J. Microbiol. Biotechnol. (2018), 28(4), 503–509
https://doi.org/10.4014/jmb.1711.11018

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Improving the Microbial Safety of Fresh-Cut Endive with a Combined Treatment of Cinnamon Leaf Oil Emulsion Containing Cationic Surfactants and Ultrasound

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Received: November 8, 2017
Revised: January 11, 2018
Accepted: January 19, 2018

First published online
February 1, 2018

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pISSN 1017-7825, eISSN 1738-8872

Endive is widely consumed in a fresh-cut form owing to its rich nutritional content. However, fresh-cut vegetables are susceptible to contamination by pathogenic bacteria. This study investigated the antibacterial activities of the combined treatment of cinnamon leaf oil emulsion containing cetylpyridinium chloride or benzalkonium chloride (CLC and CLB, respectively) as a cationic surfactant and ultrasound (US) against *Listeria monocytogenes* and *Escherichia coli* O157:H7 on endive. The combined treatment of CLC or CLB with US reduced the population of *L. monocytogenes* by 1.58 and 1.47 log colony forming units (CFU)/g, respectively, and that of *E. coli* O157:H7 by 1.60 and 1.46 log CFU/g, respectively, as compared with water washing treatment. The reduction levels of both pathogens were higher than those observed with 0.2 mg/ml sodium hypochlorite. In addition, the combined treatment showed no effect on the quality of the fresh-cut endive (FCE). In particular, the degree of browning in FCE was less for the treatment group than for the control and water washing treatment groups. Thus, cationic surfactant-based cinnamon leaf oil emulsions combined with US may be an effective washing treatment for the microbial safety of FCE.

Keywords: Cinnamon leaf oil, combined treatment, emulsion, fresh-cut endive

Introduction

The demand for fresh-cut vegetables (FCVs) has increased, owing to their advantages such as easy consumption and health benefits. However, FCVs are susceptible to microbial contamination during harvesting or distribution to consumers [1]. Endives (*Cichorium endivia* L. var. *crispum*), the fresh-cut commodity used in this study, are popularly consumed as a salad in the United States and Europe [2]. These are classified as leafy greens with high risk of foodborne illness [3]. In particular, foodborne diseases caused by *Listeria monocytogenes* in fresh-cut endive (FCE) have been reported [4]. Therefore, it is necessary to ensure the microbiological safety of FCE during storage and distribution. Chlorination is a common washing method for FCVs and used in the range of 50−200 ppm [5]. Although chlorination offers an advantage of low processing cost, negative perceptions related to some harmful substances generated during washing often limits its use. Some countries such as Belgium, Denmark, Netherlands, Germany, and Switzerland have banned the chlorination method [6] and many studies have been undertaken to replace chlorine-based sanitizers [5, 7, 8].

Essential oils (EOs) act as natural antimicrobial agents and have been applied in the food industry for a long time, as these have been classified as generally recognized as safe (GRAS) substances [9]. Of these, cinnamon leaf oil (CL), which contains eugenol as a major compound, has been widely used for its excellent antibacterial effect [10]. However, aside from its high cost, the use of a high concentration of EO for washing FCVs may negatively affect the quality of the vegetable product [11]. In addition, EOs display limitations, such as low solubility in water [12]. To resolve this issue, EO emulsion supplemented with surfactants may be used. Previously, it has been reported that EO emulsions were more inhibitory against pathogenic bacteria on FCVs than EOs only [8].

Cetylpyridinium chloride (CPC) and benzalkonium chloride (BC) are a class of quaternary ammonium compounds and cationic surfactants. Both CPC and BC are widely used in the food industry and exhibit antibacterial mechanisms [13, 14]. These compounds easily bind to the microbial membranes by electrostatic attractions [13]. The inhibitory effect of CL against foodborne pathogens was improved with the use of cationic surfactants, instead of nonionic surfactants such as Tween80, with antibacterial activity. In addition, EO emulsion may be combined with ultrasound (US) treatment to increase its inhibitory effect against pathogenic bacteria. Owing to the continuous mechanism of cavitation and sonolysis, US treatment results in disruption of the cell wall of microorganisms, thereby inactivating microbial cells [15]. However, US treatment alone fails to induce a significant effect on the inactivation of microorganisms when applied to foods [16]. Therefore, US treatment can be combined with EOs [17].

This study investigated the antibacterial activity of CL emulsion combined with US treatment against foodborne pathogens inoculated on FCE. In addition, sodium hypochlorite (NaOCl) treatment was compared with the combined treatment to determine which would have a more substantial washing effect in the FCV industry.

Materials and Methods

Materials

Endives harvested from a local farm (Korea) in July 2017 were used in this study. Upon harvest, the endives were kept at 4°C and used within 24 h for the experiment. After separated from the stalk, the endive leaves were cut into 4 × 4 cm pieces using a flame-sterilized knife. CL (eugenol 80%, trans-cinnamaldehyde 16%), CPC, and BC were obtained from Gooworl Co. (Korea), Sigma-Aldrich Co. (USA), and Alfa Aesar Co. (USA), respectively.

Preparation of Bacterial Culture and Inoculation of Pathogens on Endives

Cocktails of *L. monocytogenes* (KCTC 13064, ATCC 15313) and *Escherichia coli* O157:H7 (ATCC 43889, NCTC 12079) were prepared according to the method described by Kang and Song [7]. The population of each bacterial cocktail was approximately 8−9 log CFU/ml. The front and back sides of the FCEs at a distance of 50 cm from the 254 nm ultraviolet-C (UV-C) lamp (G15T8; Phillips, Netherland) were treated with UV-C for 10 min.

It was analyzed and confirmed that no pathogens were detected prior to inoculation. Each bacterial cocktail inoculum was spot inoculated three times on the inner side of the endive leaves at a volume of 50 μl per endive (150 μl total) and dried on a sterile aluminum foil for 1 h.

Single Treatment with CL, CPC, or BC

For single treatment, the CL concentration was adjusted to 0.05%, whereas the concentration of CPC and BC was adjusted to 0.005%. All washing solutions were homogenized with a ultrasonicator (500 W; Sonics & Materials Co., USA) at 20°C for 5 min. The inoculated endives (20 g) and the washing solution (300 ml) were placed in a sterile beaker and gently agitated for 3 min. After washing, the endives were rinsed with distilled water and dried for 1 h on a sterile aluminum foil. The dried endives (10 g) were transferred into a sterile bag containing 0.1% sterile peptone water (SPW, 90 ml) and homogenized with a stomacher for 3 min. The homogenate (1 ml) was placed in a sterile tube containing 9 ml of 0.1% SPW. After 10-fold serial dilution, 100 μl of the homogenate was plated onto Oxford medium base (OMB; Difco Co., USA) and MacConkey sorbitol agar (MSA; Difco Co., USA). All plates were incubated at 37°C for 48 h. All experiments were performed in triplicates.

Preparation and Treatment of CL Emulsions

The emulsion solution was prepared at fixed EO concentration. First, 0.005% of CPC or BC was dissolved in distilled water and 0.05% CL was added to each mixture at a final volume of 300 ml. Both emulsion solutions, CL/CPC (CLC) and CL/BC (CLB), were homogenized with an ultrasonicator (500 W, 20°C) for 5 min and prepared as a washing solution. Washing treatment was conducted in the same manner as for the single treatment described above.

Characteristics of CL Emulsion

The characteristics (Z-average and ζ potential) of the emulsion solutions (CLC and CLB) were measured with zetasizer nano ZS (Malvern Instruments Ltd., UK).

CLC or CLB Treatment Combined with US

As a combined treatment, US (Mujigae Co., Korea) at 40 KHz and 140 W was performed with the emulsion treatment (CLC or CLB). The inoculated endives (20 g) were placed in a sterilized beaker and treated with each washing solution (300 ml), followed by US treatment for 3 min. A single US treatment with distilled water was also performed in the same way. In addition, 0.2 mg/ml NaOCl treatment was performed for comparing the inhibitory activity against pathogenic bacteria.

Microbiological Analysis during Storage

After washing treatment, the treated endives were stored in a low-density polyethylene bag (25 × 30 cm, 60 μm thickness; Cleanwrap Co., Korea) at 4°C for 8 days. During storage, a homogenized sample solution was prepared using a stomacher with 0.1% SPW and plated onto OMB and MSA, and the microbial population was determined following incubation at 37°C for 48 h.

Changes in the Color and Browning Index during Storage

The color changes of the endive samples were measured with a colorimeter (Minolta Camera Co., Japan). The color values were represented by the Hunter values *L*, *a*, and *b*. To determine the degree of browning during storage, the browning index of each treated endives was determined [18]. The treated endives (5 g) were blended with 25 ml of 10% trichloroacetic acid for 1 min. After incubating at 37°C for 2 h, the blending solution was centrifuged at 10,000 ×*g* for 15 min and the supernatant was filtered. The absorbance of the filtered supernatant was measured at 420 nm wavelength using a spectrophotometer (UV-2450; Shimadzu Co., Japan).

Analysis of Texture and Total Phenolic Content (TPC) during Storage

The hardness of the endive samples was measured with a texture analyzer (TA-XT2i; Stable Micro Systems Ltd., UK). Endive samples (4 × 4 cm) were placed on the pedestal of the texture analyzer and the hardness of the endive was measured by moving the probe at 1 mm/sec. The maximum peak in the compression process was recorded as hardness. Thirty replicates per treatment were performed and the hardness was expressed in Newton unit. The changes in TPC were determined [19]. Freeze-dried endive powder (5 g) and 100 ml of methanol were added to a sterilized bottle at a ratio of 1:20 (w/v) and the extraction was performed in a shaking incubator at 170 rpm for 24 h at 25°C. The extracted solution was centrifuged at 8,000 ×*g* for 15 min. A portion of the supernatant (100 μl), 1.5 ml of distilled water, 100 μl of 2 N Folin-Ciocalteu phenol reagent, and 300 μl of 20% sodium carbonate were added to a sterilized tube in the above order and allowed to react for 1 h. After incubation, the absorbance value was measured at 765 nm wavelength using a spectrophotometer (UV-2450). Various concentrations (0.012, 0.025, 0.05, and 0.1 mg/l) of gallic acid were used as standards. The results were expressed as milligram gallic acid equivalent (GAE) per 100 g dry weight.

Statistical Analysis

Statistical analysis was performed to analyze the significant differences among the data. The data obtained from this experiment were analyzed by Duncan's multiple range test ($p < 0.05$) using the Statistical Analysis System program ver. 9.4 (SAS Institute Inc., USA).

Results and Discussion

Physicochemical Properties of CLC and CLB

Table 1 shows the particle size and zeta (ζ) potential of both emulsions. CLC and CLB had average sizes of 375.67 ± 23.9 and 344.40 ± 19.4 nm, respectively. These values were higher than those reported for an oregano oil emulsion [11]. The size of the emulsion depends on the type and amount of the surfactant used as well as the emulsion preparation method. In general, small-sized emulsions require a high concentration of surfactants [12], although these may not be applicable for washing owing to their high cost. Therefore, the appropriate size of EO emulsion is important for its application in the food industry [20]. The surface charge of CLC and CLB was positive, with a zeta (ζ) potential of 87.70 ± 2.60 and 71.87 ± 2.29 mV, respectively, attributable to the property of cationic surfactants CPC and BC. Li *et al*. [21] reported a negatively charged surface for thymol emulsion containing sodium lauryl sulfate as an anionic surfactant. It is known that the zeta potential of EO emulsions is related to the charge of the surfactants [22]. These chemical properties of cationic surfactants allow emulsions of CLC and CLB to penetrate easily into the negatively charged cell membranes.

Table 1. Characteristics of cinnamon leaf oil emulsions.

Emulsion sample	Z-average (nm)	ζ-potential (mV)
CLC	375.67 ± 23.90	87.70 ± 2.60
CLB	344.40 ± 19.35	71.87 ± 2.29

Data are presented as the mean ± SD.
CLC, cinnamon leaf essential oil 0.05% + cetylpyridinium chloride 0.005%; CLB, cinnamon leaf essential oil 0.05% + benzalkonium chloride 0.005%.

Effects of CL Emulsion Treatment

The reduction in the populations of both pathogenic bacteria expressed as log reduction was compared with water washing treatment (Fig. 1). In comparison with water washing treatment, treatment with CL (0.05%), CPC (0.005%), and BC (0.005%) reduced the population of

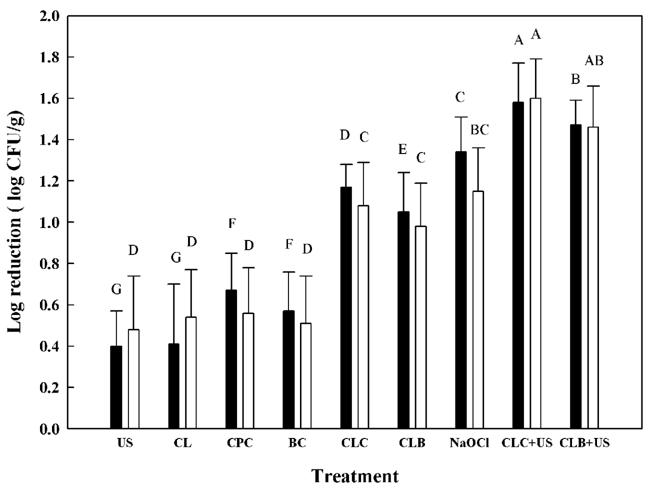

Fig. 1. Change in the populations of *Listeria monocytogenes* and *Escherichia coli* O157:H7 inoculated on fresh-cut endive.
US, ultrasound 140 W; CL, cinnamon leaf oil 0.05%; CPC, cetylpyridinium chloride 0.005%; BC, benzalkonium chloride 0.005%; CLC, CL 0.05% + CPC 0.005%; CLB, CL 0.05% + BC 0.005%; NaOCl, sodium hypochlorite 0.2 mg/ml; CLC+US, CL/CPC emulsion + US 140 W; CLB+US, CL/BC emulsion + US 140 W. ■, *L. monocytogenes*; □, *E. coli* O157:H7.

Table 2. Changes in the populations of microorganisms (log CFU/g) on fresh-cut endive during storage.

Treatment	*L. monocytogenes*				*E. coli* O157:H7			
	Storage time (day)				Storage time (days)			
	0	2	5	8	0	2	5	8
Control	6.28 ± 0.20^{Aa}	5.87 ± 0.23^{Ab}	4.98 ± 0.03^{Ac}	5.15 ± 0.30^{Ac}	6.25 ± 0.22^{Aa}	6.10 ± 0.26^{Aab}	5.90 ± 0.08^{Ab}	5.51 ± 0.08^{Ac}
Water	5.90 ± 0.23^{Ba}	5.74 ± 0.37^{Aa}	4.89 ± 0.16^{Ab}	5.01 ± 0.26^{Ab}	5.97 ± 0.03^{Aa}	5.39 ± 0.12^{Bb}	5.33 ± 0.21^{Bb}	5.33 ± 0.23^{Ab}
Combined treatment	4.44 ± 0.31^{Ca}	4.33 ± 0.20^{Ba}	3.59 ± 0.16^{Bb}	3.42 ± 0.03^{Bb}	4.82 ± 0.27^{Ba}	4.77 ± 0.09^{Ca}	4.71 ± 0.01^{Ca}	4.24 ± 0.22^{Bb}

Data are presented as the mean ± SD.

$^{A-C}$Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

$^{a-c}$Any means in the same row followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Combined treatment: cinnamon leaf oil 0.05%/cetylpyridinium chloride 0.005% emulsion + ultrasound 140 W.

L. monocytogenes by 0.41, 0.67, and 0.57 log CFU/g, respectively, and that of *E. coli* O157:H7 by 0.54, 0.56, and 0.51 log CFU/g, respectively. Treatment with both emulsions (CLC and CLB) reduced the population of *L. monocytogenes* by 1.17 and 1.05 log CFU/g, respectively, and that of *E. coli* O157:H7 by 1.08 and 0.98 log CFU/g, respectively. The antimicrobial activity of thyme EO emulsion, prepared by the addition of lauric arginate, a cationic surfactant, was higher than that of thyme EO alone [23]. Therefore, the observations in our study may be associated with the additive effect of the antibacterial activities of cationic surfactants, suggesting that the emulsion treatment is more effective against both pathogens than the CL treatment alone.

Effects of the Combined Treatment

The washing effect against both pathogens was examined by the combination of two emulsions and US treatment (Fig. 1). A single US treatment resulted in the reduction of *L. monocytogenes* and *E. coli* O157:H7 by 0.40 and 0.48 log CFU/g, respectively, as compared with water washing treatment. Thus, the microbial reduction observed was not better than the other single treatments. These results are in line with those previously reported, wherein a single US treatment was ineffective against microbial inactivation [16]. In contrast, CLC/US and CLB/US treatment reduced the population of *L. monocytogenes* by 1.58 and 1.47 log CFU/g, respectively, thereby showing an additive effect of the single US treatment. Furthermore, CLC/US and CLB/US treatments also reduced the population of *E. coli* O157:H7 by 1.60 and 1.46 log CFU/g, respectively, as compared with water washing treatment. In general, Tween 80, a polyoxyethylene sorbitan series

Table 3. Changes in Hunter color values of fresh-cut endive during storage.

Color parameter	Treatment	Storage time (days)			
		0	2	5	8
L	Control	71.70 ± 0.41^{Aa}	71.71 ± 0.67^{Aa}	71.70 ± 0.69^{Aa}	71.70 ± 0.61^{Aa}
	Water	71.41 ± 0.91^{Aa}	71.48 ± 0.79^{Aa}	71.49 ± 0.82^{Aa}	71.54 ± 0.58^{Aa}
	Combined treatment	71.52 ± 0.69^{Aa}	71.64 ± 0.51^{Aa}	71.60 ± 0.56^{Aa}	71.67 ± 0.45^{Aa}
a	Control	-1.55 ± 0.14^{Ab}	-1.50 ± 0.10^{Ab}	-1.48 ± 0.26^{Ab}	-1.01 ± 0.10^{Aa}
	Water	-1.46 ± 0.11^{Ac}	-1.46 ± 0.05^{Ac}	-1.33 ± 0.09^{Ab}	-1.05 ± 0.10^{Aa}
	Combined treatment	-1.49 ± 0.26^{Aa}	-1.49 ± 0.08^{Aa}	-1.45 ± 0.10^{Aa}	-1.34 ± 0.14^{Ba}
b	Control	4.31 ± 0.19^{Ad}	5.37 ± 0.27^{Ac}	6.25 ± 0.26^{Ab}	6.62 ± 0.37^{Aa}
	Water	4.41 ± 0.37^{Ad}	5.36 ± 0.17^{Ac}	6.13 ± 0.30^{Ab}	6.50 ± 0.45^{Aa}
	Combined treatment	4.39 ± 0.26^{Ac}	5.37 ± 0.16^{Ab}	5.66 ± 0.31^{Bab}	5.73 ± 0.18^{Ba}
ΔE	Control	-	1.28 ± 0.29^{Ac}	2.02 ± 0.34^{Ab}	2.47 ± 0.39^{Aa}
	Water	1.04 ± 0.38^{Ac}	1.26 ± 0.24^{Ac}	1.91 ± 0.13^{Ab}	2.43 ± 0.31^{Aa}
	Combined treatment	1.02 ± 0.49^{Aa}	1.24 ± 0.31^{Aa}	1.23 ± 0.37^{Ba}	1.38 ± 0.23^{Ba}

Data are presented as the mean ± SD.

$^{A-B}$Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

$^{a-c}$Any means in the same row followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Combined treatment: cinnamon leaf oil 0.05%/cetylpyridinium chloride 0.005% emulsion + ultrasound 140 W.

used for the preparation of EO emulsions, is one of the nonionic surfactants commonly used in the food industry because it is GRAS and has high hydrophilic property suitable for the preparation of emulsions [12]. However, Tween 80 has been shown to suppress the antimicrobial activity of EO [24, 25]. El-Sayed *et al.* [25] reported that the free hydroxyl group of Tween 80 forms hydrogen bonds with phenolic compounds responsible for the antimicrobial activity of garlic EO, thereby lowering the antimicrobial activity of garlic EO. Thus, cationic surfactants are considered to be more suitable than Tween 80 for EO emulsion preparation. The additive effects of CLC and CLB are mainly due to the interactions of cationic surfactant with CL, which is mainly composed of eugenol that has strong antibacterial activity [26].

In this study, NaOCl treatment at 0.2 mg/ml reduced the populations of *L. monocytogenes* and *E. coli* O157:H7 by 1.34 and 1.15 log CFU/g, respectively, as compared with water washing treatment (Fig. 1). This observation was consistent with that reported in a previous study, wherein the effect of chlorine sanitizers on bacterial log reduction was approximately <2 log CFU/g [5]. del Carmen Velázquez *et al.* [14] reported that 200 ppm of chlorine treatment resulted in reduction of the *E. coli* O157:H7 population by 1.11 log CFU/g as compared with water washing treatment. Therefore, the combination of CLC or CLB and US showed better effects against both pathogens as compared with those observed with NaOCl treatment at 0.2 mg/ml concentration.

Changes in the Population of Two Pathogens on Fresh-Cut Endive during Storage

After the combined treatment of FCE inoculated with two pathogens, changes in the microbial populations on the endive were monitored during storage at 4°C for 8 days (Table 2). After treatment, a decrease in the population of both pathogens was observed during storage for 8 days. In the case of *L. monocytogenes*, no significant difference ($p > 0.05$) was observed in the microbial count between days 5 and 8 of storage for the control and water washing treatment, although the number of bacteria increased slightly after day 5. This observation may be attributable to the characteristics of *L. monocytogenes* that grows even at low temperature [27]. In contrast, the combined treatment resulted in the steady decrease in the population of *L. monocytogenes* from 4.44 to 3.42 log CFU/g during 8 days of storage. In addition, all treatments induced a reduction in the population of *E. coli* O157:H7 by approximately 0.6 log CFU/g during storage. Of these, the combined treatment reduced the population of *E. coli* O157:H7 from 4.82 to 4.24 log CFU/g, which was lower than that observed in the control and water washing treatment groups. Francis and O'Beirne [28] reported that the growth pattern of two pathogens during storage is determined by the vegetable type and storage temperature. Overall, it should be noted

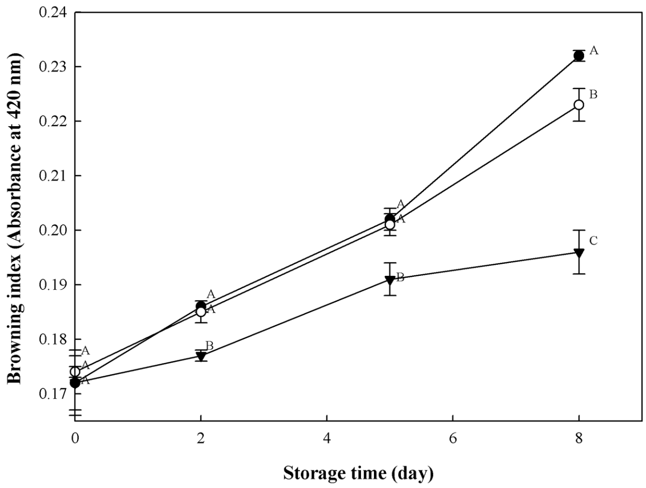

Fig. 2. Changes in the browning index of fresh-cut endive during storage.

●, Control; ○, Water; ▼, Combined treatment (cinnamon leaf oil 0.05%/cetylpyridinium chloride 0.005% emulsion + ultrasound 140 W).

Table 4. Changes in the hardness of fresh-cut endive during storage.

Treatment	Hardness (N)			
	Storage time (days)			
	0	2	5	8
Control	162.06 ± 15.90^{Aa}	162.27 ± 13.11^{Aa}	161.65 ± 12.36^{Aa}	161.86 ± 9.84^{Aa}
Water	161.72 ± 8.61^{Aa}	162.28 ± 17.92^{Aa}	161.98 ± 18.47^{Aa}	162.16 ± 17.22^{Aa}
Combined treatment	162.28 ± 13.11^{Aa}	162.48 ± 13.55^{Aa}	162.57 ± 15.64^{Aa}	161.42 ± 18.89^{Aa}

Data are presented as the mean ± SD.

^{A}Any means in the same column followed by the same letters are not significantly different ($p > 0.05$) by Duncan's multiple range test.

^{a}Any means in the same row followed by the same letters are not significantly different ($p > 0.05$) by Duncan's multiple range test.

Combined treatment: cinnamon leaf oil 0.05%/cetylpyridinium chloride 0.005% emulsion + ultrasound 140 W.

Table 5. Changes in the total phenolic content (mg GAE/100 g) of fresh-cut endive during storage.

Treatment	Total phenolic content			
	Storage time (days)			
	0	2	5	8
Control	16.00 ± 0.08^{Aa}	15.78 ± 0.05^{Aa}	15.75 ± 0.06^{Ba}	15.17 ± 0.19^{Bb}
Water	15.98 ± 0.12^{Aa}	15.87 ± 0.04^{Aa}	15.92 ± 0.03^{Aa}	15.77 ± 0.23^{Aa}
Combined treatment	16.05 ± 0.02^{Aa}	15.80 ± 0.06^{Aa}	15.77 ± 0.05^{ABa}	15.32 ± 0.13^{Bb}

Data are presented as the mean ± SD.

$^{A-B}$Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

$^{a-b}$Any means in the same row followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Combined treatment: cinnamon leaf oil 0.05%/cetylpyridinium chloride 0.005% emulsion + ultrasound 140 W.

that the combined treatment consistently controlled the growth of both pathogens during 8 days of storage.

Changes in the Color and Browning Index of Fresh-Cut Endive during Storage

Table 3 shows the color changes in FCE during storage. There was no significant difference ($p > 0.05$) in the L (lightness) value during storage. On the other hand, the values of a (redness) and b (yellowness) gradually increased, which can be explained by the browning of FCE. Among the treatment groups, the color change was the lowest in the combination treatment group during storage. These results were consistent with browning index data of FCE (Fig. 2). The combined treatment resulted in a significant retardation in the browning rate of FCE. Eugenol, a major component of CL, suppresses the activity of various enzymes that are responsible for browning, thereby inhibiting the browning in FCE. These results are similar to those of Chen *et al*. [18], wherein the browning of fresh-cut lettuce was suppressed by eugenol. Therefore, the combined treatment is considered to be suitable for washing to prevent browning in FCVs.

Changes in the Hardness and Total Phenolic Content of Fresh-Cut Endive during Storage

Tables 4 and 5 show the changes in the hardness and TPC of FCE during storage, respectively. There was no significant difference ($p > 0.05$) in hardness for all treatment groups. Chen *et al*. [18] reported that eugenol improved the texture of fresh-cut lettuce as compared with water washing treatment during storage. In addition, the TPC decreased from 15.98 to 15.77 mg GAE/100 g for 8 days in the water washing treatment, but there was no significant difference ($p > 0.05$) during storage (Table 5). On the other hand, the TPC was not significantly different ($p > 0.05$) until day 5 of storage between the control and combined treatment groups; however, a small decrease was observed thereafter. Thus, the combined treatment failed to affect the TPC of FCE.

In conclusion, the combined CL emulsion (containing the cationic surfactant CPC) and US enhanced the microbiological safety of FCE without affecting the qualities during storage. In addition, cationic surfactants and US treatment as a hurdle technology showed an additive effect on the antibacterial activities of CL. These results suggest that CL emulsion prepared with cationic surfactants combined with US is a good alternative sanitizer to NaOCl for FCVs.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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