저작자표시-비영리-변경금지 2.0 대한민국

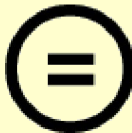
이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

Master's Thesis of Science
in Agricultural Biotechnology

Antibacterial activity of caffeic acid combined with ultraviolet-A light against foodborne pathogens : application on fresh produce washing

카페익산과 자외선A의 조합 처리를 통한
식중독균의 제어 및 신선농산물 세척 적용

August 2021

The Graduate School
Seoul National University
Department of Agricultural Biotechnology

Minyoung Park

Antibacterial activity of caffeic acid combined with ultraviolet-A light against foodborne pathogens : application on fresh produce washing

Advisor: Dong-Hyun Kang

Submitting a Master's Thesis of Science in Agricultural Biotechnology

August 2021

The Graduate School
Seoul National University
Department of Agricultural Biotechnology

Minyoung Park

Confirming the master's thesis written by Minyoung Park

August 2021

Chair	Ki Won Lee
Vice Chair	Dong-Hyun Kang
Examiner	Young Jin Choi

ABSTRACT

The aim of this study was to evaluate the antibacterial activity of caffeic acid (CA) which is a natural polyphenol, combined with ultraviolet-A (UV-A) light against the representative food-borne bacteria, *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes.* The inactivation results were obtained depending on CA concentration, light wavelength and light dose. All pathogens were significantly ($P < 0.05$) reduced when treated with CA + UV-A, inactivating *E. coli* O157:H7 and *S.* Typhimurium to detection limit. To investigate the inactivation mechanism, measurement of polyphenol uptake by bacteria, membrane damage assessment, enzymatic activity assay, and transmission electron microscopy (TEM) were conducted. It was revealed that CA was significantly ($P < 0.05$) absorbed by bacterial cells, and UV-A light allowed higher uptake of CA for both pathogens. In the enzymatic activity assay, both pathogens showed a reduction in their activity by CA and a higher reduction occurred by CA + UV-A. Moreover, TEM images indicated that CA + UV-A treatment

remarkably destructed intercellular structure. To further address the application on fresh produce washing, reusability of caffeic acid for CA + UV-A assisted washing, the effects of organic content and turbidity, and ability to inhibit cross-contamination as well as to inactivate inoculated pathogens were studied. As a result, CA was able to retain its antibacterial activity upon UV-A irradiation for three consecutive treatment cycles, by inactivating more than 6 log CFU/ml for each cycle. Also, CA + UV-A treatment was not noticeably affected by organic content and turbidity. Lastly, when apple slices were washed with CA + UV-A treatment, cross-contamination was controlled by significantly ($P < 0.05$) inhibiting *E. coli* O157:H7 and *L. monocytogenes* from attaching to surfaces and the remaining population in liquid after washing was not detected. Moreover, when both pathogens were inoculated onto surfaces and further subjected to CA + UV-A washing, they were reduced under detection limit for both apple and liquid. Therefore, this study suggests a possibility of caffeic acid + UV-A assisted washing to be applied in food industry, as it showed an effective antibacterial

activity both in liquid and in actual washing process. Moreover, the established condition for an efficient inactivation, and examination data of inactivation mechanism would provide a baseline for further research regarding photodynamic inactivation.

Keywords : **Caffeic acid, ultraviolet-A, foodborne bacteria, antibacterial activity, inactivation mechanism, fresh produce washing**

Student Number : **2019-25232**

CONTENTS

ABSTRACT……………………………………………………………….…III

CONTENTS………………………………………………………………VI

LIST OF TABLES…………………………………………..……....VIII

LIST OF FIGURES…………………………………………….…….IX

I. INTRODUCTION……………………………….………………..1

II. MATERIALS AND METHODS…………………………………………6

2.1 Bacterial strains and cell suspension…………………………………..6

2.2 Preparation of caffeic acid stock solutions………...…………………..7

2.3 Experimental setup and treatment……………………………………..7

2.4 Bacterial enumeration……………………………………..………..8

2.5 Analysis of inactivation mechanism…………………………………...9

2.5.1 Polyphenol uptake assay………………………………………10

2.5.2 Membrane damage assessment……………….………………10

2.5.3 Enzymatic activity measurement…………………………...11

2.5.4 Transmission electron microscopy (TEM) ………………………12

2.6 Application of CA + UV-A treatment on fresh produce washing……13

2.6.1 Reusability of caffeic acid for CA + UV-A treatment……………13

2.6.2 Effects of organic content and turbidity in wash-water…...………14

2.6.3 Application on fresh produce washing : apple……………………15

2.7 Statistical analysis…………………………………………………17

III. RESULTS AND DISCUSSIONS………………………………………18

3.1 Effects of various factors on the antibacterial activity of CA + UV-A treatment………………………………………………………………18

3.2 Analysis of inactivation mechanism………………………………28

3.3 Application of CA + UV-A treatment on fresh produce washing…...40

3.3.1 Reusability of caffeic acid for CA + UV-A treatment…………40

3.3.2 Effects of organic content and turbidity in wash-water………...42

3.3.3 Application on fresh produce washing : apple…………………50

3.3.3.1 Inhibition of cross-contamination during washing………...50

3.3.3.2 Inactivation of pathogen inoculated on fresh produce……..54

IV. CONCLUSION…………………………………………...………56

V. REFERENCES…………………………………….…………………57

VI. 국문초록……………………………………...…………………70

LIST OF TABLES

TABLE 1. Polyphenol uptake values of *S.* Typhimurium and *L. monocytogenes* obtained from four groups (Control, UV-A, CA, and CA + UV-A)……………………………………………………………..31

TABLE 2. Bacterial population (Log CFU/ml) of *E. coli* O157:H7 and *L. monocytogenes* subjected to CA (3 mM) + UV-A, in distilled water containing varied organic content (0, 200, 2000 ppm) ……………………46

TABLE 3. Bacterial population (Log CFU/ml) of *E. coli* O157:H7 and *L. monocytogenes* subjected to CA (3 mM) + UV-A, in distilled water with various turbidites (0, 25, 50, 100 NTU) …………………………………...47

TABLE 4. Population (Log CFU/ml) enumerated from liquid (100 ml) after washing with simulated cross-contamination……………………...……53

LIST OF FIGURES

FIGURE 1. Reduction of bacterial populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to CA, UV-A only and CA + UV-A at varying concentrations of CA…………………………..19

FIGURE 2. Reduction of bacterial populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to LED and CA + LED with LEDs of 365 nm and 408 nm……………………….…………22

FIGURE 3. Surviving populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to UV-A and CA + UV-A………..26

FIGURE 4. Membrane damage values obtained by measuring Propidium Iodide (PI) uptake values after each treatment of *S.* Typhimurium (A) and *L. monocytogenes* (B) ………………………………………………….……..34

FIGURE 5. Respiratory chain dehydrogenase activity values obtained by INF measurement of *S.* Typhimurium (A) and *L. monocytogenes* (B)……..37

FIGURE 6. TEM image of *S.* Typhimurium for control (A), UV-A (B), CA (C) and CA + UV-A (D) ……………………………………………………39

FIGURE 7. Bacterial population (Log CFU/ml) of *E. coli* O157:H7 from three consecutive cycles of CA + UV-A treatment. The error bars indicate standard deviations……………………………………………………………41

FIGURE 8. Absorption coefficients of organic content obtained at each wavelength of UV-C (220, 240, 260 and 280 nm) and UV-A (320, 340, 360 and 380 nm) ……………………………………………..………………48

FIGURE 9. Light transmittance (%) of water with varied turbidity (NTU) at each wavelength of (a) UV-C (220, 240, 260 and 280 nm) and (b) UV-A (320, 340, 360 and 380 nm) ……………………………………..…………..……49

FIGURE 10. Bacterial population (Log CFU/sample) of *E. coli* O157:H7 (A) and *L. monocytogenes* (B) enumerated from apple slices after washing with simulated cross-contamination…………………………..………………52

FIGURE 11. Bacterial population of *E. coli* O157:H7 (A) and *L. monocytogenes* (B) enumerated after washing of inoculated apple slices…..55

I. INTRODUCTION

Photodynamic inactivation (PDI), which involves light and a photosensitizer to inactivate microorganisms, has recently been recognized as a promising technology to ensure food safety, not only in medicine (Ghate, Zhou, & Yuk, 2019; Silva, Borges, Giaouris, Graton Mikcha, & Simões, 2018). The principle behind PDI is based on oxidative mechanisms which basically result in reactive oxygen species (ROS) attacking various cell structures such as cell membrane or protein, that leads to cell inactivation (Durantini, 2006). As ROS is the main mechanism, there is so far no reported antimicrobial resistance against PDI (Maisch, 2015) which makes it attractive for applicating the technique for inactivating foodborne pathogens in food processing.

The photosensitizers (PSs) in PDI mainly consist endogenous PS, usually porphyrins that naturally occur inside bacterial cells (Hessling, Spellerberg, & Hoenes, 2017), and exogenous PS. There is a wide variety of exogenous PSs, from inorganic to organic materials. For instance, in the study of Ercan et al. (Ercan, Cossu, Nitin, & Tikekar, 2016), ultraviolet-A (UV-A) irradiated Zinc oxide (ZnO) nanoparticles induced 6-log reductions of *Escherichia coli*

BL21. Besides ZnO, titanium dioxide nanoparticle is an another candidate for inorganic photosensitizer that has been widely applied in biomedical fields (Çeşmeli & Biray Avci, 2019; Ziental et al., 2020). Since involving exogenous PSs for PDI enhances its effect, there has been numerous approaches to search for a better and safer exogenous PSs which occur naturally. For example, curcumin, which is a naturally occurring compound found in *Curcuma longa*, has been applied to inactivating *E. coli* O157:H7 and *Listeria innocua* in combination with UV-A light and resulted in >5 log reduction even with low concentrations (1-10 mg L^{-1}) of curcumin (de Oliveira, Tosati, Tikekar, Monteiro, & Nitin, 2018). In addition, benzoic acid, which is a generally recognized as safe (GRAS) compound, also induced >5 log reduction of *E. coli* O157:H7 combined with UV-A light at a concentration of 15 mM (Ding, Alborzi, Bastarrachea, & Tikekar, 2018).

Caffeic acid is a phenolic compound which is naturally abundant in fruits and vegetables. Although polyphenols, including caffeic acid, are usually known as antioxidants, as well as having antimicrobial activities against pathogens (Durantini, 2006; Gülçin, 2006), it has been recently discovered that they can rather generate reactive oxygen species (ROS) upon photo-irradiation by oxidation and show bactericidal activities (Nakamura et al., 2015). For example, when 4 mM of gallic acid was exposed to blue light (400

nm), it resulted in >5 log reduction of *Staphylococcus aureus* (Nakamura et al., 2012). Similarly, caffeic acid (5 mM) with blue light (400 nm) was able to inactivate *E. coli* O157:H7 and *L. innocua* by 4 and 1 log CFU/ml, respectively (Gilbert, Alborzi, Bastarrachea, & Tikekar, 2018). In the study of Nakamura et al. (Nakamura et al., 2017), more than 5-log reduction in *Streptococcus mutans* biofilm occurred when they were treated with the combination of caffeic acid and light. Therefore, considering the natural occurrence and its ability to produce ROS when subjected to light while also acting as an antioxidant, there is a need for a study on caffeic acid as a novel photosensitizer.

One of the most important factors of photodynamic treatment is applying a light source of an appropriate wavelength that coincides with the absorption wavelength of the photosensitizer. However, despite that the absorption maximum of caffeic acid is situated near 360 nm (Balupillai et al., 2015), there is a lack of study on the combination of caffeic acid and UV-A light for inactivating foodborne pathogens. Hence, it is required to examine the antibacterial activity of caffeic acid combined with UV-A irradiation and address its efficiency over other wavelengths. In this study, therefore, the antibacterial activity of photo-irradiated caffeic acid using UV-A LED over *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* was examined due

to various factors including its concentration, light wavelength and light dose in order to establish an adequate treatment condition. Furthermore, to investigate the bactericidal mechanism, polyphenol uptake, membrane damage and enzymatic activity were measured and transmission electron microscopy (TEM) was conducted for visualization.

Meanwhile, as fresh produce is easily contaminated due to minimal processing, there has been numerous reported outbreaks regarding fresh produce. There are various sources of contamination, from the field to processing and even during storage (Carstens, Salazar, & Darkoh, 2019). To ensure the microbial safety, therefore, many preventive methods have been studied. Especially, post-harvest washing using sanitizers is considered as a crucial step (Olaimat & Holley, 2012). For example, chlorine is commonly used as a disinfectant (Banach, Sampers, Van Haute, & der Fels-Klerx, 2015) since it effectively reduces pathogens with a relatively short treatment time. However, as chlorine is found to leave disinfectant by-products (Hua & Reckhow, 2007), other alternatives that are safer and still effective for sanitation are suggested, such as excimer lamp (Kang & Kang, 2019), cold plasma water (Patange, Lu, Boehm, Cullen, & Bourke, 2019), or even recently, photodynamic treatments (de Oliveira et al., 2018; Ding et al., 2018). The challenges for fresh produce washing are whether it is safe, economical, and

effective regardless of any obstructions present in wash-water such as organic matters or turbidites. These factors should be overcome since they can block any light from transmitting, or even react with disinfectant molecules and reduce their activity. Furthermore, cross-contamination from wash-water is an another issue, as pathogens in water can unexpectedly attach to the produce surface (Gil, Selma, López-Gálvez, & Allende, 2009). Therefore, it is required and important to disinfect microbial loads in produce wash-water to avoid further cross-contamination. In this regard, CA + UV-A treatment in this study was further examined for its potential to be applied in fresh produce washing, over *E. coli* O157:H7 and *L. monocytogenes* which are common pathogenic sources of fresh produce outbreaks. For an effective application, its reusability after cyclic treatments was first investigated. Also, to figure out whether it is affected by organic content or turbidity, bacterial inactivation assays were conducted using wash-water containing various concentrations of organic materials and different levels of turbidity. Finally, actual washing process of apple slices with simulated cross-contamination or initial bacterial inoculation was studied.

In summary, the aim of this study was to examine the antibacterial activity of caffeic acid upon UV-A irradiation and illustrate its potential to serve as a novel decontamination treatment during fresh produce washing.

II. MATERIALS AND METHODS

2.1 Bacterial strains and cell suspension

Three strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S*. Typhimurium (ATCC 19585, ATCC 43971 and DT104), and *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313), acquired from the bacterial culture collection of Seoul National University (Seoul, South Korea), were used in this study. Stock culture was prepared by preserving the strains in 0.7 ml of tryptic soy broth (TSB; Difco) mixed with 0.3 ml of sterile 50% glycerol at -80°C. To make working cultures, strains were streaked onto Tryptic Soy Agar (TSA; Difco), incubated at 37°C for 24 h and stored at 4°C. A single colony of each strain from TSA plate was separately inoculated into 5 ml TSB and incubated for 24 h at 37°C in a shaking incubator. All TSB were combined and the cell pellets were harvested by centrifugation (4,000 x g, 20 min, 4°C). The obtained pellets were washed with sterile 0.2% peptone water (PW; Bacto, Becton and Dickinson company; Sparks, MD, USA) and resuspended in 9 ml of 0.2% PW, yielding a bacterial population of approximately 10^7 to 10^8 CFU/ml.

2.2 Preparation of caffeic acid stock solutions

Caffeic acid (CA; Sigma-Aldrich, St. Louis, MO) stock solution was prepared in sterile distilled water to the desired concentration, by constant stirring and heating up to 70 – 80℃ until it is completely dissolved. The concentrations used in the following treatments were determined based on its solubility in water at room temperature (Mota, Queimada, Pinho, & Macedo, 2008). The CA solution was newly prepared on the day of each experiment.

2.3 Experimental setup and treatment

A mixture of PBS (phosphate buffered saline), CA solution was filled in a petri dish (60 x 15 mm^2) and inoculated with bacterial cocktail up to a total volume of 5 ml for each treatment. The total volume of PBS and polyphenol stock solution was adjusted to obtain a desired concentration of CA at final state. The mixture was constantly stirred with a magnetic stir bar during treatments to ensure homogenous treatment, and the distance between the top of the sample and LED was fixed at 6 cm. The light dose and CA concentration used were varied, based on the objectives of each experiment which will be further mentioned.

For light treatments, four of UV-A LED (365 nm) and Blue LED (408 nm) were used. The intensity of LEDs was measured with a spectrometer (AvaSpec-ULS2048-USB2-UA-50, Avantes; Apeldoorn, Netherlands). As the distance between the LEDs and the sample was set at 6 cm as mentioned above, the irradiance values were also measured correspondingly. To ensure that a uniform irradiance to the petri dish is provided for light treatments, a petri factor was calculated by measuring the intensity for every 5 mm of the surface area. The intensity values obtained at each point were divided by the maximum value and averaged to earn the petri factor. The modified irradiance value, which was calculated by multiplying the petri factor and the maximum irradiance value, was 15.58 and 30.47 mW/cm^2 for UV-A (365 nm) and Blue LED (408 nm) each. These values were used to calculate the light dose throughout the whole experiments.

2.4 Bacterial enumeration

After each treatment, 1 ml of each sample either PBS was withdrawn and 10-fold serial dilution was followed in 9 ml of sterile 0.2% buffered PW. 0.1 ml aliquots of samples or diluents were then spread plated onto selective medium, incubated at 37℃ for 24 h and typical colonies were counted

thereafter. Sorbitol MacConkey agar (SMAC) (Difco), xylose lysine desoxycholate agar (XLD) (Oxoid), and Oxford agar base with antimicrobial supplement (OAB) (MB cell) were used to enumerate *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* respectively.

2.5 Analysis of inactivation mechanism

To investigate the inactivation mechanism of CA + UV-A LED on pathogens, measurement of polyphenol uptake, membrane damage assessment, enzymatic activity assay, and transmission electron microscopy were conducted on *S.* Typhimurium and *L. monocytogenes*. For each analysis, four groups of control, UV-A, CA, and CA + UV-A were examined for comparative data. Light dose, or the corresponding amount of time for CA treatment, was fixed at 2.5 J/cm^2 and 5 J/cm^2 for *S.* Typhimurium and *L. monocytogenes* each to equalize treatment conditions. The fluorescence or absorbance values were normalized by dividing the signal by OD_{600} of cells.

2.5.1 Polyphenol uptake assay

To measure the uptake of CA, a specific flavonoid dye, diphenylboric acid 2-aminoethyl ester (DPBA; Sigma-Aldrich) was used according to a previous method (Wang, de Oliveira, Alborzi, Bastarrachea, & Tikekar, 2017). Briefly, as DPBA can permeate the bacterial membrane, it is able to bind to flavonoids and become fluorescent. After each treatment, a 1 ml of sample was withdrawn, centrifuged at 10,000 rcf for 2 min, and washed with DW twice. For the next step, the pellet was resuspended in 450 μl of DPBA (0.2% w/v in DMSO). From the final solution, the fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA, USA) at excitation/emission wavelength of 405/465 nm.

2.5.2 Membrane damage assessment

Bacterial membrane damage was measured by using a fluorescent dye, Propidium Iodide (PI; Sigma-Aldrich). When bacterial membrane is destructed, PI can bind to cell DNA, so the value of PI uptake, measured by its fluorescence, can indicate membrane damage. After each treatment, 990 μl of treated cells were mixed with 10 μl PI solution to the final concentration of 2.9 μM and incubated in the dark for 10 min, at 37°C. After incubation,

each sample was centrifuged (10, 000 x g, 10 min) and washed twice with PBS to cease the reaction by removing the excess dye. Finally, centrifuged pellet was resuspended in PBS and its fluorescence was measured with a excitation/emission wavelength of 495/615 nm using the spectrofluorophotometer.

2.5.3 Enzymatic activity measurement

The enzymatic activity assay was conducted using a colorless iodonitrotetrazolium chloride (INT; Sigma-Aldrich) which turns to a red iodonitrotetrazolium formazan (INF) by the bacterial respiratory chain dehydrogenase. This assay was based on the preceding method (Kang, Kim, & Kang, 2018). Briefly, after each treatment, 0.9 ml of sample (either control or treated) was mixed with 0.1 ml of 0.5% INT solution and incubated in the dark at 37°C for 2 h. Next, the solution was centrifuged (10,000 rcf, 2 min) and the supernatant was discarded to obtain a cell pellet. Thereafter, 1 ml of acetone-ethanol (1:1 ratio) mixture was added to the pellet and dissolved for equivalent length of time, and 490 nm absorbance was measured with the spectrophotometer.

2.5.4 Transmission electron microscopy (TEM)

To visually examine the inactivation mechanism, TEM was conducted for *S.* Typhimurium. After each treatment, 1 ml of each sample was transferred to a 1.5 ml microtube and centrifuged (10,000 rcf, 10 min). The cell pellet was then prefixed with Karnovsky's fixative overnight at 4°C. The prefixed cells were washed with 0.05 M sodium cacodylate buffer three times for 5 min, and 1 ml of 1% osmium tetroxide diluted in 0.1 M sodium cacodylate buffer was added for post-fixation to each sample at 4°C for 2 h. After washing with distilled water for 5 min three times, the samples were immersed in 0.5% uranyl acetate overnight at 4°C for en bloc staining. The stained cells were then washed with distilled water, and dehydrated using 30, 50, 70, 80, 90, and 100% ethanol gradually, three times for 10 min. For transition, propylene oxide was added to dehydrated samples for 15 min twice and each sample was then penetrated using 1:1 and 1:2 mixture of propylene oxide and Spurr's resin for 1 h. By adding Spurr's resin, the samples were polymerized and the final samples were kept at 70°C for 24 h. The polymerized samples were subjected to ultrathin slicing, placed on copper grids, stained, and observed using a 120 kV TEM (Libra 120; Carl Zeiss, Germany).

2.6 Application of CA + UV-A treatment on fresh produce washing

To validate the potential for CA + UV-A treatment to be applied in fresh produce washing, the efficacy of CA + UV-A treatment was examined by evaluating the reusability of CA and studying the effects of organic content and turbidity on treatment. Finally, the CA + UV-A treatment was conducted for apple washing and its inactivation values were obtained.

2.6.1 Reusability of caffeic acid for CA + UV-A treatment

To evaluate the reusability of caffeic acid, three consecutive cycles of CA + UV-A treatment was performed. Bacterial suspension of *E. coli* O157:H7 was inoculated into PBS and treated with 3 mM CA and UV-A for 5 J/cm^2 following the same method stated above. After treatment, 100 μl was withdrawn for bacterial enumeration and the remaining sample was subjected to syringe filtering using a sterile 0.22 μm filter (Milllipore). 100 μl of bacterial suspension was then re-inoculated to the sample with the final bacterial population of approximately 10^8 CFU/ml, and treated with CA + UV-A in the same process. The treatment conditions and procedures were kept equally throughout the whole three cycles.

2.6.2 Effects of organic content and turbidity in wash-water

To simulate the organic content during fresh produce washing, Luria-Bertani broth (LB) (Difco) was used based on prior studies (Cossu, Ercan, Tikekar, & Nitin, 2016; Le, Zhang, Lim, McCarthy, & Nitin, 2015). To prepare the water sample containing organic content, LB broth was dissolved in sterile distilled water with the concentration of 200 and 2000 ppm LB. Additionally, to adjust the turbidity of water, bentonite (Sigma-aldrich) was dissolved in distilled water to 25, 50, and 100 NTU which were analyzed using the turbidity meter (TU-2016, Lutron Electronic, Taiwan) and sterilized before each use. For the control sample without either organic content or turbidity, distilled water was used. For CA + UV-A treatment, 3 mM CA and bacterial cocktail of *E. coli* O157:H7 and *L. monocytogenes* were added the samples and subjected to UV-A for up to 10 J/cm^2. Bacterial enumeration was conducted as described previously.

In addition to the inactivation studies, absorption coefficients of existing organic matters in water and transmittance due to turbidities were also obtained using Beer-Lambert's law (Swinehart, 1962). To calculate the absorption coefficient, absorbance of each diluent of LB broth was measured at wavelengths of UV-C (220, 240, 260 and 280 nm) and UV-A (320, 340,

360, and 380 nm). The slope from each plot of absorbance over concentration was then used as the absorption coefficient value. And transmittance was also obtained by measuring absorbance at each turbidity level, and calculated according to the equation of $A = -\log_{10}T$ (A; absorbance, T; transmittance).

2.6.3 Application on fresh produce washing : apple

To evaluate the antibacterial activity of CA + UV-A when applied to actual fresh produce washing, fresh apples were purchased from a local market, stored at 4°C and used within a week. First to examine the inhibition of cross-contamination during washing, apples were cut into disks of 2 x 2 cm^2 and three apple slices were put into a sterile beaker with 100 ml of 3 mM CA solution prepared in distilled water. After a minute of washing with constant stirring without any light treatment, 100 μl bacterial cocktail of *E. coli* O157:H7 and *L. monocytogenes* was inoculated to the solution as a simulated cross-contamination, and the final bacterial population was approximately $10^{5\text{-}6}$ CFU/ml. The inoculated solution containing three pieces of apple was then subjected to UV-A light for 30 minutes with stirring.

Next, to evaluate the bacterial inactivation of inoculated sample, spot-inoculation method was used to inoculate the bacterial cocktail onto apple

slices cut into 2 x 5 cm^2. Apple slices were placed on a sterile aluminum foil, and 100 μl of bacterial cocktail was inoculated as droplets, equally into 10 locations. After inoculation, the samples were dried in a laminar flow hood for 1 h at room temperature for sufficient bacterial attachment. A single piece of the dried apple samples was then transferred into the beaker containing 100 ml solution of 3 mM CA, and immediately subjected to UV-A light for 30 minutes with constant stirring.

For both experiments, controls and UV-A single treatments were carried out by immersing the apple slices in distilled water, and CA single treatments were conducted without light treatment. The rest of the procedures were the same as described above. For bacterial enumeration, the treated or control apple samples were individually transferred to sterile stomacher bags (Labplas Inc., Sainte-Julie, QC, Canada) containing 50 ml or 100 ml of 0.2% PW and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1 ml of homogenized solution from the stomacher bags was subjected to 10-fold serial dilution and spread-plated.

Additionally, to enumerate the remaining bacteria in liquid, 1 ml aliquot of the treated solution was withdrawn and serially diluted for spread-plating. The enumerated bacterial populations were expressed as log CFU/sample or

log CFU/ml for cross-contamination experiment, and log CFU/sample or log CFU/100 ml for inactivation experiment, respectively. Specifically for the cross-contamination experiment, the remaining population on apple slices was determined by obtaining the average population of three pieces.

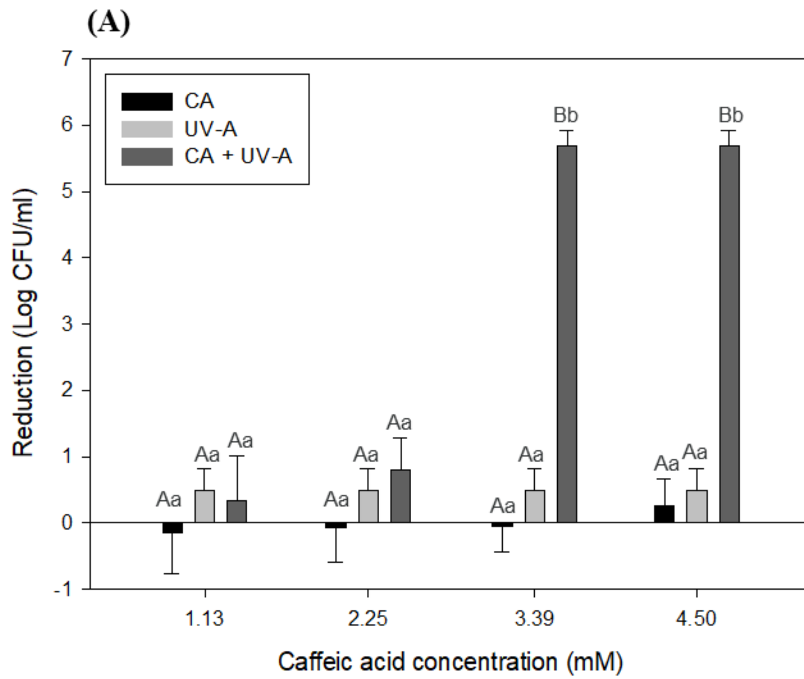
2.7 Statistical analysis

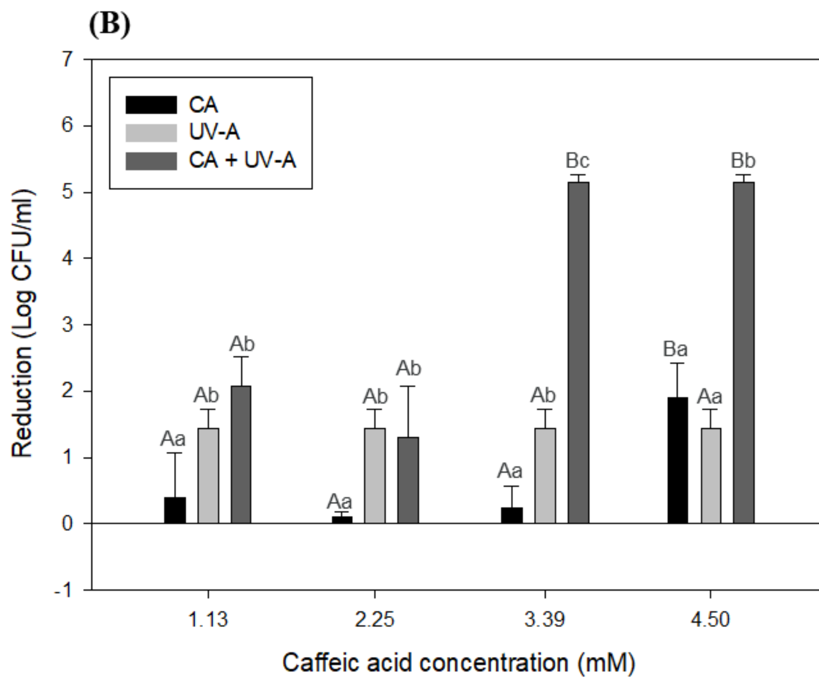
All experiments were replicated three times. All data in the present study were analyzed with SAS (ver. 9.4; SAS Institute Inc., Cary, NC, USA) using Duncan's multiple-range test. A significance level (*P*) of 0.05 was used to determine if there is a significant difference.

III. RESULTS AND DISCUSSIONS

3.1 Effects of various factors on the antibacterial activity of CA + UV-A treatment

The log reduction values or surviving populations of foodborne pathogens in PBS at varying concentrations (Fig. 1), light wavelengths (Fig. 2), and light dose (Fig. 3) are shown. As shown in Fig. 1, to examine an appropriate concentration for following treatments, caffeic acid (CA) concentrations up to 4.50 mM were tested. Caffeic acid single treatment resulted in <1 log reduction at every concentration for all three pathogens except for *S.* Typhimurium which showed 1.91 log reduction only at the highest concentration of 4.50 mM. However, when treated with 3.39 mM and 4.50 mM CA combined with UV-A, *E. coli* O157:H7 and *S.* Typhimurium were reduced to under detection limit (1 log CFU/ml), and for *L. monocytogenes* 4.88, and 5.14 log reduction values were obtained respectively. To establish a specific concentration for further experiments, 3 mM was selected for further experiments since it was in the range of the concentrations with minimum sole effect of CA and maximum synergistic effect of CA + UV-A; which means a complete inactivation of *E. coli* O157:H7 and *S*. Typhimurium.


(A)
CA
UV-A
CA + UV-A
Reduction (Log CFU/ml)
Caffeic acid concentration (mM)
1.13
2.25
3.39
4.50
Aa
Bb


(B)
CA
UV-A
CA + UV-A
Reduction (Log CFU/ml)
Caffeic acid concentration (mM)
1.13
2.25
3.39
4.50
Aa
Ab
Ba
Bb
Bc

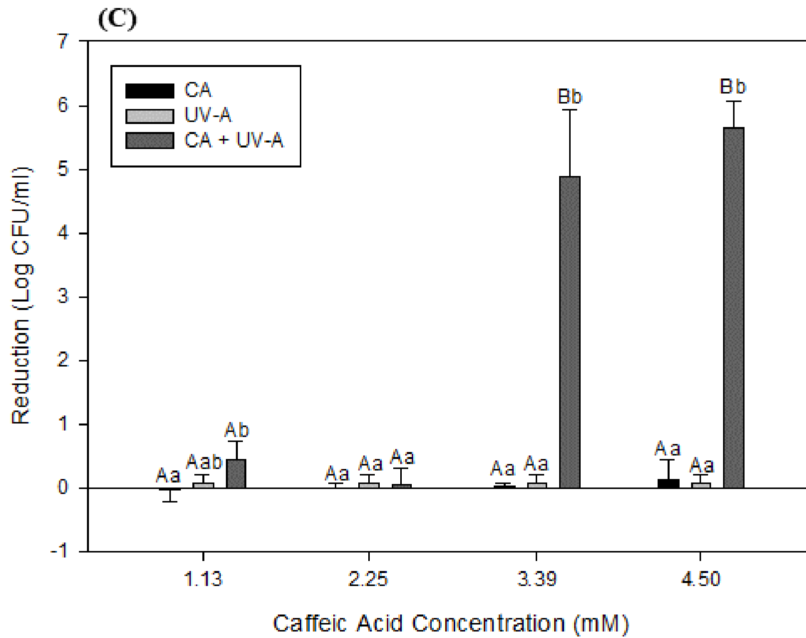
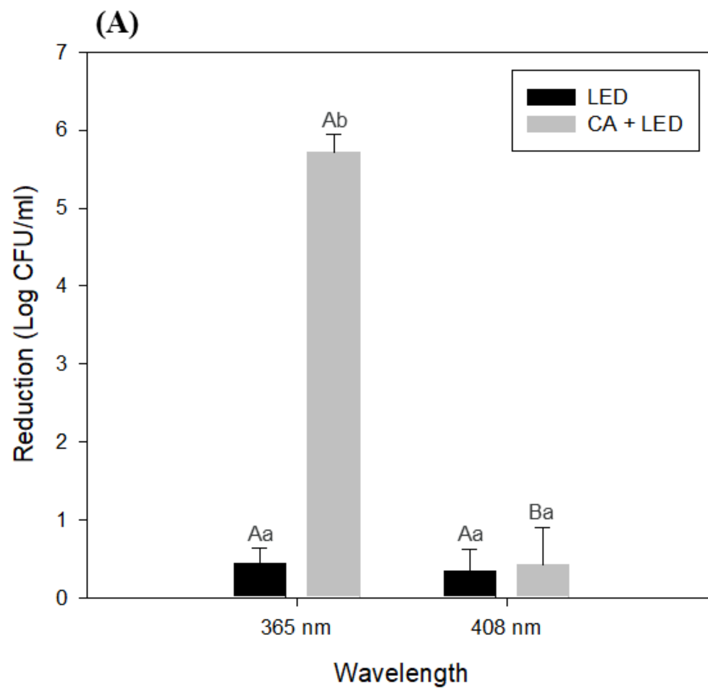
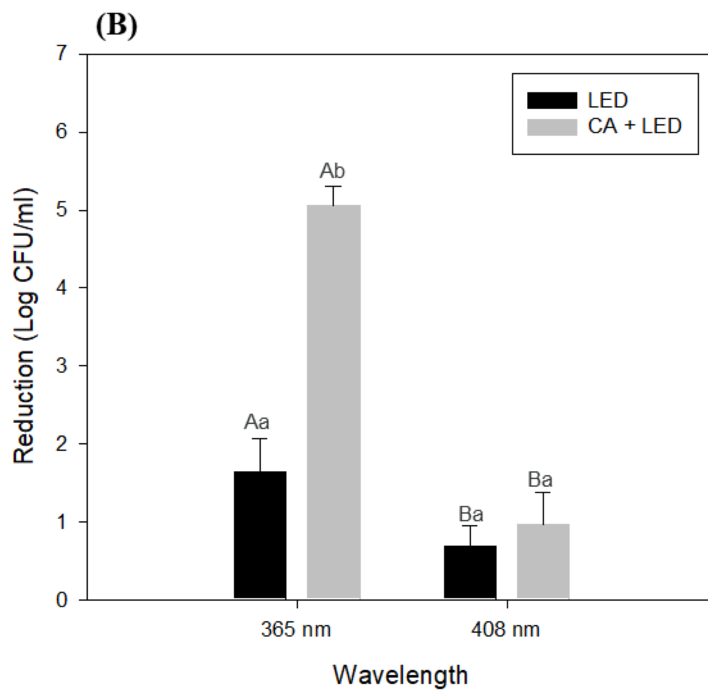


Fig 1 Reduction of bacterial populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to CA, UV-A only and CA + UV-A at varying concentrations of CA. The light dose of UV-A was fixed at 10 J/cm^2. The error bars indicate standard deviations. Different uppercase letters for cells subjected to same treatment indicate significant differences ($P < 0.05$). Different lowercase letters for the same caffeic acid concentration indicate significant differences ($P < 0.05$).

Besides concentration, another crucial factor of photodynamic treatment is applying an appropriate wavelength, which means the accordance of the absorption wavelength of photosensitizer and the emission wavelength of LED (Ghate et al., 2019). Therefore, since the absorption maximum of CA is situated near 360 nm – 370 nm (Balupillai et al., 2015), 365 nm LED was utilized in our study. To validate its advantage over blue LED, bacterial reduction values were compared over *E. coli* O157:H7, *S*. Typhimurium and *L. monocytogenes* (Fig. 2). When the pathogens were subjected to 3 mM CA and LED with light wavelengths of 365 nm (UV-A) and 408 nm (Blue light), CA + Blue LED did not show a significant reduction compared to Blue LED only. However, CA + UV-A brought a synergistic effect of 5.28, 3.42, and 5.67 log reduction for *E. coli* O157:H7, *S*. Typhimurium and *L. monocytogenes* respectively. Considering the previous studies of photodynamic treatment using polyphenols commonly apply blue light, this finding enlightens the potential of UV-A light to induce a more synergistic effect in inactivating foodborne pathogens than blue light.


(A)
Reduction (Log CFU/ml)
0
1
2
3
4
5
6
7
LED
CA + LED
Ab
Aa
Aa
Ba
365 nm
408 nm
Wavelength


(B)
Reduction (Log CFU/ml)
0
1
2
3
4
5
6
7
LED
CA + LED
Ab
Aa
Ba
Ba
365 nm
408 nm
Wavelength

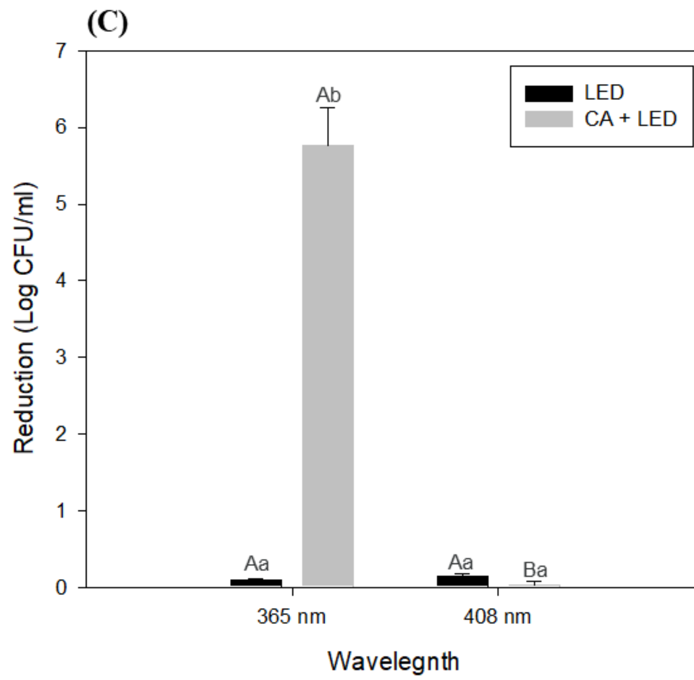
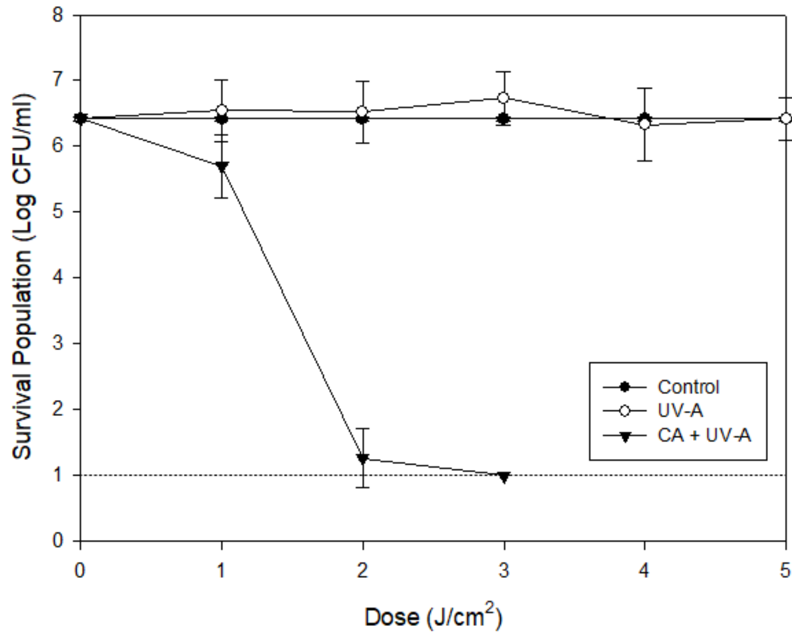


Fig 2 Reduction of bacterial populations of *E. coli* O157:H7 (A), *S.* Typhimurium (B), and *L. monocytogenes* (C) subjected to LED and CA + LED with LEDs of 365 nm and 408 nm. The light dose and CA concentration were fixed at 10 J/cm^2, 3 mM respectively. The error bars indicate standard deviations. Different uppercase letters for cells subjected to same treatment indicate significant differences (P < 0.05). Different lowercase letters for same wavelength indicate significant differences (P < 0.05).

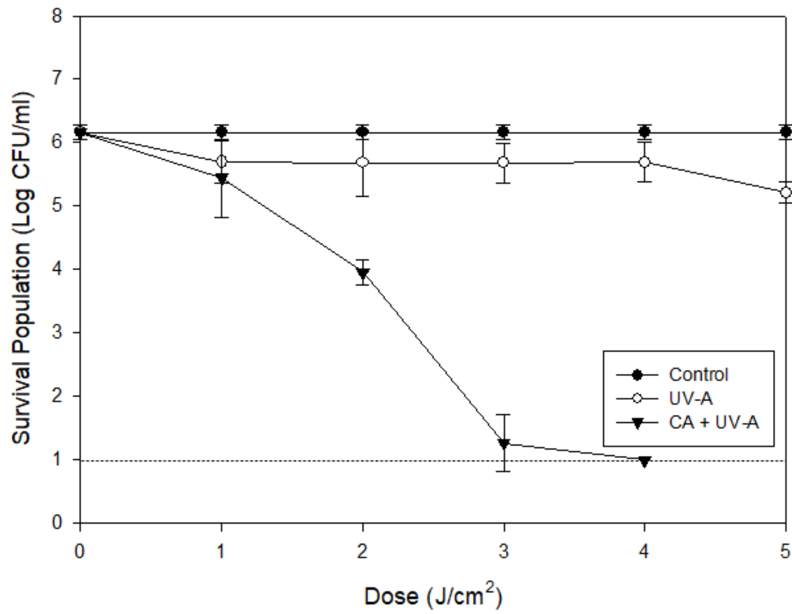
Fig. 3 depicts surviving populations of pathogens subjected to either UV-A or CA + UV-A. Although UV-A single treatment did not result in significant inactivation (<1 log reduction), *E. coli* O157:H7 and *S*. Typhimurium subjected to CA + UV-A were reduced to under the detection limit even at the low dose of 3 to 4 J/cm^2. Comparatively, *L. monocytogenes* required higher light dose for similar amount of inactivation. Generally, gram-negative bacteria are reported to be more resistant to photodynamic treatment than gram-positive bacteria since their outer membrane acts as a permeability barrier for PS molecules (F Sperandio, Huang, & R Hamblin, 2013). Therefore, additional treatments to disorganize their outer membrane are required to enhance the inactivation of gram-negative bacteria, such as ethylene diamine tetra-acetic acid (EDTA) pre-treatment or conjugating the PS with polycationic molecules (Hamblin et al., 2002; Hu et al., 2018). However, in this study, CA + UV-A treatment was even more effective towards gram-negative bacteria. Therefore, it can be inferred that CA was able to effectively penetrate the outer membrane of *E. coli* O157:H7 and *S*. Typhimurium unlike other photosensitizers. In addition to this, gram-positive bacteria are reported to be more resistant to oxidative damages than gram-negative bacteria (Bogdan, Zarzyńska, & Pławińska-Czarnak, 2015). As gram-positive bacteria possess a thick peptidoglycan layer, it could block

ROS molecules such as hydroxy radicals from entering the cells and consequently protecting them from oxidative damage. However, gram-negative bacteria have lipopolysaccharides in outer membrane which are relatively susceptible to the damage. Additionally, according to Nakamura et al. (Nakamura et al., 2015), gram-positive bacteria were more resistant to the combination treatment of polyphenols and light which matches the results in this study. To summarize, although CA could effectively enter both gram-positive and negative bacteria, gram-positive bacteria would have shown their resistance to the oxidative damage which resulted in less reduction by CA + UV-A treatment. The ability to penetrate the cell membrane of CA would be further stated.

(A)



(B)



(C)

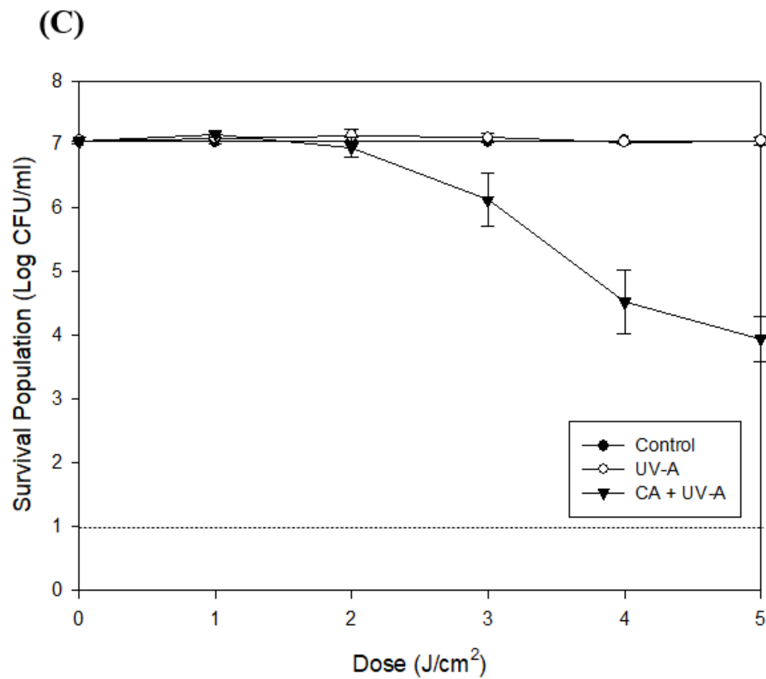


Fig 3 Surviving populations of *E. coli* O157:H7 (A), *S*. Typhimurium (B), and *L. monocytogenes* (C) subjected to UV-A and CA + UV-A. UV-A light dose was varied from 1 to 5 J/cm^2 and CA concentration was fixed at 3 mM. The error bars indicate standard deviations.

3.2 Analysis of inactivation mechanism

As UV-A and CA single treatments did not result in significant reduction for every pathogen, it is necessary to examine each action of UV-A, CA and CA + UV-A to figure out which mechanism is responsible for the synergistic antibacterial effect of CA + UV-A. It is known that the main mechanism of UV-A for inhibiting microorganisms is the generation of oxidative damage to cellular DNA (Hamamoto et al., 2007). In comparison to UV-C which directly alters the microbial DNA by producing a variety of photoproducts (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000), UV-A inactivates bacteria rather indirectly by accumulating oxidative stress to cellular components such as proteins, membrane, and DNA that leads to lethal or sublethal damage to the cells. (Bosshard et al., 2010; Cadet, Douki, & Ravanat, 2015; Moss & Smith, 1981). Therefore, it is suggested that there is a certain threshold of damage that should be exceeded to lead to a complete cell death by UV-A only (Probst-Rüd, McNeill, & Ackermann, 2017), which means that a sufficient amount of light dose is required. Since the overall maximum light dose of UV-A was only 10 J/cm^2 in this study which is a relatively low dose compared to other studies, the sole effect of UV-A might have been negligible for the cells.

Next, to examine the interaction between the cells and CA, the uptake of CA by bacterial cells was first observed (Table 1). The values of polyphenol uptake were calculated by dividing each absorbance signal by control. For both pathogens, UV-A treated group showed no significant difference ($P > 0.05$) in its value, but CA was detected in both *S*. Typhimurium and *L. monocytogenes* noticeably even when treated alone. Although no reduction in bacterial population occurred when treated by CA, it can be interpreted that CA itself can permeate the cell membrane and be absorbed by bacterial cells. Polyphenols are usually known to act as an antimicrobial agent by interacting with bacterial cell wall and cell membrane (Papuc, Goran, Predescu, Nicorescu, & Stefan, 2017). Specifically, phenolic acids such as caffeic acid have a partial lipophilic structure and are assumed to passively diffuse into cell membrane and further induce disruption even for gram-negative bacteria (Campos et al., 2009). Hence, in the same context, it can be inferred from the CA uptake values that CA was able to diffuse and permeate into the cells without any additional assistance of light. As in this experiment, however, the treatment time for CA was relatively short being up to maximum 10 m 40 s, it would have been insufficient for CA to act its antimicrobial activity by itself. Also, CA + UV-A showed even higher uptake values than CA treated group in both pathogens. This indicates that UV-A irradiation helped CA molecule

to diffuse into bacterial cells even more. When treated with CA + UV-A, the dissolved CA outside the cells would have produced reactive oxygen species (ROS) and brought a damage to cell membrane so that CA molecules can more easily move into bacterial cells than before. The results of membrane damage assessment (Fig. 4) also coincide with this interpretation.

Table 1 Polyphenol uptake values of *S*. Typhimurium and *L. monocytogenes* obtained from four groups (Control, UV-A, CA, and CA + UV-A)

Bacterial strain	Polyphenol uptake values[a]			
	Control	**UV-A**	**CA**	**CA + UV-A**
***S.* Typhimurium**	1.00±0.00 A	1.08±0.02 A	49.10±16.85 B	94.79±23.71 C
L. monocytogenes	1.00±0.00 A	0.94±0.08 A	90.43±39.88 B	131.75±31.78 C

[a] The values were calculated by dividing the obtained absorbance with control value. Data were expressed as means ± standard deviations for three independent experiments. Values in the same row with the same uppercase letter are not significantly different ($P > 0.05$).

Fig 4 shows the membrane damage values obtained by measuring Propidium Iodide (PI) uptake values after each treatment. Each value was calculated by dividing each fluorescence by control. For both pathogens, UV-A and CA single treated groups did not show any significant increase in their PI uptake values ($P > 0.05$). In contrast, the values were significantly ($P < 0.05$) increased in CA + UV-A treated groups. This indicates that a damage in cell membrane occurred when CA was assisted with UV-A. Although ROS is a well-known strong antimicrobial agent which induces cell death by causing oxidative stress (Lam et al., 2020), it has a short lifetime (Attri et al., 2015), hence the ability to diffuse long distances is limited (Forkink, Smeitink, Brock, Willems, & Koopman, 2010). For example, among the various types of ROS, hydroxyl radicals and hydrogen peroxides can penetrate bacterial cells, but superoxide anion radicals cannot due to their negative charge (Bogdan et al., 2015). Therefore, despite the uncertainty of which molecules of ROS are produced, it is likely that when ROS is produced in proximity to bacterial cells, it would be more effective in leading them to cell death. To sum up, based on the fact that CA produces ROS by photo-oxidation upon light irradiation and was able to be strongly absorbed inside cells, it would be able to produce ROS not only outside the cells but also intracellularly, making it advantageous to destruct inner cell structure directly. Thus, this would

explain why CA + UV-A treated bacteria was able to be effectively inactivated.

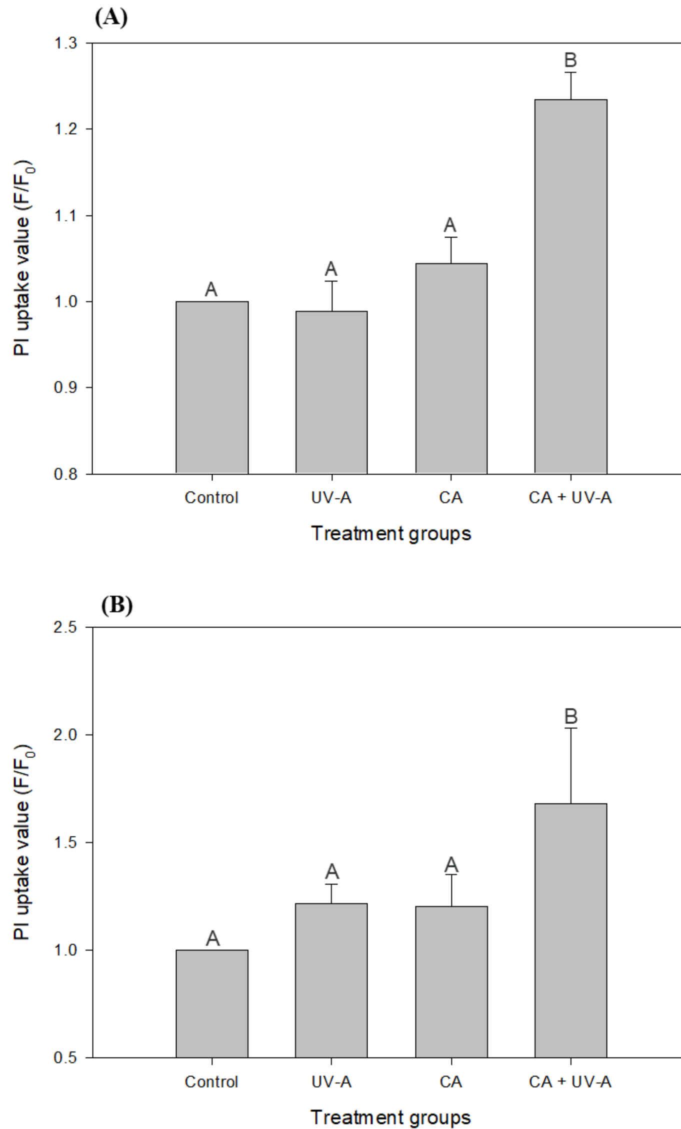


Fig 4 Membrane damage values obtained by measuring Propidium Iodide (PI) uptake values after each treatment of *S.* Typhimurium (A) and *L. monocytogenes* (B) from three replicate data. The values were calculated by dividing the obtained fluorescence (F) with control value (F_0). Different uppercase letters indicate significant differences ($P < 0.05$).

In Fig. 5, respiratory chain dehydrogenase activity values were obtained by measuring the absorbances at 490 nm (OD_{490}) of each group, which are the quantitative data of iodonitrotetrazolium formazan (INF) and were expressed as the percentage (%) against the control value. Therefore, if there is a decrease in OD_{490} value, it indicates a reduction in the respiratory dehydrogenase activity of bacteria. Although UV-A induced a slight decrease in INF measurement in *S*. Typhimurium, it did not show any significant effect ($P > 0.05$) compared to control in *L. monocytogenes*. In contrast, in all groups with CA treatment, the INF measurement (%) values were significantly ($P < 0.05$) reduced. This result is consistent with other previous reports, showing the ability of polyphenols to inhibit bacterial enzyme activity, including respiratory enzymes. Specifically, according to Haraguchi et al. (Haraguchi, Tanimoto, Tamura, Mizutani, & Kinoshita, 1998), Licochalcone A and C, which are natural phenols, were found to inhibit bacterial respiration chain. Also, Konishi et al. (KONISHI et al., 1993) showed that Tannins have an ability of reducing NADH dehydrogenase activity in various organisms such as *Paracoccus denitrificans* and *Bacillus subtilis.* Therefore, CA would have inhibited bacterial enzymes itself in both pathogens although it did not actually result in significant reduction of bacterial population, since many factors other than enzyme damage comprehensively contribute to cell death.

Furthermore, when combined with UV-A light, CA enhanced the ability of the treatment to inhibit bacterial respiratory enzymes significantly ($P < 0.05$) in *L. monocytogenes* although there was no further effect ($P > 0.05$) in *S*. Typhimurium. This suggests a possibility of the simultaneous treatment of CA and UV-A to more effectively inhibit bacterial enzymatic activity than single treatment. In this assay, INT is reduced by succinate dehydrogenase to form INF, making it possible for spectrophotometric detection (Munujos, Collcanti, Gonzalezsastre, & Gella, 1993). According to Noster et al. (Noster et al., 2019), succinate dehydrogenase is one of the most vulnerable enzymes toward ROS in the TCA cycle of bacteria. Therefore, it can be inferred that due to ROS production by CA + UV-A, the dehydrogenase activity would have been impaired, leading to a less formation of INF.

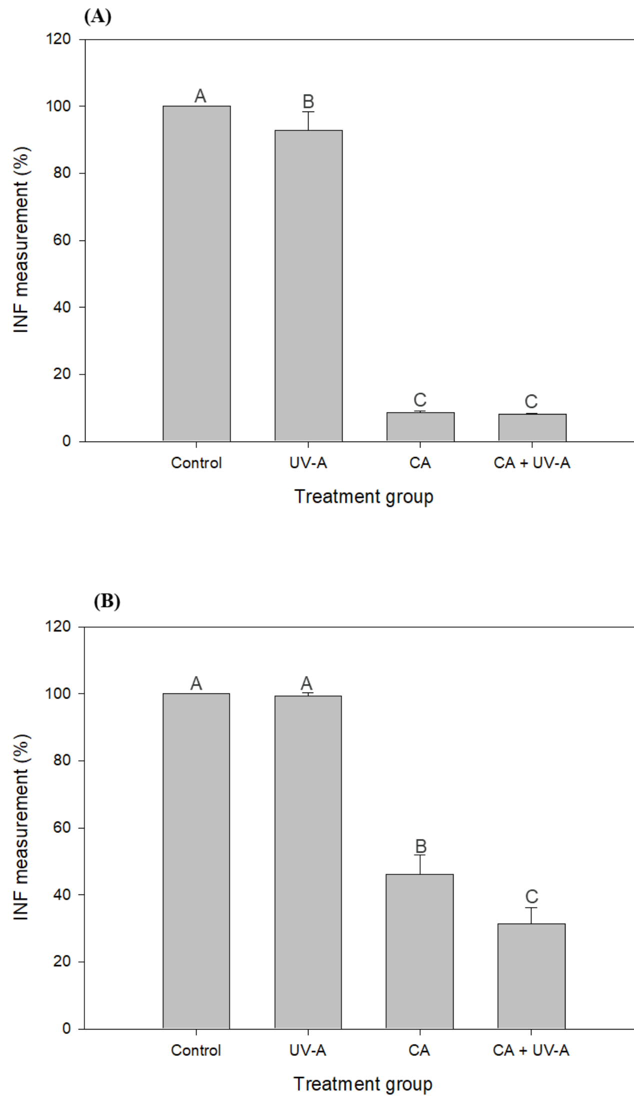


Fig 5 Respiratory chain dehydrogenase activity values obtained by INF measurement of *S.* Typhimurium (A) and *L. monocytogenes* (B). The values indicate the percentage (%) against the control group. The error bars are standard deviations from three replicate data. Different uppercase letters indicate significant differences ($P < 0.05$).

Finally, the TEM image (Fig. 6) was taken to visually analyze the inactivation mechanism. *S.* Typhimurium subjected to each treatment and its control was examined using transmission electron microscopy. Although the groups that were treated with UV-A and CA (Fig. 6B and 6C each) seemed to show a slightly wrinkled cell membrane, severe disruptions in cell morphology compared to control (Fig. 6A) was not detected visually. However, CA + UV-A treated cells (Fig. 6D) exhibited fatally destructed internal cellular materials, which are marked with arrows. Considering this, together with the explanations above, it is suggested that CA + UV-A was an effective treatment by completely destroying the inner cell structure by generating ROS from inside the cells.

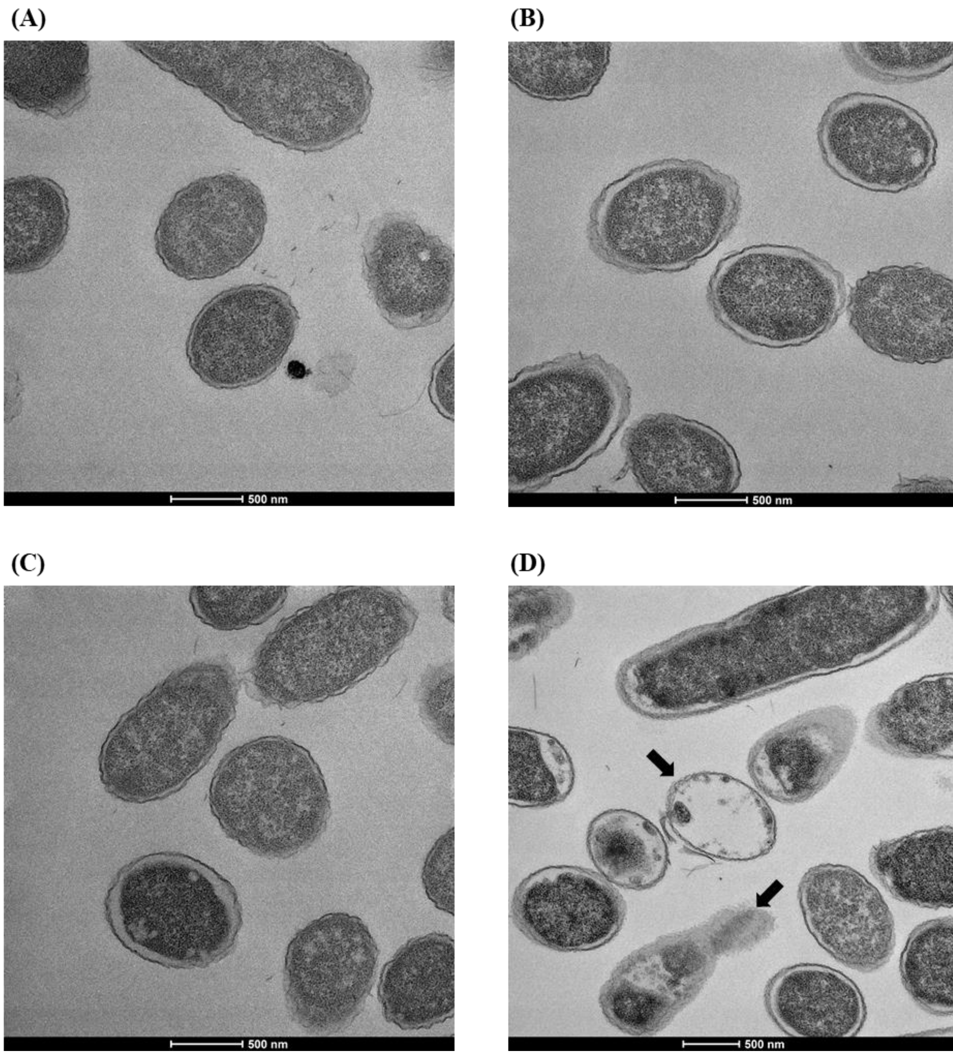


Fig 6 TEM image of *S.* Typhimurium for control (A), UV-A (B), CA (C) and CA + UV-A (D). Arrows indicate destroyed structures.

3.3 Application of CA + UV-A treatment on fresh produce washing

3.3.1 Reusability of caffeic acid for CA + UV-A treatment

Fig. 7 depicts the bacterial population of *E. coli* O157:H7 enumerated from three consecutive cycles of CA + UV-A treatment. In every cycle, CA + UV-A treatment with a dose of 5 J/cm^2, was able to inactivate more than 6 log CFU/ml of *E. coli* O157:H7. This result suggests that using CA + UV-A for fresh produce washing is efficient, in the way that it is able to retain its strong antibacterial activity for at least three cycles. It is an important issue to minimize water consumption when handling organic productions (Ölmez & Kretzschmar, 2009). Therefore, this treatment might provide a cost-effective way for fresh produce washing.

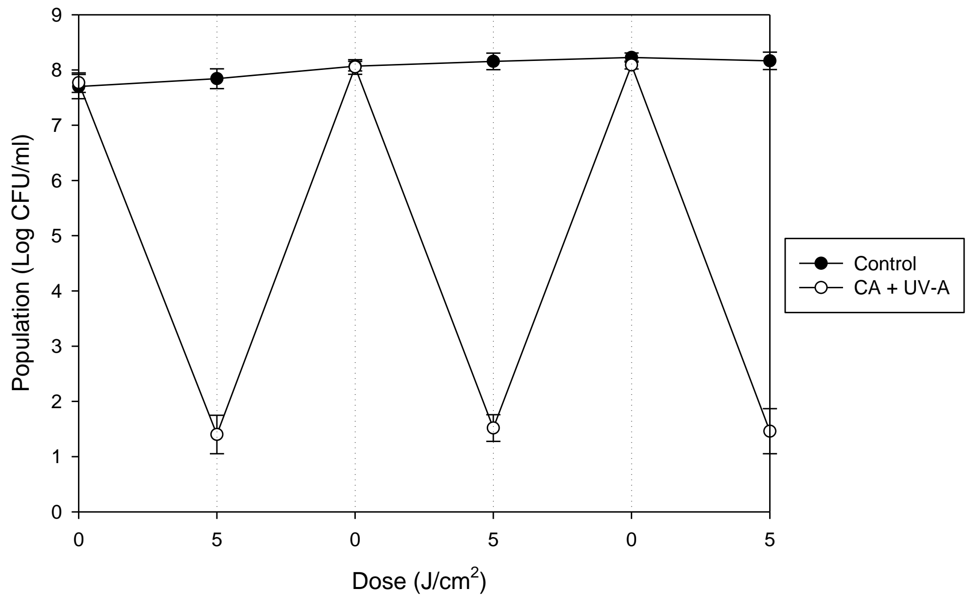


Fig 7 Bacterial population (Log CFU/ml) of *E. coli* O157:H7 from three consecutive cycles of CA + UV-A treatment. The error bars indicate standard deviations.

3.3.2 Effects of organic content and turbidity in wash-water

When it comes to fresh produce sanitation, organic content generated during washing is one of the most important factors that affect its inactivation efficacy (Ölmez & Kretzschmar, 2009). For example, chlorine, which is widely used for sanitation, is reported to rapidly react with organic matters existing in wash-water resulting in a decrease in its free chlorine concentration (Shen, Norris, Williams, Hagan, & Li, 2016). This not only limits its efficacy, but also produces chemical by-products (Cardador & Gallego, 2012). In addition to organic content, turbidity is another problem that affects the inactivation efficacy when using UV-C light for wash-water disinfection (Kim, Shin, Kang, Kim, & Kang, 2020). Therefore, to validate its efficiency of CA + UV-A in fresh produce washing, bacterial reduction was observed in simulated wash-water with varying organic content and turbidity.

Table 2 and 3 show the bacterial population of *E. coli* O157:H7 and *L. monocytogenes* subjected to CA + UV-A, in distilled water containing varied organic content and turbidity, respectively. The results of CA and UV-A single treatments were not presented, since they resulted in <1 log reduction at every organic content for the maximum dose (10 J/cm^2). In the result of organic content (Table 2), except for a slight difference in its population noticed at 4

J/cm^2 for *E. coli* O157:H7, and 8 J/cm^2 for *L. monocytogenes*, there was no significant difference between three organic contents at every light dose. Also, there was no noticeable effect of increasing turbidity, as similar amount of reductions occurred at same treatment doses (Table 3). This result indicates that, CA + UV-A treatment could maintain its inactivation efficacy regardless of the organic matters and turbidity present in the wash-water. Unlike other sanitation treatments, as CA + UV-A is a combination treatment, both elements of caffeic acid molecule and UV-A light should be considered.

The organic matters are known to entrap and protect microorganisms from disinfection or even show high reactivity towards the disinfectants (Ayyildiz, Ileri, & Sanik, 2009). However, when it comes to PDI for wash-water, this quenching effect is limited. For example, according to the results of Cossu et al. (Cossu et al., 2016), when Rose bengal was used as a photosensitizer, it exhibited 6 log CFU reduction in simulated wash-water with high organic load of 2000 ppm. Similarly, UV-A irradiated curcumin could maintain high bacterial inactivation in high organic content (de Oliveira et al., 2018). As the main principle of PDI is the action of ROS produced by the irradiated photosensitizer, it is not the reaction of photosensitizer itself that dominates the antimicrobial activity. Therefore, as caffeic acid is not a highly reactive molecule like chlorine, it is unlikely for the organic matters to react with CA.

Hence, it is supposed that CA can still generate ROS upon light irradiation. Moreover, considering the possibility of organic matters to absorb the light instead of CA, additional experiment to obtain the absorption coefficient was conducted. Fig. 8 depicts the absorption coefficients of organic content containing water, at each wavelength of UV-C and UV-A. High absorption coefficient of liquid indicates low penetration of light, due to high absorption or scattering of light by suspended solid matters (Gayán, Álvarez, & Condón, 2013; Gouma, Gayán, Raso, Condón, & Álvarez, 2015; Koutchma, Keller, Chirtel, & Parisi, 2004). As shown in Fig. 8, the absorption coefficients obtained at UV-A wavelengths were 0.05, 0.03, 0.03, and 0.02, respectively, being significantly lower than those of UV-C wavelengths, which were 6.04, 1.57, 1.18 and 0.88 each. Generally, when UV-C light is used for antimicrobial studies, the inactivation efficiency is strongly reduced due to an increase in the absorption coefficient (Gayán et al., 2013; Gouma et al., 2015). This indicates that UV-C is easily affected by the organic matters in the liquid. In this study, however, as the coefficients in UV-A were obtained near zero, it is supposed that regardless of high organic content in water, UV-A light is less likely to be disturbed by the organic matters and is able to sufficiently reach the dissolved caffeic acid molecules.

Furthermore, Fig. 9 shows the light transmittance of UV-C and UV-A at corresponding turbidities. Similar to the organic matters, turbidity decreases light transmittance by scattering the light. To overcome this reduction in its effectiveness, other processing methods such as thermal treatments are combined with UV-C to inactivate microorganisms present in turbid medium (Carrillo, Ferrario, & Guerrero, 2017). As shown in Fig. 9, the light transmittance of UV-C rapidly decreased from 100% to 4 – 12%, with a reduction rate of average 50% each time when turbidity was increased by 25 NTU. In contrast, although UV-A also showed a reduction in its transmittance, the reduction rate was average 30%, being relatively lower. To sum up, although light itself is not the crucial factor for CA + UV-A treatment, UV-A does show an advantage regarding the susceptibility to organic matters and turbidity over UV-C.

Table 2 Bacterial population (Log CFU/ml) of *E. coli* O157:H7 and *L. monocytogenes* subjected to CA (3 mM) + UV-A, in distilled water containing varied organic content (0, 200, 2000 ppm)

Bacterial strain	Dose (J/cm^2)	Organic content (ppm)[a]		
		0	200	2000
***E. coli* O157:H7**	**0**	5.61 ± 0.09 Aa	5.61 ± 0.09 Aa	5.61 ± 0.09 ABa
	2	2.86 ± 0.24 Ba	2.56 ± 0.36 Ba	2.41 ± 0.51 ABa
	4	2.59 ± 0.26 Bb	2.26 ± 0.73 Bab	1.55 ± 0.07 Ca
	6	1.22 ± 0.87 Ca	1.34 ± 0.37 Ca	0.75 ± 0.21 Ca
	8	N/D	0.30 ± 0.52 Ca	N/D
	10	N/D	N/D	N/D
L. monocytogenes	**0**	7.16 ± 0.06 Aa	7.16 ± 0.06 Aa	7.16 ± 0.06 Aa
	2	6.79 ± 0.22 Aa	6.82 ± 0.19 Aa	6.84 ± 0.14 Aa
	4	3.84 ± 0.14 Ba	4.34 ± 0.15 Ba	4.43 ± 0.74 Ba
	6	3.44 ± 0.44 BCa	3.43 ± 0.31 BCa	2.86 ± 0.77 Ca
	8	3.38 ± 0.42 BCb	3.55 ± 0.41 BCb	2.50 ± 0.18 Ca
	10	3.13 ± 0.23 Ca	2.94 ± 1.07 Ca	2.34 ± 0.32 Ca

[a]Data were expressed as means ± standard deviations. Values in the same column with the same uppercase letter are not significantly different ($P > 0.05$). Values in the same row with the same lowercase letter are not significantly different ($P > 0.05$). N/D : Not detected.

Table 3 Bacterial population (Log CFU/ml) of *E. coli* O157:H7 and *L. monocytogenes* subjected to CA (3 mM) + UV-A, in distilled water with various turbidites (0, 25, 50, 100 NTU)

Bacterial strain	Dose (J/cm^2)	Turbidity (NTU)[a]			
		0	**25**	**50**	**100**
***E. coli* O157:H7**	**0**	6.21 ± 0.29 Aa	6.21 ± 0.29 Aa	6.21 ± 0.29 Aa	6.21 ± 0.29 Aa
	2	2.95 ± 0.24 Ba	2.43 ± 0.14 Bab	1.70 ± 0.53 Bb	2.56 ± 0.64 Bab
	4	2.45 ± 0.05 Ba	1.73 ± 0.44 Cab	1.12 ± 1.04 Bab	0.83 ± 0.75 Bb
	6	1.74 ± 0.13 Ca	0.69 ± 0.60 Dab	0.69 ± 0.74 BCab	0.60 ± 0.52 BCb
	8	0.30 ± 0.52 Da	N/D	N/D	N/D
	10	N/D	N/D	N/D	N/D
L. monocytogenes	**0**	7.21 ± 0.03 Aa	7.21 ± 0.03 Aa	7.21 ± 0.03 Aa	7.21 ± 0.03 Aa
	2	6.71 ± 0.21 Aa	6.87 ± 0.23 Aa	6.92 ± 0.12 Aa	6.86 ± 0.16 Aa
	4	3.81 ± 0.66 Aa	4.20 ± 0.73 Aa	4.44 ± 0.09 Ba	4.24 ± 0.67 Ba
	6	3.18 ± 0.04 BCa	3.84 ± 0.41 BCb	4.02 ± 0.01 Bb	3.27 ± 0.32 Ca
	8	2.83 ± 0.34 Cab	3.10 ± 0.65 CDa	2.15 ± 0.49 Cb	3.23 ± 0.58 Ca
	10	3.14 ± 0.42 Ca	2.65 ± 0.48 Dab	2.08 ± 0.12 Cb	2.66 ± 0.64 Cab

[a]Data were expressed as means ± standard deviations. Values in the same column with the same uppercase letter are not significantly different ($P > 0.05$). Values in the same row with the same lowercase letter are not significantly different ($P > 0.05$). N/D : Not detected.

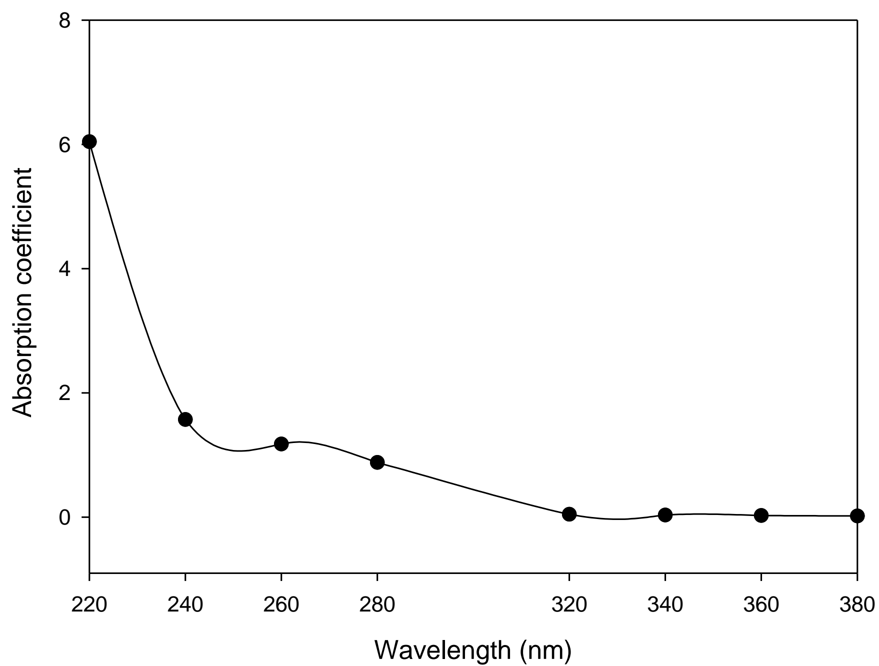


Fig 8 Absorption coefficients of organic content obtained at each wavelength of UV-C (220, 240, 260 and 280 nm) and UV-A (320, 340, 360 and 380 nm).

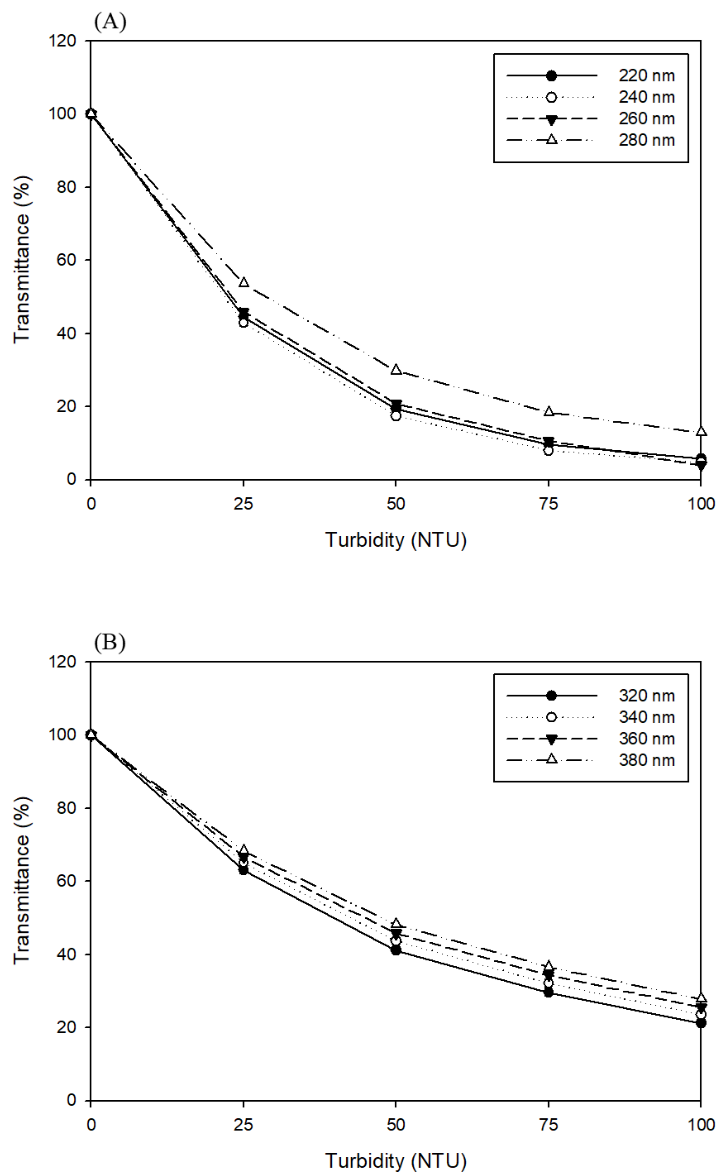


Fig 9 Light transmittance (%) of water with varied turbidity (NTU) at each wavelength of (a) UV-C (220, 240, 260 and 280 nm) and (b) UV-A (320, 340, 360 and 380 nm).

3.3.3 Application on fresh produce washing : apple

3.3.3.1 Inhibition of cross-contamination during washing

Fig. 10 depicts bacterial population of *E. coli* O157:H7 and *L. monocytogenes* enumerated from apple slices after washing with simulated cross-contamination. For *E. coli* O157:H7, UV-A single treated sample showed a reduction of 1.87 log CFU/ml than control. Although UV-A did not show any antibacterial activity previously in this study, as the treatment time was 2 before their attachment to produce surfaces, which leads to the inhibition of cross-contamination. As it is supposed that the level of bacterial contamination would be generally much lower than the inoculated population in this study, CA + UV-A treatment might provide even better efficacy for actual applications.

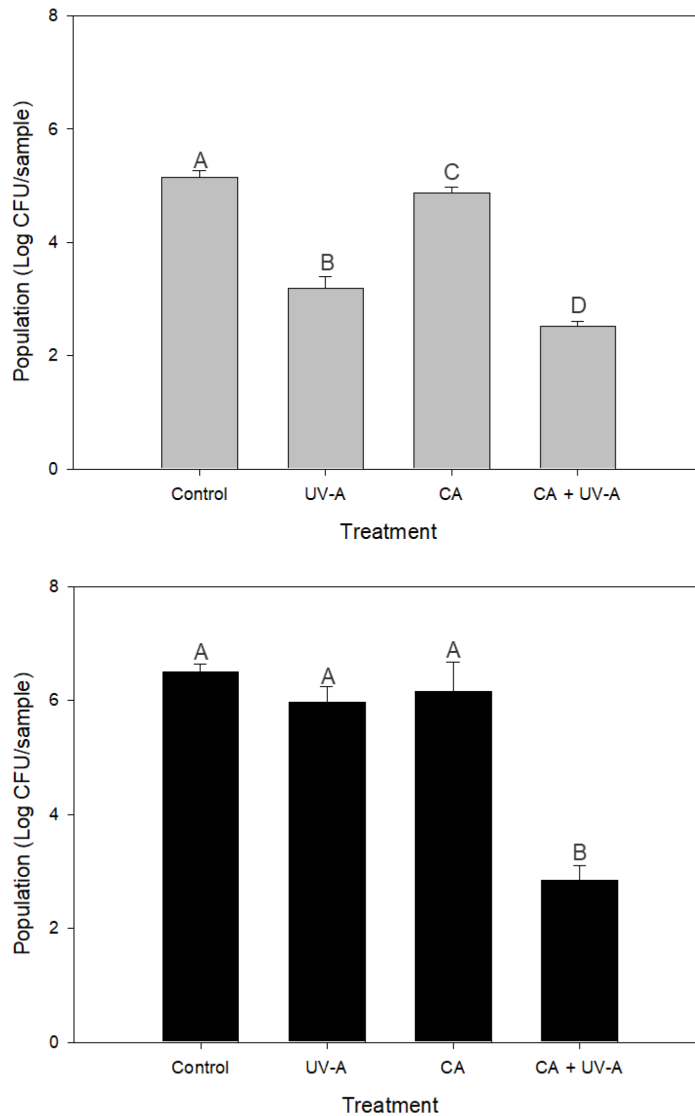


Fig 10 Bacterial population (Log CFU/sample) of *E. coli* O157:H7 (A) and *L. monocytogenes* (B) enumerated from apple slices after washing with simulated cross-contamination. The error bars indicate standard deviations. Different uppercase letters indicate significant differences ($P < 0.05$

Table 4 Population (Log CFU/ml) enumerated from liquid (100 ml) after washing with simulated cross-contamination

Bacterial strain	Population (Log CFU/ml)[a]			
	Control	UV-A	CA	CA + UV-A
***E. coli* O157:H7**	5.72 ± 0.36 A	2.72 ± 0.51 B	3.27 ± 0.59 B	N/D
L. monocytogenes	6.62 ± 0.22 A	5.36 ± 0.26 A	4.15 ± 0.21 B	N/D

[a]Data were expressed as means ± standard deviations. Values in the same row with the different uppercase letter are significantly different ($P < 0.05$). N/D : Not detected.

3.3.3.2 Inactivation of pathogen inoculated on fresh produce

In addition to the inhibition of cross-contamination, the ability of CA + UV-A to inactivate high levels of bacterial population inoculated on produce surfaces was studied. Fig 11. shows the bacterial population of *E. coli* O157:H7 and *L. monocytogenes* enumerated after the washing of inoculated apple slices. To inactivate the inoculated bacteria, bacterial detachment from the surface is involved. Since apple slices were washed with constant stirring, detachment could have occurred due to the random movement and rotation of the sample, similar to other studies of water-assisted decontamination (Huang & Chen, 2014). However, considering the possibility of cross-contamination as stated above, the detached bacteria can also be re-attached to the surfaces, resulting in no further reduction by UV-A or CA for both pathogens. In contrast, CA + UV-A combination treatment was able to inactivate both pathogens to detection limit, not only in solid but also in liquid. To sum up, as fresh produce decontamination is a challenge in food industry, CA + UV-A can act as a potential treatment for washing; in regard to its economic feasibility due to re-usability, ability to inactivate pathogens regardless of organic matters and turbidity, and effective inhibition of cross-contamination in addition to the contamination itself.

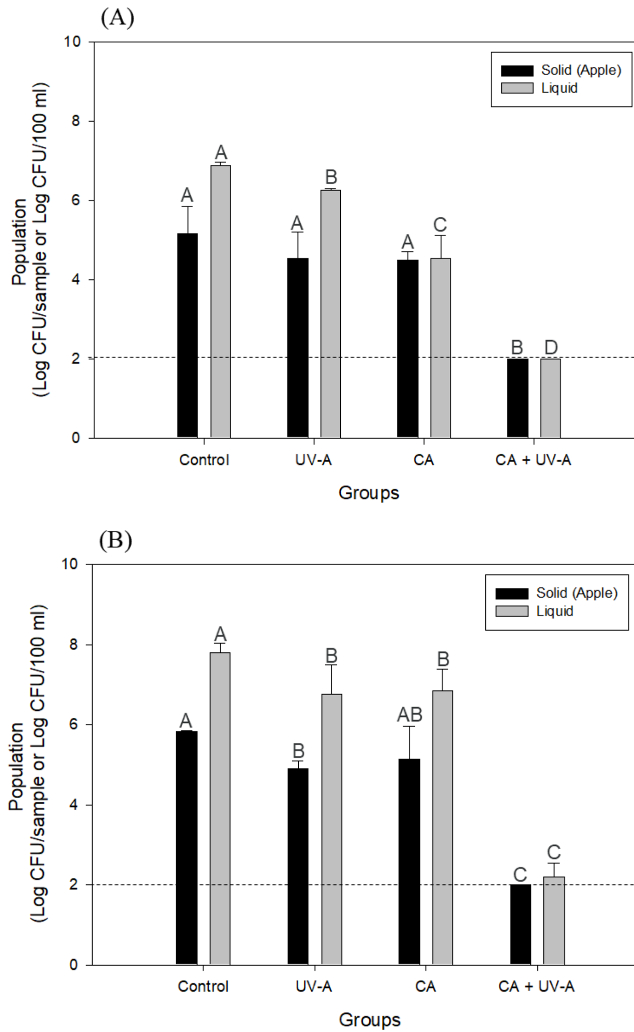


Fig 11 Bacterial population of *E. coli* O157:H7 (A) and *L. monocytogenes* (B) enumerated after the washing of inoculated apple slices. Values were expressed as log CFU/sample for solid (apple) sample or log CFU/100 ml for liquid sample, respectively. The error bars indicate standard deviations. Different uppercase letters of each sample indicate significant differences ($P < 0.05$). Limit of detection was 2 log CFU/sample or 100 ml.

IV. CONCLUSION

To sum up, this study demonstrated that the combination of caffeic acid and UV-A light is an effective antibacterial treatment, having a potential for future application in food industry. The underlying inactivation mechanism of CA + UV-A was mainly related to the uptake of CA by the cells and consequently, ROS production upon UV-A irradiation which caused a significant damage in cell membrane, enzymatic activity and inner cell structures. Furthermore, CA + UV-A was also revealed to be effective for fresh produce washing. From the results, it was proven that CA + UV-A washing is a cost-effective treatment, possessing an ability to inhibit cross-contamination and to inactivate initial contamination of bacteria to food sample, regardless of organic materials and turbidity in wash-water. Therefore, as this study includes fundamental data of CA + UV-A antibacterial PDI treatment, it can provide a guideline for further relevant studies. Moreover, considering the necessity to develop new, effective and safe techniques for fresh produce sanitation, CA + UV-A can serve as a novel treatment that can replace existing antibacterial methods.

V. REFERENCES

Attri, P., Kim, Y. H., Park, D. H., Park, J. H., Hong, Y. J., Uhm, H. S., . . . Choi, E. H. (2015). Generation mechanism of hydroxyl radical species and its lifetime prediction during the plasma-initiated ultraviolet (UV) photolysis. *Scientific reports, 5*, 9332.

Ayyildiz, O., Ileri, B., & Sanik, S. (2009). Impacts of water organic load on chlorine dioxide disinfection efficacy. *Journal of Hazardous Materials, 168*(2-3), 1092-1097.

Balupillai, A., Prasad, R. N., Ramasamy, K., Muthusamy, G., Shanmugham, M., Govindasamy, K., & Gunaseelan, S. (2015). Caffeic acid inhibits UVB-induced inflammation and photocarcinogenesis through activation of peroxisome proliferator-activated receptor-γ in mouse skin. *Photochemistry and Photobiology, 91*(6), 1458-1468.

Banach, J. L., Sampers, I., Van Haute, S., & der Fels-Klerx, V. (2015). Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. *International Journal of*

Environmental Research and Public Health, 12(8), 8658-8677.

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry–a critical review. *Journal of the Science of Food and Agriculture, 80*(6), 637-645.

Bogdan, J., Zarzyńska, J., & Pławińska-Czarnak, J. (2015). Comparison of infectious agents susceptibility to photocatalytic effects of nanosized titanium and zinc oxides: a practical approach. *Nanoscale research letters, 10*(1), 1-15.

Bosshard, F., Riedel, K., Schneider, T., Geiser, C., Bucheli, M., & Egli, T. (2010). Protein oxidation and aggregation in UVA-irradiated Escherichia coli cells as signs of accelerated cellular senescence. *Environmental microbiology, 12*(11), 2931-2945.

Cadet, J., Douki, T., & Ravanat, J. L. (2015). Oxidatively generated damage to cellular DNA by UVB and UVA radiation. *Photochemistry and Photobiology, 91*(1), 140-155.

Campos, F., Couto, J., Figueiredo, A., Tóth, I., Rangel, A. O., & Hogg, T. (2009). Cell membrane damage induced by phenolic acids on wine lactic acid bacteria. *International journal of food microbiology, 135*(2), 144-151.

Cardador, M. J., & Gallego, M. (2012). Effect of the chlorinated washing of minimally processed vegetables on the generation of haloacetic acids. *Journal of agricultural and food chemistry, 60*(29), 7326-7332.

Carrillo, M. G., Ferrario, M., & Guerrero, S. (2017). Study of the inactivation of some microorganisms in turbid carrot-orange juice blend processed by ultraviolet light assisted by mild heat treatment. *Journal of food engineering, 212*, 213-225.

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in microbiology, 10*, 2667.

Çeşmeli, S., & Biray Avci, C. (2019). Application of titanium dioxide (TiO2) nanoparticles in cancer therapies. *Journal of drug targeting, 27*(7), 762-766.

Cossu, A., Ercan, D., Tikekar, R. V., & Nitin, N. (2016). Antimicrobial effect of photosensitized rose bengal on bacteria and viruses in model wash water. *Food and bioprocess technology, 9*(3), 441-451.

de Oliveira, E. F., Tosati, J. V., Tikekar, R. V., Monteiro, A. R., & Nitin, N. (2018). Antimicrobial activity of curcumin in combination with light against Escherichia coli O157: H7 and Listeria innocua: applications for fresh produce sanitation. *Postharvest Biology and Technology, 137*, 86-94.

Ding, Q., Alborzi, S., Bastarrachea, L. J., & Tikekar, R. V. (2018). Novel sanitization approach based on synergistic action of UV-A light and benzoic acid: inactivation mechanism and a potential application in washing fresh produce. *Food microbiology, 72*, 39-54.

Durantini, E. N. (2006). Photodynamic inactivation of bacteria. *Current Bioactive Compounds, 2*(2), 127-142.

Ercan, D., Cossu, A., Nitin, N., & Tikekar, R. V. (2016). Synergistic interaction of ultraviolet light and zinc oxide photosensitizer for enhanced microbial inactivation in simulated wash-water. *Innovative*

Food Science & Emerging Technologies, 33, 240-250.

F Sperandio, F., Huang, Y.-Y., & R Hamblin, M. (2013). Antimicrobial photodynamic therapy to kill Gram-negative bacteria. *Recent patents on anti-infective drug discovery, 8*(2), 108-120.

Forkink, M., Smeitink, J. A., Brock, R., Willems, P. H., & Koopman, W. J. (2010). Detection and manipulation of mitochondrial reactive oxygen species in mammalian cells. *Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1797*(6-7), 1034-1044.

Gayán, E., Álvarez, I., & Condón, S. (2013). Inactivation of bacterial spores by UV-C light. *Innovative Food Science & Emerging Technologies, 19*, 140-145.

Ghate, V. S., Zhou, W., & Yuk, H. G. (2019). Perspectives and trends in the application of photodynamic inactivation for microbiological food safety. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 402-424.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International journal of food microbiology, 134*(1-2), 37-45.

Gilbert, A. R., Alborzi, S., Bastarrachea, L. J., & Tikekar, R. V. (2018). Photoirradiated caffeic acid as an antimicrobial treatment for fresh produce. *FEMS microbiology letters, 365*(13), fny132.

Gouma, M., Gayán, E., Raso, J., Condón, S., & Álvarez, I. (2015). Inactivation of spoilage yeasts in apple juice by UV–C light and in combination with mild heat. *Innovative Food Science & Emerging Technologies, 32*, 146-155.

Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology, 217*(2-3), 213-220.

Hamamoto, A., Mori, M., Takahashi, A., Nakano, M., Wakikawa, N., Akutagawa, M., . . . Kinouchi, Y. (2007). New water disinfection system using UVA light-emitting diodes. *Journal of applied microbiology, 103*(6), 2291-2298.

Hamblin, M. R., O'Donnell, D. A., Murthy, N., Rajagopalan, K., Michaud, N., Sherwood, M. E., & Hasan, T. (2002). Polycationic photosensitizer conjugates: effects of chain length and Gram classification on the photodynamic inactivation of bacteria. *Journal of Antimicrobial Chemotherapy, 49*(6), 941-951.

Haraguchi, H., Tanimoto, K., Tamura, Y., Mizutani, K., & Kinoshita, T. (1998). Mode of antibacterial action of retrochalcones from Glycyrrhiza inflata. *Phytochemistry, 48*(1), 125-129.

Hessling, M., Spellerberg, B., & Hoenes, K. (2017). Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths–a review on existing data. *FEMS microbiology letters, 364*(2).

Hu, J., Lin, S., Tan, B. K., Hamzah, S. S., Lin, Y., Kong, Z., . . . Zeng, S. (2018). Photodynamic inactivation of Burkholderia cepacia by curcumin in combination with EDTA. *Food Research International, 111*, 265-271.

Hua, G., & Reckhow, D. A. (2007). Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. *Water research, 41*(8), 1667-1678.

Huang, Y., & Chen, H. (2014). A novel water-assisted pulsed light processing for decontamination of blueberries. *Food microbiology, 40*, 1-8.

Kang, J.-W., & Kang, D.-H. (2019). Decontamination effect of the Spindle and 222-nanometer krypton-chlorine excimer lamp combination against pathogens on apples (Malus domestica Borkh.) and bell peppers (Capsicum annuum L.). *Applied and Environmental Microbiology, 85*(12).

Kang, J.-W., Kim, S.-S., & Kang, D.-H. (2018). Inactivation dynamics of 222 nm krypton-chlorine excilamp irradiation on Gram-positive and Gram-negative foodborne pathogenic bacteria. *Food Research International, 109*, 325-333.

Kim, S.-S., Shin, M., Kang, J.-W., Kim, D.-K., & Kang, D.-H. (2020). Application of the 222 nm krypton-chlorine excilamp and 280 nm UVC light-emitting diode for the inactivation of Listeria

monocytogenes and Salmonella Typhimurium in water with various turbidities. *LWT, 117*, 108458.

KONISHI, K., ADACHI, H., ISHIGAKI, N., KANAMURA, Y., ADACHI, I., TANAKA, T., . . . HORIKOSHI, I. (1993). Inhibitory effects of tannins on NADH dehydrogenases of various organisms. *Biological and Pharmaceutical Bulletin, 16*(7), 716-718.

Koutchma, T., Keller, S., Chirtel, S., & Parisi, B. (2004). Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Science & Emerging Technologies, 5*(2), 179-189.

Lam, P.-L., Wong, R.-M., Lam, K.-H., Hung, L.-K., Wong, M.-M., Yung, L.-H., . . . Gambari, R. (2020). The role of reactive oxygen species in the biological activity of antimicrobial agents: An updated mini review. *Chemico-biological interactions, 320*, 109023.

Le, P., Zhang, L., Lim, V., McCarthy, M. J., & Nitin, N. (2015). A novel approach for measuring resistance of Escherichia coli and Listeria monocytogenes to hydrogen peroxide using label-free magnetic resonance imaging and relaxometry. *Food control, 50*, 560-567.

Maisch, T. (2015). Resistance in antimicrobial photodynamic inactivation of bacteria. *Photochemical & Photobiological Sciences, 14*(8), 1518-1526.

Moss, S. H., & Smith, K. C. (1981). Membrane damage can be a significant factor in the inactivation of Escherichia coli by near-ultraviolet radiation. *Photochemistry and Photobiology, 33*(2), 203-210.

Mota, F. L., Queimada, A. J., Pinho, S. P., & Macedo, E. A. (2008). Aqueous solubility of some natural phenolic compounds. *Industrial & Engineering Chemistry Research, 47*(15), 5182-5189.

Munujos, P., Collcanti, J., Gonzalezsastre, F., & Gella, F. J. (1993). Assay of succinate dehydrogenase activity by a colorimetric-continuous method using iodonitrotetrazolium chloride as electron acceptor. *Analytical biochemistry, 212*(2), 506-509.

Nakamura, K., Ishiyama, K., Sheng, H., Ikai, H., Kanno, T., & Niwano, Y. (2015). Bactericidal activity and mechanism of photoirradiated polyphenols against Gram-positive and-negative bacteria. *Journal of agricultural and food chemistry, 63*(35), 7707-7713.

Nakamura, K., Shirato, M., Kanno, T., Lingström, P., Örtengren, U., & Niwano, Y. (2017). Photo-irradiated caffeic acid exhibits antimicrobial activity against Streptococcus mutans biofilms via hydroxyl radical formation. *Scientific reports, 7*(1), 1-13.

Nakamura, K., Yamada, Y., Ikai, H., Kanno, T., Sasaki, K., & Niwano, Y. (2012). Bactericidal action of photoirradiated gallic acid via reactive oxygen species formation. *Journal of agricultural and food chemistry, 60*(40), 10048-10054.

Noster, J., Persicke, M., Chao, T.-C., Krone, L., Heppner, B., Hensel, M., & Hansmeier, N. (2019). Impact of ROS-induced damage of TCA cycle enzymes on metabolism and virulence of Salmonella enterica serovar Typhimurium. *Frontiers in microbiology, 10*, 762.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: a review. *Food microbiology, 32*(1), 1-19.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food Science and*

Technology, 42(3), 686-693.

Papuc, C., Goran, G. V., Predescu, C. N., Nicorescu, V., & Stefan, G. (2017). Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. *Comprehensive Reviews in Food Science and Food Safety, 16*(6), 1243-1268.

Patange, A., Lu, P., Boehm, D., Cullen, P., & Bourke, P. (2019). Efficacy of cold plasma functionalised water for improving microbiological safety of fresh produce and wash water recycling. *Food microbiology, 84*, 103226.

Probst-Rüd, S., McNeill, K., & Ackermann, M. (2017). Thiouridine residues in tRNAs are responsible for a synergistic effect of UVA and UVB light in photoinactivation of Escherichia coli. *Environmental microbiology, 19*(2), 434-442.

Shen, C., Norris, P., Williams, O., Hagan, S., & Li, K. (2016). Generation of chlorine by-products in simulated wash water. *Food Chemistry, 190*, 97-102.

Silva, A. F., Borges, A., Giaouris, E., Graton Mikcha, J. M., & Simões, M. (2018). Photodynamic inactivation as an emergent strategy against foodborne pathogenic bacteria in planktonic and sessile states. *Critical reviews in microbiology, 44*(6), 667-684.

Swinehart, D. F. (1962). The beer-lambert law. *Journal of chemical education, 39*(7), 333.

Wang, Q., de Oliveira, E. F., Alborzi, S., Bastarrachea, L. J., & Tikekar, R. V. (2017). On mechanism behind UV-A light enhanced antibacterial activity of gallic acid and propyl gallate against Escherichia coli O157: H7. *Scientific reports, 7*(1), 1-11.

Ziental, D., Czarczynska-Goslinska, B., Mlynarczyk, D. T., Glowacka-Sobotta, A., Stanisz, B., Goslinski, T., & Sobotta, L. (2020). Titanium dioxide nanoparticles: prospects and applications in medicine. *Nanomaterials, 10*(2), 387.

VI. 국문초록

이 연구의 목적은 자연에 존재하는 폴리페놀 물질 중 하나인 카페익산과 자외선A의 조합처리기술을 주제로, 대표적인 식중독균인 *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*에 대한 항균능력을 분석하는 것이다. 저감화 결과는 카페익산의 농도, 빛의 파장과 조사량에 대하여 제시되었으며, 세 종류 병원균 모두 카페익산 + 자외선A의 조합처리에 의해 유의미하게 ($P < 0.05$) 저감화 되었다. 특히, *E. coli* O157:H7과 *S.* Typhimurium은 검출한계 밑으로 감소하는 결과를 얻었다. 저감화 기작을 분석하기 위해, 균의 폴리페놀 흡수 정도와 세포막 파괴 정도, 효소 활성을 측정하였고 투과현미경을 사용해 세포구조를 관찰하였다. 그 결과 카페익산은 세균 세포에 의해 유의미한 수준 ($P < 0.05$) 으로 흡수되었고, 자외선A를 조합하였을 때 그 흡수 정도가 증가하였다. 또한 카페익산에 의해 효소 활성의 감소가 관찰되었으며, 카페익산 + 자외선A 조합처리에 의해 더 큰 감소가 확인되었다. 투과현미경을 통해 조합처리 시 세포막 내 구조가 현저하게 파괴되는 것을 알 수 있었다.

나아가 신선식품 세척 적용을 위해, 카페익산의 재사용 가능성과, 유기물 및 탁도의 영향, 그리고 교차오염방지 및 초기에 오염된 식품에서의 저감화 능력을 조사하였다. 그 결과 카페익산은 세 번의 연속적인 처리 이후에도 항균 활성을 유지하였고, 카페익산 + 자외선A 조합처리의 저감화 효율은 세척수 내의 유기물과 탁도에도 영향받지 않았다. 실제 사과를 이용한 카페익산 + 자외선A 조합 세척 실험을 하였을 때, 균이 사과 표면에 부착되는 정도를 유의미하게 ($P < 0.05$) 감소시킴으로써 교차오염을 방지하였고 세척수 내 잔여 미생물도 검출되지 않았다. 또한 사과 표면에 높은 농도의 균을 접종한 뒤 카페익산 + 자외선A를 조합한 세척 실험을 한 결과, 사과와 세척수 모두에서 균이 검출 한계 밑으로 감소하는 것을 확인하였다.

따라서 본 연구는, 카페익산과 자외선A 조합처리 기술의 액체와 실제 식품 세척 과정 모두에서의 높은 저감화 효율을 통해, 해당 기술의 식품 산업에의 적용 가능성을 제시하는 연구이다. 또한 효율적인 저감화 조건과 저감화 기작의 분석 등은 추후 관련 연구에 기초 자료로 활용될 수 있을 것이다.

주요어 **: 카페익산, 자외선A, 식중독균, 항균활성, 저감화 기작, 신선 식품 세척**

학번 **: 2019-25232**