviation ($n = 4$). Different superscript letters indicate mean values differ significantly ($p < 0.05$).
[b] "+": Growth of bacteria was observed under film discs.

microemulsion/CBO increased in the film, while elongation% at break increased with increasing microemulsion/CBO content. The loss of CBO from films with microemulsions during ambient storage was less than a control film prepared with conventional emulsions with less Tween™ 80. Films showed strong antimicrobial activity against food-borne pathogens, particularly Gram-positive *L. monocytogenes*. Further study will be done to investigate the effects of these chitosan-based films on the microbiological quality and safety of fresh produce.

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