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# Antibacterial activity of the noni fruit extract against *Listeria monocytogenes* and its applicability as a natural sanitizer for the washing of fresh-cut produce

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ABSTRACT

This study was conducted to investigate the antibacterial activity of the noni fruit extract (NFE) against *Listeria monocytogenes* (ATCC, 19111 and 19115) and assess its applicability for the washing of fresh-cut produce. Based on the results of the disc diffusion test, *L. monocytogenes* (ATCC, 19111 and 19115) was susceptible to the activity of NFE than other pathogens studied. Additionally, results of the time-kill assay indicated that NFE at a concentration of 0.5–0.7% effectively killed *L. monocytogenes* within 7 h. Furthermore, analysis of the intracellular components such as nucleic acids and proteins released from the bacterial cells and their SEM imaging revealed that NFE could increase the membrane permeability of cells resulting in their death. Compared to their unwashed samples, washing of romaine lettuce, spinach, and kale with 0.5% NFE gave a reduction of 1.47, 2.28, and 3.38 log CFU/g, respectively against *L. monocytogenes* (ATCC, 19111 and 19115), which is significantly different to that of NaOCl. A significant correlation was observed between the antibacterial effect induced due to NFE washing with the surface roughness of the fresh-cut produce than its surface hydrophobicity. Moreover, washing with NFE was not found to affect the color of the samples. These results indicated that NFE demonstrates good antibacterial activity against *L. monocytogenes* and can be used as a natural sanitizer to ensure the microbiological safety of fresh-cut produce.

## 1. Introduction

Recently, it has become necessary to prevent outbreaks of foodborne illnesses related to fresh-cut produce because they are usually consumed raw without any decontamination process (Meireles et al., 2016; Wadamori et al., 2017; Yu et al., 2018). Fresh-cut produce is often susceptible to contamination by *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. (Goodburn and Wallace, 2013). *L. monocytogenes* is a particularly high-risk pathogen in fresh-cut produce, and cases of foodborne illnesses related to this pathogen have been reported regularly (Ajayeoba et al., 2016; Zhu et al., 2017). Unlike other pathogens, the ability of *L. monocytogenes* to grow at refrigerated temperatures is considered to be a problem in the consumption of fresh-cut produce, and listeriosis caused by this pathogen is extremely dangerous, particularly in the case of immunosuppressed individuals (Scollard et al., 2016; Zhu et al., 2017). Therefore, the development of an appropriate decontamination technique that can protect the fresh-cut produce from contamination by *L. monocytogenes* is needed.

Washing with chemical sanitizers is a common method employed for the reduction of microbial contamination in fresh-cut produce. Sodium hypochlorite (NaOCl) is particularly used due to its low cost in comparison with other sanitizers (Chardon et al., 2016; Meireles et al., 2016; Van Haute et al., 2015). However, the application of NaOCl is restricted to concentrations less than 200 ppm because it can generate carcinogenic substances and corrode food contact surfaces when used beyond that (Chardon et al., 2016; Ramos et al., 2013). Thus, many studies have been performed to find a novel sanitizer that can replace the chlorine-based ones, including NaOCl (Kang and Song, 2018; Siroli et al., 2015). Since natural antibacterial compounds are more environment-friendly than chemical sanitizers and are also preferred by the consumers, studies on the applicability of such compounds as novel sanitizing agents obtained from food or its by-products are receiving much attention in recent years (Kang and Song, 2017; Poimenidou et al., 2016; Son et al., 2017).

Noni (*Morinda citrifolia* L.) is a tropical plant used as a food item, spice, and traditional medicine in Southeast Asia for more than 2000 years (Motshakeri and Ghazali, 2015; Yang et al., 2010). Noni is majorly consumed in the form of a fermented fruit juice and contains

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approximately 200 phytochemicals such as phenolics, flavonoids, and alkaloids (Assi et al., 2017; Chan-Blanco et al., 2006; Yang et al., 2010). Thus, many functional properties of noni are known and it has been found to exhibit anticarcinogenic, anti-inflammatory, antibacterial, and antioxidant activities (Assi et al., 2017; Motshakeri and Ghazali, 2015). Particularly, noni exhibits antibacterial activity due to the presence of scopoletin, acubin, and alizarin in its fruit (Assi et al., 2017; Chan-Blanco et al., 2006; Ulloa et al., 2015). However, despite good antibacterial properties, studies on the application of the noni fruit extract as a novel sanitizer to preserve fresh-cut produce inoculated with foodborne pathogens have not been carried out. Although fermented noni fruit juice was applied for the washing of minimally processed mango (Ulloa et al., 2015), this study did not examine its antibacterial effect on major foodborne pathogens resulting from the washing.

Therefore, the objective of this study was to examine the antibacterial activity and mechanism of action of the noni fruit extract (NFE) against the foodborne pathogen *L. monocytogenes* and investigate its washing applicability as a novel natural sanitizer for the inactivation of *L. monocytogenes* on romaine lettuce, spinach, and kale. The effect of the surface properties of these vegetables on washing was also analyzed.

## 2. Materials and methods

### 2.1. Noni fruit extract (NFE) preparation

The dried noni fruit powder (NFP) used in this study was purchased from Haenafood (Seoul, Korea). In order to prepare NFE, 30 g of NFP was dissolved in 300 mL of 70% ethanol (1:10, w/v) and extracted at 25 °C for 3 h. The extracted solution was filtered using Whatman™ Nylon membrane filter papers (0.45 μm, GE Healthcare Life Sciences, Marlborough, MA, USA) and the filtrate was concentrated at 75 °C using a vacuum evaporator (Heidolph Instruments Co., Schwabach, Germany). It was then lyophilized to obtain NFE for the subsequent experiments. Optimal extraction conditions were determined based on the results of preliminary experiments.

### 2.2. HPLC analysis to determine composition of the NFE

An HPLC system was used to identify the major antibacterial compound present in NFE based on the operational conditions described by Kang and Song (2017) and Siwinska et al. (2014), with minor modifications. The HPLC system (Waters Inc., Milord, CT, USA) consisted of a dual pump (Waters 1525), a UV-visible detector (Waters 2489), an auto sampler (Waters 2707), and a Kinetex EVO C18 column (5 μm, 250 × 4.6 mm, Phenomenex Inc., Torrance, CA, USA). A solution of 0.5% formic acid in water and absolute methanol were used as solvent A and B, respectively. To analyze the chemical composition of NFE, the prepared solvents were applied as a linear gradient (90% A in B for 10 min, 60% A in B for 15 min, 40% A in B for 25 min, 15% A in B for 35 min, and 90% A in B for 36 min) at a flow rate of 0.7 mL/min. The lyophilized NFE (0.5%) and standards containing 25 ppm of scopoletin, alizarin, and acubin (Sigma-Aldrich Co., St. Louis, MO, USA), which are the major antibacterial compounds known to be present in the noni fruit, were dissolved in 80% methanol and filtered using Whatman™ PVDF filters (0.45 μm, GE Healthcare Life Sciences). The absorbance of elution at 340 nm was monitored for 35 min.

### 2.3. Pathogenic bacterial cultures and inoculum preparation

*E. coli* O157:H7 (NCTC 12079 and ATCC 43889), *S. typhimurium* (KCTC 2421 and ATCC 14028), and *L. monocytogenes* (ATCC, 19111 and 19115) were used for the preparation of each cocktail inoculum. All pathogenic bacterial cultures were kept at −70 °C in their respective selective broths with 20% glycerol. One hundred microliters of each stock culture was mixed with 25 mL of TSB or BHI broth and incubated in a shaking incubator (150 rpm, Hanbaek Scientific Co., Bucheon, Korea) at 37 °C for 24 h. The incubated bacterial cultures were centrifuged at 3800 × *g* at 4 °C for 10 min to obtain cell pellets, which were washed twice with 0.1% of sterile peptone water (SPW, Difco Co.) and re-suspended in 20 mL of SPW. In order to prepare a cocktail inoculum, 10 mL of each bacterial culture, prepared as mentioned above, was combined and further diluted with SPW to obtain an approximate cell density of 8 log CFU/mL, which was measured by the standard plate counting method (Kang et al., 2019a,b).

### 2.4. Disc diffusion test

A disc diffusion test was performed to examine the antibacterial activity of NFE against *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* (ATCC, 19111 and 19115). The diluted inoculum (7 log CFU/mL) was prepared and used to inoculate plates of Muller-Hinton agar (MHA, Difco Co.) by spreading with a sterile cotton swab impregnated with 0.2 mL of the diluted inoculum. Sterile discs (8 mm diameter, Toyo Roshi Kaisha Ltd., Tokyo, Japan) impregnated with 100 μL of each prepared NFE solution (concentration of each NFE solution: 0, 0.5, 1, 5, 10, 30, 50, and 100%; solute concentration in each disc: 0, 4, 8, 24, 40, 56, and 80 mg/disc) were placed onto the MHA plates and allowed to stand at 25 °C for 2 h, after which, they were transferred to an incubator at 37 °C to culture the pathogens for 22 h. A control plate carrying a disc that was impregnated with distilled water (D.W.) as a negative control was also incubated. Experiments for measurement of inhibition zone were carried out in triplicates using a digimatic caliper (Mitutoyo Co., Kawasaki, Japan). The extent of antibacterial activity of NFE was determined based on the dimensions of the zone of inhibition and evaluated by comparing with the susceptibility categories (susceptible (S) ≥ 15 mm, intermediate (I) = 11–14 mm, and resistant (R) ≤ 10 mm) described in literature (CLSI, 2007; Okoth et al., 2013).

### 2.5. Time-kill assay

The antibacterial activity of NFE against *L. monocytogenes* (ATCC, 19111 and 19115) was examined here. NFE at different concentrations (0, 0.3, 0.5, and 0.7%) was added to the *L. monocytogenes* cocktail inoculum containing approximately 7 log CFU/mL and incubated at 37 °C for 24 h under conditions of shaking at 150 rpm. The untreated inoculum of *L. monocytogenes* was used as a control. At each of the time points viz. 0, 1, 3, 7, 12, and 24 h, 1 mL of the NFE-treated or untreated inoculum was diluted with SPW to enumerate cell count of the *L. monocytogenes* population by the standard plate counting method. The Oxford medium base (OMB, Difco Co.) was used for microbial enumeration. Microbial analysis was conducted in triplicates and the population of *L. monocytogenes* was expressed as a log of the colony forming units (CFU)/g.

### 2.6. Analysis of the changes occurring in the cell membrane permeability

#### 2.6.1. Intracellular DNA and RNA released from the L. monocytogenes cells

Nucleic acids, such as DNA and RNA, released from the cells of *L. monocytogenes* after treatment with 0.5% NFE were analyzed according to the method described by Kang et al. (2019a,b) and Sun et al. (2018), with the introduction of some modifications. Cell pellets present in the *L. monocytogenes* cocktail inoculum were collected by centrifugation (3800 × *g*, 4 °C, 10 min), washed with SPW, mixed with 20 mL SPW containing 0.5% NFE, and cultured at 37 °C for 24 h under conditions of shaking at 150 rpm. After incubation, the cell pellets treated with or without NFE were harvested by centrifugation as described above. The supernatant was collected and its absorbance at 260 nm was measured to determine the amounts of intracellular DNA and RNA released from the cells of *L. monocytogenes* after NFE treatment. The supernatant obtained from the untreated *L. monocytogenes* inoculum was used as a control.

#### 2.6.2. SDS-PAGE of intracellular proteins

According to the method described by Wang et al. (2015) with some modifications, SDS-PAGE was performed to elucidate the changes occurring in the intracellular protein content of *L. monocytogenes* after NFE treatment. Cells of L. *monocytogenes* that were untreated or treated with NFE for 24 h were centrifuged at 3800 × *g* for 10 min and the obtained cell pellets were washed thrice with SPW. They were then disrupted using an ultrasonicator (500 W, 20 kHz, US/VCX500, Sonics & Materials, Inc., Newtown, CT, USA) with a 13 mm probe of 70% amplitude and a 2/2 s pulse on/off condition. The disrupted cells after ultra-sonication were harvested by centrifugation at 3800 × *g* for 10 min and the intracellular protein content in the supernatant was quantified (Bradford, 1976). The ultrasonicated cell suspension (80 μL) was mixed with 20 μL of sample buffer containing D.W., 1 M Tris-HCl (pH 6.8), 50% glycerol, 10% SDS, 1% bromophenol blue, and β-mercaptoethanol and boiled at 95 °C for 20 min. The boiled mixture was allowed to cool and centrifuged as described above. SDS-PAGE was carried out using 10 μL of the supernatant. The intracellular proteins of *L. monocytogenes* were separated using a 5% staking gel and 12.5% separating gel and compared with the relevant molecular weight markers (BIO-RAD, Hercules, CA, USA). After electrophoresis, the separating gel was stained using Coomassie brilliant blue R-250.

### 2.7. Scanning electron microscopy (SEM)

Changes in the morphology of *L. monocytogenes* cells treated with NFE were analyzed by microscopic imaging using low voltage field emission SEM (Zeiss MERLIN, Oberkochen, Germany). The cocktail inoculum of *L. monocytogenes* containing approximately 7–8 log CFU/mL was treated with 0.5% NFE and cultured at 37 °C for 24 h. The bacterial cells were fixed with glutaraldehyde (2.5%) dissolved in 25 mM potassium phosphate buffer (PPB, pH 7.2), rinsed thrice with PPB for 10 min, and dehydrated sequentially using 40%, 70%, and 100% ethanol. On a cover glass, 5 μL of the dehydrated inoculum was placed and dried at 25 °C until any excessive ethanol was completely removed. The cover glass attached with *L. monocytogenes* was coated with platinum and SEM images were obtained at 5 kV with 30,000× magnification.

### 2.8. Surface property of the fresh-cut samples

#### 2.8.1. Contact angle analysis

In order to analyze the surface hydrophobicity of romaine lettuce, spinach, and kale, the contact angle of each sample was measured using a contact angle analyzer (Model: Phoenix 300 Plus, Seo Co., Seoul, Korea). Here, 3 μL of distilled water was placed on the abaxial surface of fresh-cut samples (1 cm × 1 cm) and the contact angle of the droplet was determined using the Surfaceware 9 software (Seo Co.). Five measurements on the droplets were conducted.

#### 2.8.2. Surface roughness analysis

The surface roughness of romaine lettuce, spinach, and kale was measured using a confocal optical profiler (NanoFocus Inc., Seoul, Korea) with an m-Surf software (version 6.1, NanoFocus Inc.). All samples (1 $cm^2$) were attached to a glass slide and mounted on a microscope with 20× magnification, in which the moving plate was equipped with a confocal optical profiler. The surface roughness of each sample (abaxial surface) was determined by carrying out 40 scans of 80 $mm^2$ area in triplicates and expressed as the Ra (arithmetic mean roughness), which represents an average of the surface roughness.

### 2.9. Inoculation study

Fresh produce (romaine lettuce, spinach, and kale) was purchased from a local market and kept immediately at 4 °C, and used within 48 h of its procurement. All samples (3 cm × 3 cm pieces; 0.5 ± 0.05 g) were washed with 70% ethanol to remove dust, soil, and other impurities from their surfaces. Prior to inoculation, the adaxial and abaxial sides of the prepared samples were subjected to treatment with ultraviolet (UV)-C for 10 min to reduce the number of pre-existing bacteria present on each sample surface. *L. monocytogenes* was not detected on any of the samples. The abaxial surface of each sample was inoculated with 100 μL (approximately 7–8 log CFU/mL) of the cocktail inoculum of *L. monocytogenes*, which was prepared as described above, by pipetting (15–20 drops). The fresh-cut samples inoculated with *L. monocytogenes* were dried for 90 min to allow attachment of the pathogen.

### 2.10. Washing treatment

Each fresh-cut sample (3 cm × 3 cm, 2.0 ± 0.1 g; romaine lettuce, spinach, and kale) inoculated with *L. monocytogenes* was placed on the bottom of a plastic bath (12 cm × 12 cm × 5 cm, width × length × height) containing 200 mL of D.W. and 0.5% NFE or 100 ppm NaOCl and agitated using a rotator (Chang-Shin Science, Seoul, Korea) for 3 min. A solution of NaOCl (100 ppm) was prepared using sodium hypochlorite containing approximately 4–6% of free chlorine (Yuhan, Seoul, Korea). Additionally, the washing time was 3 min in this study because the maximum acceptable contact time of sodium hypochlorite on fresh-cut produce is less than 5 min (Meireles et al., 2016). Unwashed samples of romaine lettuce, spinach, and kale were used as the control.

### 2.11. Microbial enumeration

Each fresh-cut sample (2.0 ± 0.1 g) washed with D.W., NFE, and NaOCl was mixed with SPW (48 mL) and homogenized using a stomacher (AES Laboratoire, Combourg, France) for 3 min. To enumerate the population of *L. monocytogenes*, 1 mL of the sample was diluted serially up to 10-folds in 9 mL of SPW, and 100 μL of each diluted aliquot was spread onto an OMB agar plate, which is the selective medium for *Listeria* (Difco Co.) (Kang and Song, 2017). The plates were incubated at 37 °C for 48 h. Microbial analysis was carried out in triplicates, and the cell count of the *L. monocytogenes* population was presented as the log CFU/g.

### 2.12. Color measurement

The color of the abaxial surface of each fresh-cut sample (romaine lettuce, spinach, and kale) after washing was measured using a colorimeter (Minolta Co., Tokyo, Japan). The CIELAB color values of a standard white plate were L* = 97.41 ± 0.02, a* = −0.14 ± 0.03, and b* = 2.01 ± 0.03. Five replicates of the CIELAB color values of each sample were evaluated. Additionally, the total color difference (ΔE) was calculated using the equation described by Pathare et al. (2013).

### 2.13. Statistical analysis

The Duncan's multiple range test and analysis of variance (ANOVA) were performed using the SAS program (version 9.4, SAS Institute Inc., Cary, NC, USA) to analyze the statistical differences among all experimental data. A value of $p < 0.05$ was considered statistically significant. All experiments were carried out in triplicates.

## 3. Results and discussion

### 3.1. Analysis of the major antibacterial compounds in NFE

HPLC analysis was performed to identify the major antibacterial compounds in the NFE (Fig. 1). Scopoletin, asperuloside, rutin, and ursolic acid have been reported as the key bioactive compounds in the

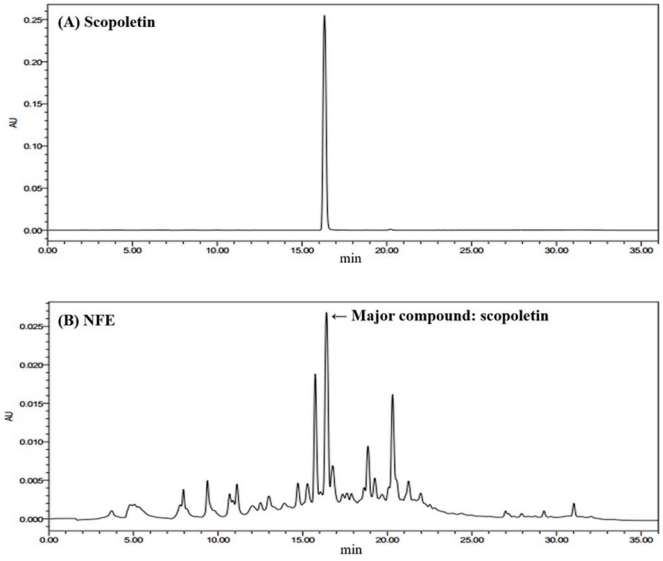


**Fig. 1.** HPLC chromatogram of noni fruit extract. (A) Scopletin (standard), (B) Noni fruit extract (NFE).

noni fruit (NF) (Yang et al., 2010). Particularly, scopoletin, alizarin, and acubin are known to demonstrate antibacterial activities against foodborne pathogens (Assi et al., 2017; Chan-Blanco et al., 2006; Ulloa et al., 2015). Based on these reports, scopoletin, alizarin, and acubin were used as the standard chemicals in this study. The HPLC analysis revealed that scopoletin was the major compound present in the NFE (Fig. 1), but not alizarin or acubin (data not shown). Compared to the results of the previous studies, scopoletin only was detected in the NFE as an antimicrobial compound in this study, probably owing to differences in cultivation area and harvest time (Assi et al., 2017; Motshakeri and Ghazali, 2015). Scopoletin is a type of phenolic coumarin, which is an effective antibacterial agent against *Pseudomonas aeruginosa*, *Bacillus cereus,* and *Staphylococcus aureus* (Gnonlonfin et al., 2012; Napiroon et al., 2018). De La Cruz-Sánchez et al. (2019) have reported that scopoletin obtained from the noni seed could potentially inhibit methicillin-resistant *S. aureus*. Napiroon et al. (2018) also reported that scopoletin extracted from *Lasianthus lucidus* Blume demonstrated antibacterial activity against multidrug-resistant *P. aeruginosa*.

### 3.2. Antibacterial activity of NFE against foodborne pathogens

The disc diffusion test was performed to examine the antibacterial activity of NFE against *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* (Table 1). The antibacterial activity of NFE against *L. monocytogenes* was found to be better than that against other pathogens employed in this study. The average dimension of zone of inhibition resulting from NFE application at a concentration of 80 mg/disc against *L. monocytogenes* was 22.43 mm, whereas that against *E. coli* O157:H7 and *S. typhimurium* at the same concentration was 9.70 and 10.35 mm, respectively (Table 1). In general, the antibacterial activity of phenolic compounds like scopoletin is known to be better against Gram-positive bacteria than Gram-negative bacteria owing to a difference in their cell wall structure (Gyawali and Ibrahim, 2014). Although antibacterial activities of scopoletin against Gram-negative bacteria like *P. aeruginosa* and *E. coli* (Napiroon et al., 2018) have been reported, in this study very low antibacterial activities of NFE against *E. coli* O157:H7 and *S. typhimurium* were observed (Table 1). These results indicated that the antibacterial activity of NFE containing scopoletin is better against Gram-positive pathogens like *L. monocytogenes*, a finding that is consistent with other reports (De La Cruz-Sánchez et al., 2019; de Souza et al., 2005). Therefore, *L. monocytogenes* was chosen as the model pathogen to examine the antibacterial effect of NFE washing on fresh-cut produce.

**Table 1**
Antibacterial activity of noni fruit extract against *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium.

| NFE[a] concentration (mg/disc) | Inhibition zone[b] (mm) | | |
|---|---|---|---|
| | *L. monocytogenes* | *E. coli* O157:H7 | *S.* Typhimurium |
| 0 | –[c] | – | – |
| 4 | – | – | – |
| 8 | – | – | – |
| 24 | 15.61 ± 0.21$^{S}$ | – | – |
| 40 | 18.75 ± 0.32$^{S}$ | – | – |
| 56 | 20.26 ± 0.41$^{S}$ | 8.87 ± 0.10$^{R}$ | 9.41 ± 0.07$^{R}$ |
| 80 | 22.43 ± 0.36$^{S}$ | 9.70 ± 0.13$^{R}$ | 10.35 ± 0.13$^{R}$ |

There was no microbial growth around the control disc (8 mm) without NFE. S represents complete susceptibility and R represents resistance.
[a] NFE: Noni fruit extract.
[b] Inhibition zone includes diameter (8 mm) of disc used.
[c] No antibacterial activity (−); no inhibition zone.

The time-kill assay was performed to determine the optimal concentration of NFE to be used for washing the fresh-cut produce. After treatment with NFE at various concentrations (0.3, 0.5, and 0.7%) for 24 h, changes in the cell densities of *L. monocytogenes* populations were determined (Fig. 2). In addition, the bactericidal effect of NFE against *L. monocytogenes* was confirmed by the assay. Bactericidal effect of antibacterial agents is defined as a 3 log-reduction or 99.9% kill on specific pathogens within 24 h of treatment, which is equivalent to a 90% kill within 6 h of treatment (Balouiri et al., 2016; Konaté et al., 2012). In case of the untreated control, no change in the cell densities of *L. monocytogenes* were observed until 24 h of incubation. On the other hand, it was found that the growth of *L. monocytogenes* was inhibited corresponding to the increasing concentrations of NFE (Fig. 2). It was also observed that NFE had the bactericidal effect against *L. monocytogenes* regardless of NFE concentrations because NFE treatment at all concentrations decreased the population of *L. monocytogenes* by more than 3 log CFU/mL within 1 h of treatment, compared to the untreated control (Fig. 2). Furthermore, after 7 h of the treatment with 0.5% or 0.7% NFE, the population of *L. monocytogenes* decreased below the detection limit (< 1 log CFU/mL). Thus, 0.5% was chosen as the optimal concentration of NFE to be used for the washing application because no significant differences were observed between the antibacterial activities of NFE at 0.5% and 0.7% (Fig. 2). The antibacterial effects of NFE on *L. monocytogenes* at these concentrations were also similar to those of other natural sanitizing agents. Kang and Song (2017) reported that the extract of pomegranate pomace (PPE)

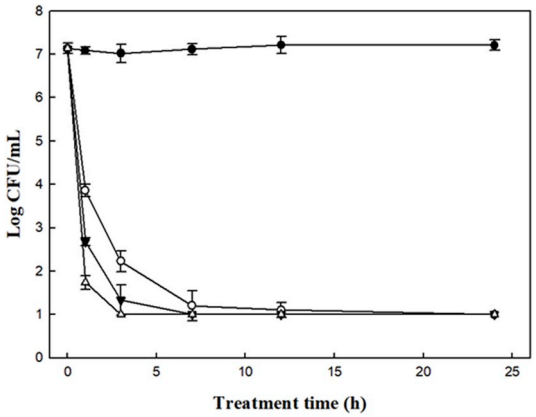


**Fig. 2.** Time-kill assay of *L. monocytogenes* treated with noni fruit extract at different concentrations. (●) Control, (○) 0.3% NFE, (▼) 0.5% NFE, (△) 0.7% NFE.

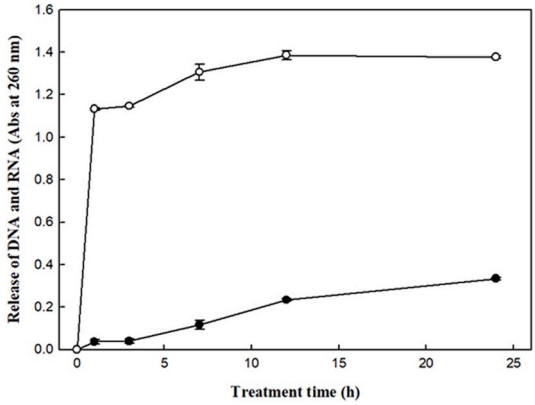


**Fig. 3.** Changes in intracellular compounds (DNA and RNA) released from *L. monocytogenes* treated with 0.5% noni fruit extract. (●) Control, (○) 0.5% NFE.

containing punicalagin and ellagic acid as major antibacterial substances exhibited good antibacterial activity and washing effect against *L. monocytogenes* at similar concentrations. Fermented noni juice could also inhibit the growth of aerobic mesophilic bacteria on mango cubes during storage for 15 days (Ulloa et al., 2015). Therefore, along with these results, it is suggested that natural plant extracts such as PPE and NFE with antibacterial compounds can be used as a sanitizer for the washing of fresh-cut produce.

To examine the antibacterial mechanism of 0.5% NFE, which was chosen as the optimal concentration for the washing application, we analyzed the change occurring in the cell membrane permeability of *L. monocytogenes* after treatment with NFE (Figs. 3 and 4, and Table 2). Additionally, SEM images of *L. monocytogenes* cells treated with NFE were analyzed to observe the effect of NFE on the cell membrane structure (Fig. 5). Changes in the amounts of intracellular DNA and RNA released from the cells of *L. monocytogenes* after treatment with 0.5% NFE for 24 h are shown in Fig. 3. The amount of compounds released from the cells increased significantly after NFE treatment for 1 h compared to that of the untreated sample, and this observation was consistent even at 24 h after the treatment (Fig. 3). These results were also consistent with those of the time-kill assay. Fig. 4 and Table 2 present the SDS-PAGE profile and results of the protein assay, respectively, showing the intracellular protein content of the *L. monocytogenes* cells after NFE treatment. The content (44.67 μg/mL) of intracellular proteins in the NFE-treated *L. monocytogenes* was much lower than that of the untreated control (598.56 μg/mL) (Fig. 4 and Table 2), indicating that NFE treatment damaged the cell membrane and increased its permeability, and the antibacterial mechanism was similar to that of other natural antimicrobial agents (Gyawali and Ibrahim, 2014; Lin et al., 2018; Wang et al., 2015). From the SEM images, it was observed that the morphology of *L. monocytogenes* cells treated with NFE was severely altered compared to that of the untreated cells. The altered cells exhibited wrinkled cell walls, micro-pores, and released intracellular compounds on the surface (Fig. 5). Similarly, the morphology of *P. aeruginosa* treated with scopoletin from *Lasianthus lucidus* Blume had changed remarkably exhibiting swollen cell walls and leakage of intracellular compounds (Napiroon et al., 2018). Thus, these results suggest that NFE demonstrates good antibacterial activity against *L. monocytogenes* and causes microbial death due to cell membrane damage and disruption.

### 3.3. Applicability of NFE for washing of the fresh-cut produce

To confirm the applicability of NFE as a natural sanitizer for washing, its effects against *L. monocytogenes* inoculated on romaine lettuce, spinach, and kale were analyzed along with their surface properties (Tables 3 and 4). Table 3 shows the contact angle (CA),

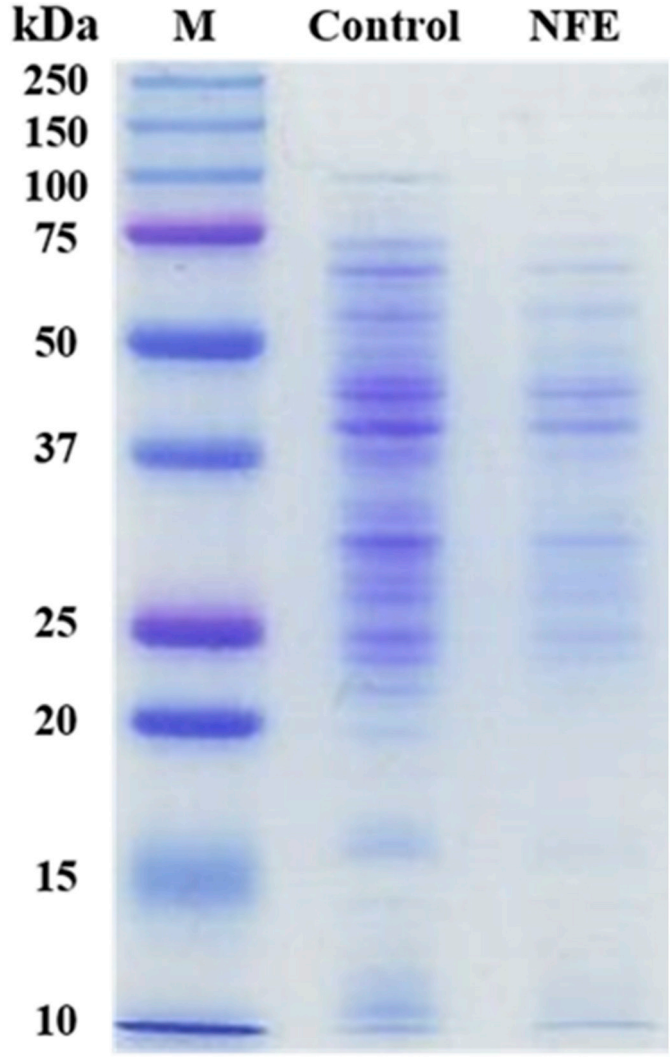


**Fig. 4.** SDS-PAGE profiles of intracellular proteins in *L. monocytogenes* after treatment with 0.5% noni fruit extract for 24 h. Control indicates intracellular proteins in non-treated *L. monocytogenes*.

**Table 2**
Changes in intracellular protein content in *L. monocytogenes* after treatment with 0.5% noni fruit extract for 24 h.

| Treatment[a] | Intracellular protein content (μg/mL) |
|---|---|
| Control | 598.56 ± 0.60[b] |
| NFE | 44.67 ± 1.03[c] |

[a] Control: non-treated *L. monocytogenes*, NFE: *L. monocytogenes* treated with 0.5% noni fruit extract.
[b] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[c] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

which is an indication of the surface hydrophobicity and surface roughness of romaine lettuce, spinach, and kale. A surface is known to be hydrophilic when its CA is less than 65° and is closely correlated with microbial adhesion (Park and Kang, 2017). Decrease of the surface hydrophobicity increases the likelihood of adhesion of the pathogens to the surfaces of vegetables, but the washing effect of sanitizers can increase on surfaces with greater hydrophilicity owing to their high resultant wettability (Kang et al., 2019a,b; Park and Kang, 2017). The CA values indicate that the surface of romaine lettuce (57.60°) is

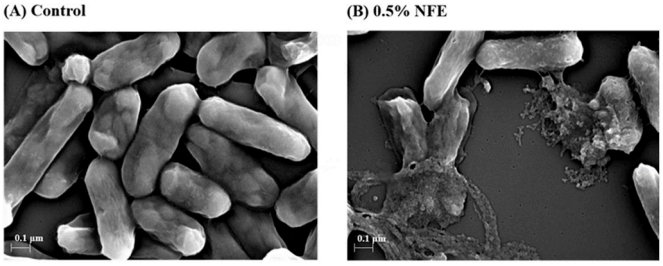


**Fig. 5.** Scanning electron microscopic images of *L. monocytogenes* after treatment with 0.5% noni fruit extract for 24 h. (A) Control: non-treated *L. monocytogenes* cells, (B) 0.5% NFE: *L. monocytogenes* cells treated with 0.5% NFE.

**Table 3**
Surface properties (contact angle and surface roughness) of romaine lettuce, spinach, and kale.

| Fresh-cut produce (abaxial side) | Contact angle (°) | Surface roughness (μm) |
|---|---|---|
| Romaine lettuce | 57.60 ± 0.97[b] | 5.09 ± 0.43[a] |
| Spinach | 109.57 ± 4.68[a] | 2.81 ± 0.14[b] |
| Kale | 109.84 ± 3.93[a] | 0.94 ± 0.07[c] |

[a] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[b] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[c] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

**Table 4**
Changes in the counts of *L. monocytogenes* inoculated on three different fresh-cut produce after washing with noni fruit extract.

| Treatment[a] | *L. monocytogenes* (log CFU/g) | | |
|---|---|---|---|
| | Romaine lettuce | Spinach | Kale |
| Control | 6.61 ± 0.08[b] | 6.80 ± 0.08[b] | 6.88 ± 0.05[b] |
| Water | 6.08 ± 0.03[c] | 5.99 ± 0.02[c] | 5.92 ± 0.20[c] |
| NFE | 5.14 ± 0.15[d] | 4.62 ± 0.19[D] | 3.50 ± 0.17[d] |
| NaOCl | 5.21 ± 0.13[d] | 4.89 ± 0.12[d] | 3.12 ± 0.26[D] |

[a] Control: no washing, Water: distilled water washing, NFE: 0.5% noni fruit extract washing, NaOCl: 100 ppm sodium hypochlorite washing.
[b] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[c] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
[d] Any means in the same column followed by different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

hydrophilic, whereas that of spinach (109.57°) and kale (109.84°) is hydrophobic (Table 3). These results are consistent with those of the previous reports (Lazouskaya et al., 2016; Lu et al., 2015). Thus, more cells of *L. monocytogenes* can attach to the surface of romaine lettuce than that of spinach and kale. In addition, the washing effect of NFE on romaine lettuce could be consequently higher because the surface hydrophilicity of lettuce is greater than that of spinach or kale.

The surface roughness (SR) values of romaine lettuce, spinach, and kale were 5.09, 2.81 and 0.94 μm, respectively, indicating that the surface of lettuce is relatively rough while that of kale is smooth (Table 3). These results are in good agreement with those of the previous reports (Lazouskaya et al., 2016; Lu et al., 2015). In general, the effect of washing with sanitizers is reduced on rough surfaces (Sridhar and Adithan, 2012). Additionally, it has been reported that surfaces with less roughness are relatively more hygienic (Fransisca and Feng, 2012).

Table 4 shows the changes in the average cell population of *L. monocytogenes* on three different fresh-cut produce after washing with NFE. The counts of *L. monocytogenes* cells remaining on romaine lettuce, spinach, and kale after washing with 0.5% NFE were 5.14, 4.62, and 3.50 log CFU/g, respectively, with log differences of 1.47, 2.18, and 3.38 compared to their respective unwashed samples (Table 4). These effects were similar to those of our previous studies in which the washing effect of various natural sanitizers, such as the extracts of pomegranate pomace, chestnut shell, and safflower seed meal were examined (Kang and Song, 2017; Park et al., 2016; Son et al., 2017). Moreover, in this study, the effect of washing with NFE was higher or similar to that of NaOCl (100 ppm). Chlorine-based sanitizers including NaOCl are usually applied to fresh-cut produce in the range of 50–200 ppm to prevent manifestation of their harmful effects, and the antimicrobial effect due to washing is typically less than 2 log reductions (Goodburn and Wallace, 2013). Therefore, these results indicated that NFE is a novel natural sanitizer that can potentially replace the chlorine-based sanitizers.

Regardless of the type of sanitizer, the antibacterial activity against *L. monocytogenes* inoculated on fresh-cut produce increased in the order of romaine lettuce, spinach, and kale (Table 4). Along with these results, it is apparent that the surface roughness of fresh-cut produce plays a more important role in the effectiveness of washing by sanitizers than the surface hydrophobicity. Thus, washing effect of NFE applied in this study had more correlation with surface roughness than surface hydrophobicity of fresh-cut produce. Similarly, Kang and Kang (2017) reported that the effect of washing with malic acid by vacuum impregnation against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* inoculated on king oyster mushrooms and muskmelons with greater relative surface roughness was lower than that of paprika or carrots. Additionally, Wang et al. (2009) reported that the antibacterial effects of washing with acidic electrolyzed water and peroxyacetic acid against *E. coli* O157:H7 inoculated on several fruits decreased with an increase in their surface roughness. These results suggested that surface properties, particularly roughness, of fresh-cut produce play an important role in the extent of antimicrobial effect observed as a result of washing with sanitizing agents.

To investigate the impact of NFE on the quality of fresh-cut produce, changes in the surface color of romaine lettuce, spinach, and kale were analyzed after washing with 0.5% NFE (Table 5). Regardless of their type, color changes due to washing with NFE were not observed in all leafy vegetables employed in this study (Table 5). Similar to our results, it has been reported that the color change in fresh-cut produce was not a result of washing with various natural sanitizers (Kang and Song, 2018; Son et al., 2017; Yossa et al., 2012). Particularly, the total color difference (△E), which represents the color difference between the unwashed and washed samples, was not significantly different among the samples after NFE washing. A △E value between 6 and 12 is indicative of quality deterioration (Rizzo and Muratore, 2009). In this study, however, the △E values of romaine lettuce, spinach, and kale were found to be 1.05, 1.23, and 1.10, respectively, regardless of whether the washing treatment was conducted or not, and their colors were consequently maintained well (Table 5). Therefore, based on these results, it can be suggested that the NFE employed in this study can be used as a natural sanitizer to secure the microbial safety of fresh-cut produce without affecting its quality. However, further studies are needed because the resultant effect of NFE washing varied depending on the surface roughness of the fresh-cut produce.

## 4. Conclusion

This study examined the antibacterial activity of NFE against foodborne pathogens and its applicability as a natural agent for the sanitization of fresh-cut produce. Higher antibacterial activities of NFE were observed against *L. monocytogenes* compared to those against *S. typhimurium* and *E. coli* O157:H7. NFE effectively inhibited the growth of *L. monocytogenes* and induced cell death by damaging its membrane. After washing with 0.5% NFE, the log reductions of *L. monocytogenes*

**Table 5**
Changes in the surface color of fresh-cut produce after washing with noni fruit extract.

| Fresh-cut produce (abaxial side) | Color parameter | Treatment[a] | | | |
|---|---|---|---|---|---|
| | | Control | Water | NFE | NaOCl |
| Romaine lettuce | **L*** | 47.52 ± 0.40[b] | 47.43 ± 0.32[b] | 47.41 ± 0.79[b] | 47.49 ± 0.95[b] |
| | **a*** | −16.86 ± 0.34[b] | −16.82 ± 0.39[b] | −16.73 ± 0.21[b] | −16.80 ± 0.26[b] |
| | **b*** | 25.35 ± 0.37[b] | 25.31 ± 0.57[b] | 25.68 ± 0.51[b] | 25.64 ± 0.39[b] |
| | **ΔE** | – | 1.05 ± 0.12[b] | 1.05 ± 0.10[b] | 1.05 ± 0.10[b] |
| Spinach | **L*** | 41.27 ± 0.77[b] | 41.68 ± 1.33[b] | 41.17 ± 0.87[b] | 41.61 ± 0.88[b] |
| | **a*** | −14.70 ± 0.53[b] | −14.58 ± 0.38[b] | −14.28 ± 0.30[b] | −14.50 ± 0.29[b] |
| | **b*** | 20.60 ± 1.05[b] | 20.23 ± 0.76[b] | 20.25 ± 0.63[b] | 20.12 ± 0.48[b] |
| | **ΔE** | – | 1.24 ± 0.22[b] | 1.23 ± 0.17[b] | 1.23 ± 0.18[b] |
| Kale | **L*** | 46.19 ± 0.70[b] | 46.26 ± 0.78[b] | 46.12 ± 0.91[b] | 46.24 ± 0.64[b] |
| | **a*** | −13.64 ± 0.16[b] | −13.74 ± 0.37[b] | −13.57 ± 0.19[b] | −13.63 ± 0.38[b] |
| | **b*** | 18.47 ± 0.58[b] | 18.25 ± 0.69[b] | 18.37 ± 0.20[b] | 18.23 ± 0.46[b] |
| | **ΔE** | – | 1.10 ± 0.13[b] | 1.11 ± 0.13[b] | 1.10 ± 0.12[b] |

[a] Control: no washing, Water: distilled water washing, NFE: 0.5% noni fruit extract washing, NaOCl: 100 ppm sodium hypochlorite washing.
[b] Any means in the same row followed by same letter are not significantly different ($p > 0.05$) by Duncan's multiple range test.

inoculated on romaine lettuce, spinach, and kale were 1.47, 2.28, and 3.38 respectively, which were either similar or higher to those observed upon treatment with NaOCl. However, there is a need to further investigate the economical aspect and sensorial changes in fresh-cut produce by NFE treatment in order to compare with NaOCl treatment. It was found that lower surface roughness of the fresh-cut produce corresponded to an increase in the microbial reduction upon NFE washing. However, there was no significant relationship between the surface hydrophobicity of the fresh-cut produce and the effect of NFE washing. The surface color of fresh-cut produce did not change after NFE washing. These results suggested that, due to its high antibacterial activity, NFE can be applied as a natural sanitizer to improve the microbial safety of fresh-cut produce. However, further studies are needed to determine ways for enhancing the antimicrobial effect of NFE upon washing, because its microbial reduction was found to be similar to that of NaOCl.

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