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Inhibitory effect of plant essential oil nanoemulsions against *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium on red mustard leaves

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ABSTRACT

In this study, essential oil (EO) nanoemulsions as an alternative to chlorine-based sanitizers for red mustard leaves were prepared, and their inhibitory effects against pathogenic bacteria were examined. Red mustard leaves spot-inoculated with *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium were treated with EOs alone, surfactants (SFs) (Tween 80 (T80), non-ionic; cetylpyridinium chloride (CPC), cationic) alone, and nanoemulsions formulated with EOs (0.02%) and SFs (0.002%). The results indicated that nanoemulsions containing CPC exhibited a higher inhibitory effect than those including T80. The reductions in all pathogens after treatment with nanoemulsions containing EOs and CPC were higher (0.1–0.6 log reductions) than those of 0.02% NaOCl. In addition, nanoemulsion treatment did not affect the sensory qualities of red mustard during storage. Therefore, nanoemulsions with EOs and CPC can become an effective substitute for chlorine-based sanitizers for fresh-cut produce if regulations allow the use of CPC.

Industrial relevance: Foodborne-illness outbreaks related to fresh-cut produce have increased globally. The use of chlorine-based sanitizers in the fresh-cut produce industry has been decreasing due to health-related problems. Thus, an alternative washing treatment is needed to control pathogenic bacteria contaminating fresh-cut produce. Nanoemulsions with EOs and cationic SF developed in this study can be a suitable washing agent for inactivating foodborne pathogens on fresh-cut produce.

1. Introduction

One reason for increased demand worldwide for fresh-cut produce is greater consumer interest in health and well-being (Meireles, Giaouris, & Simões, 2016). Fresh-cut produce can offer many benefits, such as being a good source of vitamins, minerals, and fiber (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). Red mustard belongs to the family Brassicaceae, and it is economically important in the fresh-cut produce industry (Santos, Oliveria, Ibáñez, and Herrero, 2014). In particular, it contains various phenolic compounds compared to other Brassicaceae vegetables. Thus, red mustard is a popular fresh-cut produce to the consumers because of its high nutritional value (Lin, Sun, Chen, & Harnly, 2011; Santos, Oliveria, Ibáñez, & Herrero, 2014). However, the incidence of foodborne-illness outbreaks associated with fresh-cut produce has also increased steadily (Wadamori, Gooneratne, & Hussain, 2017). In particular, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. have been regarded as the major foodborne pathogens that can easily contaminate fresh-cut produce (Goodburn & Wallace, 2013). Thus, proper disinfection methods are needed for these foodborne pathogens contaminating fresh-cut produce.

The washing process is a general technique to reduce pathogenic bacteria and other residuals, such as soil, insects, and pesticides on the surface of fresh-cut produce (Gil, Selma, López-Gálvez, & Allende, 2009). Particularly, for improving the microbial safety of fresh-cut produce, chlorine has been commonly used in the fresh-cut produce industry due to its low cost and wide range of usage compared to other chemical sanitizing agents (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009). However, washing with chlorine for fresh-cut produce can cause the production of carcinogenic by-products (Ramos et al., 2013). Therefore, many studies have been performed on appropriate alternatives for fresh-cut produce (Meireles et al., 2016).

Natural antimicrobial agents from plants are regarded as a novel substitute for the use of chlorine in the fresh-cut produce industry (de Medeiros Barbosa et al., 2016). Especially, essential oils (EOs) originating from various parts of plants, such as leaves, barks, buds, flowers, and roots have emerged as suitable antimicrobial agents because of their antimicrobial effect against foodborne pathogens related to fresh-

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cut produce (Patrignani, Siroli, Serrazanetti, Gardini, & Lanciotti, 2015). EOs are a group of natural antimicrobial agents recognized as safe substances by the Food and Drug Administration and have received attention as an effective alternative to chemical sanitizers such as chlorine (Zhang, Ma, Critzer, Davidson, & Zhong, 2016). However, there is a critical limitation such as the low water solubility of EOs to apply as a washing agent for fresh-cut produce (Donsi and Ferrari, 2016). In addition, when EOs are applied to foods, it is necessary to use a large amount to obtain the antimicrobial activity. In addition, high concentration of EOs may affect the food quality such as flavor negatively (Burt, 2004). Therefore, a proper technique is required to overcome these problems.

Emulsion systems are general techniques for combining hydrophobic substances such as EOs in a water system using a surfactant (SF) and special equipment, such as a high shear homogenizer, ultrasonicator, and high-pressure homogenizer (Donsi and Ferrari, 2016). As a synthetic SF, Tween 80 has been used for formulating EOs emulsions (Donsi and Ferrari, 2016). However, there are inadequate aspects, such as high cost and strong flavor, for developing a washing agent for fresh-cut produce (Komaiko & McClements, 2016). Thus, developing EOs nanoemulsions requires optimal amounts of EOs and SFs. In addition, several studies have reported that polysorbate-based SFs such as Tween 80 can reduce the antimicrobial activity of EOs (El-Sayed, Chizzola, Ramadan, & Edris, 2017). To resolve this problem of Tween 80, a more suitable SF should be found to increase the antimicrobial activity of EOs when applied to washing agents for fresh-cut produce. Recently, there have been studies on the antimicrobial activity of EOs nanoemulsions formulated with a cationic SF (Hilbig, Ma, Davidson, Weiss, & Zhong, 2016; Ma, Michael Davidson, Critzer, & Zhong, 2016). However, these studies are focused on antimicrobial properties only, not washing application for fresh-cut produce.

Quaternary ammonium compounds (QAC) are cationic surfactants that can be applied to sanitize the food surface (Meireles et al., 2016). QAC as a sanitizing agent has many advantages, such as being stable in water, eco-friendly, odorless, and effective under various pH and temperature conditions (Meireles et al., 2016; Tan et al., 2015). In particular, it has been reported that cetylpyridinium chloride (CPC), one of QAC, exhibits a powerful antimicrobial activity against many foodborne pathogens including L. *monocytogenes*, *E. coli* O157:H7, and *Salmonella* (Phua, Neo, Khoo, & Yuk, 2014). In addition, according to the report of Yang, Cheng, Swem, and Li (2003), CPC has the potential to be applied as a sanitizing agent for fresh-cut produce. Some studies associated with the sanitizing effect of CPC on fresh-cut produce have been reported (Phua et al., 2014; Tan et al., 2015; Yang et al., 2003). However, there are no studies on washing effect of EOs nanoemulsions formulated by CPC for fresh-cut red mustard leaves.

Therefore, the objective of this study was to examine the washing effect of EO nanoemulsions with different types of SFs (non-ionic T80 or cationic CPC) against L. *monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium inoculated on red mustard leaves. In addition, their effects on the changes in sensory qualities, such as color and tissue of the red mustard leaves during storage, were examined.

2. Materials and methods

2.1. Bacterial culture and inoculum preparation

Two strains of L. *monocytogenes* (ATCC 19111 and 19,115), *E. coli* O157:H7 (ATCC 43889 and NCTC 12079), and *S.* Typhimurium (ATCC 14028 and KCTC 2421) were used in this study for inoculation. All strains of each pathogen were streaked onto tryptic soy agar (Difco Co., Detroit, MI, USA) and incubated overnight at 37 °C. After incubation, single colonies obtained from each strain of three pathogens were transferred to sterile disposable corning tubes with 25 mL of each selected broth (brain heart infusion broth for L. *monocytogenes*, tryptic soy broth for *E. coli* O157:H7 and *S.* Typhimurium), and then cultured in a shaking incubator (150 rpm) at 37 °C for 24 h. All bacterial cultures were harvested by centrifugation at 3000 × *g* for 10 min (4 °C). The resulting cell pellets were washed twice using sterile peptone water (SPW, 0.1%) and then re-suspended with 0.1% SPW. Ten milliliters of bacterial suspensions were combined using a sterile serological pipette for preparing the cocktail inoculum of each pathogen. For subsequent experiments, the inoculum was diluted with 0.1% SPW at a cell concentration of approximately 8–9 log CFU/mL.

2.2. Determination of essential oils MIC

Minimum inhibitory concentrations (MIC) of 20 different essential oils (EOs; oregano, cinnamon-bark, cinnamon-leaf, thyme, clove bud, clove leaf, coriander seed, coriander leaf, geranium, lemon grass, rosewood, marjoram, tea tree, basil, sage, eucalyptus, myrtle, bergamot, rosemary, and ginger) against L. *monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium were examined using a broth micro-dilution method in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2012). All EOs (4%, 100 μL) in distilled water were two-fold serially diluted in a 96-well plate containing 100 μL of Mueller-Hinton broth (Difco Co.). Each pathogen inoculum (100 μL, approximately 8–9 log CFU/mL) prepared as described above was added to each well to prepare final concentrations of EOs at 0, 0.001, 0.002, 0.0039, 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1, and 2%. Ninety-six well plates of each pathogen were incubated overnight at 37 °C, and growth was measured using a Microplate reader (Model US/680, Bio-Rad Laboratories, Inc., USA). To determine the MIC values of all EOs, the lowest concentration inhibiting the microbial growth was selected with the absorbance showing below 0.05 at 595 nm.

2.3. Preparation of washing solution

Based on the MIC test, cinnamon-bark, cinnamon-leaf, and oregano EOs were selected for the washing application because these EOs have the lowest MIC among 20 EOs. The concentration (0.02, 0.03, and 0.05%) of a single EOs treatment was chosen in the range of their MIC (0.0125–0.0625%). Especially, 0.02% of each EO was applied for formulating nanoemulsions to compare the washing effect with sodium hypochlorite (SH, NaOCl, 0.02%) because the maximal used concentration of SH is 0.02% (200 ppm) for fresh-cut produce washing. The concentration (0.002%) of SFs (Tween 80 [T80, non-ionic] and cetylpyridinium chloride [CPC, cationic]; Sigma-Aldrich Chemical Co., St. Louis, USA) used in this study was established in order to avoid using excess SFs (surfactants to essential oils ratio; 0.1 [SFs]:1 [EOs]). To prepare each single washing solution, 100 mL of distilled water containing the amount of each EO and SF as described above was stirred with 500 rpm for 10 min before the experiments. For formulating EO nanoemulsions, the amount of SFs (T80 and CPC) equal to 0.002% was added to distilled water (100 mL) and then stirred with 500 rpm for 1 min. After stirring, 20 μL of EOs (0.02%) was sequentially combined with this prepared solution and mixed at a speed of 500 rpm for 10 min.

2.4. Characterization of essential oil nanoemulsions

The average particle size (*Z*-average), distribution (PDI, polydispersity index), and ζ-potential of the EO nanoemulsions were measured by dynamic light scattering with Nano ZS (ZetaSizer, Malvern Instruments, Malvern, UK). Measurements were conducted with at least 10 runs following 2 min of an equilibrium period.

2.5. Sample preparation and inoculation

Red mustard leaves were purchased from a local market in Daejeon, Korea. Red mustard samples were washed with running tap water to remove soil and dust, drained using an absorbing paper, and then cut into 5 × 10 cm pieces with similar weight (1.5 ± 0.2 g) by a sterile