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Comparison of effectiveness of edible coatings using emulsions containing lemongrass oil of different size droplets on grape berry safety and preservation

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ABSTRACT

Effects of lemongrass oil (LO) droplet size in the LO/chitosan emulsion on the emulsion's coating effectiveness in improving microbiological safety and preserving grape berries (*Vitis vinifera* L. × *V. labruscana* Bailey) were investigated. LO was homogenized with a chitosan solution (1 g/100 g) and Tween 20 (0.25 g/100 g) by high shear mixing (HSM; 10,000 rpm, 1 min) or dynamic high pressure processing (DHP; 103–172 MPa, 1–3 passes), forming LO coating emulsions. The particle sizes of the HSM and DHP emulsions were 461.9–632.6 nm and 204.2–378.8 nm, respectively. DHP emulsions were more stable than HSM emulsions. Compared with the HSM emulsion, the DHP emulsion coating resulted in higher initial inhibition of *Salmonella typhimurium*; greater growth inhibition of total mesophilic aerobes, yeasts, and molds; and retention of color, total soluble solid content, and antioxidant activity during storage at 25 °C ($P < 0.05$). The sensory results suggest that the DHP emulsion minimized differences in sensory attributes compared with uncoated grape berries more effectively than did the HSM emulsion coating. The nanoemulsion coating with smaller LO droplets demonstrated greater efficiency in increasing microbial safety against *Salmonella* and preserving grape berries, compared with the coating with larger droplets.

1. Introduction

Essential oils, including lemongrass oil (LO), are effective natural antimicrobial agents (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014). Essential oil-incorporating coating in an emulsion form has been reported to improve microbial safety of fresh fruits (Jo et al., 2014). Forming oil emulsions in nanometric sizes has been suggested to increase accessibility of more emulsions per unit volume to microorganism and passive cellular absorption of the oil emulsion by microorganisms (Donsi, Annunziata, Sessa, & Ferrari, 2011).

Submicron or nanoemulsions of biopolymer materials and essential oils have been produced in water using dynamic high pressure processing (DHP) (Kim, Oh, Lee, Song, & Min, 2014). Oil droplets introduced into a dynamic high pressure processor are accelerated to a high velocity within microchannels. Intense forces are generated in the microchannels by high pressure difference, high shear, and cavitation, causing the droplets to become disrupted and leading to the formation of nanoemulsions (Qian & McClements, 2011). Edible coatings formed on fruits with essential oil nanoemulsions successfully decontaminated microorganisms on the fruit surfaces and reduced the rates of microbial growth and quality deterioration (Kim et al., 2013; 2014). However, the advantage of using emulsions with smaller oil droplets for fruit coating on fruit safety and preservation has not been elucidated by directly comparing effectiveness of coatings formed from emulsions with different emulsion droplet sizes.

Grape berries are popular freshly consumed ready-to-eat food, but they are highly perishable and vulnerable to contamination with foodborne pathogens (Myles et al., 2011; Pastor et al., 2011). In 2014, 1 outbreak, 27 illnesses, and 10 hospitalizations were caused

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by *Salmonella* in grapes (CDC, 2016). Antimicrobial edible coatings have been developed as a postharvest technology to improve microbiological safety and extend the shelf life of grape berries (Kim et al., 2014). However, further research on the effects of antimicrobial coatings in terms of improved grape berry safety and preservation is warranted. Thus, the objectives of this study were to develop a stable emulsion of LO and chitosan for coating and to investigate the effects of LO droplet size in the emulsion on microbiological safety and physicochemical properties of emulsion-coated grape berries during storage at 4 and 25 °C.

2. Materials and methods

2.1. Materials

The table grapes (*Vitis vinifera* L. × *V. labruscana* Bailey) used in this study were produced in Yeongcheon, South Korea, in 2014. Harvested grapes were transported to the laboratory, de-stemmed, and washed with running tap water and distilled water. Flawless grape berries free of visible defects were selected based on uniformity of appearance, size, and color. LO (Now Foods, Bloomingdale, IL, USA), medium molecular weight chitosan (450 kDa; Sigma-Aldrich, St. Louis, MO, USA), glacial acetic acid (Sigma-Aldrich), and Tween 20 (Ilshinwells Co., Seoul, Korea) were used to prepare the coating emulsions.

2.2. Coating emulsion preparation

The chitosan solution was prepared by following the method of Zivanovic, Chi, and Draughon (2005) with modifications. Chitosan was stirred overnight in 1 mL/100 mL glacial acetic acid (1 g/100 g) at room temperature until it was completely dispersed. The pH of the solution was 3.5. The solution was then filtered using Whatman filter paper (no. 4; Whatman Ltd., Maidstone, UK) and autoclaved at 121 °C for 15 min. LO (1 g/100 g) and Tween 20 (0.25 g/100 g) were added to the autoclaved chitosan solution. A high shear probe mixer (Model T25; IKA Works, Inc., Wilmington, NC, USA) or a dynamic high pressure processor (D. O. S, Siheung, Korea) was used to emulsify the mixture. High shear mixing (HSM) using the high shear probe mixer was performed at 10,000 rpm for 1 min. DHP was run at 138 MPa with 1 pass, which resulted in the smallest droplet size of LO-chitosan emulsion and insignificant change in the droplet size for 10 days at room temperature in our preliminary study. Prior to DHP, the chitosan-LO mixture was pre-homogenized using the high shear probe mixer at 2500 rpm for 30 s. The homogenate from HSM or DHP was degassed under vacuum at room temperature to form an emulsion for coating of the grape berries.

2.3. Emulsion stability determination

Emulsions prepared by HSM and DHP were stored for 0, 1, 2, 4, 7, and 10 days at 23 ± 2 °C. Particle size was determined using a particle size analyzer (Zetasizer Nano-ZS; Malvern Instruments Ltd., Worcestershire, UK). The stability of the emulsions was examined according to the method of Chauvierre, Labarre, Couvreur, and Vauthier (2004) by determining light backscattering using an emulsion stability analyzer (Turbiscan AGS; Formulaction, Toulouse, France). Light backscattered by the emulsion sample was analyzed in zones I (19–22 mm from the bottom of the detection cells containing the emulsion sample) and zone II (37–40 mm).

2.4. Grape berry coating

Grape berries were dip-coated by immersion for 2 min in the emulsion prepared by HSM (HSM emulsion) or DHP (DHP emulsion) to prepare HSM emulsion-coated or DHP emulsion-coated grape berry samples, respectively, or in sterile distilled water to prepare the uncoated control samples. Dip-coating was repeated twice. For preparing *Salmonella*-inoculated coated samples, grape berries were coated after ultraviolet-sterilization at 40 W (130 kJ/m^2) for 30 min, prior to microbial inoculation. Coated berry samples were dried for 2 h under atmospheric conditions (23 ± 2 °C and 26 ± 3% relative humidity (RH)). The coatings were confirmed to be uniform and continuous across the entire surface. This confirmation was made using a coating solution containing a blue dye (FD&C Blue No. 1, Sensient Technologies Corp., St. Louis, MO, USA) (0.7 g/100 g). The blue coating solution was spread on the surfaces of the berries and the resulting coated surfaces were visually examined.

2.5. S. typhimurium inoculation

Inoculum was prepared with *S. typhimurium* DT 104, obtained from the Agricultural Biotechnology Culture Collection at Seoul National University (Seoul, Korea). Tryptic soy agar (BD Difco, Sparks, MD, USA) and tryptic soy broth (TSB; BD Difco) were used as growth media. Cells of each overnight (18-h) culture were collected by centrifugation (4000 × *g*, 15 min, 22 °C; GyroSpin, Gyrozen, Seoul, Korea) and suspended in peptone water (0.1 g/100 mL). The suspension (~9 log CFU/mL) was diluted in 0.1 g/100 mL peptone water to produce the desired inoculum concentration (~6 log CFU/mL). *S. typhimurium* was inoculated on either coated or uncoated samples by dipping for 10 min inside a laminar-flow biohazard hood at 23 ± 2 °C (30 ± 2% RH). After drying for 1.5 h in the hood, each berry sample was placed in a polyethylene sterile filter bag (30 mL; Nasco Whirl-Pak®, Fort Atkinson, WI, USA). The inoculation level of *S. typhimurium* on uncoated samples was ~3 log CFU/g.

2.6. Sample storage

The *S. typhimurium*-inoculated samples were stored separately from the berries used to determine the growth of background microorganisms and the physicochemical properties of berry samples during storage. Berry samples were placed in the sterile bags and stored at 4 ± 1 °C or 25 ± 1 °C for up to 28 days. The RH in the berry-containing bags during storage at 4 and 25 °C was 90–98%.

2.7. Microbial analysis

On each day of sampling, each sample in the bag was diluted with 0.1 g/100 mL peptone water (20 mL), and hand-rubbed and mashed for 3 min. Uninoculated grape berry samples were analyzed to determine viable counts of total mesophilic aerobes, yeasts, and molds during storage. *Salmonella* viable counts were determined by plating on xylose-lysine-deoxycholate agar (Difco™; Becton Dickinson, Detroit, MI, USA), and culturing the agar plates at 37 °C for 48 h. Total mesophilic aerobes and yeasts and molds were counted using plate count agar (PCA; Difco™) and PDA, respectively. PCA and PDA plates were incubated at 37 °C for 2 days and 25 °C for 5 days, respectively.

2.8. Determination of color, total soluble solid content, and antioxidant activity

Grape berry surface color was measured using a colorimeter (Chroma Meter CR-400; Minolta Camera Co., Osaka, Japan) and expressed in L^*, a^*, and b^* values based on CIELAB coordinates. The colorimeter was calibrated using a white standard tile (Illuminate D 65) and a 10° standard observer.

Grape juice was obtained from the berries using a food