**Organic thyme oil emulsion as an alternative washing solution to enhance the microbial
safety of organic cantaloupes**

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Abstract

Emulsions of organic essential oils may be used as postharvest alternative washing solutions in fresh produce production. In the present study, organic thyme oil was emulsified with whey protein concentrate, gum arabic, lecithin, or their equal mass mixtures without using specialized equipment. The stability of these emulsions was monitored by measuring hydrodynamic diameter during ambient storage up to 7 days. The antimicrobial activity of these emulsions against *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All emulsions had lower MIC and MBC than free thyme oil pre-dissolved in ethanol. Thyme oil emulsified with gum arabic had the smallest and most stable hydrodynamic diameter (156-239 nm) and was chosen as the washing solution to evaluate its efficacy in reducing pathogens on organic cantaloupes. Cantaloupes inoculated with pathogens were immersed in 0.1%, 0.2%, or 0.5% emulsified or free thyme oil for 2 min. The counts of the three cultures inoculated on cantaloupes were reduced by either 0.2% or 0.5% thyme oil and the emulsions were more effective than free thyme oil ($P < 0.05$). Organic load (2% or 5%) had no effect on their antimicrobial efficacy ($P > 0.05$). During ambient storage (21 °C) up to 10 days, the counts for all three bacteria gradually declined for all treatments and the emulsion treatment had consistently lower populations than unwashed and water-washed treatments. Therefore, emulsions of organic essential oils have potential applications as postharvest washing solutions to improve the microbiological safety of organic fresh produce.

Key words: organic thyme oil emulsion; organic cantaloupe; washing solution; antimicrobial activity; foodborne pathogens

1. Introduction

Foodborne pathogens account for up to 47.8 million illnesses annually in the United States, many of which are linked to consumption of fresh fruits (Callejón, et al., 2015; Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011; Scallan, Hoekstra, et al., 2011). Cantaloupe is one of the most popular fruits worldwide but it has also been linked to at least 34 foodborne disease outbreaks in the United States between 1973 and 2011 (Danyluk, Friedrich, & Schaffner, 2014). Cantaloupe is rich in sugars and has a near neutral acidity, both of which are favorable conditions for the growth of foodborne pathogens (Gil, Aguayo, & Kader, 2006; Golden, Rhodehamel, & Kautter, 1993).

Many sanitizers have been studied to reduce pathogens inoculated on cantaloupes, including ozone, chlorine dioxide gas, and chlorine (Mahmoud, Vaidya, Corvalan, & Linton, 2008; Rodgers, Cash, Siddiq, & Ryser, 2004). However, the regulated chlorine level (up to 200 ppm) in the production of organic fresh produce and the introduction of organic material during washing that neutralizes the chlorine make it ineffective in reducing pathogens (Beuchat & Ryu, 1997; Fukuzaki, 2006; USDA, 2011). Generation of carcinogenic materials after chlorine sanitation is another concern (Chen & Zhu, 2011). Additionally, the rough surface of cantaloupes can entrap bacteria in cavities on the rind surface and further reduce the effectiveness of washing treatments. Alternative technologies like steam treatment and UV irradiation have also been studied (Kozempel, Radewonuk, Scullen, & Goldberg, 2002; Manzocco, Da Pieve, & Maifreni, 2011), but the practicality of these technologies is questionable in the commercial production.

Essential oils (EOs) distilled from plants or plant parts (leaves, buds, seeds, etc.) have exhibited excellent activities against a broad spectrum of pathogenic and spoilage microorganisms (Davidson, Critzer, & Taylor, 2013; Dorman & Deans, 2000; Ma, Davidson, &

Zhong, 2013). EOs and their components have been studied as natural antimicrobial preservatives to improve safety of organic fresh produce because of their antimicrobial properties and their generally-recognized-as-safe (GRAS) regulatory status (Moore - Neibel, Gerber, Patel, Friedman, & Ravishankar, 2012; Zhang, Critzer, Michael Davidson, & Zhong, 2014). However, the limited water solubility and high volatility of EOs limit their application as washing solutions for fresh produce. Emulsions are common choices to incorporate hydrophobic compounds in aqueous systems and have been used to disperse EOs (Chen, Zhang, & Zhong, 2015). Synthetic surfactants are commonly used to prepare emulsions using high pressure homogenization. However, the use of synthetic surfactants is limited by potential toxicity and prohibited in organic production, while the high capital and operating costs of traditional high pressure homogenization methods may have limitations, especially at the farm level. In a previous study, we prepared emulsions of organic clove bud oil without using specialized equipment (Luo, et al., 2014). The principle was based on the deprotonation of the hydroxyl group of EO components (eugenol in clove bud oil) in alkaline conditions. The dissolved eugenol precipitated and self-emulsified *in situ* with emulsifiers when the mixture was acidified to neutral pH. Emulsions of clove bud oil were prepared using emulsifiers permitted in the production of organic foods, including whey protein concentrate (WPC), gum arabic (GA), lecithin, and their equal mass mixtures. This novel self-emulsifying technique can be applied to other EOs which have major components containing hydroxyl groups, such as thyme oil with the major components of thymol and carvacrol (Juven, Kanner, Schved, & Weisslowicz, 1994). These novel EO emulsions may be used as alternative washing solutions in the production of fresh produce like cantaloupes.

Therefore, the first objective of the present work was to characterize physical and antimicrobial properties thyme oil emulsions prepared with the self-emulsifying technique using

WPC, GA, lecithin, and their mixtures. The second objective was to evaluate the efficacy of the chosen thyme oil emulsion in inhibiting foodborne pathogens on organically grown cantaloupes.

2. Materials and methods

2.1. Materials

Organic-certified thyme oil was purchased from Sigma-Aldrich Corp. (St. Louis, MO). Organic cantaloupes were purchased from a local retail grocery store. WPC-34 and soy lecithin Sunlipon™ 50 were provided by Grande Cheese Company (Grande, WI) and Perimondo, LLC (New York, NY), respectively. GA was purchased from Fisher Scientific (Pittsburg, PA). The fat globules in WPC were removed to obtain a transparent dispersion before emulsion preparation (Liu & Zhong, 2014). Other analytical grade chemicals and reagents were purchased from either Sigma-Aldrich or Fisher Scientific (Pittsburgh, PA).

2.2. Preparation of emulsions

The preparation of emulsions was based on the self-emulsification technique as described in our previous study (Luo, et al., 2014). Nine mL of 3 M NaOH was mixed with 1 mL thyme oil in a glass vial that was placed in a glycerol bath for heating at 120°C for 30 min. The 1 mL of this mixture at 120°C was added to 9 mL of the aqueous solution with a total of 1%w/v emulsifier consisting of WPC only, GA only, lecithin only, or their equal mass mixtures (0.5% each for two emulsions; 0.33% each for three emulsifiers). After mixing for 5 min on a magnetic stir plate at room temperature (21°C), the pH of the above mixtures was adjusted to 7.0 using 3 M citric acid.

2.3. Determination of droplet size of emulsions during ambient storage

The hydrodynamic diameter (D_h) and polydispersity index (PDI) of emulsions in fresh dispersions and during storage up to 7 days was measured using a dynamic light scattering instrument (Delsa Nano C particle size/zeta potential analyzer, Beckman Coulter, Fullerton, CA) with a scattering angle of 165°. Measurements were done in triplicate for each sample.

2.4. Preparation bacteria cultures and determination of MICs and MBCs

The bacterial strains were obtained from the culture collection of the Department of Food Science and Technology at the University of Tennessee (Knoxville, TN). Cocktails of cultures were prepared from 5 strains each of *E. coli* O157:H7 (H1730, F4546, K3995, CDC658 and 932) and *L. monocytogenes* (ENV2011010804-1 (390-1), ENV2011010804-2 (390-2), 310, Scott A, and V7 for) and five serovars (Agona, Montevideo, Gaminara, Michigan, and Saint Paul) of *S. enterica*. These strains/serovars were associated with produce outbreaks, with *L. monocytogenes* ENV2011010804-1 (390-1) and ENV2011010804-2 (390-2) and *S. enterica* Michigan being linked to cantaloupe outbreaks. Each test strain or serovar stored in glycerol at -20°C was transferred in tryptic soy broth (TSB) and incubated at 37°C for *E. coli* O157:H7 and *S. enterica* or 32°C for *L. monocytogenes* for 2 consecutive days. All further tests involving the three strains were incubated at the same respective optimum temperatures. The 5 test strains or serovars were combined to yield a cocktail containing equal proportions of each test strain/serovar and diluted to $\sim 10^6$ CFU/mL as the working culture.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of emulsions and free thyme oil were determined using the microbroth dilution method (Branen & Davidson, 2004; Ma, et al., 2013). Free thyme oil samples were prepared by dissolving 10% w/v thyme oil in 50% aqueous ethanol, followed by dilution in TSB to a thyme oil concentration of 100, 200, 400, 600, 800, 1000, 1200, and 1600 ppm. The emulsion samples

were diluted in TSB directly to the same thyme oil concentrations. The working culture (120 μ L) was added into wells of a 96-well microtiter plate and was mixed with 120 μ L of TSB with or without 5% ethanol, or an antimicrobial sample diluted in TSB. The microtiter plates were incubated at optimum temperatures for 24 h. The absorbance at 630 nm (OD_{630}) of each well was acquired before and after incubation using a microtiter plate reader (Titertek Multiscan MC, Labsystems, Helsinki, Finland). The MIC was defined as the lowest antimicrobial concentration corresponding to an OD_{630} change of <0.05 . To determine MBC, 10 μ L of the mixture from negative wells (with $OD_{630} < 0.05$) was spread on tryptic soy agar (TSA) and incubated for another 24 h at optimum temperatures. MBC was determined as the lowest antimicrobial concentration corresponding to no detectable colonies on TSA after incubation. The measurements were taken from two samples and independently replicated twice ($n = 4$).

2.5. Inoculation of cantaloupes

The strains and serovars listed in Section 2.4 were made nalidixic acid (NA) resistant by gradually introducing NA to the cultures over a period of time so that the pathogens could be differentiated from the background microflora on inoculated cantaloupes (Niemira & Lonczynski, 2006). All cultures were resistant to 40 ppm NA. The NA resistant strain or serovar was grown and recovered in TSB with 40 ppm NA (TSBNA) by incubating at optimum temperatures for 24 h. After being transferred twice, 0.3 mL of each serovar and strain was plated onto TSANA and incubated at optimum temperatures for 24 h to create a lawn of each strain or serovar. The lawn of each strain or serovar was re-suspended in 5 mL of phosphate-buffered saline (PBS) with 0.2% Tween 80 and mixed to obtain a cocktail with 10.0 ± 0.1 log CFU/mL *L. monocytogenes*, 10.7 ± 0.1 log CFU/mL *E. coli* O157:H7, or 10.9 ± 0.3 log CFU/mL *S. enterica*. Then 1.0 mL suspension was taken from each bacterial mixture and diluted twice in 9 mL PBS to reach

approximately 9.0 log CFU/mL *E. coli* O157:H7, *S. enterica* or 8.0 log CFU/mL *L. monocytogenes* as the working culture for inoculation.

The surface of cantaloupes was marked with several 2.5 cm× 2.5 cm squares and the squares were excised using a sterile knife (Parnell, Harris, & Suslow, 2005; Zhang, Ma, Critzer, Davidson, & Zhong, 2015). The pulp attached to the square was carefully removed to obtain a thin cantaloupe rind. The squares were washed by sterile water and dried in the bio-safety cabinet for 1 h. A total of 100 µL culture was spot-inoculated over one square at 10 µL each on 10 locations to facilitate drying over 2-3 h (Chen & Zhu, 2011).

2.6. Procedures of treating cantaloupe rind squares inoculated with bacteria

Treatment solutions containing 0 (control), 0.1%, 0.2% and 0.5% (w/v) free or GA-emulsified thyme oil in sterile water were prepared by dilution in sterile water. For each treatment, two inoculated squares (~5 g) from one cantaloupe were immersed in 20 mL treatment solution for 2 min at room temperature (21°C). After treatment, the squares were removed from the washing solution and placed into a sterile stomacher bag containing 20 mL sterile PBS (10 mM, pH 7.4) with 0.2% Tween 80 and rubbed by hand for 1 min. A 1.0 ml sample of the rinsate was removed, serially diluted in PBS, surface-plated on TSANA plates and incubated at the optimum temperatures of bacteria for 24 h, followed by enumeration of colonies. Two inoculated squares without treatment were used to determine the population of inoculated bacteria on squares (Zhang, et al., 2015). Each treatment was replicated twice, analyzing two samples per replicate. To study the effect of organic load on the activity of antimicrobials, 5 g cantaloupe pulp was blended and the puree was added at 2 or 5% w/w into the washing solution right before introducing the inoculated cantaloupe rinds.

2.7. Transfer of bacteria to washing solutions and clean cantaloupe rind squares

To determine the transfer of bacteria from contaminated washing solution to clean cantaloupes, two uninoculated samples were immersed into each treatment solution after washing and removing the inoculated samples. After 2 min, the population of bacteria transferred to the uninoculated samples was determined using the same method described above. After washing inoculated and uninoculated squares, the bacteria population in the treatment solution was determined immediately by surface plating on TSANA. The experiment was repeated in two independent replicates, each with two squares ($n = 4$).

2.8. Survival of bacteria on whole cantaloupes after washing and during ambient storage

One hundred μL culture containing $\sim 10^8$ CFU/mL *S. enterica* or *L. monocytogenes* was inoculated on each of four 2.5 cm $\times$ 2.5 cm squares marked on the surface of whole cantaloupes (Parnell, et al., 2005). The inoculum was applied in approximately equal volumes at 10 locations over the marked area and dried overnight. After drying, each cantaloupe was placed into a plastic bucket with 3000 mL of sterile distilled water or wash solution with 0.2% free or GA-emulsified thyme oil. After 2 min, cantaloupes were removed from wash solution, placed in a dry sterile box, and stored at ambient conditions (21°C) for up to 10 days. The inoculated cantaloupes without treatment were used as controls. For enumeration, all marked squares were cut by a sterile knife to obtain the rind for enumeration as described in Section 2.6. The marked rind squares after washing without storage were treated as day zero. Each treatment had four replicates based on four squares on each of two cantaloupes treated with two independent washing solutions ($n = 4$).

2.9. Statistical analysis

All results were reported as means $\pm$ standard deviations. Statistical analyses were performed using the SPSS 16.0 statistical analysis system (SPSS Inc., Chicago, IL). The one-way

analysis of variance (ANOVA) of means was separated at a significance level (P) of 0.05 using the least significant difference method.

3. Results and discussion

3.1. The appearance and stability of emulsions

Figure 1 shows the appearance of alkaline-dissolved thyme oil mixed with 1% emulsifier solutions before (Fig. 1a) and after (Fig. 1b) neutralization to pH 7.0. The sample emulsified with WPC, GA, or their blend showed a lighter color and was less turbid than those with lecithin, which can be attributed to water insolubility of lecithin with pigments. There was no visible free oil or creaming for any of the six samples, indicating the successful self-emulsification. After being diluted 5 times to 0.2% thyme oil, the sample emulsified with GA was transparent, those with WPC and WPC-GA blend were translucent, while others with lecithin remained turbid (Fig. 1c).

The D_h of fresh emulsions and during storage at ambient conditions up to 7 days was measured (Table 1). The freshly prepared emulsions showed an average D_h in the range of 155 - 450 nm. The D_h varied significantly with emulsifiers used in sample preparation. The sample emulsified with GA had a significantly smaller ($P < 0.05$) D_h than other emulsions, followed by those with GA and WPC or lecithin blends, whereas samples with WPC, WPC+lecithin and the combination of three emulsifiers had much larger D_h ($P < 0.05$). The D_h of emulsions with WPC and its mixtures with other emulsifiers increased significantly during 7 d storage, while the emulsions with GA and GA+lecithin had better stability, with the GA emulsion showing no significant change of D_h after 7 d. Similar trends were observed for PDI. Overall, the emulsion

prepared with GA showed the smallest D_h and had no significant change in D_h or PDI during storage.

The parameters for GA-emulsified thyme oil were different than a previous study where clove bud oil emulsions prepared with WPC had smaller and more stable D_h than those prepared with GA and GA blends (Luo, et al., 2014). Eugenol, the major component in clove bud oil, is more polar than thymol and carvacrol in thyme oil (Sundt, 1961; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005). The polarity difference may cause different affinity of EOs with emulsifiers and therefore water/oil interfacial tension, resulting in variations of D_h at different EO-emulsifier combinations. The same affinity may also have resulted in differences of D_h increase after storage due to coalescence and Ostwald ripening. Emulsion droplets prepared by GA can be stabilized by repulsive steric and electrostatic interactions against aggregation, the prerequisite of coalescence, while electrostatic repulsion is the major stabilization mechanism of droplets emulsified by whey proteins (Chanamai & McClements, 2002). The relatively lower solubility of thymol and carvacrol in water than eugenol can reduce the diffusion of thyme oil components between droplets through the continuous phase and hence improve the stability of emulsion against Ostwald ripening (Chen, Davidson, & Zhong, 2014; Wooster, Golding, & Sangsri, 2008). Both mechanisms can contribute to the better stability of thyme oil emulsion prepared with GA in the present study than the clove bud oil emulsion reported previously (Luo, et al., 2014).

3.2. MICs and MBCs of emulsions in TSB

MICs and MBCs of free and emulsified thyme oil in TSB against *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica* are listed in Table 2. The MIC of free thyme oil against all three bacteria was 400 ppm, whereas the MBCs were 600, 400, and 600 ppm against *E. coli* O157:H7,

S. enterica and *L. monocytogenes*, respectively. In a previous study, the emulsifiers alone did not show inhibition of the three bacteria (Luo, et al., 2014). The MICs and MBCs of thyme oil emulsions were lower than those of free thyme oil, which suggested an improved antimicrobial activity of thyme oil after emulsification. For all three bacteria, all emulsions exhibited similar antimicrobial activity. The enhanced antimicrobial activity of thyme oil after emulsification is likely due to the improved solubility (Donsi, Annunziata, Vincensi, & Ferrari, 2012).

3.3. Reduction of bacteria on cantaloupes after washing

As discussed previously, the emulsion prepared with GA showed the smallest and most stable droplets and had good antimicrobial activity. Thus, this emulsion was diluted to three thyme oil concentrations for washing cantaloupes. Table 3 shows the surviving bacteria on cantaloupes after washing treatments by 0, 0.1, 0.2 or 0.5% thyme oil pre-dissolved in ethanol or emulsified by GA. From an inoculum of 10^8 - 10^9 CFU/cm², 6.62 log CFU/cm² of *E. coli* O157:H7, 6.89 log CFU/cm² of *S. enterica*, and 5.85 log CFU/cm² of *L. monocytogenes* were attached on the surface of the cantaloupes based on the untreated rinds. Washing with water did not cause a significant reduction ($P > 0.05$) of the three bacteria. The result of washing with water is inconsistent to that of whole cantaloupe studies that more than one log reduction can be achieved because much less water was used in this case (Parnell, et al., 2005). Both free and emulsified thyme oil showed effectiveness in reducing the surviving bacteria. The populations of three bacteria decreased to a greater extent after treatment by higher concentrations of thyme oil. The thyme oil emulsion showed better effectiveness with varying degrees against the three bacteria than free thyme oil after the washing treatment ($P < 0.05$), which was in accordance with the observations based on MICs and MBCs (Table 2). At the lowest thyme oil concentration (0.1%) with no organic load, the emulsified thyme oil reduced the population of *E. coli* O157:H7,

S. enterica and *L. monocytogenes* by approximately 1 log CFU/cm², while free thyme oil reduced the *E. coli* O157:H7 by only 0.4 log CFU/cm² and did not show significant reduction of other two bacteria ($P > 0.05$). After washing by 0.5% of emulsified thyme oil, *E. coli* O157:H7 and *L. monocytogenes* were decreased by 2.3 and 1.7 log CFU/cm², respectively, while the reduction of *S. enterica* was much higher (3 log CFU/cm²). The more significant reduction on *S. enterica* agreed with the lower MBC value of the emulsion prepared with GA against *S. enterica* than that of other two bacteria (Table 2).

Influence of organic loads on the efficiency of washing treatments was measured by adding cantaloupe pulp puree at 2% and 5% w/w in washing solutions (Table 3). The organic load showed no significant effect on the reduction of all three bacteria by three concentrations of emulsified thyme oil. For free thyme oil, the organic load showed no effects on the antimicrobial efficacy against *E. coli* O157:H7 and *L. monocytogenes* but a slight improvement against *S. enterica*. This improvement may have resulted from some pulp components improving the susceptibility of *S. enterica* to thyme oil, but the exact mechanism would need further investigation. Because the sanitization effectiveness of oxidizing chemical sanitizers like chlorine is greatly reduced by organic matter (Park, Alexander, Taylor, Costa, & Kang, 2009), activity of the thyme oil emulsions in the presence of organic matter is an important advantage for use as postharvest washing solution for fresh produce.

3.4. Transfer of bacteria to clean cantaloupes and washing solutions

To study cross-contamination of bacteria, uninoculated cantaloupe rind squares were introduced in the washing solutions after treating and removing inoculated rind squares. The bacteria populations detected on clean cantaloupe rind squares by different washing treatments are shown in Fig. 2. The transfer of the pathogens to the wash water by the inoculated cantaloupe

rinds and subsequent cross contamination to the uninoculated squares was confirmed by the presence of 3.87 log CFU/cm² *E. coli* O157:H7, 3.80 log CFU/cm² *S. enterica*, and 3.42 log CFU/cm² *L. monocytogenes* on the uninoculated squares in the absence of a sanitizer. For all of the thyme oil treatments except 0.1% free thyme oil, **no bacteria were detected after direct plating on TSANA**. With 0.1% free thyme oil, 1.68 log CFU/cm² *S. enterica* and 2.08 log CFU/cm² *L. monocytogenes* were transferred to uninoculated squares while no bacteria were detected **at the studied conditions** for treatments with 0.1% emulsified thyme oil.

The effects of organic load on the transfer of bacteria were also studied (Fig. 2). No detectable transfer of bacteria was detected **at the studied conditions** for any treatment with emulsified thyme oil in the presence of organic matter. For 0.1% free thyme oil, cross contamination did not show a consistent pattern, with bacteria detected on some uninoculated squares mixed with 2% or 5% organic load, which may be attributed to the heterogeneous distribution of free thyme oil in the washing solutions with organic matter.

The populations of bacteria in washing solutions after treating and removing inoculated and uninoculated squares were also determined (Fig. 3). For water alone, 5.62 log CFU/mL of *E. coli* O157:H7, 6.07 log CFU/mL of *S. enterica* and 5.13 log CFU/mL of *L. monocytogenes* were detected, indicating the transfer of bacteria from the original contaminated squares as a source for cross contamination to uninoculated squares (Fig. 2). For the treatments with GA-emulsified thyme oil, no surviving bacteria **after direct plating on TSANA** were detected in emulsions after washing inoculated cantaloupes, which indicated that bacteria transferred from inoculated squares were killed quickly (less than 5 min) by thyme oil in emulsions. Organic load did not influence bacterial transfer to emulsions ($P < 0.05$). For treatments with 0.1% free thyme oil, *S. enterica* and *L. monocytogenes* were detected in washing solutions with and without 2% organic

load, which was in agreement with the cross-contamination of uninoculated cantaloupe rind squares observed for this treatment. Similarly, the treatment with 0.1% free thyme oil and 5% organic load resulted in the transfer of *E. coli* O157:H7 cross-contamination. At 0.2 and 0.5% free thyme oil, no transfer of bacteria to washing solutions was observed after direct plating on TSANA. The antimicrobial activity of chlorine can be greatly reduced by the organic load and a higher concentration is needed to prevent the cross-contamination which may exceed the limitation of chlorine that can be used under the Safe Drinking Water Act (USDA, 2011). The elimination of cross-contamination by thyme oil emulsion however requires the verification for viable cells by the enrichment method.

The inactivation of bacteria transferred from inoculated cantaloupe rind squares to washing solutions is in agreement with the MBCs for free thyme oil of 600, 400, and 600 ppm for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, respectively. The binding of thyme oil components with the cantaloupe surface likely reduced the availability of antimicrobials leading to survival of bacteria in the washing solution and transfer to uninoculated cantaloupes when there was 0.1% (1000 ppm) free thyme oil present. For 0.1% emulsified thyme oil, the improved solubility and potentially reduced binding to cantaloupe together with a lower MBC may have resulted in the elimination of bacteria transfer. With 0.2% and 0.5% free or emulsified thyme oil, the concentrations of antimicrobials in the washing solutions were likely above MBC, corresponding to no detectable bacteria in washing solutions at the studied conditions.

3.5. Survival of bacteria on whole cantaloupes after washing and during ambient storage

Figure 4 shows the survival of *S. enterica* and *L. monocytogenes* on cantaloupes during ambient storage for 10 days after washing with sterile water, 0.2% free or GA-emulsified thyme oil. The populations of the two bacteria on cantaloupes without washing treatment or washed by

water decreased gradually during storage, which may be due to the drying on the surface of the cantaloupes leading to increased bacteria inactivation (Behrsing, et al., 2003; Knudsen, Yamamoto, & Harris, 2001).

With water treatment, the population of *S. enterica* and *L. monocytogenes* decreased only 0.4 and 0.9 log, respectively, after washing, while the reductions were more significant for thyme oil treatments, especially the emulsion (Fig. 4). The 0.2% free thyme oil reduced *S. enterica* and *L. monocytogenes* to 4.70 ($P < 0.05$, compare to control) and 2.19 log CFU/cm² at day 0, respectively, after washing. In contrast, 2.59 log CFU/cm² *S. enterica* and 1.10 log CFU/cm² *L. monocytogenes* remained on cantaloupes after treatment by 0.2% emulsified thyme oil. Despite the incomplete inhibition, the emulsion treatment showed a relatively better antibacterial effectiveness against these two bacteria on whole cantaloupes than free thyme oil. The results suggest that thyme oil emulsion can provide a relatively long-term effectiveness in controlling foodborne pathogens contaminating cantaloupes.

4. Conclusions

In summary, thyme oil emulsions were successfully prepared using WPC, GA, soybean lecithin, and their combinations using the self-emulsification technique developed recently. The physical stability varied with the emulsifier. The MICs and MBCs of these emulsions against *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* were similar and lower than free thyme oil pre-dissolved in ethanol due to the improved solubility. Thyme oil emulsified with GA had the best physical characteristics for use as alternative washing solution. The emulsion had better activities than with free thyme oil pre-dissolved in ethanol in decontaminating pathogens inoculated on cantaloupes. The insensitivity of sanitation effectiveness to organic loads, the effectiveness of

reducing bacteria in washing solutions, and long-term effectiveness in inhibiting pathogens after washing showed the potential of thyme oil emulsions as alternative washing solutions to improve the safety of cantaloupes.

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Table 1. Hydrodynamic diameter (D_h) and polydispersibility index (PDI) of thyme oil emulsions during 7-day storage at 21 °C.*

Parameters	Day	Emulsifier(s)					
		WPC	GA	WPC+GA	GA+lecithin	WPC+lecithin	WPC+GA+lecithin
D_h	0	387.3±18.5 ^d	156.5±11.2 ^b	225.0±6.1 ^d	267.0±1.4 ^c	451.5±20.0 ^c	434.0±13.9 ^c
	1	707.7±24.5 ^c	212.0±6.0 ^a	326.5±3.3 ^c	372.5±7.1 ^a	477.5±16.7 ^c	446.3±26.0 ^{bc}
	3	1028.3±29.0 ^b	239.0±15.1 ^a	425.3±4.9 ^b	354.4±11.3 ^{ab}	709.6±29.9 ^b	523.0±22.6 ^b
	7	1291.0±22.4 ^a	196.1±11.3 ^{ab}	546.4±9.2 ^a	336.9±4.0 ^b	939.0±15.0 ^a	753.0±18.1 ^a
PDI	0	0.217 ± 0.040 ^d	0.337±0.060 ^a	0.272±0.010 ^d	0.233±0.010 ^c	0.267 ± 0.010 ^d	0.345 ± 0.020 ^c
	1	0.332±0.010 ^c	0.310±0.010 ^b	0.288±0.010 ^b	0.289±0.030 ^a	0.329±0.010 ^c	0.345±0.030 ^c
	3	0.377±0.070 ^b	0.309±0.050 ^b	0.282±0.020 ^c	0.282±0.070 ^{ab}	0.346±0.010 ^b	0.382±0.040 ^b
	7	0.454±0.050 ^a	0.327±0.050 ^{ab}	0.307±0.010 ^a	0.270±0.010 ^b	0.391±0.010 ^a	0.414±0.000 ^{01a}
472	*Emulsions with 1%v/v thyme oil were prepared with 1%w/v emulsifiers consisting of WPC, gum arabic (GA),						
473	lecithin individually or their combinations with equal mass. Numbers are means ± standard deviations (n = 3).						
474	Means which share letters within a column for each parameter are not significantly different ($P \geq 0.05$).						

Table 2. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) in ppm of free[‡] and emulsified* thyme oil against *Salmonella enterica*, *Escherichia coli* O157:H7 at 37 °C and *Listeria monocytogenes* at 32 °C.

Sample	<i>E. coli</i> O157:H7		<i>S. enterica</i>		<i>L. monocytogenes</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
Free thyme oil	400	600	400	400	400	600
Emulsified thyme oil*						
WPC	200	400	200	200	200	400
GA	200	400	200	200	200	400
WPC+GA	200	200	200	200	200	400
WPC+lecithin	200	200	200	200	200	400
GA+lecithin	200	200	200	200	200	400
WPC+GA+lecithin	200	200	200	200	200	400

[‡] Pre-dissolved at 10% w/v in 50% ethanol, corresponding to 5% ethanol after dilution to 1% thyme oil. The 5% ethanol did not show inhibition against all bacteria.

*Emulsions were prepared with 1%v/v thyme oil self-emulsified with 1%w/v surfactants composed of WPC, gum arabic (GA), lecithin individually or their combinations with equal mass.

Table 3. Surviving bacteria on the surface of cantaloupes (log CFU/cm²) after treatment by 0-0.5% free^ζ and emulsified thyme oil (TO) for 2 min at 21°C, with and without organic load.*

Bacteria	Treatment	Organic Load		
		0%	2%	5%
<i>E. coli</i> O157:H7	None	6.62±0.14 ^{Aa}	6.19±0.12 ^{Aba}	6.19±0.12 ^{Aba}
	Water	6.37±0.04 ^{ABb}	6.10±0.07 ^{ABCab}	5.94±0.26 ^{ABCab}
	0.1% Free TO	6.20±0.08 ^{ABb}	5.68±0.22 ^{BCDab}	5.98±0.15 ^{ABCab}
	0.2% Free TO	5.32±0.20 ^{CDEd}	5.73±0.16 ^{BCDab}	5.03±0.02 ^{DEFGc}
	0.5% Free TO	4.70±0.12 ^{EFGHe}	4.52±0.58 ^{EFGHc}	4.99±0.07 ^{DEFGc}
	0.1% Emulsified TO	5.66±0.02 ^{BCDc}	5.58±0.13 ^{BCDb}	5.69±0.06 ^{BCDb}
	0.2% Emulsified TO	4.68±0.19 ^{EFGHe}	4.74±0.32 ^{EFGHc}	5.10±0.42 ^{DEFc}
	0.5% Emulsified TO	4.36±0.12 ^{FGHf}	3.98±0.64 ^{Hd}	4.29±0.47 ^{GHd}
<i>Salmonella</i> <i>enterica</i>	None	6.89±0.06 ^{Aba}	6.97±0.09 ^{Aa}	6.74±0.01 ^{ABCa}
	Water	6.30±0.22 ^{BCDabc}	6.25±0.08 ^{BCDEb}	6.38±0.13 ^{ABCDa}
	0.1% Free TO	6.53±0.14 ^{ABCa}	5.64±0.10 ^{EFGHc}	5.00±0.20 ^{HIJb}
	0.2% Free TO	6.39±0.16 ^{ABCDab}	5.26±0.30 ^{GHIJc}	4.96±0.26 ^{IJb}
	0.5% Free TO	5.35±0.43 ^{GHIId}	4.67±0.44 ^{JKd}	4.24±0.88 ^{KLc}
	0.1% Emulsified TO	5.75±0.36 ^{DEFGbc}	6.23±0.14 ^{BCDEb}	6.08±0.18 ^{CDEFa}
	0.2% Emulsified TO	5.58±0.77 ^{FGHIcd}	5.34±0.20 ^{GHIc}	4.93±0.26 ^{IJb}
	0.5% Emulsified TO	3.83±0.02 ^{Le}	3.66±0.13 ^{Le}	4.12±0.41 ^{KLc}
<i>L.</i> <i>monocytogenes</i>	None	5.85±0.12 ^{ABCa}	6.20±0.10 ^{Aa}	6.05±0.11 ^{Aba}
	Water	5.84±0.30 ^{ABCa}	6.04±0.06 ^{ABCa}	6.02±0.01 ^{ABCa}
	0.1% Free TO	5.71±0.19 ^{ABCDa}	5.95±0.05 ^{ABCab}	5.57±0.05 ^{BCDEb}
	0.2% Free TO	5.69±0.10 ^{ABCDa}	5.65±0.10 ^{ABCDc}	5.48±0.11 ^{CDEc}
	0.5% Free TO	4.36±0.32 ^{GHd}	4.84±0.22 ^{FGd}	4.90±0.23 ^{FGd}
	0.1% Emulsified TO	5.19±0.31 ^{DEFb}	5.72±0.21 ^{ABCDbc}	5.72±0.10 ^{ABCDb}
	0.2% Emulsified TO	4.78±0.07 ^{FGc}	4.91±0.07 ^{FGd}	5.04±0.05 ^{EFd}
	0.5% Emulsified TO	4.19±0.12 ^{Hd}	4.40±0.19 ^{GHe}	4.38±0.13 ^{GHe}

^ζPre-dissolved at 10% w/v in 50% ethanol, corresponding to 5% ethanol in 1% thyme oil. The 5% ethanol did not show inhibition of all bacteria in TSB.

*Numbers are means ± standard deviations (n = 4). Numbers sharing different lowercase letters for same column and same bacteria or capital letters for same bacteria with three organic loads are significantly different ($P < 0.05$). The samples without treatment indicate the attachment of inoculum. Detection limit is 1.2 log CFU/ cm².

Figure 1. Appearance of samples (a) after adding 1% v/v alkaline-predissolved thyme oil in pH 7 aqueous solutions with 1% w/v emulsifier composed of (1) WPC only, (2) gum arabic (GA) only, (3) equal mass of WPC and GA, (4) equal mass of GA and lecithin, (5) equal mass of WPC and lecithin, and (6) equal mass of WPC, GA and lecithin, followed by (b) acidification to pH 7.0 and (c) further 5-fold dilution to 0.2% v/v thyme oil in deionized water.

Figure 2. *E. coli* O157:H7 (A), *S. enterica* (B) and *L. monocytogenes* (C) detected on uninoculated cantaloupe rind squares after mixing with washing solutions (water, free or emulsified thyme oil (TO), with and without 2% and 5% organic load) that were previously used to wash inoculated cantaloupe rind squares. Numbers are means $\pm$ standard deviations ($n = 4$). Data at the detection limit line indicate no detectable cells at the studied conditions that will require the enrichment method to verify the presence of viable cells.

Figure 3. Surviving *E. coli* O157:H7 (A), *S. enterica* (B) and *L. monocytogenes* (C) detected in washing solutions (water, free or emulsified thyme oil (TO), with and without 2% and 5% organic load) after treating inoculated cantaloupe rind squares. Numbers are means $\pm$ standard deviations (n = 4). Data at the detection limit line indicate no detectable cells at the studied conditions that will require the enrichment method to verify the presence of viable cells.

Figure 4. Surviving *S. enterica* (A) and *L. monocytogenes* (B) on whole cantaloupes after washing by sterile water and 0.2% free or emulsified thyme oil (TO) and subsequent ambient (21 °C) storage for up to 10 days. Numbers are means $\pm$ standard deviations (n = 4).