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Investigation of methods for inactivating foodborne pathogens in black pepper

후춧가루에 있는 식중독 균을 저감화하는 효과적인 방법

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Department of Agricultural Biotechnology

Seoul National University

Won Jeong Kim

석사학위논문

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이 논문을 석사학위 논문으로 제출함

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위원장 문태화 (인)

부위원장 강동현 (인)

위원 하남출 (인)

ABSTRACT

This study evaluated the efficacy of the simultaneous application of NIRUV (Near-infrared and Ultraviolet light) with organic acid (OA), peracetic acid (PAA) and Carvacrol (CAR) treatment for inactivating food-borne pathogens, including *E. coli* O157:H7 and *S.* Typhimurium on black pepper. A cocktail of three strains of *Escherichia coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *Salmonella* Typhimurium (ATCC 19585, ATCC 43971, DT014) was inoculated onto black pepper and then treated by spraying with organic acid (2 % malic acid, 2 % citric acid), 80 ppm peracetic acid or 1 mM carvacrol and exposing to NIRUV for 7 min. A quartz halogen infrared heating lamp (radiation intensity, 141.75 μW/cm2/nm at the sample location) and a UV germicidal lamp (radiation intensity, 2.62 mW/cm2 at the sample location) were used. Also, the effect of the treatment on quality was determined by measuring changes in color. NIRUV treatment for 7 min achieved approximately 2 log CFU reductions in

E. coli O157:H7 and *S.* Typhimurium. Organic acid (malic acid, citric acid) and peroxyacetic acid treatment with NIRUV for 7 min showed approximately 3 log CFU reductions of *E. coli* O157:H7 and *S.* Typhimurium. After 1 min, *E. coli* O157:H7 and *S.* Typhimurium treated by the carvacrol and NIRUV were not detected. The inactivation mechanisms were evaluated by the propidium iodide (PI) uptake test and transmission electron microscopy (TEM) analysis. The result of PI uptake and that of TEM showed similar patterns. NIRUV-combined treatment caused disruption of the bacterial cell membrane and cell wall. Moreover, NIRUV-CAR treatment showed severe cell disruption. These results mean that major factor of synergistic lethal effect was cell membrane damage. Ialso measured quality of black pepper treated by each combination treatment. The color values of the NIRUV combined treatment were not significantly ($P > 0.05$) different from those of the control. In case of the injured cell, the value of microbial cell and that of injured cell were not significantly ($P > 0.05$)

different. These results suggests that NIRUV and OA, PAA, CAR combined treatment can be applied as an alternative to other interventions for spices.

In conclusion, NIRUV-combined system can be expanded to food industry because of its safety and easy utility.

Keywords: **NIRUV system, food-borne pathogens, organic acid, peracetic acid, carvacrol**
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CONTENTS

LIST OF TABLE

LIST OF FIGURES

I. INTRODUCTION

Spices have been mainly used as a flavoring and seasoning agents by the food industry. However, even though only small amounts of spices are used (Fowels, Mitchell, & McGrath, 2001), food products supplemented with spices have encountered the problems. Especially, when spices contain large number of microorganisms which may cause food spoilage or human illness (I. Kiss and J. Farkas., 1988).

In 2009, a multistate outbreak of *Salmonella* Montevideo infections occurred in the USA traced to salami products seasoned with contaminated black and red pepper spices (CDC, 2010). April of 2010, 272 persons in 44 states and DC suffered foodborne illness due to this outbreak (CDC, 2010). This outbreak was the biggest issue of the food manufacturing business and it suggested the importance of spice safety. Therefore, it is positively necessary to decontaminate black pepper before consuming or using in food processing.

Several methods have been used to reduce bacterial loads of spices, such as irradiation and ozone treatment. Song et al. (2013) reported that gamma irradiation of black pepper and red pepper resulted in 4.4-5.2 log CFU/g reductions of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium.

These results show that gamma irradiation easily inactivates these bacteria. However, negative consumer perception of this technology makes it currently unattractive to the spice industry. Zhao and Cranston (1995) reported that ozone treatment (40 mg/min for 60 min) of black pepper resulted in 2-3 log CFU/g reductions of *E. coli* and *Salmonella*. These results show that ozone treatment is time-consuming as well as producing undesirable sensory changes in volatile compounds.

There were none of methods has showed to be completely satisfactory, so I was striving against decontaminating foodborne pathogens in the black pepper. Finally, I found the infrared (IR) heating and ultraviolet (UV) irradiation system. That system was the safe and efficient method and showed infrared (IR) heating and UV-C irradiation as a possible intervention for decontaminating dried spices (Ha and Kang, 2013).

IR radiation is part of the electromagnetic spectrum, with wavelengths intermediate between UV and microwave radiation, and is distinguished as near infrared (0.76 to 2 um), medium infrared (2 to 4 um), and far infrared (4 to 1,000 um). UV-C light, wavelength 250-260 nm, has the highest germicidal power (Cheon HL, 2015) due to formation of pyrimidine dimers and induction of DNA damage in cellular microorganisms. Moreover, UV-C does not produce any residues in products (Chang et al., 1985).

Ha and Kang (2013) reported that the combination of IR heating and UV-C irradiation was found to be effective in inactivation of *E. coli* O157:H7 and *Salmonella enterica Serovar* Typhimurium in powdered red pepper. However, these treatments were not sufficient to control these bacteria on black pepper.

In my previous studies (Table 1) showed that low sanitizing efficiency of single treatment of NIR heating and UV irradiation. There were only approximately 1.54 log CFU/g and 1.47 log CFU/g reductions in *E. coli* O157:H7 and *S.* Typhimurium respectively, treated by NIR heating treatment. And 0.45 and 0.16 log CFU/g reductions in *E. coli* O157:H7 and *S.* Typhimurium respectively, treated by UV irradiation treatment. Moreover, there were only approximately 2 log CFU/g reductions in *E. coli* O157:H7 and *S.* Typhimurium treated by NIRUV treatment. Therefore, to enhance efficiency of sanitizing property, it is necessary to combine other treatment with NIRUV system.

Table 1. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimuriumin black pepper treated with NIR, UV, and NIR-UV (NU)

Treatment time	Log reduction [$\log_{10}$ (N_0/N)] by treatment type					
	E. coli O157:H7			*S.* Typhimurium		
	NIR	UV	NIRUV	NIR	UV	NIRUV
1 min	0.17±0.07Aa	0.41±0.07Aa	0.18±0.03Ab	0.23±0.05Aa	0.2±0.21Aa	0.37±0.04Aa
3 min	0.39±0.06Aa	0.53±0.12Aa	0.58±0.05Ba	0.46±0.01Ba	0.12±0.08Ab	0.61±0.04Bc
5 min	1.13±0.13Ba	0.35±0.17Ab	1.54±0.13Cc	0.96±0.06Ca	0.22±0.09Ab	1.47±0.07Cc
7 min	1.54±0.01Ba	0.45±0.20Ab	2.19±0.04Dc	1.46±0.10Da	0.16±0.03Ab	1.92±0.02Dc

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Various chemical compounds have been developed by the food industry to control microorganism decontamination. Chlorine dioxide is generally more effective than chlorine for inactivating surface microorganisms (Benarde, M. A., 1965), but it can induce discoloration of raw almonds surface at high concentrations (Wihodo M, 2005). Sodium hypochlorite (NaOCl) is commonly used to sanitize food. However, it is limited at the consumer level, and residual chlorine and tis reaction products would be harmless to health (Allende et al., 2009).

In order to improve the decontamination efficiency, I chose to combine organic acid (OA), peracetic acid (PAA) and carvacrol (CAR) spray treatment with NIRUV (NU) treatment. All of these compounds are admitted as generally recognized as safe (GRAS) by FDA. A number of chemical compounds and their usages have been developed by the food industry for decontaminating microbes on surfaces of raw food.

Pao et al. (2006) reported an acid spray method for decontaminating *Salmonella* enterica on raw almonds. After spraying with 10% citric acid, a 5-log reduction was achieved. However, due to concerns about food quality, acidic solution should be used at lower concentration. Moreover, Ha and Kang (2015) used 2% lactic acid and NIR to eliminate *S. enterica* serovar Enteritidis (NCC) 1226, NCCP 12243, and NCCP 14771) on the nuts, and

achieved approximately 4 log CFU/g reductions without quality change. Therefore, I used 2 % organic acid to concern about food quality.

Peracetic acid (PAA) is the peroxide of acetic acid. PAA has shown good disinfection efficiency against enteric bacteria in wastewaters (J. Koivunen and H. Heinonen-Tanski, 2005) and has shown effective antimicrobial activity on apples, lettuce, strawberries and cantaloupe at 80 ppm (Stephanie L. et al., 2004). However, there is no published information on treatment of spices with PAA.

Sara Burt (2004) reported that antibacterial activity of essential oils (EOs) against *Listeria monocytogenes, Salmonella* Typhimurium, *Escherichia coli* O157:H7 at levels between 0.2 and 10 ul ml-1. And Gram-negative organisms are slightly less susceptible than gram-positive bacteria. In the United States, using the chemical antimicrobials are very limited. To be used as a food ingredients, studying the use of natural antimicrobials is needed. Carvacrol is generally recognized as safe (GRAS) ingredients for human consumption, so can be used as food additives to control foodborne pathogen contamination on food produce (L. Zhu et al., 2013). Moon and Rhee (2015) reported that the use of EOs, especially carvacrol is limited because of unpleasant smells, when using high concentration of carvacrol. So, we used

the small amount of carvacrol (1mM). This concentration was less than the minimal inhibitory concentration (MIC) (Burt, 2004).

The aims of this study were to investigate the efficiency of the simultaneous combination of NU treatment and spray application of OA, PAA and CAR for reducing populations of food-borne pathogens, including *E. coli* O157:H7 and *Salmonella enterica serovar* Typhimurium, in whole black pepper and to determine the effect of the combination treatment on quality indicators of whole black pepper.

II. MATERIALS AND METHODS

2.1. Bacterial strains and preparation of pathogen inoculum

2.1.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890) and *S.* Typhimurium (ATCC 19585, ATCC 43971, and DT 104) obtained from the bacterial culture collection of Seoul National University (Seoul, South Korea), were used in this experiment. Stock cultures were kept frozen at -80℃ in 0.7 ml of tryptic soy broth (TSB; Difco Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol (vol/vol). Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37℃ for 24 h, and stored at 4℃.

2.1.2. Preparation of pathogen inoculum

All strains of *E. coli* O157:H7, *S.* Typhimurium were cultured individually in 5ml of TSB at 37℃ for 24 h, harvested by centrifugation (4,000 × g for 20 min at 4℃) and washed three times with 0.2% peptone water (PW; Difco). The final pellets were resuspended in PW, corresponding to approximately 10^7 to 10^8CFU/ml. Subsequently, suspended pellets of each strain of the

three pathogenic species (six strains total) were combined to construct mixed culture cocktails. These cell suspensions with a final concentration of approximately 10^8CFU/ml were used in this study. To analyze the mechanism of inactivation, each final pellet of *E. coli* O157:H7 or *S.* Typhimurium was resuspended in 5 ml of phosphate-buffered saline (PBS; 0.1 M) and transferred to a sterile glass petri dish (16mm [height]).

2.2. Experiments of spraying with OA, PAA, CAR and NIR-UV irradiation

2.2.1. Sample preparation and inoculation

Black pepper was purchased from a local grocery store (Costco, Gyeonggi province, Korea Rep.). 5 ml of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium suspension were inoculated into approximately 250 g of black pepper.

2.2.2. Spraying with OA, PAA and CAR

For spraying the 2% OA, 80 ppm PAA or 1 mM CAR solution on black pepper samples, a hand-operated sprayer (650 ml, Apollo, Siheung-si, South Korea) was used. The sprayer was held on the top of the treatment chamber (at a distance of 13 cm from the samples). An approximate volume of 5 ml of

2% OA, 80 ppm PAA, or 1 mM CAR was sprayed evenly over inoculated black pepper (250 g) during simultaneous operation of the rotational mixer (23 rpm).

2.2.3. NIR-UV system

A stainless steel chamber (concave-upwards base, 380 by 205 by 158 mm) with a rotational mixer was used for combined NU irradiation and OA, PAA or CAR spray treatment. A quartz halogen infrared heating lamp (NS-104; 350 mm; NSTECH, South Korea) with a maximum power of 500 W (radiation intensity, 141.75 μW/cm2/nm at the sample location) with a 230-V input was used as a NIR-emitting source. A UV germicidal lamp (G10T5/4P; 357 mm; Sankyo, Japan) with a nominal output power of 16W (radiation intensity, 2.62 mW/cm2 at the sample location) was used as a UV-C-emitting source. The radiation intensities generated from the NIR and UV lamps were measured and recorded by a NIR fiber optic spectrometer (AvaSpec-NIR256-1.7; Avantes, Eerbeek, Netherlands) and a UV fiber optic spectrometer (AvaSpec-ULS2048; Avantes). Since both lamps radiate in all directions, they were placed within aluminum reflectors to redirect the radiation waves and enhance the efficiency of NIR and UV radiation. After the outputs of the NIR and UV lamps were stabilized (following 2 min of run

time), the two lamps were placed on the treatment chamber for the inactivation experiments (NU with 2% OA spray, NU with 80 ppm PAA spray, NU with 1 mM CAR spray, NU without supplemental spray, and OA, PAA or CAR spray without NU). All treatments were accompanied by stirring (23 rpm) by means of the rotational mixer.

For the inactivation mechanism study, 5-ml cell suspensions kept in glass petri dishes were treated with OA, PAA, CAR NU, NU-OA, NU-PAA and NU-PAA for 7 min under identical conditions. The treatment time (7 min) was selected based on the temperatures of black pepper observed during NU treatment

2.3. Bacterial enumeration

At selected time intervals, 25 g treated samples were removed and immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of 0.2% PW (detection limit, 5 CFU/g) and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9-ml blanks of PW, and 0.1 ml of sample or diluent was spread-plated onto selective medium. Sorbitol MacConkey agar

(SMAC; Difco) and xylose lysine desoxycholate agar (XLD; Difco) were used as selective media for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Where low numbers of surviving cells were anticipated, 1 ml of undiluted sample was equally distributed onto four plates to lower the detection limit. All agar media were incubated at 37℃ for 24 to 48 h before colonies were counted.

2.4. Enumeration of injured cells

To enumerate injured cells of *E. coli* O157:H7 and *S.* Typhimurium, the liquid repair method was used. One mL aliquots of treated samples were 10-fold serially diluted in 9 mL of tryptic soy broth (TSB; Difco Becton Dickinson, Sparks, MD, USA), and incubated at 37℃ for 2 h to resuscitated injured cells. After the recovery step, 0.1 mL of selected diluent was spread onto the selective media. Sorbitol MacConkey agar (SMAC; Difco) and xylose lysine desoxycholate agar (XLD; Difco) were used as selective media for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. All agar plates were incubated 37℃ for 22 h, and colonies were counted.

2.5. Effect of spraying with OA, PAA, CAR and NIR-UV irradiation

2.5.1. Measurement of propidium iodine (PI) uptake

Cell membrane damage was quantitatively measured by using the fluorescent dye PI (Sigma-Aldrich). Treated *E. coli* O157:H7 and *S.* Typhimurium were centrifuged (10,000 × g for 10 min), and the pellets were resuspensed and diluted in PBS to an optical density at 680 nm (OD 680) of approximately 0.2 and then mixed with PI solution to a final concentration of 2.9 μM. After incubation for 10 min, the cell suspensions were centrifuged at 10,000 × g for 10 min and washed by PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 493 nm and an emission wavelength of 630 nm. Adjusted fluorescence values were obtained by the subtraction of fluorescence values of untreated cells from those of treated cells, and the date for each sample was normalized with OD 680 of the cell suspensions.

2.5.2. Transmission electron microscopy (TEM) analysis

Transmission electron microscopy (TEM) analysis was used to analysis structural damages in pathogen cells caused by OA-, PAA-, CAR-, NU-,

NU-OA, NU-PAA and NU-CAR treatments. *S.* Typhimurium in PBS was subjected to OA, PAA, CAR, NU, NU-OA, NU-PAA and NU-CAR treatments and collected by centrifugation at 4,000 × g for 20 min. The pellets were fixed in modified by Karnovsky's fixation (2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer) at 4℃ for 2 to 4 h. After primary fixation, samples were wahsed three times with 0.05M sodium cacodylate buffer. Then the cells were postfixed in 2% osmium tetroxide and 0.1 M cacodylate buffer at 4℃ for 2 and briefly washed twice in distilled water. After that, the cells were en bloc stained by 0.5% uranyl acetate 4℃ for 24 hours then dehydrated using a graded ethanol series of 30, 50, 70, 80, 90 and three changes of 100% for 10 min each. After dehydration, cells were transited twice by propylene oxide at room temperature for 10 min each. And then, cells were infiltrated in 1:1 propylene oxide and Spurr's resin for 2 h, and placed in Spurr's resin overnight at room temperature. Infiltrated samples were polymerized at 70℃ for 24 h. These cells were sectioned (into 70 nm thick slices) using an ultramicrotome (MT-X; RMC, Tucson, AZ) and then stained with 2% uranyl acetate for 7 min and Reynold's lead citrate for 7 min. The dried sections were examined by TEM (Libra 120; Carl Zeiss, Heidenheim, Germany) and photographed.

2.6.Color measurement

Color values (L*, a*, b*) were measured at randomly selected locations within the samples using a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan). L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively. All measurements were performed in triplicate.

2.7. Statistical analysis

All experiments were duplicate-plated and replicated three times. All data were analyzed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA) and mean values were separated using Duncan's multiple-range test. Significant differences in the processing treatments were determined at a significance level of $p = 0.05$.

III. RESULTS

3.1. Effect of spraying with OA, PAA, CAR and NIR-UV irradiation for inactivation of foodborne pathogens

3.1.1. Inactivation of pathogenic bacteria by spraying with OA, PAA, CAR and NIR-UV irradiation

My study showed that the efficiency of NU-OA is similar to that of NU-PAA treatment, and significantly different microbial reductions on NU-CAR. Initial populations of *E. coli* O157:H7 and *S.* Typhimurium on inoculated whole black pepper were approximately 10^7 to 10^8 CFU/g. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimurium in black pepper during NU irradiation and spraying methods are shown in Tables 2, 3, 4 and 5.

Table 2 shows the bactericidal effect of CA, NU and NU-CA treatment against *E. coli* O157:H7 and *S.* Typhimurium on black pepper. NU treatment for 7 min achieved 2.19- and 1.92- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively. CA treatment for 7 min obtained 0.44- and 0.28-log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively. NU-CA treatment for 7 min showed 3.31- and 2.94- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively. These results showed that

simultaneous application of both technologies could produce the synergistic effect.

In case of malic acid, reductions of 2.89- and 3.07- log units were observed in *E. coli* O157:H7 and *S.* Typhimurium, respectively, after NU-MA treatment for 7 min (Table 3). MA treatment for 7 min obtained 0.32- and 0.36- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively (Table 3).

There were 3.35- and 3.07- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively, after treatment with NU-PAA (Table 4). PAA treatment for 7 min obtained 0.36- and 0.40- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively (Table 4).

Table 5 shows the reductions of *E. coli* O157:H7 during CAR, NU and NU-CAR treatment. CAR treatment alone for 7 min achieved 1.68 log reduction, and NU treatment showed 2.19 log reduction for 7 min, respectively. However, NU-CAR treatment showed dramatical bactericidal effect, so any cells were not observed after 3 min. In case of the *S.* Typhimurium, the results showed the similar reduction patterns. There were 1.82 log reduction during CAR treatment for 7 min, 1.92 log reduction during NU treatment for 7 min, and 5.94 log reduction during NU-CAR treatment for 3 min, respectively.

Table 2. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimuriumin black pepper treated with NIR-UV (NU), Citric acid (CA), and both technologies simultaneously (NU-CA)

	Log reduction [$\log_{10}$ (N_0/N)] by treatment type					
	E. coli O157:H7			*S.* Typhimurium		
Treatment time	NU	CA	NU-CA	NU	CA	NU-CA
1 min	0.18±0.03Aa	0.33±0.06Ab	0.42±0.05Ab	0.37±0.04Aa	0.25±0.02Ab	0.38±0.03Aa
3 min	0.58±0.05Ba	0.49±0.01ABa	1.19±0.21Bb	0.61±0.04Ba	0.28±0.03Ab	0.65±0.09Ba
5 min	1.54±0.13Ca	0.35±0.05BCb	1.97±0.07Cc	1.47±0.07Ca	0.24±0.02Ab	1.57±0.19Ca
7 min	2.19±0.04Da	0.44±0.04Cb	3.31±0.06Dc	1.92±0.02Da	0.28±0.03Ab	2.94±0.05Dc

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table 3. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimuriumin black pepper treated with NIR-UV (NU), Malic acid (MA), and both technologies simultaneously (NU-MA)

	Log reduction [$\log_{10}$ (N_0/N)] by treatment type					
	E. coli O157:H7			*S.* Typhimurium		
Treatment time	NU	MA	NU-MA	NU	MA	NU-MA
1 min	0.18±0.03Aa	0.22±0.99Aa	0.30±0.02Aa	0.37±0.04Aa	0.22±0.06Ab	0.43±0.04Aa
3 min	0.58±0.05Ba	0.14±0.03ABb	0.75±0.09Bc	0.61±0.04Bab	0.33±0.15Aa	0.88±0.22Bb
5 min	1.54±0.13Ca	0.31±0.10Bb	1.51±0.12Ca	1.47±0.07Ca	0.35±0.13Ab	1.93±0.28Cc
7 min	2.19±0.04Da	0.32±0.02Bb	2.89±0.09Dc	1.92±0.02Da	0.36±0.04Ab	3.07±0.17Dc

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table 4. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimuriumin black pepper treated with NIR-UV (NU), Peracetic acid (PAA), and both technologies simultaneously (NU-PAA)

	Log reduction [$\log_{10}$ (N_0/N)] by treatment type					
	E. coli O157:H7			*S.* Typhimurium		
Treatment time	NU	PAA	NU-PAA	NU	PAA	NU-PAA
1 min	0.18±0.03Aa	0.34±0.02Ab	0.33±0.04Ab	0.37±0.04Aa	0.27±0.05Aa	0.31±0.04Aa
3 min	0.58±0.05Ba	0.34±0.02Ab	0.66±0.03Ba	0.61±0.04Ba	0.28±0.05Ab	0.87±0.19Ba
5 min	1.54±0.13Ca	0.34±0.04Ab	1.63±0.22Ca	1.47±0.07Ca	0.41±0.02Bb	1.84±0.10Cc
7 min	2.19±0.04Da	0.36±0.04Ab	3.35±0.18Dc	1.92±0.02Da	0.40±0.03Bb	3.07±0.09Dc

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table 5. Viable-count reductions of *E. coli* O157:H7 and *S.* Typhimuriumin black pepper treated with NIR-UV (NU), Carvacrol (CAR), and both technologies simultaneously (NU-CAR)

	Log reduction [$\log_{10}$ (N_0/N)] by treatment type					
	E. coli O157:H7			*S.* Typhimurium		
Treatment time	NU	CAR	NU-CAR	NU	CAR	NU-CAR
1 min	0.18±0.03Aa	1.47±0.83Aa	2.18±0.23Ab	0.37±0.04Aa	0.87±0.41Aa	2.59±0.36Ab
3 min	0.58±0.05Ba	1.59±0.71Aa	>5.90±0.25*Bb*	0.61±0.04Ba	1.34±0.10Ba	>5.94±0.62*Bb*
5 min	1.54±0.13Ca	1.83±0.10Ab	>5.90±0.25*Bb*	1.47±0.07Ca	1.77±0.15Bb	>5.94±0.62*Bb*
7 min	2.19±0.04Da	1.68±1.34Ab	>5.90±0.25*Bb*	1.92±0.02Da	1.82±0.03Bb	>5.94±0.62*Bb*

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

3.1.2. Recovery of pathogenic bacteria by spraying with OA, PAA, CAR and NIR-UV irradiation-injured cells

Table 6 and 7 show levels of sub-lethally injured *E. coli* O157:H7 and *S.* Typhimurium cells in black pepper following NU-OA, NU-PAA and NU-CAR treatment. Determining the difference between inactivation of samples subjected to injured-cell recovery methods and those plated directly on selective media revealed that there were no significant ($P > 0.05$) differences.

Table6. Levels of surviving cells and cells including injured *E. coli* O157:H7 on black peppers following NU-CA, NU-MA, NU-PAA, NU-CAR treatment

	E. coli O157:H7							
	CA		MA		PAA		CAR	
Treatment time	SA	SAR	SA	SAR	SA	SAR	SA	SAR
1 min	0.36±0.10Aa	0.40±0.05Aa	0.25±0.06Aa	0.29±0.19Aa	0.33±0.04Aa	0.31±0.08Aa	2.18±0.23Aa	2.40±0.21Aa
3 min	1.19±0.21Ba	0.71±0.43Ba	0.75±0.08Ba	0.65±0.17Ba	0.66±0.03Ba	0.56±0.16Ba	>5.90±0.25*Bb*	>6.03±0.26*Bb*
5 min	1.84±0.20Ca	1.63±0.20Ca	1.50±0.12Ca	1.58±0.31Ca	1.63±0.21Ca	1.51±0.16Ca	>5.90±0.25*Bb*	>6.03±0.26*Bb*
7 min	3.30±0.06Da	3.10±0.04Da	2.89±0.08Da	3.08±0.19Da	3.35±0.18Da	3.24±0.20Da	>5.90±0.25*Bb*	>6.03±0.26*Bb*

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row are not significantly different ($P > 0.05$). SA, plating directly on selective agar; SAR, plating on selective agar preceded by a resuscitation step (liquid repair method).

Table7. Levels of surviving cells and cells including injured *Salmonella* Typhimurium on black peppers following NU-CA, NU-MA, NU-PAA, NU-CAR treatment

	S. Typhimurium							
	CA		MA		PAA		CAR	
Treatment time	SA	SAR	SA	SAR	SA	SAR	SA	SAR
1 min	0.38±0.03Aa	3.33±0.05Aa	0.43±0.04Aa	0.33±0.04Aa	0.31±0.04Aa	0.38±0.07Aa	2.59±0.36Aa	2.31±0.16Aa
3 min	0.68±0.04Ba	0.87±0.04Ba	0.88±0.22Ba	0.61±0.06Ba	0.87±0.19Ba	0.92±0.44Ba	>5.94±0.62*Bb*	>5.60±0.40*Bb*
5 min	1.57±0.18Ca	1.94±0.18Ca	1.75±0.39Ca	1.42±0.19Ca	1.84±0.10Ca	1.52±0.23Ca	>5.94±0.62*Bb*	>5.60±0.40*Bb*
7 min	2.94±0.05Da	2.82±0.09Da	3.07±0.17Da	2.81±0.15Da	3.07±0.09Da	3.03±0.29Da	>5.94±0.62*Bb*	>5.60±0.40*Bb*

Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row are not significantly different ($P > 0.05$). SA, plating directly on selective agar; SAR, plating on selective agar preceded by a resuscitation step (liquid repair method).

3.2.Effect of spraying with OA, PAA, CAR and NIR-UV irradiation

3.2.1. Determination of membrane damage by PI uptake

As a quantitative analysis of cell membrane damage, OA-, PAA, CAR, NU-, NU-OA- NU-PAA and NU-CAR treated cells were stained with the fluorescent dye PI, which only passes through damaged cell membranes. Figure 1 and 2 show the PI uptake values of *E. coli* O157:H7 and *S.* Typhimurium after each treatment.

The overall pattern of results for *E. coli* O157:H7 was similar to that of *S.* Typhimurium. Cells subjected to NU, NU-OA, NU-PAA and NU-CAR treatment showed significantly ($P< 0.05$) higher PI uptake values than did cells subjected to OA, PAA and CAR spraying alone. Moreover, the degree of membrane damage between NU-, NU-OA-, NU-PAA and NU-CAR treated cells was significantly ($P< 0.05$) different.

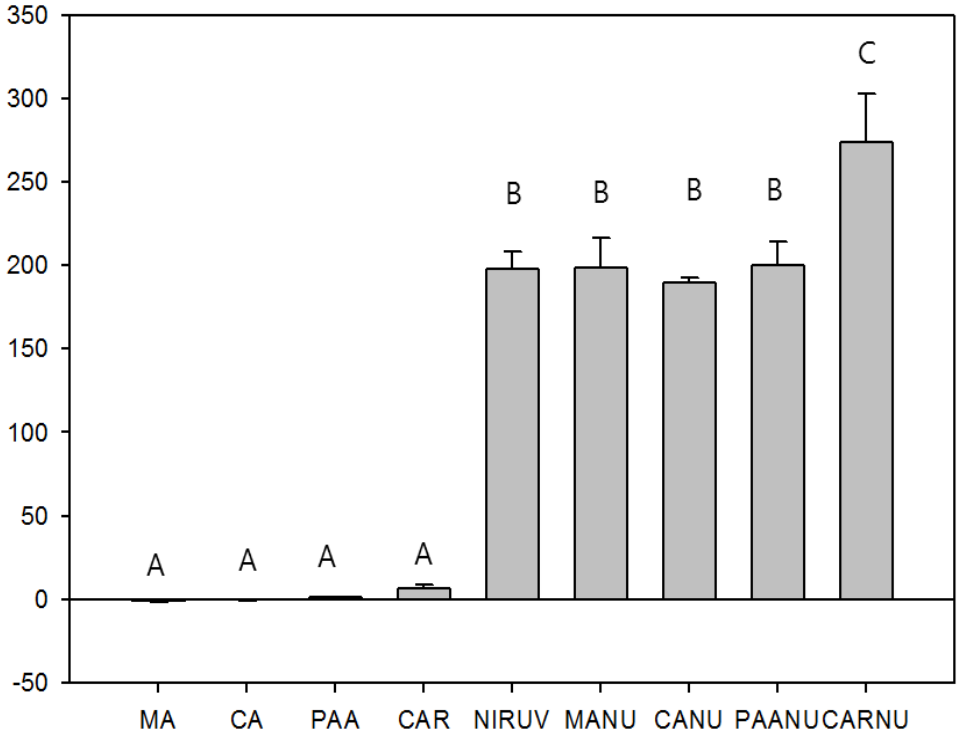

Fig. 1. Levels of membrane damage of MA-, CA-, PAA-, CAR-, NU-, NUCA-, NUMA-, NUPAA- and NUCAR- treated cells (*E. coli* O157:H7) obtained from the propidium iodine (PI) uptake test

Values are means from three replications ± standard deviations.

Values followed by the same letter are not significantly different ($P > 0.05$).

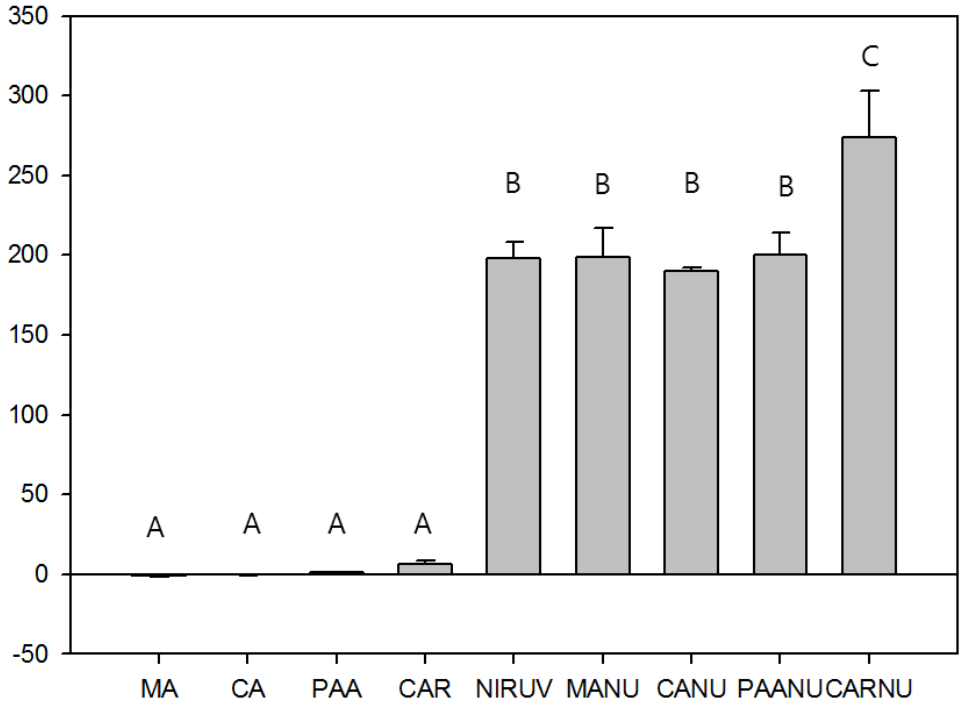

Fig. 2. Levels of membrane damage of MA-, CA-, PAA-, CAR-, NU-, NUCA-, NUMA-, NUPAA- and NUCAR- treated cells (*Salmonella* Typhimurium) obtained from the propidium iodine (PI) uptake test

Values are means from three replications ± standard deviations.

Values followed by the same letter are not significantly different ($P > 0.05$).

3.2.2. Microscopic evaluation of cellular damages

Selected TEM images (Fig. 3) showed ultrastructural changes in *S.* Typhimurium following treatment with OA, PAA, CAR, NU, NU-OA, NU-PAA and NU-CAR. Cells treated with OA, PAA and CAR showed cell wall damage, but there was no significant difference between non-treated cells and treated cells. This indicates that OA, PAA and CAR treatments alone did not substantially contribute to cell destruction. However, NU, NU-OA, NU-PAA and NU-CAR treated cells experienced significant cell wall damages, which led to leakage of cellular contents from the cytoplasm. More specifically, NU-OA and NU-PAA treated cells showed more cytoplasmic shrinkage and aggregation than NU treated cells, and NU-CAR treated cells were seriously destructed than other treatments (OA, PAA, CAR, NU, NU-OA and NU-PAA).

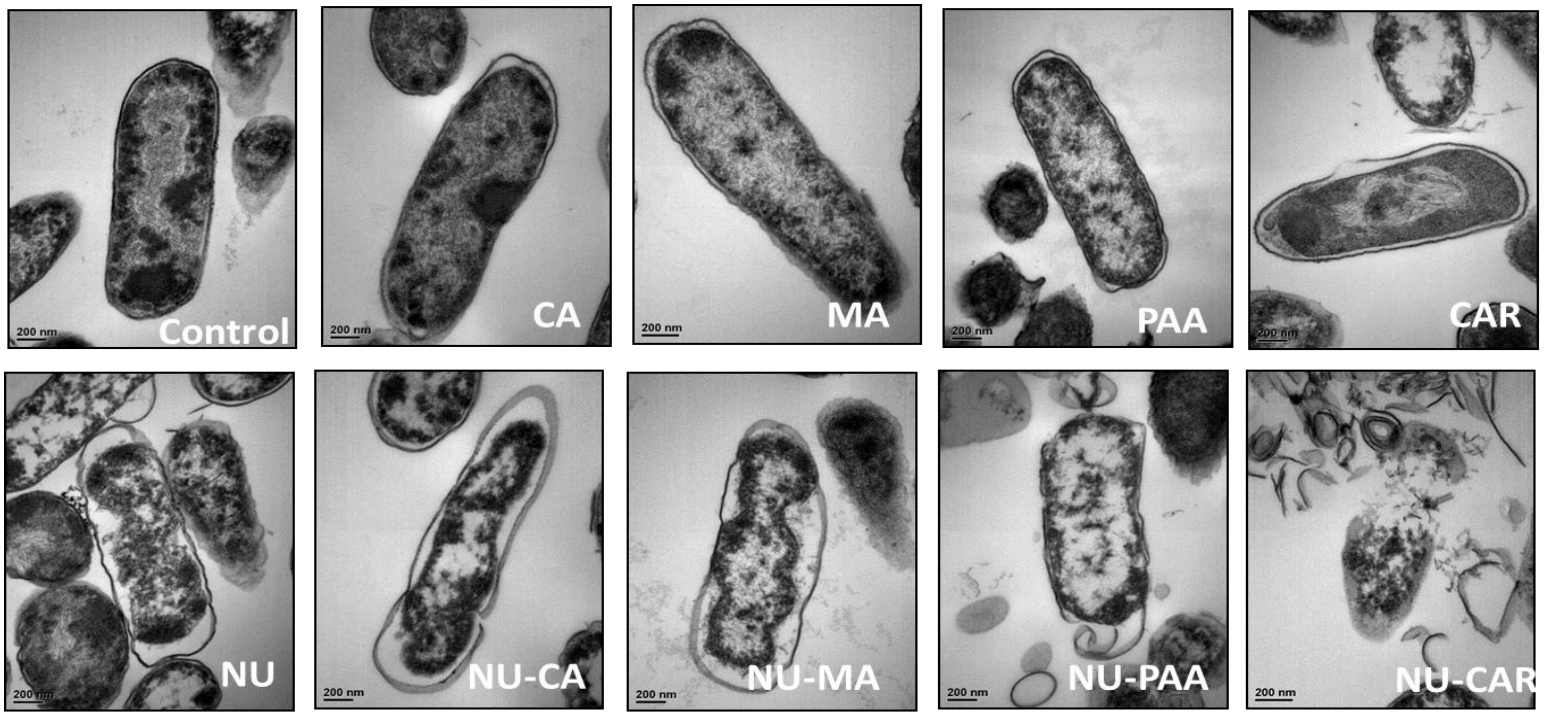

Fig. 3. Comparison of damaged induced by OA-, PAA-, CAR-, NU-, NU-OA-, NU-PAA- and NU-CAR- treatment (for 7 min) in *Salmonella* Typhimurium cells, observed by TEM.

(A) Control sample; (B) Citric acid treated sample; (C) Malic acid treated sample; (D) Peracetic acid treated sample; (E)Carvacrol treated sample; (F) NIRUV treated sample; (G) NU-CA treated sample; (H) NU-MA treated sample; (I) NU-PAA treated sample; (J) NU-CAR treated sample

3.3. Effect of spraying with OA, PAA, CAR and NIR-UV irradiation treatment on product quality

The color values of black pepper after NU-OA, NU-PAA and NU-CAR treatment are summarize in Table 8. The L*, a* and b* values of NU-OA, NU-PAA and NU-CAR treated (7 min) black pepper were not significantly ($P > 0.05$) different from those of non-treated samples.

Table 8. Color L (lightness), a (redness), and b (yellowness) values of black pepper treated by NU-OA, -PAA and –CAR

Treatment time	Parameter and treatment type											
	L*				a*				b*			
	NU-CA	NU-MA	NU-PAA	NU-CAR	NU-CA	NU-MA	NU-PAA	NU-CAR	NU-CA	NU-MA	NU-PAA	NU-CAR
Control	18.43±0.40A	18.43±0.40A	18.43±0.40A	18.43±0.40A	4.52±0.33A	4.52±0.33A	4.52±0.33A	4.52±0.33A	8.25±0.03A	8.25±0.03A	8.25±0.03A	8.25±0.03A
1 min	18.50±0.97A	18.20±0.51A	18.69±0.34A	17.60±0.58A	5.04±0.58A	4.86±0.06A	5.28±1.01A	4.85±0.06A	9.73±0.47A	9.35±0.14A	9.84±0.15A	8.33±0.26A
3 min	18.32±0.81A	18.14±0.98A	18.31±0.17A	17.10±1.03A	4.98±0.20A	4.51±0.49A	4.81±0.20A	4.44±0.26A	9.85±0.66A	9.62±4.82A	9.82±0.35A	8.52±0.39A
5 min	18.24±0.42A	17.78±0.28A	18.47±0.38A	17.76±0.26A	4.90±0.20A	4.81±0.02A	4.74±0.12A	4.81±0.03A	9.57±0.32A	9.4±0.07A	9.59±0.34A	8.47±0.33A
7 min	18.66±0.06A	18.26±0.13A	18.54±0.29A	17.61±0.61A	4.81±0.49A	4.65±0.08A	4.85±0.49A	4.75±0.25A	9.57±0.02A	9.38±0.18A	9.88±0.21A	8.59±0.40A

Values are means from three replications ± standard deviations. No values are significantly different within a column per parameter (P> 0.05). L*, lightness; a*, redness; b*, yellowness.

IV. DISCUSSION

After NU treatment only for 7 min, there were 2.19- and 1.92- log reductions in *E. coli* O157:H7 and *S.* Typhimurium, respectively. I used kinetics modeling to analysis of NU system (Table 9, 10 and 11). According to value of mean square error and regression coefficient, the appropriate modeling of NU system is Weibull model. Based on the calculated parameters (Table 9) of the Weibull model (R^2 =0.98), to achieve >3-log reductions in the NU system 8.66 min would be needed for *E. coli* O157:H7 (R^2 =0.99) and 10.42 min would be needed for *S.* Typhimurium (R^2 =0.99).

However, NU treatment over 7.5 min, black pepper was burnt out visually, so it is not efficient treatment for black pepper sanitizing method. As these results indicate, NU treatment alone was not effective in reducing microbial loads in the black pepper considering the product quality. Therefore, to inactivate foodborne pathogens on black pepper while simultaneously maintaining product quality, another technology was needed.

Table 9. Comparison of goodness of fit of the various models for the survival curves of *E. coli* O157:H7 and *S.* Typhimurium on black pepper treated with NU, NU-MA, NU-CA, NU-PAA, and NU-CAR

Pathogen	Treatment type	Log-Linear		Weibull model		Biphasic model	
		MSE	R-square	MSSE	R-square	MSSE	R-square
E. coli O157:H7	NU	0.0126	0.9928	0.017	0.9903	0.0905	0.9742
	NU-MA	0.0929	0.9479	0.0226	0.9935	0.1393	0.9479
	NU-CA	0.0367	0.9841	0.018	0.9933	0.1100	0.9841
	NU-PAA	0.2041	0.8881	0.0214	0.9941	0.6123	0.9161
	NU-CAR	2.6916	0.7421	1.5938	0.8982	0.2361	0.9925
S. Typhimurium	NU	0.0214	0.9747	0.0292	0.9770	0.0641	0.9747
	NU-MA	0.0516	0.9748	0.026	0.9906	0.1549	0.9748
	NU-CA	0.1197	0.9351	0.0207	0.9933	0.3592	0.9351
	NU-PAA	0.0552	0.9735	0.0066	0.9979	0.1657	0.9735
	NU-CAR	2.7269	0.7266	1.0654	0.9288	0.0683	0.9977

MSSE, mean square error; R-square, regression coefficient

Table 10. Model parameters of *E. coli* O157:H7 onblack pepper treated with NIR heating and combined treatments

Model	Treatment type	δ	p	MSSE	RMSSE	R-square	t_{3d}	t_{5d}
Weibull	NU	3.88	1.37	0.02	0.13	0.99	8.66	12.59
	NU-MA	2.89	1.30	0.02	0.15	0.99	6.71	9.94
	NU-CA	4.02	1.82	0.02	0.13	0.99	7.35	9.72
	NU-PAA	4.11	2.19	0.02	0.15	0.99	6.80	8.59

Model	Treatment type	f	kmax1	kmax2	MSSE	RMSSE	R-square	t_{3d}	t_{5d}
Biphasic	NU-CAR	1.00	5.47	0.28	0.24	0.48	0.99	1.20	2.01

Table 11. Model parameters of *S. Typhimurium* onblack pepper treated with NIR heating and combined treatments

Model	Treatment type	δ	p	MSSE	RMSSE	R-square	t_{3d}	t_{5d}
Weibull	NU	3.95	1.13	0.03	0.17	0.98	10.42	16.36
	NU-MA	4.13	1.95	0.03	0.16	0.99	7.27	9.45
	NU-CA	3.30	1.45	0.02	0.14	0.99	7.05	10.04
	NU-PAA	3.39	1.51	0.01	0.08	1.00	7.01	9.82

Model	Treatment type	f	kmax1	kmax2	MSSE	RMSSE	R-square	t_{3d}	t_{5d}
Biphasic	NU-CAR	1.00	5.51	0.13	0.07	0.26	0.99	1.25	2.09

Because of the consumers' perception, minimally processed foods with reduced chemical additives have preferred and led to developments in non-thermal food preservation technologies (Mertens& Knorr, 1992). Ha and Kang (2015) reported on the potential use of the combination of NIR and lactic acid on nuts, and an described an estimated 3.92- and 4.12- log reductions of *E. coli* O157:H7 and *S.* Typhimurium, respectively. However, the efficiency of the NU and acid combination on spices to date has not been reported. Therefore, I chose to investigate combinations of OA and PAA with NU treatment.

Moreover, Essential oils have been proven to be used in food product to control bacteria dependent on their concentration (Stewart and Fyfe, 1998). Singh et al. (2003) reported that essential oil showed the most effective sanitizing effect on *E. coli* O157:H7 on alfalfa seeds among the chlorine dioxide, ozonated water, and essential oil treatment. The major essential oil is carvacrol, citral and geraniol. Moreover, antibacterial activity of carvacrol was most effective against *Salmonella* typhimurium in culture medium and on fish cubes (Kim et al.,1995). Also, Friedman et al. (2002) reported that antibacterial activity of carvacrol was powerful against *Campylobacter jejuni*, *Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica.*

Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is one of the most useful essential oil as a safe food additive (Kisko and Roller, 2005). In recent study, Knowles and Roller(2001) said that carvacrol can be used to decontaminate *Salmonella* Typhimurium, *Listeria* monocyotenes and *Saccharomyces* cerevisiae attached to stainless steel. However, the application of carvacrol was limited because of its strong flavor. So, it is important to use the appreciate concentration of carvacrol not to damage food quality. Therefore, in this study I used 1 mM carvacrol on 250-g black pepper.

The difference in inactivation efficacy between NU and NU-OA,-PAA-CAR combination treatments may be attributed to the interaction of organic acids, peracetic acid, and carvacrol with NU irradiation.

After single treatment of *E. coli* O157:H7 with OA, PAA and CAR for 7 min, there were 0.32-, 0.44-, 0.36- and 1.68- log reductions of MA, CA, PAA and CAR treatment, respectively, and for *S.* Typhimurium, 0.36-, 0.28-, 0.40- and 1.82- log reductions following MA, CA, PAA and CAR treatment, respectively. In the case of *E.coli* O157:H7, 2.19-, 3.31-, 2.89-, and 3.35- log reductions were observed after NU, NU-MA, NU-CA, and NU-PAA treatment at 7 min, respectively and. For *S.* Typhimurium, 1.92-, 2.94-, 3.07-, and 3.07- log reductions were obtained following NU, NU-MA, NU-CA, and NU-PAA treatment for 7 min, respectively. Moreover, microbial cells (*E.*

coli O157:H7 and *S.* Typhimurium) were not detected in treated by NU-CAR for 7 min. As these results indicate, we can observe the synergistic effect with NU-OA, NU-PAA and NU-CAR treatment.

In previous studies, NU treatment caused membrane damage to *E. coli* O157:H7 and *S.* Typhimurium cells. NU combined treatment significantly damaged cell envelopes of both pathogens (Ha and Kang, 2013). To clarify the synergistic lethal mechanism of NU-OA, NU-PAA and NU-CAR combined treatments, membrane damage to *E. coli* O157:H7 and *S.* Typhimurium cells caused by OA, PAA, CAR NU, NU-OA, NU-PAA and NU-CAR treatment was evaluated by qualitative (TEM analysis) and quantitative (PI uptake test) methods.

PI is a fluorogenic compound that binds stoichiometrically to nucleic acids (Wallen et al., 1982). So, when cell membranes are damaged, PI can binds to nucleic acids and fluoresce. In results, the PI value of NU-combined system was significantly ($P< 0.05$) higher than that of single treatment (MA, CA, PAA and CAR) in *E. coli* O157:H7 and *S.* Typhimurium cells. Especially, NU-CAR treatment showed the highest PI value than all treatments in *E. coli* O157:H7 and *S.* Typhimurium cells.

TEM analysis revealed, severe membrane damage to *S.* Typhimurium cells caused by NU, NU-OA, NU-PAA and NU-CAR treatments. However,

OA, PAA and CAR treatments alone did not cause serious membrane damage. In overall, quantitative results of cell membrane damage measured by PI uptake showed a similar tendency to that of qualitative observations obtained from TEM analysis.

As is well known, low-pH exposure (OA treatment) also can cause sub-lethal injury to cell membranes, which in turn can cause disruption of the proton motive force across cell membranes, owing to loss of H^+-ATPase (Lin et al., 2004). This could result in more serious cell membrane damages when combined with NU irradiation. However, in the present study, the level of membrane damage that can be inferred from the degree of PI uptake in NU-OA-treated cells was not significantly higher than that of NU-treated cells (Fig 1 and 2). Therefore, it can postulated that NU treatment might disrupt the outer membrane system of the cells, allowing them to become permeable to the sprayed organic acid solution rather than increasing damages to the cell membrane.

In case of the carvacrol, Ultee and others (2002) reported that carvacrol dissolves phospholipid bilayer of cells and interacts with the cell membrane. This could cause the destabilization of the cell membrane and would increase membrane fluidity and permeability. In overall, the target mechanism of

carvacrol and NU is membrane damage, so there were effective microbial reductions (Table 5).

Also, in the kinetics modeling of each treatment (Table 10 and 11) showed thatt_{3d} and t_{5d}value.t_{3d} means the time required to decrease microbial population by 99.9 % (3 log) and t_{5d}indicates the time required to decrease microbial population by 99.999 % (5 log).$t_{3d}$and t_{5d}value (*E. coli* O157:H7 and *S*. Typhimurium) were lowest in NU-CAR treatment among the other NU-combined treatment.

Various systems have been used for inactivating foodborne pathogens in spices. However, there are some disadvantages in using super-heated steam to decontaminate foodborne pathogens in the spices. More specifically, super-heated steam treatment of red pepper caused color changes and lower sensory scores (Rico, W. C., Kim, G. R., Ahn, J.J., Kim, H. K., Furuta, M.,& Kwon, J. H., 2010). Conventional steam treatment of spices is also difficult to apply because moisture condensed on the surface of the particles needs to be removed after treatment to prevent unwanted mold growth (Schweiggert, U., Carle, R., &Schieber, A., 2007). Overall, quality changes in spices, especially color change is an important factor when applying a sterilizing process using heat treatment. Therefore, a combination treatment can be an alternative to heat treatment alone. Cheon et al. (2015) reported that UV-C

irradiation combined with mild heat treatment applied to powdered red pepper is more effective than treating with UV-C irradiation alone.

In conclusion, it is necessary to use combination treatment to decontaminate foodborne pathogens in the spices, especially black pepper. In this study, it is more effective to combine NU with OA, PAA, CAR for inactivating *E. coli* O157:H7 and *S.* Typhimurium than to treat with NU alone. Moreover, this combined treatment did not cause quality deterioration of black pepper. Thus, NU-OA,-PAA-CAR treatment can be utilized by the food industry.

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VI.국문초록

근적외선 (near-infrared, NIR) 과 자외선 (ultraviolet, UV) 동시처리 살균은 기존 선행연구들을 통하여 상대적으로 낮은 가열 강도로 식품의 품질변화 없이 미생물을 저감화하는데 우수한 살균법임을 인정받았다. 특히나, 기존에 있던 살균 방법으로 어려웠던 분말 식품에 있는 병원균을 저감화 할 수 있는 대체 기술로서의 활용 가능성을 보여주었다. 하지만, 후춧가루에서는 근적외선과 자외선 동시처리가 낮은 저감화 효율을 나타내었다. 근적외선과 자외선 동시처리만으로 후춧가루에 있던 대표적인 병원균인, *Escherichia coli* O157:H7 와 *Salmonella enterica* serovar Typhimurium 에서 각 각 2.19 log CFU/g (7 분처리), 1.92 log CFU/g (7 분처리)의 저감화 효율을 확인할 수 있었다. 따라서, 본 연구에서는 적용 가능한 허들 기술을 통하여 살균 효율을 높여보고자 하였다. 이러한 허들 기술로써, 2% 구연산 (citric acid, CA), 2% 말산 (malic acid, MA), 80ppm 과산화아세트산 (peracetic acid, PAA), 1mM 카바크롤 (carvacrol, CAR) 을 각각 수용액 형태로 식품에 분사 처리하고 근적외선과

자외선 동시처리 살균을 적용하는 조합 시스템을 구축하였고 후춧가루의 살균 가능성을 검토해보았다. 그 결과, 유기산 (구연산, 말산) 과 과산화아세트산을 처리하고 근적외선과 자외선 동시 처리 (7 분 처리)한 후춧가루에서는 약 3 log CFU/g 의 저감화 효율을 보였으며, 카바크롤을 처리한 후 근적외선과 자외선 동시 처리 (1 분 처리) 한 후춧가루에서는 *Escherichia coli* O157:H7 와 *Salmonella enterica* serovar Typhimurium 가 관찰되지 않았다. 이러한 원인을 찾아보고자 저감화 기작을 확인하였다. 저감화 기작은 프로피디움 아이오딘화물 (propidiumiodide, PI) 흡착 정도 측정 (정량적 분석) 과 투과전자현미경 (Transsmission electron microscope, TEM)의 관찰 (정성적 분석)을 통해서 확인하였다. 그 결과, 적외선과 자외선을 동시 처리한 살균법은 세포의 막에 손상을 입힌다는 것을 확인할 수 있었고, 특히나 카바크롤을 처리한 것에서는 세포막 손상이 다른 처리군들에 비해 그 정도가 유의적으로 높았다. 품질변화에 있어서는 적외선과 자외선을 동시 처리한 처리군, 유기산, 과산화아세트산, 카바크롤을 각각 사용 한 후 적외선과 자외선을 처리한 처리군과 대조군과의 색도 변화에서

유의적인 차이를 보이지 않았다 (p> 0.05). 이러한 결과는 본 실험에서 진행한 처리 방식들이 후춧가루의 품질 (색도)를 저하시키지 않고 식품에 있는 병원균을 사멸하는데 효과적으로 이용될 수 있음을 보여주었다. 또한 위와 같은 근적외선과 자외선의 조합 살균 시스템은 단독처리에 비하여 에너지 투입 수준을 낮출 수 있기에 공정비용의 절감이 가능하다는 장점을 가진다. 또한, 처리가 간편하며 연속식 공정으로 처리가 가능하기에 이를 기반으로 쉽게 산업화와 대형화가 가능할 것이다. 결론적으로 근적외선과 자외선의 조합 살균 시스템은 식품 산업에서 안전하면서도 낮은 비용으로 품질이 높은 식품을 생산하기 위한 새로운 살균 공정으로 활용될 수 있을 것이다.

주요어: 적외선, 자외선, 유기산, 과산화아세트산, 카바크롤

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