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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.

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, 2011a, 2011b). 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,

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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 SunliponTM 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 *Escherichia coli* O157:H7 (H1730, F4546, K3995, CDC658 and 932) and *Listeria 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 *Salmonella 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. monocyto*genes 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 ~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 μL) was added into wells of a 96-well microtiter plate and was mixed with 120 μL 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 μL 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