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Project Title
Novel coating systems with sustained release of food antimicrobials to improve safety of cantaloupe

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Objectives
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1) Identify conditions of preparing nanoemulsions of EO
2) Characterize physical properties of films and release properties of EO
3) Evaluate antimicrobial effectiveness of coatings against spoilage and pathogenic microorganisms on cantaloupes.
4) Assess the cost of antimicrobial coatings and impacts on the quality of cantaloupes.

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Abstract

Cantaloupes have been linked to outbreaks of foodborne illnesses due to contamination by pathogens. The irregular surface of cantaloupes can protect pathogens from sanitation, and the contaminating foodborne pathogens can survive during the long-term storage of cantaloupes. Therefore, novel strategies are needed to improve the safety of cantaloupes. The overall goal of this project was to improve the safety and quality of whole cantaloupes using novel coatings formed with polysaccharides and essential oils (EOs).

Because EOs are volatile, soybean oil (SBO) was studied to reduce their evaporation during storage. The first objective was to formulate and characterize mixtures with oil, water, and emulsifiers. We successfully formulated clear mixtures (microemulsions) with polysorbate 80 (Tween 80) as a surfactant and EOs and SBO at mass ratios of 1:0, 2:1, or 4:1. We developed phase diagrams summarizing these formulations and studied physical bases of forming these clear mixtures. We characterized the dimension and stability of droplets in these mixtures. These clear mixtures were shelf-stable for more than 3 months. We also characterized antimicrobial activities of these mixtures and observed the reduction of activities when compared to free EOs dissolved in ethanol, with cinnamon bark oil (CBO) maintaining the highest antimicrobial activities. We additionally studied another emulsifier, lauric arginate (LAE) that is also an effective antimicrobial. We observed the synergistic antimicrobial activities of LAE and cinnamon oil (CO) with the presence of ethylenediaminetetraacetate (EDTA).

To understand and predict the effectiveness of antimicrobial coatings on cantaloupes, the second objective was devoted to study physical, mechanical, and antimicrobial activities of films prepared from polysaccharides, antimicrobials, and emulsifiers. Two sets of films were prepared and characterized. The first set of clear films was prepared from clear oil/water/emulsifier mixtures and chitosan and was compared to opaque films prepared from turbid mixtures with less emulsifiers. Clear films had favorable physical properties for coating applications and were more effective than opaque films in retaining CBO during storage. The second set of films was prepared with 1%w/w sodium alginate, 0.5%w/w Tween 80, 0-2%w/w CBO and 0-1% SBO. These films also had desirable physical properties, and the addition of SBO improved the retention of CBO in films during storage. Films had good antimicrobial activities when tested against bacteria cocktails made of five strains/serovars each of *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* O157:H7.

In the third objective, three coating mixtures were studied for inhibition of pathogens inoculated on whole cantaloupes and native microflora: (1) 1% chitosan and microemulsions with 2% CBO, (2) 1% alginate and 2% CBO with 0 or 0.5% SBO, and (3) 1%w/w chitosan, 0.1%w/w LAE, 0.1% w/w EDTA, and 0, 0.5, or 1% w/w cinnamon oil (CO). Overall, coating treatments were effective in inhibiting the inoculated pathogens and the growth of native molds and yeasts, especially after addition of SBO in alginate-based films. The first formulation, as originally planned, was the most expensive and had the lowest antimicrobial activity. The second and third formulations were studied for impacts of coatings on cantaloupe quality in the fourth objective. Antimicrobial coatings slowed the color change and improved the firmness of cantaloupes during storage, and had no impacts on other quality parameters. Additionally in the fourth objective, costs of the second and third coating formulations were estimated to be less than 1 cent per cantaloupe. The project findings showed the potential application of the two coating mixtures to improve the quality and safety of cantaloupes.

Background

Cantaloupes have been linked to several outbreaks of foodborne illnesses. A total of 1434 people became ill after consumption of contaminated cantaloupes in 1983-2002 (Bowen et al., 2006). A recent multistate outbreak of listeriosis associated with cantaloupes caused 84 cases of infections in 28 states, 33 deaths, and 1 miscarriage in 2011 (CDC, 2011).

Many strategies have been investigated to improve the safety of fresh produce. Chemical sanitizers such chlorine and peroxyacetic acid are commonly studied, but the interference of organic matters originating from fresh produce during washing reduces the sanitation effectiveness (Rodgers et al., 2004). Surface roughness of fresh produce is another important factor that affects the effectiveness of sanitation. Surface roughness was positively and linearly correlated to the adhesion rate of bacteria while negatively and linearly correlated to inactivation efficiency of acidic electrolyzed water and peroxyacetic acid, as demonstrated for the lower efficiency on cantaloupes than avocado, oranges, and apples (Wang et al., 2009). Surfactants can facilitate sanitizer solution to reach cavities harboring pathogens and therefore sanitation effectiveness, as we recently reported (Xiao et al., 2011). Other strategies including pulsed electric field, UV radiation, e-beam irradiation, chlorine dioxide gas, and ozone gas (Selma et al., 2008; Trinetta et al., 2011) have also been studied, but these technologies have not yet been used in the fresh produce industry.

Because of lacking a single technology that can completely inactivate pathogens on produce, the ability of pathogens to recover after sanitation is a big concern. For example, washing treatments using 5% hydrogen peroxide or 1,000 ppm chlorine significantly reduced *Salmonella* Stanley on cantaloupes, but the bacterium recovered after storage above 4°C (Ukuku and Sapers, 2001). Novel strategies are needed to ensure the safety of cantaloupes throughout shelf-storage.

Edible antimicrobial films and coatings are promising strategies to improve safety and quality of fruits and vegetables. EOs are natural antimicrobials that are effective against a broad spectrum of foodborne pathogens. EOs are volatile and their loss from coatings can reduce the antimicrobial effectiveness during storage. The loss/release of EOs can be controlled by encapsulation in colloidal particles, further controlled by blending with non-volatile lipids such as SBO. Surfactants used to prepare these colloidal systems can reduce the interference of surface roughness to improve the antimicrobial effectiveness of coatings.

The overall project goal was to improve the safety and quality of cantaloupes by polysaccharide coatings with EOs. The first objective was to identify conditions of preparing clear oil/water/surfactant mixtures (nanoemulsions) of EOs. The rationale of using clear nanoemulsions is to prepare clear films/coatings that will not interfere the appearance of cantaloupes. We formulated transparent thermodynamically stable oil/water/surfactant mixtures (microemulsions) by simple mixing and characterized physical and antimicrobial properties of microemulsions. The second objective was to characterize physical properties of films and release properties of EOs to predict properties of coatings on cantaloupes. Two sets of clear films were studied for clear microemulsions and turbid emulsions. The third objective was to evaluate effectiveness of coatings inhibiting spoilage and pathogenic microorganisms on cantaloupes. Besides clear microemulsions, two coating mixtures with turbid emulsions were studied. The fourth objective was to assess the cost of antimicrobial coatings and impacts on cantaloupe quality.

Research Methods and Results

A. Methods

A1. Preparation of fully-dilutable microemulsions of EOs or their components (EOCs)

Surfactant (Tween 80) and oil phase were mixed at a mass ratio from 1:1 to 9:1, with the oil phase comprised of EO or EOCs (CBO, eugenol or thymol) and SBO at a mass ratio of 1:0, 2:1, or 4:1. These microemulsions are abbreviated as microemulsion 1:0, 2:1 and 4:1, respectively, hereafter. The polar phase with equal mass of water and propylene glycol (PG) was added into the oil/surfactant mixture to obtain a final concentration from 10 to 90% w/w. After magnetic stirring till no visual change in turbidity, the mixtures were placed on the bench for at least 24 h equilibrium at room temperature (21°C) and the sample appearance was recorded by photographing. Transparent samples after incubation and confirmation by cross-polarized light microscopy as being isotropic were treated as microemulsions. The compositions corresponding to microemulsion formations were plotted in pseudo-ternary phase diagrams. Microemulsions remaining transparent and isotropic after diluting the oil/surfactant mixture with 90% w/w of polar phase were considered as fully-dilutable.

A2. Droplet dimension measured by dynamic light scattering (DLS)

To characterize the stability of microemulsions, hydrodynamic diameters (D_h) of microemulsions with the highest surfactant efficiency (Q_m, eq. 1) in each group were measured during 90–day storage at 21 °C. The DLS instrument was a Delsa Nano analyzer (Beckman Coulter, Atlanta, GA) with a scattering angle of 165°. All samples were diluted with deionized water to the instrument sensitivity range, and experiments were repeated for triplicate samples, each tested twice.

$$Q_m = [W]/([W]+[S]) \quad (1)$$

where [*W*] and [*S*] are the respective mass concentrations of polar phase and surfactant at the failure point (becoming turbid or phase separation) upon dilution (Mehta et al., 2009). Q_m indicates the maximum capability of surfactants to incorporate the polar phase.

A3. Antimicrobial activity of microemulsions

Microemulsions were prepared as described in Section A1. For free EO/EOC treatments, EOs/EOCs were pre-dissolved in ethanol to a concentration of 5%w/w. Bacteria cocktails were used in the microbiological studies. Each bacterium cocktail consisted of equal populations of 5 test strains/serovars as shown in Table 1. Before mixing, each strain was cultured in tryptic soy broth (TSB) supplemented with yeast extract (TSBYE) for *L. monocytogenes* or in TSB for *S. enterica* and *E. coli* O157:H7 for 24 h at 32°C (*L. monocytogenes*) or 37°C (*S. enterica* and *E. coli* O157:H7) and transferred for at least 2 times. Then 2 mL culture from each strain was mixed together to yield the cocktail for testing.

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using an agar dilution method (Thongson et al., 2005). Different amounts of microemulsions or free EO/EOCs were added into 60 mL tryptic soy agar (TSA) to obtain a final concentration from 156 to 5,000 ppm. The mixtures were then stirred vigorously until TSA was just about to solidify, followed by dividing at equal volumes into 3 petri dishes. The 10 μL aliquots of bacterial culture with about 10^4 CFU/mL bacteria were inoculated at 3 spots on TSA plates. MIC was defined as the lowest concentration of EO/EOC corresponding to no growth of bacteria after 2-day incubation at 32°C for *L. monocytogenes* and 37°C for *S.*

enterica and *E. coli* O157:H7. The lowest EO/EOC concentration corresponding to no growth of bacteria after 4-day incubation was defined as MBC.

To study the growth of bacteria in TSB, 1 mL culture with about 10^7 CFU/mL *L. monocytogenes* or *E. coli* O157: H7 was added into 9 mL TSB to obtain a final population of about 10^6 CFU/mL bacteria. The free CBO or microemulsion with CBO was added into the TSB-bacteria mixture at an overall concentration of 625 ppm and incubated at room temperature. The same concentration of ethanol as in the free CBO treatment was studied as an ethanol control. The formulation of the microemulsion with CBO:SBO mass ratio of 1:0 (with the highest PG content) was used to prepare a mixture without CBO and SBO as a microemulsion control. Viable cells were enumerated after 0, 24, 48, 72, 96, and 120 h by pour plating method for *L. monocytogenes* or surface plating method for *E. coli* O157:H7. The detection limit was 1 log CFU/mL. Experiments were repeated at least twice.

A4. Antimicrobial activity of mixtures with LAE, CO and EDTA

This group of antimicrobials was additionally studied because LAE is an effective antimicrobial and also a surfactant.

A4.1. Microbial growth kinetics in TSB

Growth curves of bacteria were studied using 96-well microtiter plates by measuring optical density (OD) at 630 nm (Synergy HT MultiMode Microplate Reader, BioTek, Winooski, VT). 120 µL culture with ca. 10^7 CFU/mL bacteria and 120 µL of an antimicrobial solution were added into each well, and the OD was recorded with an interval of 30 min for 10 h during incubation at 37°C (for *S.* Enteritidis and *E. coli* O157:H7 ATCC 43895) or 35°C (for *L. monocytogenes* Scott A). Stock solutions of LAE and EDTA were prepared at 500ppm and 4%w/w, respectively, in deionized water and adjusted to pH 6.8 using 1 M NaOH or HCl. CO stock solution was prepared at 5%w/w in 90% aqueous ethanol. Same ethanol concentration used in CO samples was studied as an ethanol control. Wells without antimicrobial were compared as positive controls. For *S.* Enteritidis and *E. coli* O157:H7, concentrations of LAE, EDTA, and CO were 5, 500, and 200 ppm, respectively, while these respective concentrations were 2.5, 100, and 100 ppm for *L. monocytogenes* treatments. Experiments were performed in triplicate.

A4.2. Microbial survivability studied for end-point analysis

To test the microbial survivability of bacteria in TSB, stock solutions were added alone or in combination in TSB to overall concentrations of 5 ppm LAE, 200 ppm CO, and 500 ppm EDTA. One mL culture with ca. 10^7 CFU/mL bacteria was added to 9 mL TSB containing the above antimicrobials to obtain a bacterial population of ca. 10^6 CFU/mL. After incubating the mixtures at 32°C (for *L. monocytogenes*) or 37°C (for *S.* Enteritidis and *E. coli* O157:H7) for 2 h, survival bacteria were enumerated using surface plating method on TSA (for *S.* Enteritidis and *E. coli* O157:H7) or TSA supplemented with yeast extract (TSAYE, for *L. monocytogenes*). The detection limit was 1 log CFU/mL, and experiments were done in triplicate.

A5. Preparation of chitosan-based films

A 2% chitosan stock solution was prepared with low molecular weight chitosan in 1% acetic acid solution after stirring overnight at room temperature. The impurities were removed by filtrating the chitosan stock solution through a microcloth (Calbiochem-Novabiochem Corp., San Diego, CA). Microemulsions with 40%w/w of polar phase and a 2:1 mass ratio of Tween 80 and CBO were prepared as Section A1, and mixed with the chitosan stock solution, glycerol (20% mass of chitosan) and deionized water to final mixtures with 1, 2 and 3% w/w CBO, 1% w/w

chitosan, and 0.5% w/w acetic acid. Films were prepared by casting 30 g film-forming mixture on a 17.8cm×17.8cm glass plate and drying at room temperature for 24 h. Control chitosan film was prepared similarly, without microemulsions. Dried films were conditioned in desiccators filled with saturated NaBr solution (57% relative humidity at 25°C) for >48 h before analyses.

A6. Preparation of alginate-based films

The alginate solution was prepared by dissolving 8 g sodium alginate in 400 mL sterile distilled water at 70°C. After 30 min stirring for complete dissolution, glycerol (0.3 g/g alginate) was added as a plasticizer while $CaCl_2$ (0.05g/g alginate) was added to strengthen the film. CBO was mixed with Tween 80, followed by mixing with the alginate solution with and without SBO. Water was added to make up the total volume to 800 mL. The final mixtures contained 1% w/v alginate, 0.05% $CaCl_2$, 0.3% glycerol, 0.5% w/v Tween 80, 0, 1, or 2% w/v CBO, and 0, 0.5, or 1% w/v SBO. After stirring at room temperature (21°C) for 30 min, 40 mL of the mixture was casted on a 17.8cm×17.8cm glass plate. After drying at 21°C for 40 h, films were peeled and stored in desiccators until being used. Two replicates were prepared for each formulation.

A7. Physical properties of films

The thickness of films was measured using a digital microcaliper (Mitutoyo, Japan) to the nearest 0.001 mm, and 12 locations were measured for each of two film replicates. The color of films was measured for *L* (lightness), *a* (red-green), and *b* (yellow-blue) parameters in the CIELab scale using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Measurements were performed over the standard white plate in sextuplicate and average *L*, *a*, *b* values were reported. Color differences (ΔE) were also calculated using the following equation:

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

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

To determine the moisture content (Eq. 2) and swelling ratio (Eq. 3) of films, films were cut into 2cm×2cm squares and weighted (w_1). Moisture contents of films were determined by drying the film squares at 105°C for 24 h and weighing after cooling down to room temperature in a desiccator filled with anhydrous calcium chloride (w_2). Swelling ratio was measured by immersing the film squares in deionized water for 24 h. Wet samples were wiped with filter paper to remove surface free water and weighted (w_3).

$$\text{Moisture}\ (\%) = \frac{(w_1 - w_2)}{w_1} \times 100\% \quad (2)$$

$$\text{Swelling}\ (\%) = \frac{(w_3 - w_1)}{w_1} \times 100\% \quad (3)$$

Water vapor permeability (*WVP*) was determined by measuring the mass of Fisher/Payne permeability cups (Fisher Scientific, Pittsburgh, PA) over time. Cups were filled with 5.0 g deionized water, sealed with films, and placed in a desiccator filled with saturated NaBr solution (57% relative humidity at 25°C). Cups were weighed hourly for 8 h or every 1.5 h for 9 h and changes of mass were plotted as a function of time. Each film was measured three times. Water vapor permeation ratio (*WVPR*) was determined based on the mass loss (*m*), time (*t*), and effective film area (*A*) (Eq. 4, Pelissari et al., 2009), followed by determining WVP (Eq. 5):

$$WVPR = \frac{m}{t \times A} \quad (4)$$

$$WVP = \frac{WVPR \times Film\ thickness}{sp \times (RH_1 - RH_2)} \quad (5)$$

where *sp* is water vapor saturation pressure at 25°C (Pa), RH_1 *and* RH_2 are the relative humidity inside and outside the cups.

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

Surface morphology of films was studied with a LEO 1525 scanning electron microscope (LEO Electron Microscopy, Oberkochen, Germany). Films were mounted on the specimen holder and observed at a voltage of 1.00 kV without gold coating.

A8. Residual content of CBO in films during ambient storage

The loss of CBO from films during ambient storage (21°C) was quantified. Films incubated for up to 7 days were cut into 2×2 cm^2 squares that were placed in 20 mL vials with 10 mL hexane. After extraction by stirring overnight at 21°C, the supernatant after centrifugation at 13,000rpm for 5 min was measured for absorbance at 280 nm (Evolution 201, Thermo Scientific, Waltham, MA) to determine CBO concentration based on a standard curve constructed with standard CBO solutions in hexane. The amounts of CBO before (C_{original}) and after ($C_{\text{remaining}}$) storage for a certain duration from three film replicates were used to calculate the loss:

$$\text{Loss}\,(\%) = \frac{(C_{\text{original}} - C_{\text{Re}\,maining})}{C_{original}} \times 100\% \quad (6)$$

A9. Antimicrobial activity of films

Antimicrobial properties of films were evaluated by the disk diffusion method. Films were cut into circular discs with a diameter of 10 or 15 mm. 200 μL bacteria culture with ~10^6 CFU/mL bacterial cocktail (as in Section A3) was spread uniformly on the surface of TSA or TSAYE (for *L. monocytogenes*) plates. Two discs of each film were laid on each plate and inoculated for 24 h at 32°C (*L. monocytogenes*) or 37°C (*E.coli* O157: H7 and *S. enterica*), followed by measuring inhibition zone diameter (d_{iz}).

A10. Inhibition of pathogens inoculated on whole cantaloupes after antimicrobial coatings

Coating mixtures were prepared as the film-forming mixtures in Sections A4 and A5. The microemulsion-based coatings contained 1% w/w chitosan, 0.5% w/w acetic acid, glycerol (20% mass of chitosan), and microemulsion 1:0, 2:1 or 4:1 containing 2% w/w CBO. The alginate-based formulations contained 1% w/v alginate, 0.5% w/v Tween 80, 0 or 2% w/v CBO, and 0 or 0.5% w/v SBO. The chitosan-based coatings with LAE were studied for mixtures with 1% of chitosan, 0.5% acetic acid, 0.1% w/w of LAE, 0.1% w/w of EDTA, and 0%, 0.5% or 1% w/w CO. Controls included no washing or washing with a solution with polysaccharide only.

Coating experiments followed a literature method (Chen et al., 2012). Cantaloupes were washed using deionized water with 0.5% Tween 80 and rinsed with tap water. After drying at 21°C overnight, 100 μL culture (40 ppm nalidixic acid adapted) with about 10^8 CFU/mL bacteria cocktail in Table 1 was spread on each of two 2.5cm×2.5cm squares on each cantaloupe. After inoculation, cantaloupes were dried for another 6 h at 21°C to allow the attachment of bacteria.

400 μL of a coating mixture was spread uniformly on the inoculated square using small painting brushes and dried overnight at 21°C.

For enumeration, the inoculated squares were excised using sterile knife after ambient storage for 1 (after coating), 3, 7, 10 and 14 days. The squares were placed into a sterile blender bag (Thermo Fisher Scientific, Inc., Waltham, MA) containing 25 mL sterile 10 mM phosphate-buffered saline (PBS, pH 7.4) with 0.2% w/w Tween 80 and hand-massaged for 1 min. The homogenate was then serially diluted in 0.1% peptone water and surface-plated on nalidix acid (40 ppm) acidified TSA plates or selective media for the wild type bacteria: XLT4 agar plates for *S. enterica*, MOX for *L. monocytogenes*, and CT-SMAC plates for *E. coli* O157:H7. After incubation at 37°C (*S. enterica* and *E. coli* O157:H7) or 32°C (*L. monocytogenes*) for 48 h, colonies were enumerated.

A11. Impacts of coatings on cantaloupe quality

Whole cantaloupes without washing were immersed in 2 L of a coating mixture for 10 s and dried at 21°C for 24 h. Uncoated cantaloupes were treated as controls. During ambient storage, various quality parameters were determined, with replications detailed in Table 2. The percentages of weight loss were determined with respect to the sample weight after coating on day 0. The color of cantaloupe surface was measured using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). For the firmness test, each cantaloupe was cut into four pieces. For each piece, the flesh in the center was prepared to a 4.52 cm^2× 1 cm cylinder that was tested using a TA.XTplus Texture Analyzer in the compress mode (Texture Technologies Corp., Scarsdale, NY) with a cylindrical probe (TA-57R, 7mm-1”R) traveling at 50 mm/min. The content of total soluble solids of the flesh was measured with a refractometer directly (model TS400, Reichert Analytical Instruments, Depew, NY).

A12. Inhibition of yeasts and molds on cantaloupes after coating treatments

Whole cantaloupes were treated as in Section A11. The total populations of molds and yeasts on cantaloupe surface with and without coatings were determined for three cantaloupe replicates. Four pieces of cantaloupe rind squares (4.52 cm^2) from different locations were excised using a sterile knife. The samples were placed into sterile blender bags and treated using the same procedures in Section A10, and the colonies on dichloran rose Bengal chloramphenicol agar (DRBC) were enumerated. Visible molds on cantaloupes were also recorded during storage at room temperature for up to 14 days.

A13. Estimation of costs of coatings

The cost of coating mixtures for each cantaloupe was estimated based on the price of each ingredient used (Table 3) and the mass of coating mixture needed for each cantaloupe. The amount of coating mixture needed was determined by weighing six cantaloupe replicates for each mixture, before coating and after draining extra coating mixture.

A14. Statistical analysis

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

B. Results

B1. Properties of fully-dilutable microemulsions

B1.1. Partial phase diagrams for microemulsion regimes

Pseudo-ternary phase diagrams are shown in Fig. 1 for formulations corresponding to microemulsion formation. Without SBO, microemulsions of CBO, thymol, and eugenol were fully-dilutable at the surfactant:oil mass ratio of ≥4:1, 9:1, and ≥3:1, respectively. At the 4:1 mass ratio of EO/EOC: SBO, fully-dilutable microemulsions of CBO, thymol, and eugenol were observed at surfactant:oil mass ratios of ≥4:1, ≥7:1, and ≥2:1, respectively. At the EO/EOC: SBO mass ratio of 2:1, fully-dilutable microemulsions were only observed for thymol at a surfactant:oil mass ratio of ≥7:1, while microemulsions of CBO and eugenol can only be prepared with up to 80% polar phase at a surfactant:oil mass ratio of ≥8:1 and ≥3:1, respectively. Therefore, under the studied conditions, fully-dilutable microemulsions of CBO, thymol and eugenol can be successfully prepared by titrating the polar phase with equal mass of water and PG into the oil-surfactant mixture. Furthermore, the use of SBO at 4:1 mass ratio of EO: SBO reduced the amount of surfactants needed to formulate fully-dilutable microemulsions of thymol and eugenol, while this improvement was only observed for microemulsions with thymol at the EO: SBO mass ratio of 2:1. For dilution lines that did not reach 90% mass of polar phase, the addition of SBO increased the maximum amount of polar phase in microemulsions. Overall, microemulsion dilutability can be enhanced after blending a certain amount of SBO, but the extent of enhancement is a function of EO chemistry.

B1.2. Hydrodynamic diameter (D_h) and storage stability of microemulsions

The D_h of microemulsions with the highest Q_m for each EO at each EO:SBO mass ratio was measured over 90 days during ambient storage to study the stability of microemulsions. As shown in Fig. 2A, the D_h of microemulsion 2:1 with thymol was about 14 nm, and those of microemulsions 1:0 and 4:1 with thymol were similar ($p > 0.05$), about 15 nm. For eugenol treatments (Fig. 2B), the D_h of microemulsions 1:0, 2:1, and 4:1 was about 22, 12 and 15 nm, respectively. Microemulsions 1:0, 2:1, and 4:1 with CBO had D_h of about 22, 12, and 14 nm, respectively (Fig. 2C). The results showed that all samples had stable D_h within 90 days, which indicated microemulsions were stable. Overall, microemulsions with a larger amount of SBO had significantly smaller D_h ($p < 0.05$).

B1.3. Antimicrobial activities of microemulsions

MICs and MBCs of microemulsions and free EOs/EOCs (pre-dissolved in ethanol) are presented in Table 4. The MIC of free CBO against *L. monocytogenes* was 313 ppm, while the MIC of CBO microemulsions was 625 ppm, one level up in the dilution scheme. The free CBO and CBO microemulsions however had same MBCs (625 ppm) against *L. monocytogenes*. Same MICs (625 ppm) and MBCs (625 ppm) of free CBO and CBO microemulsions were observed for *S. enterica*. For *E. coli* O157:H7, both MICs and MBCs of CBO microemulsions were one dilution higher than free CBO. The content of SBO in microemulsions did not impact the MIC and MBC of CBO in TSB. As for eugenol and thymol, MICs and MBCs of microemulsions (≥2,500 ppm) were remarkably higher than those of free EOCs (313-625 ppm). This group of studies indicates that microemulsions of CBO were more effective than those of eugenol and thyme and the microemulsion preparation had the least effects on antimicrobial activities of CBO.

The growth of foodborne pathogens inhibited by 625 ppm free CBO and CBO in microemulsion form is shown in Fig. 3. About 2.5 log reduction of *L. monocytogenes* was observed for the free CBO treatment in the first 24 h and the bacteria population decreased to below the detection limit after 72 h (Fig. 3A). Conversely, *L. monocytogenes* was decreased gradually by the CBO microemulsions, from only about 0.2 log reduction after 24 h to about 1 log reduction after 120 h (Fig. 3A). The difference between free CBO and CBO microemulsions

was less significant for *E. coli* O157:H7. Although free CBO was more effective in the first 72 h, the population of *E. coli* O157:H7 was decreased to below the detection level after 96 h by microemulsion treatments (Fig. 3B). The presence of SBO in microemulsions did not have significant impacts on antimicrobial activities of CBO at the studied conditions.

B2. Antimicrobial activity of mixtures with LAE, CO, and EDTA

Growth curves of bacteria measured for OD 630 nm in 96-well plates are shown in Fig. 4. For *E. coli* O157: H7 (Fig. 4A), the combination of LAE and EDTA with and without CO resulted in no increase of OD, while other treatments showed gradual increases in OD. The combination of LAE, EDTA and CO was the most efficient in inhibiting the growth of *S.* Enteritidis, followed by combinations of LAE and EDTA, and CO and EDTA (Fig. 4B). Similar results were observed for *L. monocytogenes* Scott A (Fig.4C). Overall, the combination of LAE, EDTA and CO was more effective than treatments with two or one antimicrobials, which indicates the potential of utilizing synergistic antimicrobial activities.

The microbial survivability end-point analysis was further studied (Table 5). The log-reduction of Gram-negative bacteria in the treatment with LAE, EDTA and CO combination was much higher than other treatments, achieving respective reductions of 4.70 and 5.01 log CFU/mL for *E. coli* O157: H7 and *S.* Enteritidis after 2 h. For Gram-positive *L. monocytogenes*, the triple combination was also the most effective, but a reduction of only 1.7 log CFU/mL was observed.

B3. Physical and antimicrobial properties of chitosan-based films with microemulsions

B3.1. Physical properties of films

The film thickness is compiled in Table 6. The control chitosan film was significantly thinner (0.012mm, $p < 0.05$) than those with microemulsions. Overall, an increase in microemulsion content significantly increased the thickness of films. For films prepared with 1% CBO, no significant differences in film thickness were found among treatments with microemulsions. For treatments prepared with 2% CBO, the thickness of film with microemulsion 2:1 (0.069 mm) was significantly higher than that with microemulsion 1:0 (0.063 mm), while no significant difference was found between the film with microemulsion 4:1 (0.066 mm) and the other two. For those prepared with 3% CBO, the film thickness followed the order of microemulsion 2:1 (0.106 mm), 4:1(0.097 mm), and 1:0 (0.089 mm). Therefore, the thickness of films increased with the increase of SBO content.

No significant differences in lightness (*L*, around 89) were found among all films that were visually transparent (Table 6). Generally speaking, as the content of CBO used in film preparation increased from 1% to 3%, the yellowness of films increased from 1.40-2.80 to 3.60-6.30, due to the increased amount of Tween 80 or CBO.

The control chitosan film had the highest moisture content (22%), and no significant differences in moisture content were observed for films prepared with a same CBO:SBO mass ratio but different CBO contents (Table 7). The control chitosan film also had the highest swelling ratio (>800%), and the highest swelling ratio (~58%) among films with microemulsions was observed for the treatment with microemulsion 4:1 and 2% CBO. For films with microemulsions at the same CBO:SBO mass ratio, the swelling ratio followed the order of 2% (above 50%), 3% (23%-35%) and 1% (about 9-19%) CBO treatments.

WVP of films had the same trend as the thickness of films, as shown in Table 7. As the amount of microemulsion in the films increased, WVP significantly increased. WVP of control chitosan film (1.012×10^{-10} g/Pa m s) was significantly lower than those prepared with

microemulsions. At a same level of CBO, WVP of films prepared with 1 and 2% CBO showed no significant differences. However, WVP of films prepared with 3% CBO varied with the SBO content, with the microemulsion 2:1 treatment (8.005×10^{-10} g/Pa m s) being significantly higher than those of microemulsion 4:1 (6.995×10^{-10} g/Pa m s) and 1:0 (6.627×10^{-10} g/Pa m s).

B3.2. Mechanical properties of films

As shown in Table 8, control chitosan film had a much higher tensile strength (627 MPa) and lower elongation% (4.2%) than films with microemulsions. With the amount of CBO in film-forming mixtures increasing from 1% to 3%, the tensile strength of films significantly decreased from 71-121 MPa to 27-36MPa, elongation% significantly increased from 13.2-23.7% to 32.7-42.8%, and these properties were not significantly affected by SBO content. No significant differences in tensile strength and elongation% were found between films prepared from microemulsions 4:1 or 2:1 with 2% and 3% of CBO.

B3.3. Surface structure of films studied for scanning electron microscopy (SEM)

SEM images are shown in Fig. 5. The control chitosan film had a smooth surface (Fig. 5A) with some pores due to evaporation of acetic acid (Fig. 5B). Films prepared from microemulsion 1:0 at 1% and 2% CBO levels had very smooth surfaces (Fig. 5C,D), while some particulates were observed for those prepared at 3% CBO (Fig. 5E). Films prepared with microemulsion 2:1 at 1% and 2% CBO levels showed porous surface structures that indicated CBO evaporation during drying (Fig. 5F,G), while the film prepared at 3% CBO had a smoother surface (Fig. 5H). The surface of films prepared with microemulsion 4:1 at 1% CBO (Fig. 5I) was less porous than that with microemulsion 1:0 (Fig. 5J), and the film with microemulsion 4:1 at 2% CBO (Fig. 1K) had smoother surface than that with microemulsion 2:1 (Fig. 5G).

B3.4. Loss of CBO in films during ambient storage

The residual content of CBO in films during storage (Fig. 6) was determined and compared to control chitosan films prepared with literature conditions of 0.5% Tween 80 and 2% or 3% of CBO (Chi et al., 2006; Wang et al., 2011; Zivanovic et al., 2005). About 50% CBO was detected in films prepared with microemulsions and 2% CBO at day 1 (after drying), while only 30% CBO was detected in the control film. Similarly for treatments prepared with 3% CBO, the films with microemulsions had a higher CBO retention (~70%) than the control film (~50%) after drying. A significantly higher amount of CBO was retained in films prepared with microemulsions than the control films during storage. The results confirmed the effectiveness of microemulsion in reducing the loss of EOs during film preparation and storage.

B3.5. Antimicrobial properties of films

The d_{iz} of film discs is listed in Table 9. No inhibition was found for samples prepared with 1% CBO or without CBO. The d_{iz} of film discs prepared with 3% CBO (15.3-16.8 mm for *E. coli* O157:H7, 18.3-20.0 mm for *S. enterica*, 22.0-23.5 mm for *L. monocytogenes*) was significantly larger than those prepared with 2% CBO (11.5-11.8 mm for *E.coli* O157:H7, 11.4-12.0 mm for *S. enterica*, 13.0-14.6 mm for *L. monocytogenes*).

B4. Physical and antimicrobial properties of alginate-based films with CBO and SBO

B4.1. Appearance, mechanical and physical properties of films

All alginate films were visually homogeneous without bubbles and easy to be removed from the cast plates. The thickness of films, between 0.021 and 0.045 mm (Table 10), increased with the amounts of CBO and SBO, as a result of the increased solids content (Benavides et al., 2012). The incorporation of CBO affected the total color difference (ΔE) that was significantly

greater than the control film without CBO, due to the decreased *b* value (Table 10). The reduced transparency of alginate films with CBO was alleviated after further addition of SBO, and no difference was noted between the control film and that with 1% CBO and 0.5% SBO (Fig. 7).

The incorporation of CBO produced stronger and more flexible alginate films, as indicated by higher values of tensile strength and elongation% (Table 11). However, the addition of SBO reduced tensile strength and elongation%. The control alginate film showed a very high solubility in water (~99.5%) that was reduced after incorporation of CBO and SBO (Table 11). The incorporation of CBO increased the WVP of films that was not affected by SBO content (Table 11). The determined moisture content was the lowest for films prepared with 1% CBO but was not affected by SBO content (Table 11).

B4.2. Antimicrobial properties of films

Table 12 shows the d_{iz} of film discs. No inhibition was observed for the control alginate film. The d_{iz} of CBO-containing discs was the smallest against *S. enterica* after 24 h incubation and was the smallest against *L. monocytogenes* after 48 h incubation, indicating the possible recovery of *L. monocytogenes*. Films prepared with 1 and 2% CBO had no obvious difference in d_{iz}. For films prepared with 1% CBO, the addition of SBO decreased the d_{iz} significantly, which was opposite to those prepared with 2% CBO. After 48 h incubation, only film discs prepared with 2% CBO and 0.5% SBO showed clear inhibition on *L. monocytogenes*, and these film discs remained effective against *E. coli* O157:H7 and *S. enterica*.

B5. Inhibition of pathogens on cantaloupes treated by antimicrobial coatings

B5.1. Chitosan-based coatings with CBO microemulsions

Viable bacteria cell counts on whole cantaloupes after coating and during storage at 21°C are presented in Fig. 8. For *E. coli* O157:H7 (Fig. 8A), significantly lower viable cell counts were observed for microemulsion treatments than uncoated cantaloupes during 14-day storage, with exceptions for the treatment with microemulsion 1:0 on day 10 and microemulsion 4:1 on day 14. No significant differences in viable cell counts were observed for the coating treatment of chitosan only and the uncoated controls up to day 10. During storage, viable cell counts of *S. enterica* on uncoated cantaloupes were significantly higher than those on coated cantaloupes (Fig. 8B). Viable cell counts of *S. enterica* on cantaloupes coated with chitosan only were significantly higher than the ones coated with microemulsion 1:0 or microemulsion 4:1 on day 1 and day 3. However, no significant difference was found in viable *S. enterica* between the treatment with chitosan only and that containing microemulsion 2:1 on day 1 and day 3. After 7-day storage, no significant differences in viable *S. enterica* were found among coating treatments. For *L. monocytogenes*, coating treatments reduced the viable cell counts on day 1, and no significant difference was observed among different coating treatments.

B5.2. Chitosan-based coatings with LAE, CO, and EDTA

As shown in Fig. 9A, coating treatments significantly reduced the viable cell counts of *E. coli* O157:H7. However, only the coating treatment with 0.1% LAE, 0.1% EDTA and 1% CO effectively inhibited the recovery of *E. coli* O157:H7 after day 3. For *S. enterica* (Fig. 9B), all coating treatments with LAE and/or CO reduced the viable cell counts to the detection limit after day 1 without recovery during storage, while some recovery was observed in the treatment of chitosan only on day 7 and day 14. For *L. monocytogenes* (Fig. 9C), viable cell counts were significantly reduced after coating treatment (day 1), and the treatment with 0.1% LAE, 0.1% EDTA and 1% CO showed the best inhibition during storage, followed by treatment with 0.1%

LAE, 0.1% EDTA and 0.5% CO. Overall, the chitosan coating with 0.1% LAE, 0.1% EDTA and 1% CO was the most effective in inhibiting the growth of tested pathogens on cantaloupes.

B5.3. Alginate-based coatings with CBO and SBO

Fig. 10 shows the growth of bacteria on cantaloupes during ambient storage for 15 days after treatments by mixtures containing 1% alginate without (control) and with 2% CBO and 0 or 0.5% SBO. The populations of three bacteria without coating treatment or treated with alginate only decreased gradually, especially for *E. coli* O157:H7 showing only ca. 2.0 log CFU/cm^2 after 15 day storage, which can be attributed to the dry surface of cantaloupes. For *E. coli* O157:H7, the CBO coating treatments reduced the pathogen to the detection limit after 1-day enumeration, followed by no recovery during 15-day storage. For *S. enterica*, a recovery of ca. 2.5 Log CFU/cm^2 was detected at day 7 after treatment by CBO. For the treatment with CBO, *L. monocytogenes* also recovered to ca. 3.0 log CFU/cm^2 after 7-day storage and maintained a relatively high level during the following days. In contrast, no recovery of bacteria was detected for all coating treatments with both CBO and SBO.

B6. Effects of antimicrobial coatings on quality parameters of cantaloupes

B6.1. Chitosan-based coatings with LAE, CO, and EDTA

Effects of coating treatments on the color of cantaloupes during storage are shown in Fig. 11. The redness (*a*) and yellowness (*b*) of uncoated cantaloupes were significantly higher than that of coated cantaloupes from day 6, while no significant difference was found among coating treatments ($p > 0.05$). The lightness (*l*) of uncoated cantaloupes was much lower than that of coated ones from day 2. The results indicate that coatings, especially those with LAE and CO, can delay the ripening of whole cantaloupes.

Correspondently, the firmness of uncoated cantaloupes was lower than the coated cantaloupes on day 6 and the differences became significant ($p < 0.05$) on day 10 (Fig. 12). Cantaloupes coated with chitosan only were softer than those with additional LAE, EDTA, and/or CO from day 10. However, no significant difference was found in weight loss (Fig. 13) and total solids content (Fig. 14) of cantaloupes among all treatments during storage.

B6.2. Alginate-based coatings with CBO and SBO

Table 13 shows weight loss of cantaloupes during storage. Throughout 15-day storage, all samples showed a gradual loss of weight, and no significant difference was found between uncoated controls and the coating treatments. The cantaloupes became significantly lighter in color after 1 day storage for all groups, with *L* values increasing from 59.4 to ~64, and no significant difference in *L* values was observed after day 1 among all treatments (Table 14). For redness (*a*) and yellowness (*b*), no difference was observed for all treatments on day 1, but the increases in *a* and *b* values during 15 day storage were significantly higher for the control than coatings with CBO or CBO+SBO. The results suggest that these coatings can help maintain the quality of cantaloupes during postharvest storage by delaying the ripening.

The flesh of all cantaloupes became softened after the third day of storage (Table 15). No significant difference was observed between the control and the treatment coated with alginate during 15-day storage. The flesh of coating treatments with CBO was significantly firmer than the control and the coating without CBO up to 10-day storage, especially for those coated with CBO and SBO. After 15 days, there was no significant difference among all treatments.

The total solids content of flesh (Fig. 15) increased after 3-day storage due to ripening and loss of water, but decreased slightly during the following 12 days due to respiration. The

coated cantaloupes had slightly lower total solids contents than the control up to 10-day storage, and no significant difference was observed between different coating formulations.

B7. Growth of yeasts and molds on cantaloupes treated by antimicrobial coatings

B7.1. Chitosan-based coatings with LAE, CO and EDTA

Coating treatments reduced native molds and yeasts to below the detection limit on day 0 (Fig. 16). Molds were visible on uncoated cantaloupes from day 2 and appeared on day 4 and day 5 for treatments with chitosan only and those containing 0.1% LAE, 0.1% EDTA and 1% CO (Fig. 17). The chitosan coating containing 0.1% LAE, 0.1% EDTA and 1% CO significantly reduced the growth of native molds and yeasts on cantaloupes during 14-day storage (Fig. 17), which is significant in improving the microbial quality (inhibition of spoilage) of cantaloupes.

B7.2. Alginate-based coatings with CBO and SBO

Effects of coating systems on the native molds and yeasts on cantaloupes after room temperature storage for 5 days are shown in Fig. 18. Coating treatments with CBO significantly reduced the populations of native molds and yeasts that were ca.1.8 and 2.6 log CFU/cm^2 lower than the control for coatings with CBO and CBO+SBO, respectively. The alginate coating showed no inhibition but a slight increase in the population of microflora due to the increased moisture content on the surface benefiting the growth of yeasts and molds.

B8. Estimated costs of coatings

About 5 g of coating mixtures was estimated for each cantaloupe. Based on the costs in Table 3, and the material cost to coat 100 cantaloupes is about $0.410 for the coating mixture containing 1% w/v alginate, 0.5% Tween 80, 0.3% glycerol, 0.05% $CaCl_2$, 2% w/v CBO, and 0.5% w/v SBO. This cost is about $0.637 for 100 cantaloupes using the formulation containing 1% chitosan, 0.5% acetic acid, 0.1% LAE, 0.1% EDTA, and 1% CO. Considering other costs, we expect the cost for coating each cantaloupe to be less than 1 cent for these two formulations.

Outcomes and Accomplishments

1. Formulations dissolving EOs and SBO at a wide range of oil contents.

Microemulsions of EOs were successfully prepared by mixing Tween 80, a polar phase with equal mass of PG and water, and an oil phase with various mass ratios of CBO and SBO. These microemulsions can be prepared at a wide range of EO concentrations, and the addition of SBO can reduce the amount of Tween 80 required to dissolve oil. Microemulsions of CBO maintained the antimicrobial activity. Microemulsions are thermodynamically stable, which provides shelf-stability when they are used in the production.

2. Novel formulations utilizing synergistic antimicrobial activities of LAE and EOs.

We additionally studied the mixtures with multiple antimicrobials - CO and LAE. LAE can also replace Tween 80 as a generally-recognized-as-safe emulsifier. The addition of EDTA enables synergistic activities of the antimicrobials, which can lower their usage level and cost. Although chitosan was studied as the coating polysaccharide, we expect similar performances when alginate is used to incorporate LAE, EDTA, and EO to reduce the cost.

3. Antimicrobial coatings improving safety and quality of cantaloupes and beyond.

The studied antimicrobial coating formulations, especially with SBO, effectively reduced the loss of EOs during storage. This provides antimicrobial activities of coatings that inhibit the growth of not only foodborne pathogens potentially contaminating cantaloupes but also native microflora causing spoilage. Additionally, coatings with EOs delay the ripening of cantaloupes

to maintain the appearance (color) and firmness. Costs of these novel coating mixtures are about 1 cent/cantaloupe. These findings are significant to extend the shelf-life of cantaloupes and possibly other fresh produce products.

Summary of Findings and Recommendations

Microemulsion formulations were identified to dissolve both EOs and SBO at various mass ratios using Tween 80 as a surfactant and equal mass of PG and water as the polar phase. These microemulsions have been characterized for fundamental antimicrobial activities and physical properties including viscosity, droplet dimension, and stability. When chitosan was used as the film forming polysaccharide, the mixtures of chitosan and microemulsions were used to prepare films to predict properties of coatings when applied on cantaloupes. These films were transparent, had suitable physical, mechanical, and antimicrobial properties, and retained CBO better than conventional formulations during storage. Transparent films were also prepared using the CBO and SBO blend and alginate. These films were also found to be suitable for coatings and were less costly than those of chitosan microemulsions. To reduce the amount of EOs and costs of coatings, LAE was studied as an emulsifier with excellent antimicrobial activity, and synergistic antimicrobial activity was observed using the mixture of LAE, CO, and EDTA.

Three mixtures were chosen to study impacts of coatings on safety and quality of whole cantaloupes: (1) 1% chitosan and microemulsions with 2% CBO, (2) 1% alginate and 2% CBO with 0 or 0.5% SBO, and (3) 1%w/w chitosan, 0.1%w/w LAE, 0.1% w/w EDTA, and 0, 0.5, or 1% w/w CO. These coatings, especially after addition of SBO, showed the effectiveness of inhibiting common foodborne pathogens *L. monocytogenes*, *S. enterica* and *E. coli* O157:H7 inoculated on cantaloupes, as well as native microflora. Antimicrobial coatings also slowed the color change and improved the firmness of cantaloupes during storage, and had no impacts on other quality parameters. Costs of coatings were estimated to be less than 1 cent per cantaloupe. Due to time limitation, the third coating mixture was not studied for alginate as the film-forming polysaccharide for possible further reduction of cost.

Based on antimicrobial activities and effects on storage quality, two coating formulations studied in this project are recommended. The first mixture is recommended to contain 1% w/v alginate, 0.05% w/v $CaCl_2$, 0.3% w/v glycerol, 0.5% w/v Tween 80, 2% w/v CBO, and 0.5% w/v SBO that can be prepared by simple mixing. The material cost of this coating mixture is $0.410 for 100 cantaloupes. The second mixture is formulated with 1% w/w chitosan, 0.5% w/w acetic acid, 0.1% w/w LAE, 0.1% w/w EDTA and 1% w/w CO, and the estimated material cost for 100 cantaloupes is $0.637. Chitosan may be replaced by less expensive alginate to additionally reduce the cost. The costs of these coating mixtures can be offset by the reduced number of recalls and the extended shelf-life of cantaloupes.

APPENDICES

Publications and Presentations

Ma, Q. and Q. Zhong. Formulating fully-dilutable microemulsions of plant essential oils blended with soybean oil. *Food Research International*. Revision submitted.

Ma, Q., Y. Zhang, F. Critzer, P.M. Davidson, and Q. Zhong. Quality attributes and microbial growth on whole cantaloupes with antimicrobial coatings containing chitosan, lauric arginate, and cinnamon oil. The 2015 IFT Annual Meeting, July 13-16, Chicago, IL. (Submitted)

Ma, Q., Y. Zhang, and Q. Zhong. Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil and ethylenediaminetetraacetic acid. The 2015 IFT Annual Meeting, July 13-16, Chicago, IL. (Submitted)

Ma, Q., Y. Zhang, F. Critzer, P.M. Davidson, and Q. Zhong. Synergistic antimicrobial activity of lauric arginate and cinnamon oil against foodborne pathogens in the presence of ethylenediaminetetraacetic acid. The 2015 IFT Annual Meeting, July 13-16, Chicago, IL. (Submitted)

Zhang, Y., Q. Ma, F. Critzer, P.M. Davidson, and Q. Zhong. Effects of alginate coatings with cinnamon bark oil and soybean oil on quality and safety of cantaloupe. The 2015 IFT Annual Meeting, July 13-16, Chicago, IL. (Submitted)
Zhang, Y., Q. Ma, F. Critzer, P.M. Davidson, and Q. Zhong. Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil. The 2015 IFT Annual Meeting, July 13-16, Chicago, IL. (Submitted)

Ma, Q., M.F. Critzer, P.M. Davidson, S. Zivanovic, and Q. Zhong. 2014. Physical properties of chitosan films with microemulsions of incorporated cinnamon bark oil. The 2014 IFT Annual Meeting, June 21-25, New Orleans, LA. Paper # 206-40 (poster presentation).

Ma, Q., M.F. Critzer, P.M. Davidson, and Q. Zhong. 2013. Formulating fully-dilutable microemulsions of plant essential oils by blending with soybean oil. The 2013 IFT Annual Meeting, July 13-16, Chicago, IL. Paper # 255-146 (poster presentation).

Six manuscripts are being prepared for submission around April, 2015.

Funding spending

The breakdown of budget is as follows and has been mostly spent.

Category	Total
Personnel - salaries	$118,000
Personnel - benefits	$34,800
Travel	$9,696
Supplies and materials	$32,195
Other costs	$29,328
Indirect costs	$7,640
Total costs	$231,659

Because coating experiments were done in the second year only, we did not have enough time and budget to study other EO and polysaccharide combinations that may be more practical. We also were not able to test coatings in a plant and on other produce products. We also have not had time to publish all papers.

Tables and Figures

Table 1. The make-up of bacteria cocktails used in the study.

Bacteria	Strain/serovar
Escherichia coli O157:H7	H1730, F4546, K3995, 658, 932
Salmonella enterica	Agona, Montevideo, Gaminara, Michigan, Saint Paul
Listeria monocytogenes	LM1, LM2, 310, Scott A, V7

Table 2. Numbers of cantaloupes and samplings/cantaloupe (A×B) used to determine quality parameters.

Parameter	Chitosan-based coatings	Alginate-based coatings
Color	4×3	3×3
Weigh loss (%)	4×1	6×1
Firmness	3×4	3×4
Total soluble solids	3×4	3×4

Table 3. Price of ingredients used to estimate costs of coating mixtures.

Ingredient	Price ($/kg)	Source
Chitosan	62	ebay.com
Acetic acid	12	ebay.com
Lauric arginate	41.5	Vedeqsa Inc. (New York, NY)
Cinnamon (leaf) oil	53.5	100pureessentialoils.com
EDTA	17.4	essentialwholesale.com
Sodium alginate	15.4	ebay.com
Cinnamon bark oil	28.9	wfmed.com
Soybean oil	5.2	essentialwholesale.com
$CaCl_2$	1.57	soapgoods.com
Glycerol	3.1	bulkapothecary.com
Tween 80	10.4	essentialwholesale.com

Table 4. MIC (ppm) and MBC (ppm) of free EOs (pre-dissolved in ethanol) and their microemulsions.

Antimicrobial	*L. monocytogenes*		*S. enterica*		*E. coli* O157: H7	
	MIC	MBC	MIC	MBC	MIC	MBC
Free cinnamon bark oil (CBO)	313	625	625	625	313	313
Free eugenol	625	1250	625	625	625	625
Free thymol	625	625	313	313	313	313
Microemulsions of EO/EOC, with the EO/EOC: soy bean oil mass ratio in parentheses						
CBO (1:0)	625	625	625	625	313-625	313-625
CBO (2:1)	625	625	625	625	625	625
CBO (4:1)	625	625	625	625	625	625
Eugenol (1:0)	>5000	>5000	2500	2500	2500	2500
Eugenol (2:1)	>5000	>5000	5000	5000	5000	5000
Eugenol (4:1)	>5000	>5000	2500	2500	2500	2500
Thymol (1:0)	>5000	>5000	>5000	>5000	>5000	>5000
Thymol (2:1)	>5000	>5000	>5000	>5000	>5000	>5000
Thymol (4:1)	>5000	>5000	>5000	>5000	>5000	>5000

Table 5. Log-reduction of *E. coli* O157:H7 ATCC 43895 (initial count of 6.17 log CFU/mL, 37°C), *S.* Enteritidis (initial count of 6.23 log CFU/mL, 37°C) and *L. monocytogenes* Scott A (initial count of 6.41 log CFU/mL, 32°C) in tryptic soy broth after 2 h.

Treatment [#]	Log reduction (CFU/mL)*		
	E. coli O157:H7	*S.* Enteritidis	*L. monocytogenes*
LAE	- 0.16±0.05^{c}	-0.60±0.18^{d}	-0.31±0.16^{c}
EDTA	-0.18±0.08^{c}	-0.51±0.15^{d}	-0.07±0.44^{bc}
CO	0.03±0.05^{c}	-0.49±0.10^{d}	-0.42±0.51^{c}
LAE+EDTA	0.91±0.27^{b}	0.34±0.18^{b}	0.10±0.30^{bc}
CO+EDTA	0.44±0.11^{bc}	0.07±0.17^{bc}	-0.06±0.16^{bc}
LAE+CO	0.42±0.12^{bc}	-0.24±0.16^{cd}	0.76±0.20^{b}
LAE+CO+EDTA	4.70±0.53^{a}	5.01±0.26^{a}	1.71±0.08^{a}

[#] Antimicrobial concentrations were 5 ppm LAE, 200 ppm CO, and 500 ppm EDTA.
* Numbers are mean ± standard deviation (n = 3). Different superscript letters in each column indicate significant differences ($p < 0.05$).

Table 6. Thickness (n = 24) and color (n = 6) of chitosan films prepared with microemulsions at an overall CBO concentration of 1-3% w/w and three CBO:SBO mass ratios. [*]

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%	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%	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%	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}

[*] Numbers are mean ± standard deviation. Different superscript letters suggest that means in the same column differ significantly at $p \leq 0.05$.

Table 7. Water content, swelling ratio, and water vapor permeability (WVP) of chitosan films (n = 3) prepared with microemulsions at an overall CBO concentration of 1-3% w/w and three CBO: SBO mass ratios. *

Overall CBO concentration	CBO:SBO mass ratio	Water content (%)	Swelling ratio (%)	WVP ($\times 10^{-10}$ g/Pa m s)
0% (chitosan only)		22.47 ± 5.13^{a}	871.01 ± 53.29^{h}	1.012 ± 0.003^{e}
1%	1:0	16.96 ± 2.52^{ab}	8.98 ± 3.44^{g}	2.913 ± 0.113^{d}
	2:1	12.73 ± 0.85^{bc}	19.74 ± 1.29^{de}	3.022 ± 0.027^{d}
	4:1	10.34 ± 1.87^{c}	15.15 ± 2.06^{ef}	2.663 ± 0.058^{d}
2%	1:0	11.91 ± 2.50^{bc}	50.23 ± 2.27^{b}	4.674 ± 0.020^{c}
	2:1	10.13 ± 1.03^{c}	50.72 ± 3.50^{b}	4.912 ± 0.106^{c}
	4:1	9.62 ± 0.33^{c}	58.07 ± 2.00^{a}	4.657 ± 0.123^{c}
3%	1:0	16.68 ±0.27^{ab}	35.70 ± 0.46^{c}	6.627 ± 0.341^{b}
	2:1	13.84 ± 1.66^{bc}	23.88 ± 1.47^{d}	8.005 ± 0.139^{a}
	4:1	14.87 ± 0.70^{bc}	31.61± 1.62^{c}	6.995 ± 0.065^{b}

* Numbers are mean ± standard deviation. Different superscript letters suggest that means in the same column differ significantly at $p \leq 0.05$.

Table 8. Mechanical properties of chitosan films ($n \geq 5$) prepared with microemulsions at an overall CBO concentration of 1-3% w/w and three CBO:SBO mass ratios. *

Overall CBO concentration[B]	CBO:SBO mass ratio	Tensile strength (MPa)	Elongation (%)
0% (chitosan only)		627.5 ± 20.5^{f}	4.2 ± 0.7^{g}
1%	1:0	121.3 ± 7.8^{a}	23.7 ± 4.1^{de}
	2:1	71.2 ± 9.0^{bc}	13.2 ± 3.9^{fg}
	4:1	94.8 ± 8.9^{ab}	15.9 ± 3.2^{ef}
2%	1:0	42.2 ± 11.6^{cd}	24.5 ± 9.9^{de}
	2:1	56.0 ± 2.8^{cd}	36.7 ± 1.3^{abc}
	4:1	44.4 ± 5.9^{cd}	30.2 ± 3.3^{cd}
3%	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}

* Numbers are mean ± standard deviation. Different superscript letters suggest that means in the same column differ significantly at $p \leq 0.05$.

Table 9. Inhibition zone diameters (n = 4) of chitosan films prepared with microemulsions at an overall CBO concentration of 1-3% w/w and three CBO:SBO mass ratios. [A]

Overall CBO concentration	CBO:SBO mass ratio	Inhibition zone diameter (mm)		
		E. coli O157:H7	*S. enterica*	*L. monocytogenes*
0% (chitosan only)		-	-[B]	-
1%	1:0	-	-	-
	2:1	-	-	-
	4:1	-	-	-
2%	1:0	11.8 ± 0.5^{c}	12.0 ± 0.8^{b}	14.6 ± 0.8^{b}
	2:1	11.5 ± 0.6^{c}	11.8 ± 0.5^{b}	13.3 ± 0.9^{b}
	4:1	11.6 ± 0.5^{c}	11.4 ± 0.5^{b}	13.0 ± 0.8^{b}
3%	1:0	15.3 ± 0.5^{b}	18.5 ± 1.1^{a}	22.0 ± 0.0^{a}
	2:1	16.8 ± 1.0^{a}	20.0 ± 1.6^{a}	23.5 ± 2.1^{a}
	4:1	15.3 ± 0.5^{b}	18.3 ± 0.9^{a}	23.5 ± 1.7^{a}

[A] Different superscript letters suggest differences in the same bacterium group ($p \leq 0.05$); [B] "-": bacteria growth observed under film discs.

Table 10. The thickness and color of alginate films prepared from different contents of CBO and SBO.*

Composition	Thickness	*L*	*a*	*b*	*ΔE*
Control	0.021 ± 0.004^{D}	62.98 ± 0.61^{D}	38.66 ± 0.44^{A}	58.90 ± 1.71^{A}	3.45
1% CBO	0.029 ± 0.004^{C}	64.96 ± 0.14^{C}	36.59 ± 0.61^{B}	52.71 ± 2.11^{A}	9.56
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
1% CBO, 1% SBO	0.034 ± 0.004^{BC}	65.08 ± 1.41^{C}	37.33 ± 1.71^{AB}	60.32 ± 1.43^{A}	2.30
2% CBO	0.035 ± 0.004^{B}	68.80 ± 0.43^{A}	29.28 ± 0.45^{D}	32.53 ± 0.89^{C}	31.27
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% CBO,1% SBO	0.045 ± 0.002^{A}	66.49 ± 0.62^{B}	34.10 ± 1.45^{C}	43.57 ± 4.62^{B}	19.12

*Mean values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 11. Mechanical and physical properties of alginate films prepared with CBO and SBO.*

Composition	*Tensile strength* (MPa)	*Elongation* (%)	WVP (g/m h Pa×10^{-7})	Moisture (%, dry basis)	Water solubility (%)
Control	6.51 ± 1.04^{C}	11.99 ± 2.39^{D}	4.36 ± 0.34^{D}	22.49 ± 1.40^{B}	99.50 ± 0.50^{A}
1% CBO	16.03 ± 3.14^{A}	36.32 ± 3.38^{B}	5.08 ± 0.12^{C}	18.75 ± 0.35^{C}	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}	17.26 ± 1.04^{CD}	91.76 ± 0.26^{D}
1% CBO, 1% SBO	6.67 ± 1.92^{BC}	16.11 ± 0.97^{D}	5.50 ± 0.20^{BC}	15.54 ± 0.34^{D}	90.68 ± 0.71^{D}
2% CBO	15.94 ± 1.08^{A}	45.97 ± 8.82^{A}	6.38 ± 0.05^{AB}	27.43 ± 1.12^{A}	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}	27.23 ± 0.95^{A}	96.39 ± 1.40^{BC}
2% CBO, 1% SBO	7.31 ± 0.88^{BC}	25.13 ± 1.28^{C}	6.88 ± 0.21^{A}	25.79 ± 1.29^{A}	96.04 ± 0.27^{C}

*Mean values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 12. Inhibition zone diameters of alginate film discs with CBO and SBO on TSA. [a]

Bacteria	Composition	Diameter (mm)		Inhibition [b]
		24 h	48 h	
L. monocytogenes	Control	0	0	-
	1% CBO	19.7 ± 5.5^{C}	0	+
	1% CBO, 0.5%SBO	17.3 ± 2.3^{C}	0	+
	1% CBO, 1% SBO	0	0	+
	2% CBO	22.6 ± 5.6^{C}	0	+
	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 ± 3.4^{C}	+
S. enterica	Control	0	0	-
	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	0	0	+
	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	Control	0	0	-
	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	0	0	+
	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] Mean values with different superscript letters in the same bacterium group are significantly different ($p < 0.05$). [b] (-): No inhibition after 24 h incubation; (+): inhibition underneath discs.

Table 13. Effects of alginate-based coatings on weight loss (% of mass at day 0) of cantaloupes during ambient storage for up to 15 days.

Coating	Day 1	Day 5	Day 12	Day 15
None	1.93 ± 0.34^{E}	4.47 ± 0.44^{D}	11.29 ± 1.42^{C}	12.89 ± 1.58^{ABC}
Alginate	1.94 ± 0.27^{E}	5.12 ± 0.26^{D}	11.54 ± 3.58^{C}	12.45 ± 0.82^{ABC}
CBO	2.11 ± 0.33^{E}	4.50 ± 0.80^{D}	12.83 ± 1.24^{ABC}	13.99 ± 1.40^{A}
CBO+SBO	1.72 ± 0.44^{E}	4.12 ± 0.56^{D}	11.71 ± 1.19^{BC}	13.39 ± 1.03^{AB}

*Mean values with different superscript letters are significantly different ($p < 0.05$).

Table 14. Effects of alginate-based coatings on the *L*, *a*, and *b* values of cantaloupes stored at room temperature for up to 15 days.*

Coating	Day 1	Day 3	Day 5	Day 7	Day 10	Day 15
L (before coating: 59.40±2.54^{d})						
Control	64.05±2.58^{abcd}	66.91±1.60^{abc}	66.65±3.15^{abc}	68.42±2.92^{a}	67.46±2.53^{abc}	65.59±1.63^{abc}
Alginate	63.48±2.84^{bcd}	68.14±1.84^{ab}	63.10±1.54^{cd}	66.34±1.83^{abc}	66.02±2.08^{abc}	63.81±3.35^{abcd}
CBO	63.90±2.20^{abcd}	65.07±1.55^{abc}	65.69±2.37^{abc}	67.57±2.44^{abc}	66.40±2.34^{abc}	66.36±1.94^{abc}
CBO+SBO	63.51±2.66^{bcd}	63.54±3.39^{bcd}	64.90±2.18^{abc}	65.63±0.3^{abc}	65.93±3.11^{abc}	66.48±0.71^{abc}
a (before coating: 1.36±0.41^{i}*)*						
Control	2.07±0.92^{hi}	4.68±1.70^{cde}	5.07±1.23^{cd}	6.43±0.96^{ab}	7.35±2.02^{a}	7.28±1.65^{a}
Alginate	2.54±0.83^{fghi}	3.44±1.62^{efgh}	3.72±2.00^{defg}	4.14±1.73^{cde}	5.22±1.10^{ab}	3.96±2.14^{cdef}
CBO	1.24±0.97^{i}	1.98±1.24^{hi}	2.00±0.56^{hi}	2.36±0.52^{ghi}	2.37±0.61^{ghi}	2.71±0.41^{fghi}
CBO+SBO	1.86±0.97^{i}	2.43±0.78^{ghi}	2.31±1.13^{ghi}	2.13±0.82^{hi}	2.62±0.87^{fghi}	1.93±0.51^{hi}
b (before coating: 20.74±1.21^{cdef})						
Control	21.26±1.49^{bcde}	25.32±1.75^{a}	26.16±1.64^{a}	26.29±0.96^{a}	25.70±1.89^{a}	25.42±1.36^{a}
Alginate	21.00±1.99^{cde}	22.04±2.44^{bc}	21.68±2.49^{bcd}	22.46±2.75^{bc}	23.23±1.51^{b}	20.73±2.72^{cdef}
CBO	18.60±1.51^{f}	20.24±2.43^{cdef}	20.52±1.48^{cdef}	19.52±1.18^{def}	19.34±1.00^{ef}	20.24±1.38^{cdef}
CBO+SBO	20.93±2.62^{cde}	20.81±1.80^{cdef}	20.30±1.64^{cdef}	19.19±0.87^{ef}	19.29±2.06^{ef}	18.90±1.05^{f}

*Mean values with different superscript letters in the same parameter are significantly different ($P < 0.05$).

Table 15. Effects of alginate-based coating on the firmness (N) of cantaloupe flesh after storage at room temperature for up to 15 days.*

Treatment	Day 0	Day 3	Day 5	Day 7	Day 10	Day 15
Control	12.61±3.85^{a}	4.65±1.37^{de}	3.66±0.61^{ef}	2.73±1.01^{fg}	3.16±0.81^{fg}	2.09±0.31^{g}
Alginate	-	4.97±1.64^{de}	3.66±0.67^{ef}	3.00±0.50^{fg}	2.53±0.40^{fg}	2.64±0.56^{fg}
CBO	-	5.71±1.07^{cd}	4.75±1.47^{de}	5.35±2.24^{cd}	4.75±1.57^{de}	2.64±0.49^{fg}
CBO+SBO	-	8.45±1.04^{b}	5.56±0.32^{cd}	6.76±1.61^{c}	4.68±1.79^{de}	3.14±0.56^{fg}

*Mean values with different superscript letters are significantly different ($P < 0.05$).

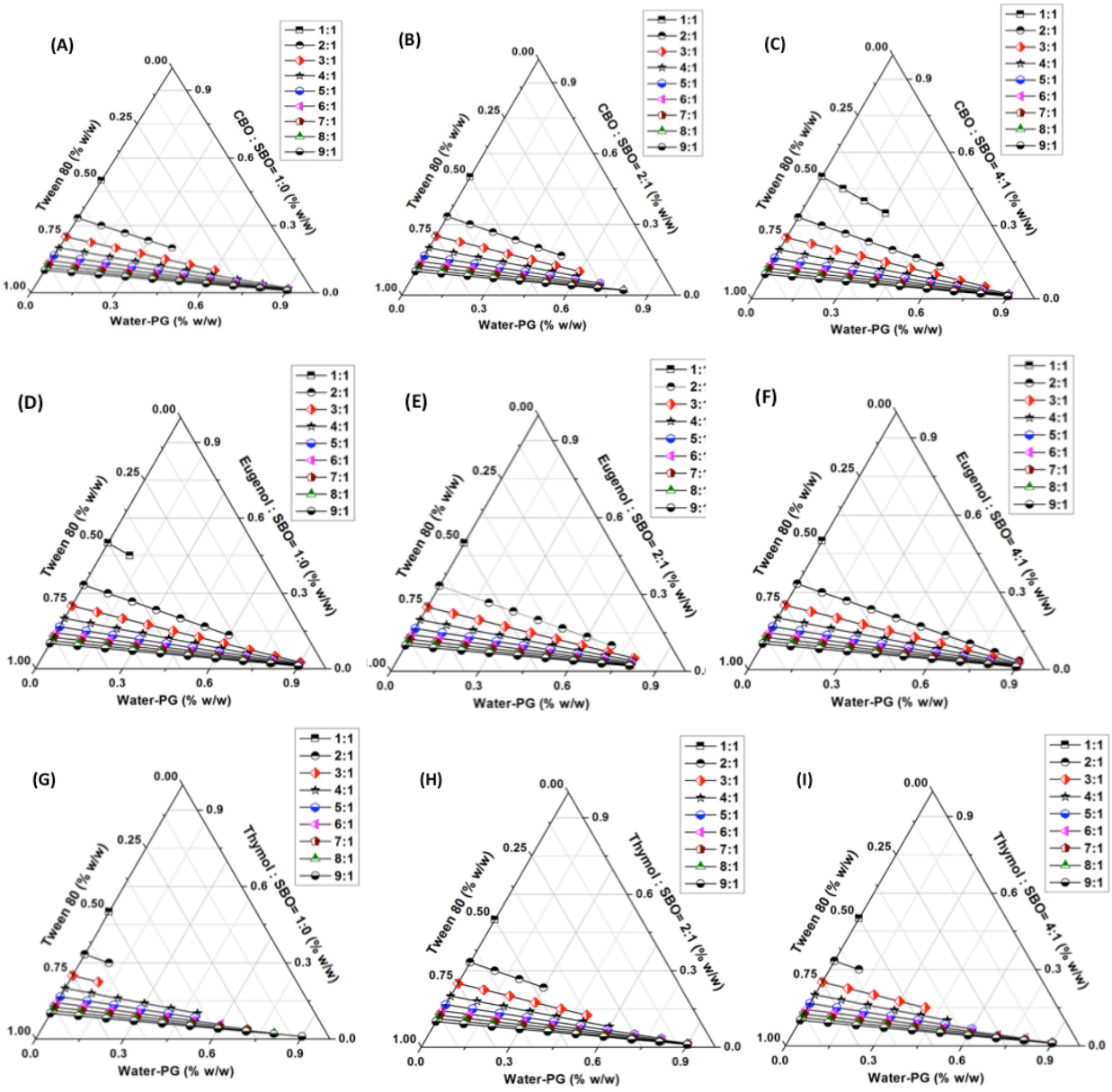


Fig. 1. Pseudo-ternary phase diagrams at 21 °C showing formulations corresponding to transparent microemulsions with the oil phase prepared with essential oil and soybean oil (SBO) at 1:0, 2:1, and 4:1 mass ratios and the polar phase formulated with equal mass of water and propylene glycol (PG).

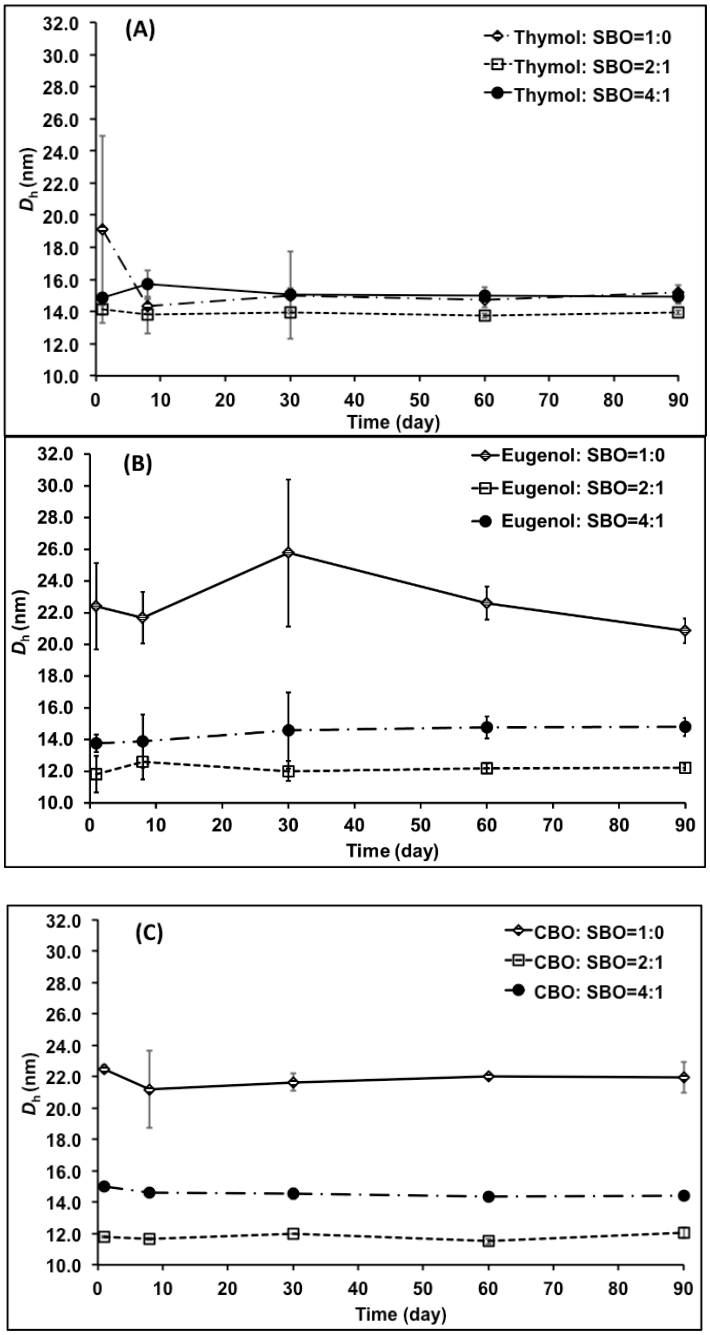


Fig. 2. Average hydrodynamic diameters (D_h) of microemulsions with the highest Q_m for each EO at a specific EO:SBO mass ratio during storage at room temperature (21 °C). Microemulsions were formulated with the oil phase prepared with thymol (A), eugenol (B) or cinnamon bark oil (CBO, C) blended with soybean oil (SBO) at 1:0, 2:1, and 4:1 mass ratios and the polar phase with equal mass of water and propylene glycol. Error bars are standard deviations from six measurements.

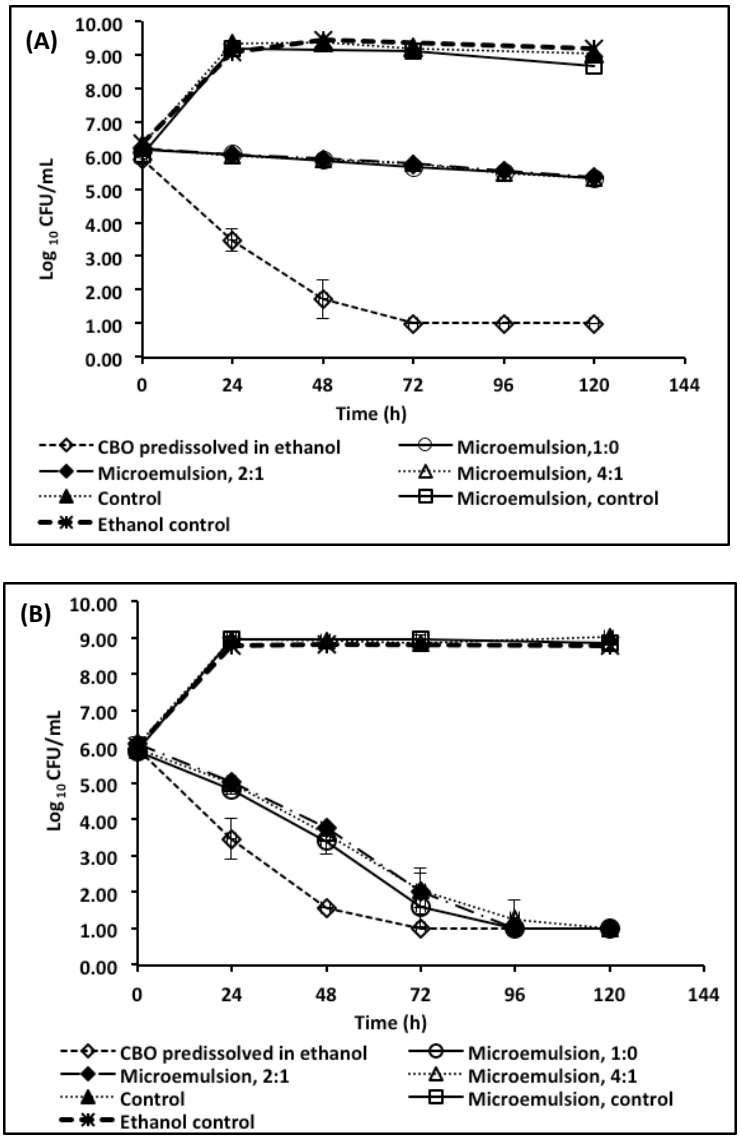


Fig. 3. Growth of *Listeria monocytogenes* (A) and *Escherichia coli* O157:H7 (B) cocktail in tryptic soy broth at 21°C as impacted by 625 ppm cinnamon bark oil (CBO) pre-dissolved in ethanol or microemulsions prepared with an oil phase with CBO and soybean oil at mass ratios of 1:0, 2:1 and 4:1. The microemulsion control was prepared without CBO, while the ethanol control was studied for the same amount of ethanol as that of pre-dissolved CBO.

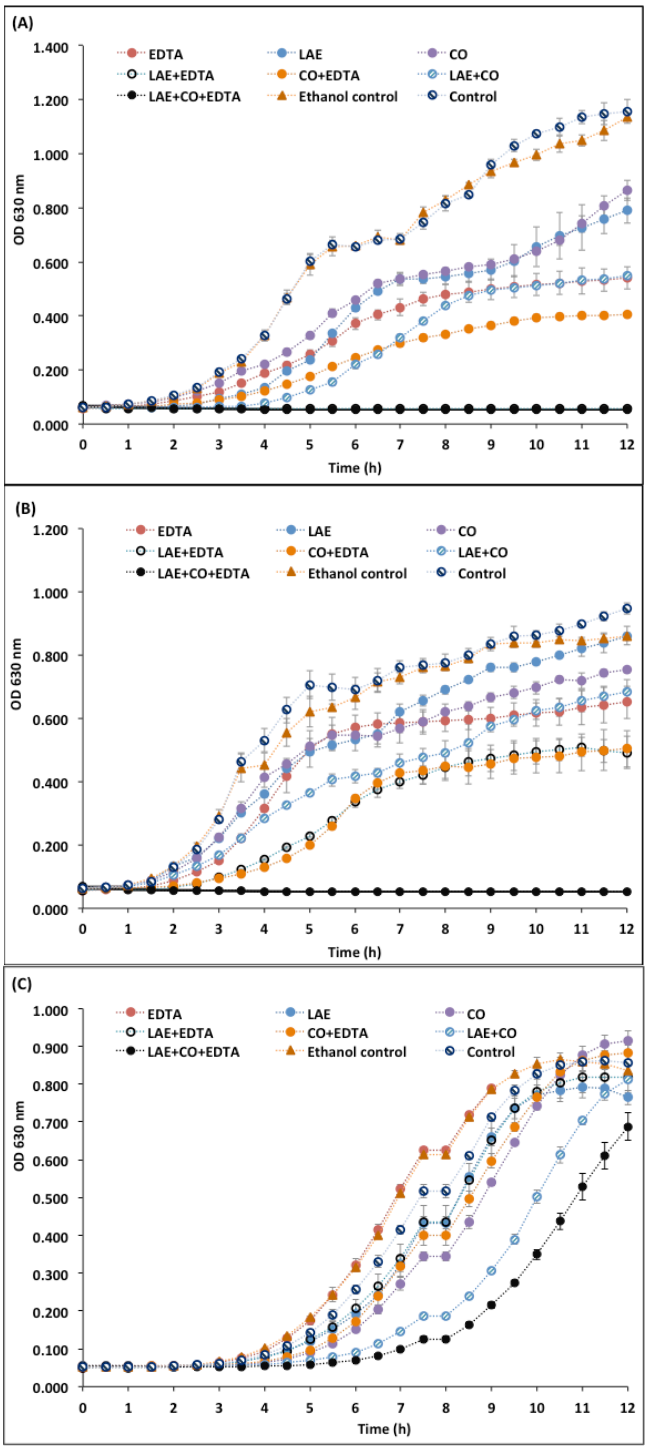


Fig. 4. Growth curves of *Escherichia coli* O157:H7 ATCC 43895 (A), *Salmonella* Enteritidis (B) and *Listeria monocytogenes* Scott A (C) in tryptic soy broth at 37°C (for *E.coli* O157:H7 and *S.* Enteritidis) or 35°C (for *L. monocytogenes*). Treatments for *E. coli* O157:H7 and *S.* Enteritidis contained 5 ppm lauric arginate (LAE), 500 ppm EDTA, 200 ppm cinnamon oil (CO) alone or in combinations. Treatments for *L. monocytogenes* contained 2.5 ppm LAE, 100 ppm EDTA, and 100 ppm CO alone or in combination.

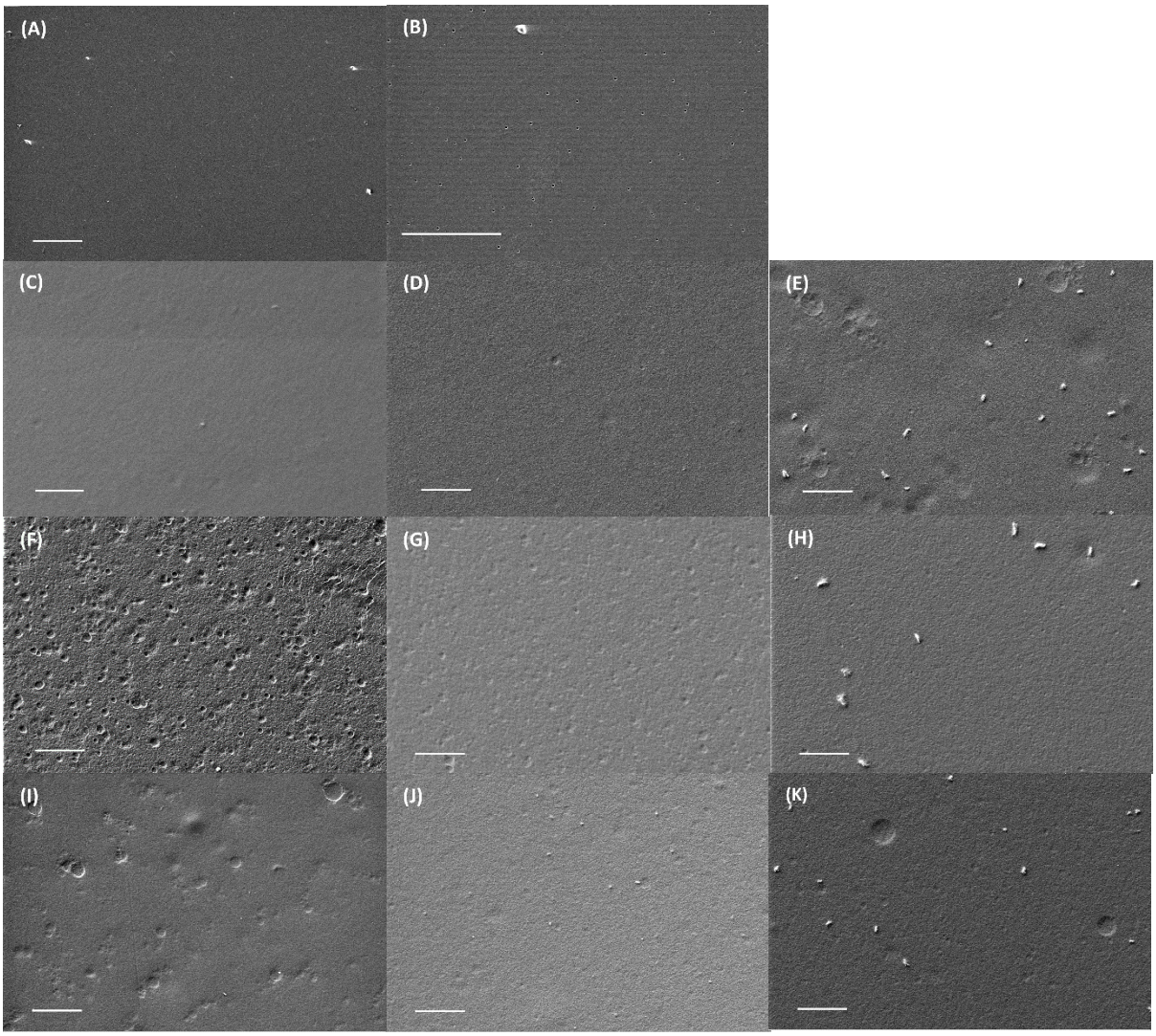


Fig. 5. SEM images of films prepared from mixtures containing 1% w/w chitosan and microemulsions formulated with CBO:SBO mass ratios of 1:0 (C-E), 2:1 (F-H) and 4:1 (I-K) and overall CBO concentrations of 1, 2, and 3% w/w (left, middle, and right in each row). Control chitosan film is compared in A and B at two magnifications. Bar = 10 μm.

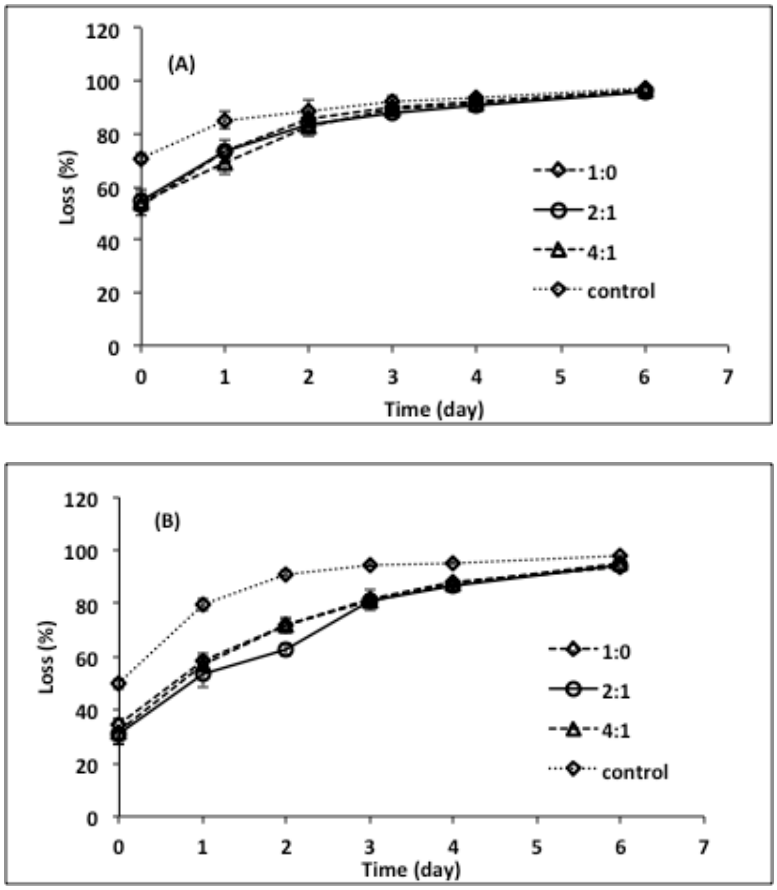
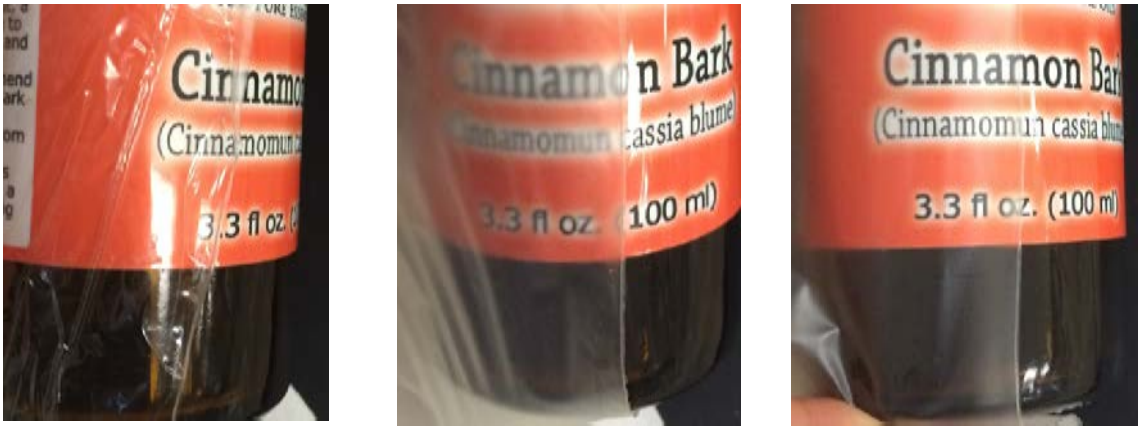


Fig. 6. Loss% of CBO, with respect to the CBO mass in film-forming mixture, from films during ambient storage (21°C, n = 3). Films were prepared with microemulsions with CBO:SBO mass ratios of 1:0, 2:1 and 4:1 at an overall CBO level of (A) 2% and (B) 3%. The control films were prepared with conventional emulsions with 0.5% Tween 80 and same CBO concentrations.



(A) (B) (C)

Fig. 7. Transparency of alginate films prepared without (A) and with (B) 2% CBO or (C) 2% CBO and 0.5% SBO.

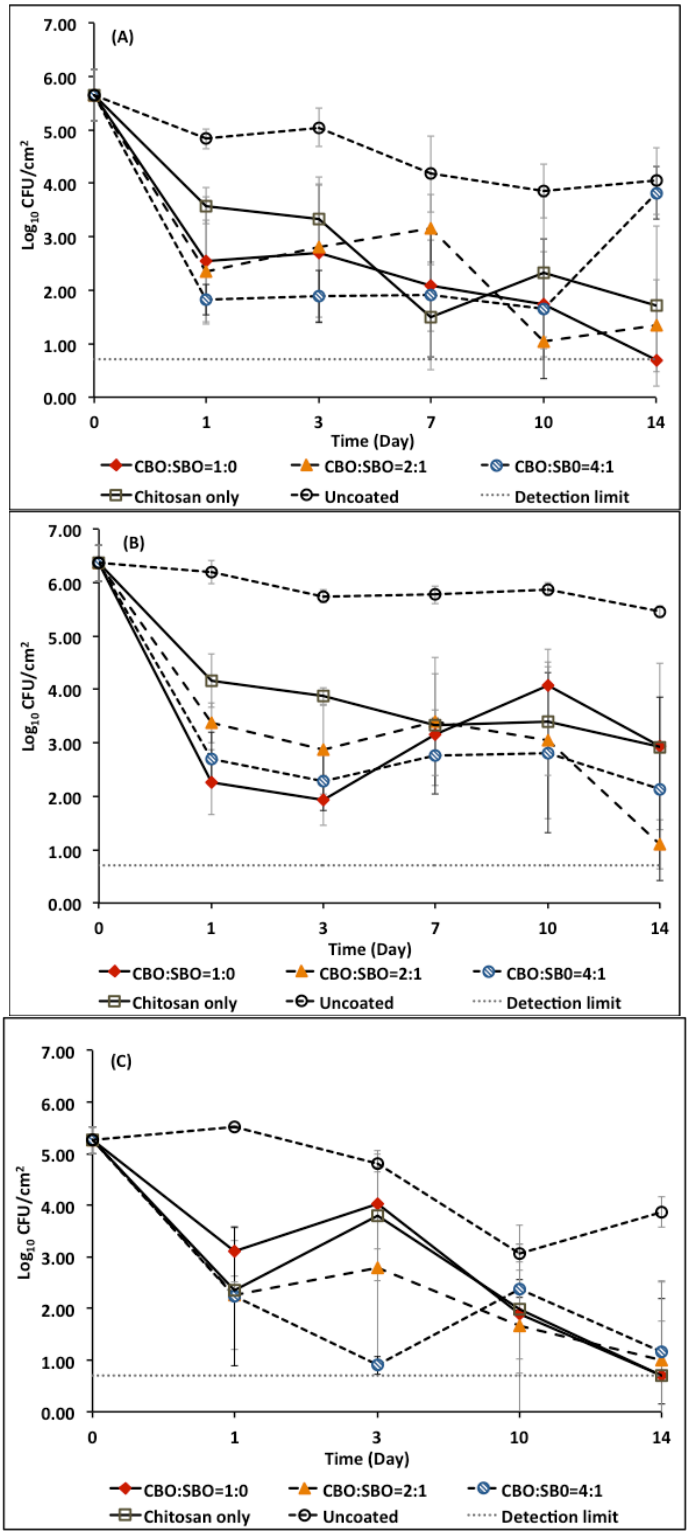


Fig. 8. Growth kinetics of *Escherichia coli* O157:H7 (A), *Salmonella enterica* (B), and *Listeria monocytogenes* (C) on the surface of whole cantaloupes stored at room temperature (21°C) up to 14 days. The inoculated cantaloupes were coated with chitosan only or with additional microemulsions at an overall CBO concentration of 2% and different CBO:SBO mass ratios.

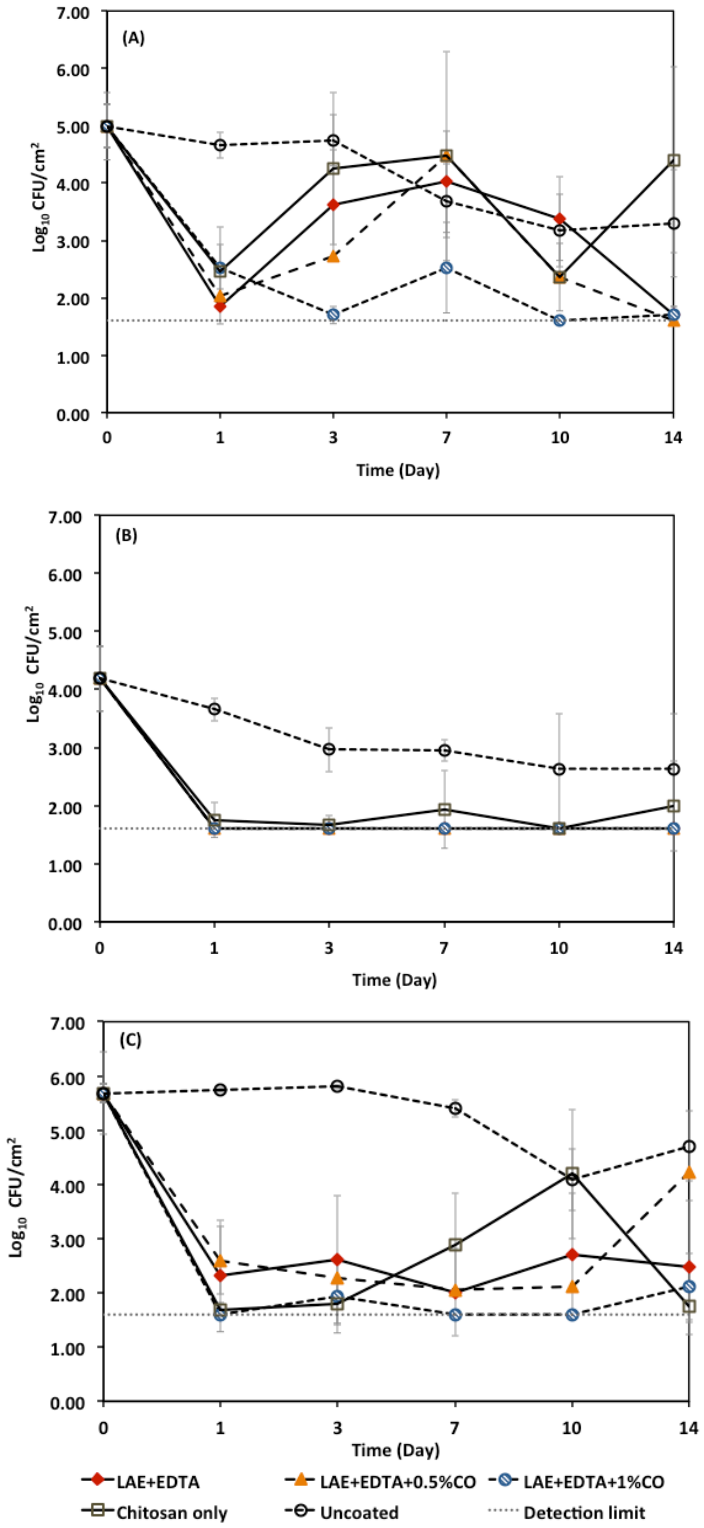


Fig. 9. Growth kinetics of *E. coli* O157:H7 (A), *S. enterica* (B) and *L. monocytogenes* (C) on cantaloupes during storage at room temperature (21°C) up to 14 days. The inoculated cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA and 0.5% or 1% w/w CO.

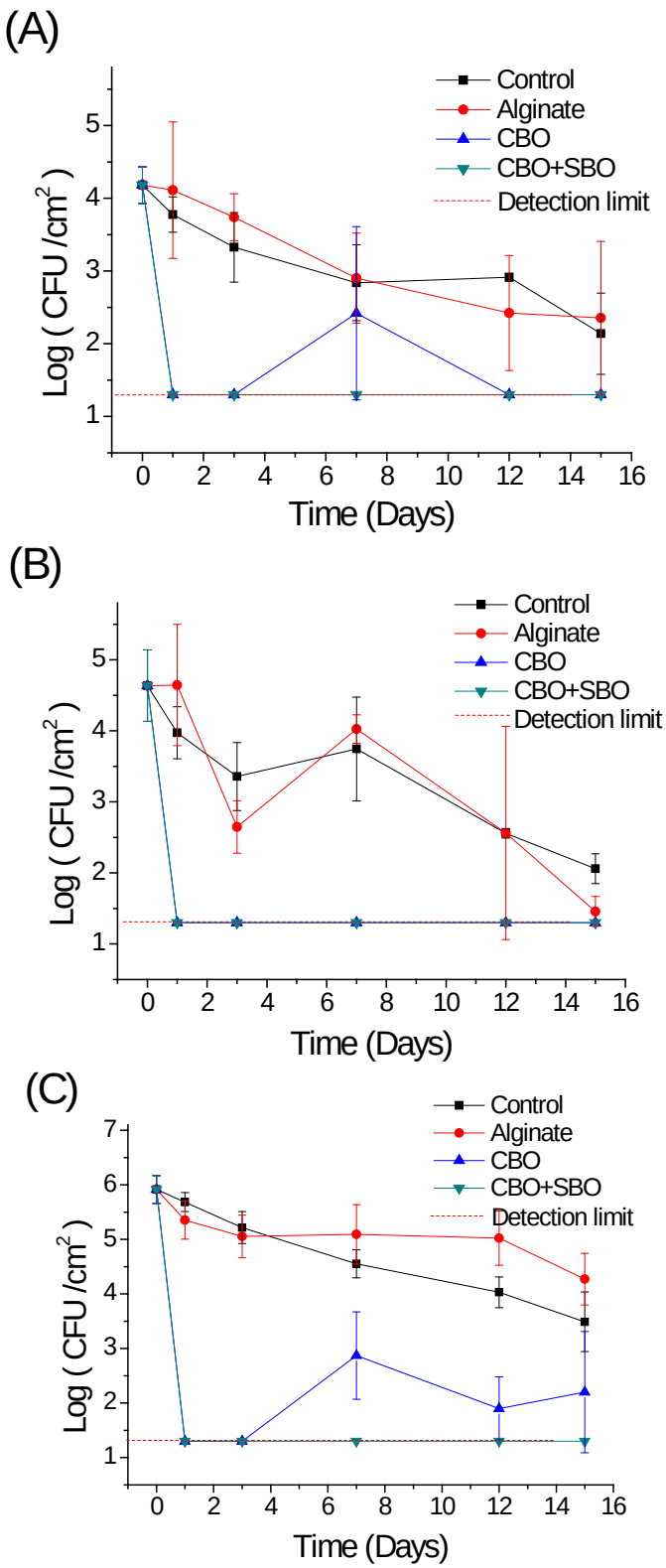


Fig. 10. Growth of *S. enterica* (A), *E. coli* O157:H7 (B) and *L. monocytogenes* (C) on cantaloupes at 21°C after different coating treatments. The inoculated cantaloupes were coated with 1% alginate only or with additional 2% CBO and 0.5% SBO. Numbers at day 0 are the bacteria population before coating.

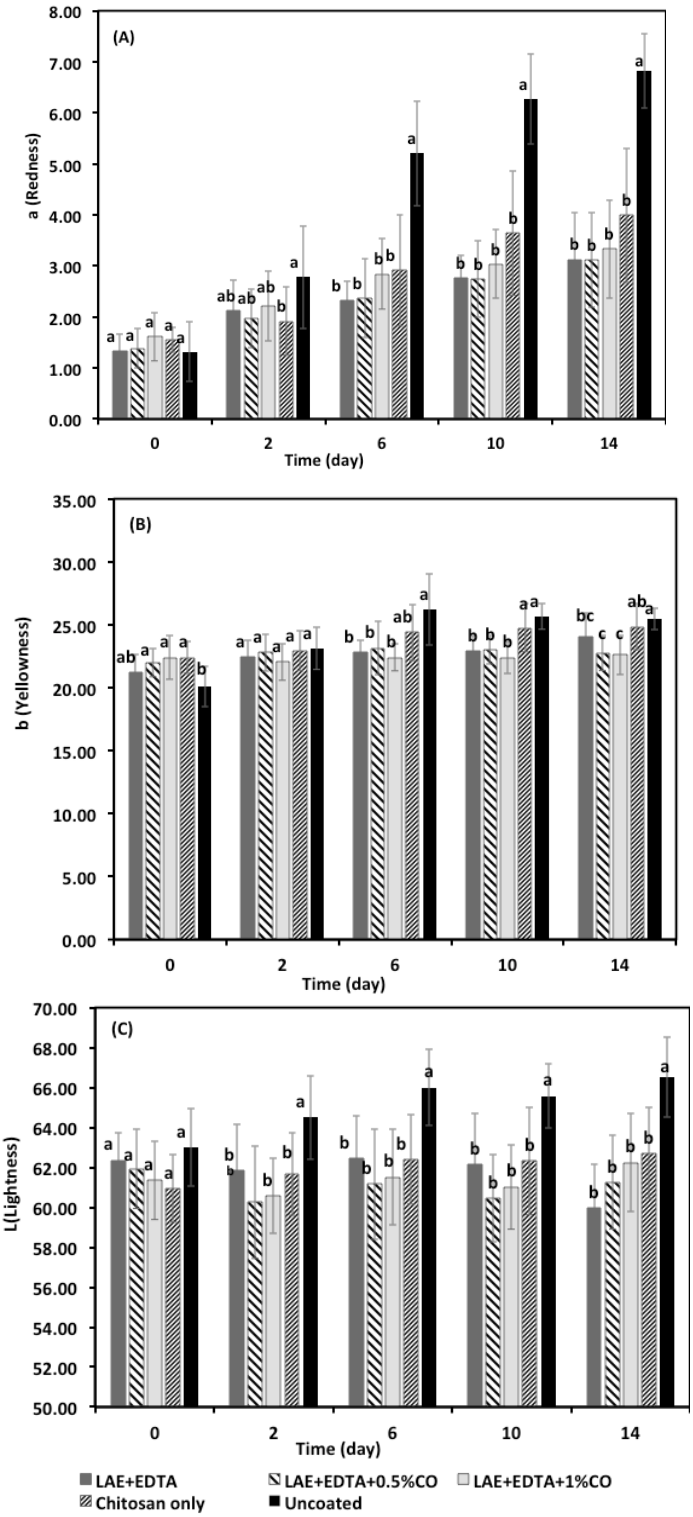


Fig. 11. Changes of cantaloupe colors during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

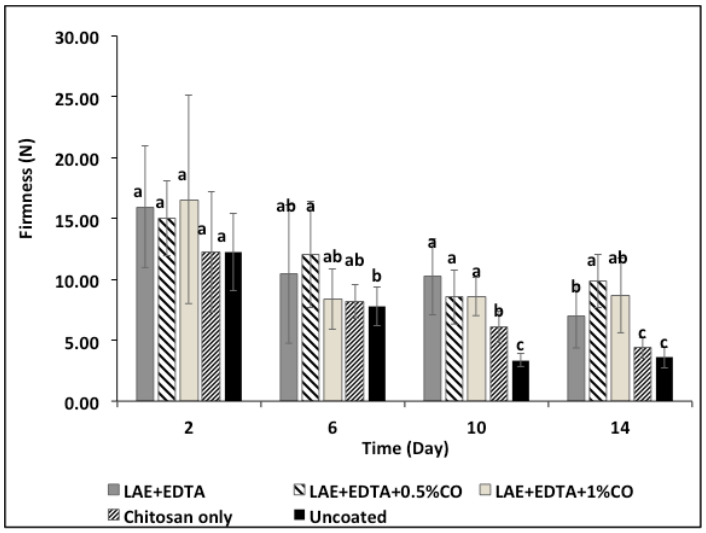


Fig. 12. Changes of cantaloupe firmness during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

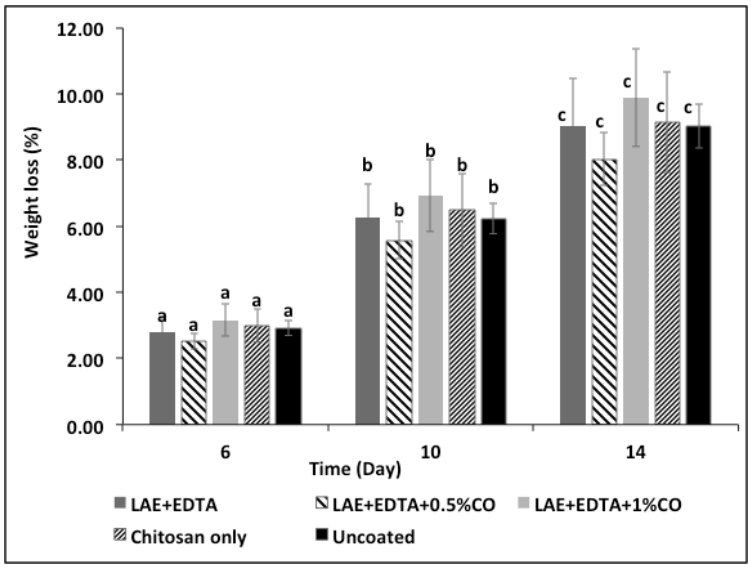


Fig. 13. Changes of weight loss (%) of cantaloupes during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

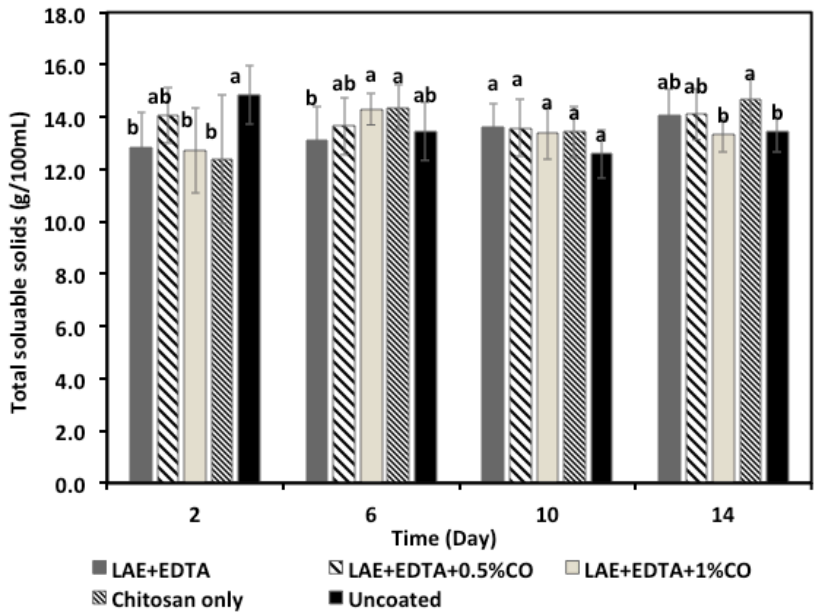


Fig. 14. Changes of total soluble solids contents of cantaloupe flesh during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

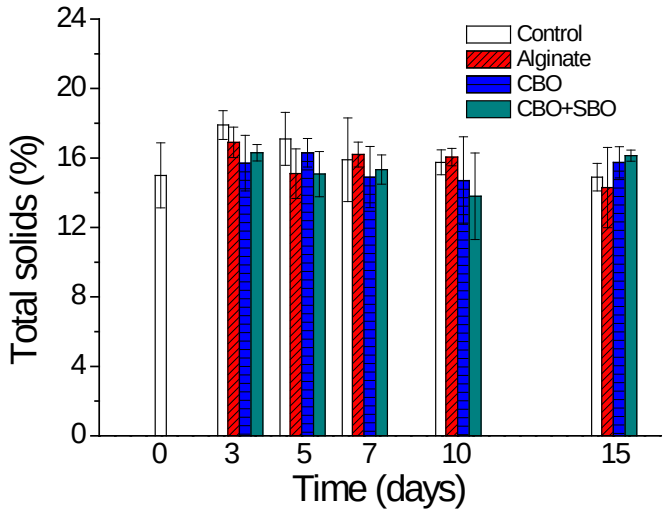


Fig. 15. Changes of total solids content (%) of cantaloupe flesh after storage at room temperature for up to 15 days. Cantaloupes were coated with 1% alginate only or with additional 2% CBO and 0.5% SBO.

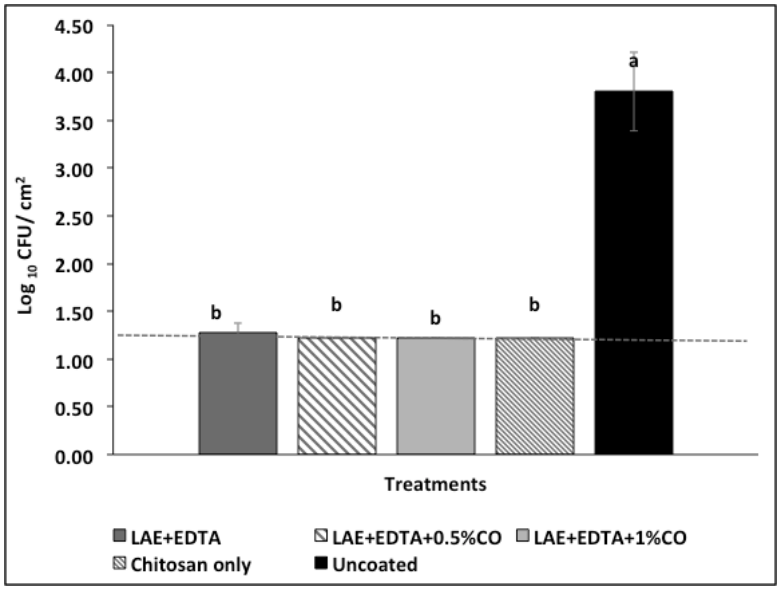


Fig. 16. Effects of coating treatments on the population of total molds and yeasts on cantaloupe surfaces after coating (day 0) with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

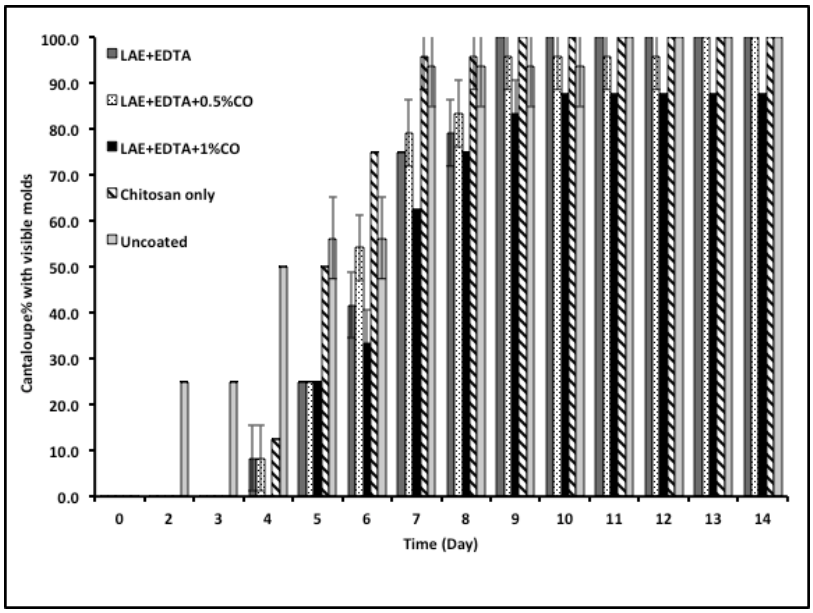


Fig. 17. Percentages of cantaloupes with visible molds during storage at room temperature (21°C). Cantaloupes were coated with chitosan only or with additional 0.1% w/w LAE, 0.1% w/w EDTA, and 0, 0.5 or 1% w/w CO.

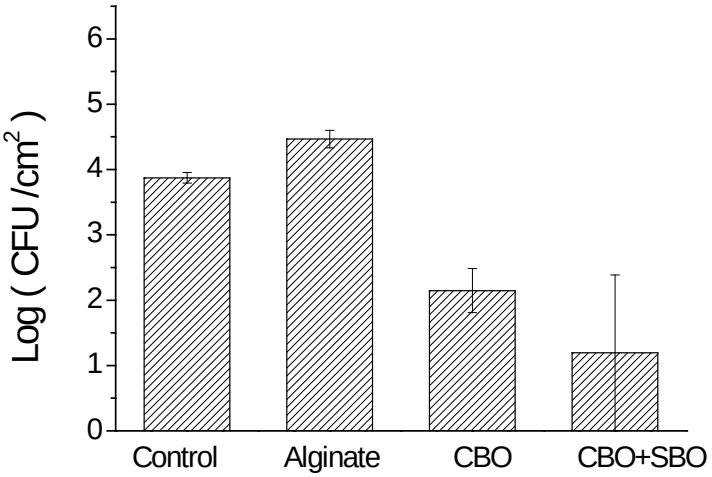


Fig. 18. Effects of coating treatments on the population of total molds and yeasts on cantaloupe surfaces after storage at room temperature for 5 days. Cantaloupes were coated with 1% alginate only or with additional 2% CBO and 0.5% SBO.

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