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Combined treatment of cinnamon bark oil emulsion washing and ultraviolet-C irradiation improves microbial safety of fresh-cut red chard

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ABSTRACT

Cationic surfactant (cetylpyridinium chloride (CPC))-based trans-cinnamaldehyde (TC) or cinnamon bark oil (CB) emulsions (TCE and CBE, respectively) were used to wash fresh-cut red chard (FCR). To enhance the washing effect, CBE (0.02% CB + 0.002% CPC) was combined with ultraviolet-C treatment (1.2 kJ/m^2), and the combined treatment reduced the populations of *Listeria monocytogenes* and *Salmonella* Typhimurium on FCR by 1.29 and 1.40 log CFU/g, respectively, as compared to populations after water washing. The log reduction levels of the combined treatment against these two pathogens were higher than those of NaOCl (0.2 g/L) treatment (0.68 and 1.13 log CFU/g, respectively). After the combined treatment, the membranes of both pathogens on the red chard surface were observed to be damaged through scanning electron microscopic images. In addition, the microbial reductions by the combined treatment were maintained during storage, and sensory evaluation results showed that it did not affect the quality of the red chard. Therefore, a CBE/UV-C combined treatment can be considered a satisfactory washing method for FCR to replace chlorination.

1. Introduction

The demand for fresh-cut vegetables (FCVs) has been increasing (Kang & Song, 2018). Due to the characteristics of FCVs consumed in a raw state, foodborne illness outbreaks related to FCVs are of concern (Yu, Neal, & Sirsat, 2018). Red chard (*Beta vulgaris* cv. *cicla*) is rich in vitamin C and phenolic compounds (Tomás-Callejas, Boluda, Robles, Artés, & Artés-Hernández, 2011), and it is commonly consumed as a mixed salad in a FCV form (Tomás-Callejas et al., 2012). Minimally processed FCVs have a short shelf life compared to that of whole vegetables, and they are susceptible to major foodborne pathogens, such as *Listeria monocytogenes* and *Salmonella* spp. (Ma, Zhang, Bhandari, & Gao, 2017). Recently, FCVs have become a major mediator of salmonellosis caused by *Salmonella* spp. (Mishra, Guo, Buchanan, Schaffner, & Pradhan, 2017). In addition, according to the Food and Drug Administration (FDA) report in 2016, 19 cases of listeriosis caused by *L. monocytogenes* in FCVs have occurred in the U.S. (Mishra et al., 2017). Thus, it is essential to inactivate the pathogens in FCVs using a novel sanitizer (Kang & Song, 2018).

In general, chlorination is widely used as a washing method for FCV because of its low cost and good washing effect against various bacteria (Kang & Song, 2018). However, chlorination can generate harmful substances (Artés-Hernández, Martínez-Hernández, Aguayo, Gómez, & Artés, 2017, pp. 7–44). For this reason, in some European countries, chlorination has been banned for FCV washing, and a new washing agent is needed to replace it (Artés-Hernández et al., 2017, pp. 7–44).

Due to their strong antibacterial properties, essential oils (EOs) have been used to sanitize FCVs instead of chlorination (Kang & Song, 2018). Among them, cinnamon bark oil (CB) and its main component, trans-cinnamaldehyde (TC), have been used as antimicrobial agents owing to their high antibacterial properties (Park, Kang, & Song, 2018; Zhang, Chen, Critzer, Davidson, & Zhong, 2017). Although the antibacterial mechanisms of EOs have not been fully revealed yet (Kang & Song, 2018), most EOs penetrate the cytoplasmic membranes of bacteria and cause cell lysis to inactivate the bacteria (Artés-Hernández et al., 2017, pp. 7–44).

Cetylpyridinium chloride (CPC), a kind of quaternary ammonium compound, is a cationic surfactant (SF) and has strong antibacterial properties (Park et al., 2018). Several studies have examined the application of CPC as a washing agent for FCVs (Park et al., 2018; Tan et al., 2015). Recently, Kang and Song (2018) reported that EO emulsions containing low concentrations of CPC were effective in inactivating pathogens in fresh-cut red mustard compared to the use of Tween 80 as a surfactant. In addition, cinnamon EO emulsions containing CPC showed synergistic antibacterial activity against *E. coli* O157:H7 and *S.* Typhimurium inoculated on basil leaves in a previous study (Park et al., 2018), although the concentration used was somewhat high (EOs, 0.25%; CPC, 0.05%).

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Ultraviolet-C (UV-C) irradiation is mainly applied as a type of non-thermal processing, which inactivates bacteria by damaging their DNA (Chun, Kim, Kim, & Song, 2009). In particular, low-dose UV-C irradiation has been recommended because high-dose irradiation can cause undesirable effects on the surface of FCVs (Kim & Song, 2017). Low-dose UV-C irradiation is free of residue after treatment, and it does not affect FCV surfaces and improves the shelf life (Artés-Hernández et al., 2017, pp. 7–44). Therefore, we investigated the effect of washing with an EO emulsion prepared by adding CPC to CB or TC, and examined the antimicrobial activity of the combined treatment with low-dose UV-C irradiation against *L. monocytogenes* and *S*. Typhimurium inoculated on fresh-cut red chard (FCR). In addition, we examined the effect of the combined treatment on FCR quality.

2. Materials and methods

2.1. FCR and bacterial cocktail inoculum

The red chard samples were purchased from a local market (Daejeon, Korea), and cut into 10 × 5 cm pieces (2.5 ± 0.1 g) using a sterile knife. Two strains of *L. monocytogenes* (ATCC 15,313 and KCTC 13,064) and *S*. Typhimurium (ATCC 14,028 and KCTC 2421) were inoculated on FCR surfaces, and bacterial cocktail inoculums were prepared by the method described by Kang and Song (2018). The population of bacterial cocktail inoculums was approximately 7–8 log CFU/mL.

2.2. Spot-inoculation of pathogens on FCR surface

To reduce pre-existing microorganisms on FCR, the abaxial and adaxial sides of FCR were treated with UV-C treatment for 10 min, respectively. It was confirmed that both pathogens were not detected on FCR after UV-C treatment. Each bacterial cocktail inoculum was spot-inoculated 5 times with 50 μL (total 250 μL) per FCR, and the inoculated FCR samples were dried on a clean bench for 1 h to allow pathogen attachment.

2.3. Preparation and washing treatment of EO emulsion solutions

The concentration of CB (TC; approximately 70%, Gooworl Co., Daegu, Korea) and TC (99%, Sigma-Aldrich Co., St. Louis, MO, USA) was fixed at 0.02% in order to compare the washing effect with 200 ppm sodium hypochlorite (NaOCl), which is the maximum concentration that can be applied to FCV (Park et al., 2018). To avoid excessive use of SF (CPC, Sigma-Aldrich Co.), the ratio of EOs (CB and TC) and a SF was set to 10:1 (EOs; 0.02%, CPC; 0.002%). All solutions were dissolved in distilled water and homogenized by ultrasonication (500 W, Sonics & Materials Co., Danbury, CT, USA) at 20 °C for 5 min. The inoculated FCR samples (10 g) were immersed in a sterile beaker containing the prepared washing solution (300 mL) and gently agitated on a rotator (Changshin Co., Seoul, Korea) for 3 min. After washing, the samples were rinsed with distilled water and dried for 30 min on sterile aluminum foil placed on a clean bench. Water washing treatment was used as a control to compare the antimicrobial activity of the emulsion solutions.

2.4. Physicochemical properties of CB or TC emulsion

The Z-average and ζ-potential of CB or TC emulsion (CBE and TCE, respectively) solution were examined using a Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK).

2.5. Measurement of wettability of solutions on the FCR surface

The wettability of the washing solutions (Water, CB, TC, CPC, CBE, and TCE) on the FCR (2 × 2 cm) surface was measured using a contact angle analyzer (Phoenix 300 Plus, SEO Co., Ansung, Korea). One drop (3 μL) of each solution was dropped onto the FCR surface using a syringe and the contact angle (°) was measured three times at 25 °C.

2.6. Combined treatment with UV-C

After washing with the EO emulsion solutions, the samples (10 g) were further treated with UV-C irradiation. This was carried out using a UV-C device with UV germicidal lamps (G15T8, Phillips, Eindhoven, Netherland) as described by Chun et al. (2009) with minor modifications (Kim & Song, 2017). Prior to treatment, the UV-lamps were turned on for 30 min, and the UV-C intensity (10 W/m^2) was measured using a UV radiometer (UV-340, Lutron Electronic Co., Taipei, Taiwan). The FCR samples were then placed on the plate in the UV-C device and irradiated from top and bottom sides. The UV-C irradiation time at 1.2 kJ/m^2 was 2 min. For comparison, a single UV-C and a NaOCl (0.2 g/L) treatment were also carried out. After treatment, the FCR samples (control, water washing, and combined treatment) were stored in LDPE (25 × 30 cm, 60 μm thickness) bags at 4 °C, 62 ± 1% RH for 9 days.

2.7. Microbiological analysis

The FCR samples (5 g) were put into a sterile bag containing 0.1% sterile peptone water (SPW, 45 mL) and homogenized with a stomacher for 3 min. The homogenized solution (1 mL) was placed in a sterile tube containing 0.1% SPW (9 mL) for serial dilution. The diluted solution (100 μL) was plated onto Oxford Medium Base and Xylose lysine deoxycholate agar (Difco Co., Detroit, MI, USA), and plates were placed in an incubator at 37 °C for 48 h. Microbial enumeration was performed in triplicate.

2.8. Scanning electron microscopy (SEM) analysis

Low-voltage field-emission SEM (FE-SEM, Zeiss MERLIN, Oberkochen, Germany) was used to investigate the morphology of both pathogens on FCR. Samples (1 × 1 cm) were immersed in sodium phosphate buffer (SPB, pH 7.0, 0.1 M) containing 2.5% glutaraldehyde at 4 °C for 12 h to fix the pathogens to the FCR surface, followed by washing with SPB. The samples were dehydrated by immersing in different concentrations (50, 70, and 100%) of ethanol for 5 min successively, and the dehydrated samples were completely dried on a clean bench. The samples were then attached to carbon tape and coated with platinum for 2 min. FE-SEM images were measured at 5 kV with 5,000× magnification.

2.9. Color changes and total phenolic contents (TPC)

The color changes in FCR samples during storage were expressed by Hunter color values (L, a, and b) using a colorimeter (Minolta Camera Co., Osaka, Japan). The FCR leaf surface area of the green part except the red leaf vein was measured ten times. In addition, total color differences (ΔE) were calculated using Hunter color values.

The TPC changes in FCR were determined according to the method described by Rumbaoa, Cornago, and Geronimo (2009) with minor modifications. Lyophilized FCR samples (0.5 g) were placed in a sterilized bottle containing methanol (30 mL) and extracted at 20 °C for 24 h using a shaking incubator at 200 rpm. The extracted solution (100 μL), distilled water (4.5 mL), 2 N Folin-Ciocalteu's phenol reagent (100 μL, Sigma-Aldrich Co.), and 20% sodium carbonate (300 μL, Sigma-Aldrich Co.) were added into a sterilized tube and reacted at 20 °C for 30 min. Gallic acid was used as a standard, and five replications were performed. The results were expressed as milligrams gallic acid equivalent (GAE) per 100 g dry weight of the sample.