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Grape berry coatings of lemongrass oil-incorporating nanoemulsion

In-Hah Kim [a], Yoon Ah Oh [a], Hanna Lee [a], Kyung Bin Song [b], Sea C. Min [a,*]

[a] Department of Food Science and Technology, Seoul Women's University, Seoul 139-774, Republic of Korea
[b] Department of Food Science and Technology, Chungnam National University, Daejeon 305-764, Republic of Korea

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ABSTRACT

Grape berries are highly perishable and vulnerable to contamination with foodborne pathogens. Nanoemulsions of lemongrass oil (LO) were developed for coating of grape berries (*Vitis labruscana* Bailey) to improve the shelf life and microbiological safety. Vortex mixing, high-shear probe mixing, and dynamic high pressure (DHP) processing were tested as methods of incorporating LO into a carnauba-based solution (0.5–4.0 g/100 g LO). The coating solutions produced by DHP demonstrated the highest emulsion stability and resulted in uniform and continuous coatings on grape berries. The coating on the berries with 3.0 g/100 g LO initially inhibited *Salmonella typhimurium* and *Escherichia coli* O157:H7 inoculated on the berries by more than 3.2 and 2.6 log CFU/g, respectively. The coatings did not significantly alter the flavor of the berries and improved their glossiness. Antimicrobial effects against *S. typhimurium* and *E. coli* O157:H7 were exhibited during storage at 4 and 25 °C for 28 days. The coatings were also effective on reducing losses of weight, firmness, phenolic compounds, and antioxidant activity and delaying increases in total anthocyanin concentration in the berries. The LO-nanoemulsion coatings have demonstrated the potential to inhibit foodborne pathogen contamination of grape berries, and prolong their shelf life.

1. Introduction

The table grape is a highly perishable non-climacteric fruit (Meng, Li, Liu, & Tian, 2008) that undergoes various postharvest deteriorations such as changes in color and loss of weight, firmness, and nutritional and functional compounds, even during cold storage (Pastor, et al., 2011; Serrano et al., 2006). Like many other fresh produce, grapes are vulnerable to sporadic contamination with foodborne pathogens, either directly or indirectly, via animals, soil, water, dirty equipment, or human handling via cross-contamination (Alegre et al., 2012; Pinto, Lichter, Danshin, & Sela, 2006). Microorganisms on the surface or damaged areas of fruits can multiply rapidly after failures in temperature control and re-storage after opening. Contaminated fresh produce and salads cause safety problems because they are typically consumed without undergoing a lethal microbiological treatment (Badosa, Trias, Parés, Pla, & Montesinos, 2008). The incidence of foodborne disease from fruit sources has increased (Berger et al., 2010; Brassard, Gagné, Généreux, & Côté, 2012). Thus, a treatment that improves both overall quality during storage and microbiological safety against foodborne pathogens is desirable (Serrano et al., 2006).

Interest in the application of edible coatings for fruits has increased since they can create a modified atmosphere around each piece of fruit, which reduces the respiration rate and metabolic processes (Rojas-Graü, Tapia, & Martín-Belloso, 2008). Antimicrobial edible coatings include those incorporating antimicrobial substances that diffuse to the coated food and the surface of the coating (Min, Rumsey, & Krochta, 2008). Fruits are potential targets for antimicrobial edible coatings. Antimicrobial edible coatings have been investigated as measures to inhibit foodborne pathogens that contaminate strawberries (García, Martino, & Zaritzky, 2001), apples (Rojas-Graü et al., 2007), melon (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008), pears (Krasaekoopt & Mabumrung, 2008), and pineapples (Montero-Calderón, Rojas-Graü, & Martín-Belloso, 2008).

Some research has focused on the incorporation of natural or biologically derived antimicrobial materials in edible coatings (Min, Harris, & Krochta, 2005). Essential oils are effective natural antimicrobial agents (Sánchez-González et al., 2011). Several studies have reported antimicrobial properties of lemongrass oil (LO) (Azarakhsh, Osman, Ghazali, Tan, & Adzahan, 2014; Moore-Neibel, Gerber, Patel, Friedman, & Ravishankar, 2012). The use of essential oils is, however, limited due to their high volatility, cost, odor,

* Corresponding author. Tel.: +82 2 970 5635; fax: +82 2 970 5977.
E-mail addresses: smin@swu.ac.kr, scmin2@gmail.com (S.C. Min).

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and flavor (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Nevertheless, incorporating them into edible coatings could reduce their volatility and sensory impact (Serrano et al., 2006).

Nanoemulsions can result in higher stability in terms of gravitational separation, flocculation, and coalescence of oil droplets and enhanced bioactivity of emulsified oils compared with microemulsions due to the smaller droplet size and a higher surface area to droplet volume ratio (Magnuson, Jonaitis, & Card, 2011). In addition, formulation as a nanoemulsion may improve the barrier properties of the coating due to the reduced droplet size and higher homogeneity compared with microemulsions (Rao & McClements, 2012). Dynamic high pressure (DHP) processing, which involves application of emulsions to instant pressure drop, high shear, and cavitation, is used to form oil nanoemulsions (Qian & McClements, 2011). We hypothesized that DHP treatment could be used to form stable nanoemulsions of LO with a carnauba wax-based coating solution for coating grape berries and that this nanoemulsion coating would improve the microbiological safety and stability of grape berries during storage. Thus, the objectives of this study were to develop nanoemulsions of LO using DHP, which is stable and organoleptically suitable for coating grape berries, and investigate the effects of the nanoemulsion coating on microbiological safety against *Salmonella typhimurium* and *Escherichia coli* O157:H7, physicochemical qualities, including weight, color, total soluble solid content, pH, firmness, the concentrations of phenolic compounds and total anthocyanin, and the antioxidant activity of the berries during storage at 4 and 25 °C.

2. Materials and methods

2.1. Materials

Table grapes (*Vitis labruscana* Bailey) were harvested in Hwaseong (Korea) in 2012. The harvested grapes were immediately transported to the laboratory, de-stemmed, washed in an 8 g/100 g sodium hydrogen carbonate solution, and then drained and dried at 23 ± 2 °C for 2 h. The grape berries were selected carefully for uniformity in appearance, size, and color, and were free from mechanical damage or fungal decay. LO (100%) was purchased from Now Foods (Bloomingdale, IL, USA). A commercial carnauba wax-based solution for fruit coating (Safepack Products, Kfar Saba, Israel), used as the coating base material, was composed of water (75.8 g/100 g), carnauba wax (18.1 g/100 g), morpholine salts of fatty acids (3.8 g/100 g), white shellac (1.5 g/100 g), and silicon resin (0.8 g/100 g). The carnauba wax-based solution complies with the EU and USA (FDA) regulations for food additives. Tween 80, used as an emulsifier, was purchased from Ilshinwells Co., Ltd., (Seoul, Korea). All the coating materials were food grade.

2.2. Development of antimicrobial coating emulsions

2.2.1. Preparation

The 0.5, 2.0, 3.0, or 4.0 g/100 g-LO emulsion was incorporated into the carnauba wax coating solution along with Tween 80 (25 g Tween 80/100 g LO) using a vortex mixer (Vortex-genie2, Model G560, Scientific Industries Inc., New York, NY, USA), a high shear probe mixer (Ultra-Turrax, Model T25, IKA-Works, Inc., Wilmington, NC, USA), or a DHP processor (D.O.S., Siheung, Korea). Vortexing was conducted for 1 min. Homogenization using the high shear probe mixer was performed at 10,000 rpm for 60 s. DHP treatment was conducted at 172 MPa with one pass. Prior to the DHP treatment, LO and the wax coating solution were pre-homogenized using the mixer at 5000 rpm for 30 s.

2.2.2. Emulsion stability

The stability of emulsions containing 0.5, 2.0, 3.0, and 4.0 g/100 g LO, which were prepared by the three mixing methods, was analyzed after 0, 1, 2, 4, and 7 days of storage at 23 ± 2 °C. The stability was evaluated microscopically using an optical microscope (CETI, Medline Scientific Limited, Oxfordshire, UK) at a magnification of 200×. The stability of the DHP-treated emulsions was also examined by determining coalescence kinetics, measuring the mean values of backscattering of the emulsions as a function of storage time in the 4–34 mm zone of the entire length of the sample (37 mm) using an emulsion stability analyzer (Turbiscan AGS, Formulaction, Toulouse, France). The LO droplet sizes in the emulsions containing 0.5 and 4.0 g/100 g LO were determined using a particle size analyzer (Zetasizer Nano-ZS, Malvern Instruments Ltd., Worcestershire, UK).

2.2.3. Sensory evaluation

Sensory attributes of flavor and glossiness of uncoated grape berry samples and samples coated with DHP-treated emulsions at 0, 0.5, and 3.0 g/100 g LO were evaluated. The evaluation was conducted using grape berry samples prepared freshly on the day of study. Panelists were recruited from students in the Department of Food Science and Technology at Seoul Women's University (Seoul, Korea). The panelists were screened initially for the frequency that they consumed grapes. A total of 30 female panelists with ages ranging from 21 to 27 participated in the sensory evaluation and were asked to rate the berry attributes. A 9-point intensity scale was used for each attribute where 1 indicated extreme dislike and 9 indicated extreme like. The samples, coded with three digit random numbers, were served in random order. A cup of water and unsalted cracker were provided to cleanse the panelists' palates between samples.

2.2.4. Antimicrobial effects of the coating emulsion

The antimicrobial test was conducted with grape skins following the method of Kim et al. (2013). The skins of berries were trimmed to make continuous 1.00 ± 0.03 g disks (1 g each) using a sterile knife. Both sides of each skin disk were ultraviolet-sterilized at 40 W (130 kJ/m^2) for 30 min. The coating emulsion (100 μL) was spotted and spread on the smooth outer skin surface after (I + C) or before (C + I) inoculating with *S. typhimurium* (~5 log CFU/g) or *E. coli* O157:H7 (~4 log CFU/g) inside a laminar flow biohazard hood at 23 ± 2 °C at RH 30 ± 2%. Approximately 2.5 h lapsed from the time of contact between microorganisms and the coating emulsion during the I + C or C + I treatment. The disk samples were placed aseptically in a sterile bag (6.5 cm × 19 cm, Nasco WHIRL-PAK®, Fort Atkinson, WI, USA). A solution of 0.1 g/100 g peptone was dispensed into the bag, and the disk was subsequently destroyed in the bag by scraping with a spreader. The content was transferred into a centrifuge tube, serially diluted, and plated on either xylose-lysine-deoxycholate agar (XLD) (Difco™, Becton and Dickinson, Detroit, MI, USA) for enumerating *S. typhimurium* or MacConkey sorbitol (Difco™) for enumerating *E. coli* O157:H7. Both XLD and MacConkey sorbitol plates were incubated at 35 °C for 24 h before the colonies were counted.

2.3. Grape treatments

De-stemmed grape berries were dip-coated by immersion for 1 min either in the coating solution containing 0, 0.5, or 3.0 g/100 g LO to prepare the coated fruit samples or in distilled water for the uncoated control samples. The dipping was repeated twice and excess coating was drained. Coated berries were dried for 10 h at 23 ± 2 °C and 25 ± 4% relative humidity (RH) under natural convection. After drying, the integrity of the coating on each berry,