Master's Thesis of Science
in Agricultural Biotechnology

Inactivation of foodborne pathogens by combination of UVA – LED and riboflavin

UVA-LED와 리보플라빈을 이용한 식중독 균의 저감화

August 2020

The Graduate School
Seoul National University
Department of Agricultural Biotechnology

Hyun Sik Kim

Inactivation of foodborne pathogens by combination of UVA – LED and riboflavin

Advisor: Dong-Hyun Kang

Submitting a Master's Thesis of Science
in Agricultural Biotechnology

August 2020

The Graduate School
Seoul National University
Department of Agriculture Biotechnology

Hyun Sik Kim

Confirming the master's thesis written by
Hyun Sik Kim
August 2020

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

ABSTRACT

Although the low hydrogen ion concentration (pH) and water activity (A_w) have a significant adverse effect on the survival of bacteria, there have been several reports of foodborne outbreak caused such conditions. To inactivate these foodborne pathogens, in this experiment, I investigated inactivation efficacy using reactive oxygen species (ROS) using UVA-LED as a light source and riboflavin under change of pH and A_w.

The wavelength of UVA-LED was 398 nm, and the riboflavin was dissolved in an aqueous solution of 0.33 g/L and the concentration of in sample was diluted to 50 μmol/L for each experiment. The amount of light was treated as 10 J/cm^2, 20 J/cm^2, 30 J/cm^2. As a result of PBS sample, combination treatment of UVA and riboflavin showed further inactivation than UVA treatment in both *E. coli* O157:H7 and *S.* Typhimurium. As a result of variation of riboflavin concentration test, *E. coli* O157:H7 was confirmed to show an increased inactivation between 0.005 μmol/L and 0.05 μmol/L, S. Typhimurium was confirmed to show an increased inactivation

efficacy between 0.5 μmol/L and 5 μmol/L. It was confirmed that *E. coli* O157:H7 is more sensitive to the increase of reactive oxygen species than *S.* Typhimurium. And, I investigated the inactivation under changes of intrinsic factor of food, pH, and A_w. When pH lowered, UVA treatment did not show any further inactivation, but the further inactivation was confirmed in the UVA-riboflavin combination treatment. In the case of A_w test, the inactivation of UVA treatment was shown to increase as the A_w lowered, and the inactivation of UVA-riboflavin treatment was confirmed to decrease as the A_w lowered. In the apple juice, In *E. coli* O157:H7, the inactivation of UVA treatment was confirmed to increase, and the inactivation of UVA-riboflavin treatment was shown to decrease compared to the PBS treatment. For *S.* Typhimurium, there was no difference from treatment in PBS. As a result of measuring cell membrane damage, free radical species, and redox potential using PI, DCFH-DiOxyQ, and ORP, the results showed same tendency as inactivation result. In this study, it was found that all UVA-riboflavin treatments had a further inactivation than UVA treatment.

Keywords: **UVA-LED, Riboflavin, vitamin B_2, pH, water activity, reactive oxygen species**
Student Number: **2018-25630**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Fig. 6. Normalizing of redox potential in the apple juice for 30 J/cm^2. The error bars indicate standard deviations

Fig. 7. TEM Image analysis *E. coli* O157:H7, Show different morphological characteristics for (a) Control (b) Riboflavin (c) UVA (d) UVA + Riboflavin treatment

I. INTRODUCTION

The major bacterial causes of foodborne outbreaks are generally known to be *E. coli* O157:H7, *S.* Typhimurium. The low acidity food has been recognized to be an inhibitor against survival and growth of foodborne pathogens, as well as the low A_w food. However, there were numerous outbreaks related to low pH and low A_w food (1, 4, 7, 24, 33), *E. coli* O157:H7 has been shown to survive for several days in fresh juice (25). For *S.* Typhimurium, not only survived, but also grew on chopped tomatoes even though its relative low pH (4.0-4.5) (36). It has also been shown the ability of *E. coli* O157:H7 and *S.* Typhimurium to grow on temperature-abused fresh-cut mangoes (pH 4.2) (31). By April 2009, 714 persons infected with the outbreak associated occurred in peanut butter associated with strain of Salmonella Typhimurium have been reported from 46 states (10) Outbreaks of salmonellosis occurred in low A_w food, such as snack, peanut butter, dried coconut have been reported too (9).

To inactivate these pathogens, fruit juice is typically pasteurized by heating juice to 92 °C to 105 °C for 15 to 30 s. But even heat treatment is effective method to ensure the microbiological safety of fruit juices, it is unrecommended because of thermal damage to sensory quality, long processing times, high energy consumption, and low heating efficiency (25).

To overcome these disadvantages, novel way to inactivate pathogens is needed, as an alternative method.

Ultraviolet (UV), which consists of electromagnetic spectrum from 100 to 400 nm, is classified as UVA (315 to 400 nm), UVB (280 to 315 nm), and UVC (100 to 280 nm) (34). Especially, some studies have investigated the germicidal effects of UVA against bacteria (3, 8, 14). It has been reported that UVA light inactivates the bacteria by creating reactive oxygen species (e.g. H_2O_2, OH^-, O_2^-) via the photosensitization of endogenous photosensitizer (flavin, porphyrin, NAD^+/$NADP^+$), resulting in oxidative damage to DNA, whereas UVC directly causes DNA damage (3, 8, 14, 16, 30). UVA is generally thought to be a less bactericidal than UVC (16). However, this limitation can be overcome through the reaction with riboflavin.

Riboflavin, vitamin B_2, is a yellowish compound present in most living systems and granted as GRAS by FDA (32). Riboflavin can be easily reduced and oxidized by accepting and donating hydrogen or an electron by certain wavelength of light. When irradiated with visible or UVA light, riboflavin can produce reactive oxygen species such as superoxide anion radicals, singlet oxygen, hydroxy radical, and hydrogen peroxide in the presence of atmospheric oxygen (11, 28). The objective of this study is

identifying the combination effect of UVA with riboflavin under various conditions, using UVA-LED as a light-emitting source.

II. MATERIALS AND METHODS

2.1. UVA-LED system

Four UVA-LED modules (LG Innotek Co., Seoul, Republic of Korea) were connected to an electronic printed circuit board (PCB) to get a constant electric current of 0.5 A from a DC power supply (TPM series; Toyotech, Incheon, Republic of Korea). Four LED modules were arranged at center as a distance of 2.1 cm from each other and placed at 4.5 cm distance between the LEDs and a petri dish. This setting showed equal intensity throughout the whole petri dish (55 mm diameter). The petri dish was located directly below the LEDs to receive maximum UV exposure. The average peak wavelength of the spectrum was 398 nm.

2.1.1 Irradiance measurement

Radiation intensities were measured by a spectrometer (AvaSpec-ULS2048-USB2-UA-50; Avantes, Apeldoorn, Netherlands) calibrated for a 200 to 1100 nm range within the UV spectrum. To identify the irradiance at the same distance between LEDs and the sample, an optical probe was placed 4.5 cm above the LED and the peak irradiance, peak wavelength of the spectrum was read. The petri factor which indicates the area of even

distribution of irradiated light on a petri dish, was higher than 0.9, means a nearly uniform exposure of the whole petri dish to UV treatment (5). To calculate the petri factor, the probe scanned the surface of the petri dish every 10 mm. The intensity of each point was divided by the maximum intensity, and the petri factor was calculated as the average ratio of the intensity. The maximum intensity value was multiplied by the petri factor to obtain the corrected irradiance, which indicates the average fluence (UV dose) rate

2.2. Bacterial suspension

Three strain of *S.* Typhimurium (DT 104, ATCC 19585, and ATCC 43971) and *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890) were obtained from Seoul National University (Seoul, South Korea). Stock cultures were grown in tryptic soy broth (TSB; Difco, Becton, Dickinson and Company, Sparks, MD, USA) and stored at -80℃ in 70 ml TSB and 0.3 ml of 50 % glycerol. Working cultures were gained by streaking bacteria in stock into tryptic soy agar (TSA) and incubated at 37℃ for 24 h and stored at 4℃. Each strain of *S.* Typhimurium and *E. coli* O157:H7 was cultivated in 5 ml of TSB at 37℃ for 24 h in the shaking incubator. The pellet of bacteria was collected by centrifugation (4000 × g at 4℃ for 20 min) and then

resuspended by 9 ml of 0.2 % peptone water (PW; Bacto, Becton, Dickinson and Company, Sparks, MD, USA). Resuspended pellets were used in the form of cocktail mixed culture of two pathogen, and the final concentration of each strain of *E. coli* O157:H7 and *S.* Typhimurium was approximately 10^7 CFU/ml and 10^8 CFU/ml.

2.3. Sample preparation and inoculation

For control and UVA treatment in PBS, mixed-cultured cocktail (0.1 ml) was inoculated into 4.9 ml of PBS. For riboflavin treatment and UVA-riboflavin treatment in PBS, mixed-cultured cocktail (0.1 ml) was inoculated into the mixture of 4.618 ml of PBS, and riboflavin solution(Vitamin B_2; SIGMA-ALDRICH, USA) was prepared by dissolving riboflavin in D.W. at a concentration of 0.33 g/L (20), and 0.282 ml of riboflavin solution was added to 4.718 ml of sample to adjust final concentration of riboflavin at 50 μmol/L. To identify the effect of concentration of riboflavin, after inoculating mixed-cultured cocktail (0.1 ml) into 4.618 ml of PBS, I diluted 0.282 ml of the riboflavin solution dissolved at a concentration of 0.33 g/L by one tenth, adjusted the concentrations of 50 μmol/L, 5 μmol/L, 0.5 μmol/L, 0.05 μmol/L, 0.005 μmol/L and inoculated into the solution. For pH test, I adjusted pH (pH 3 - 6) using pH meter (Seven multi 8603, Mettler Toledo, Greifensee, Switzerland) by adding 6N-hydrochloric acid solution

(HCl; Samchun Chemical Co., Pyeongtaek-si, South Korea). And mixed-cultured cocktail (0.1 ml) was inoculated into 4.9 ml of PBS at different pH for control and UVA treatment, and mixed-cultured cocktail (0.1 ml) and 0.282 ml of riboflavin solution (0.33 g/L) was inoculated into the mixture of 4.618 ml of PBS at different pH for UVA-riboflavin and riboflavin treatment. The concentration of riboflavin added to each sample was 50 μmol/L. For A_w test, I adjusted A_w (0.975, 0.950, 0.942) of D.W. by adding sucrose (MB Cell, Seoul, Republic of Korea) (13) using A_w meter (AQUA Lab 4TE, Decagon Inc., Pullman, WA). And a mixed-cultured cocktail (0.1 ml) was inoculated into 4.9 ml of PBS at different A_w for control and UVA treatment, and a mixed-cultured cocktail (0.1 ml) and 0.282 ml of riboflavin solution (0.33 g/L) was inoculated into the mixture of 4.618 ml of PBS at different A_w for UVA-riboflavin treatment and riboflavin treatment. The concentration of riboflavin added to each sample was 50 μmol/L. For apple juice test, apple juice was purchased from a local grocery store. Mixed-cultured cocktail (0.1 ml) was inoculated into 4.9 ml of apple juice for control and UVA treatment, and mixed-cultured cocktail (0.1 ml) and 0.282 ml or riboflavin solution (0.33 g/L) was inoculated into the mixture of 4.618 ml of apple juice for UVA-riboflavin treatment and riboflavin treatment. The concentration of riboflavin added to each sample was 50 μmol/L.

2.4. UV treatment

Inoculated samples were treated with 398 nm UVA-LEDs at 0, 10, 20, 30 dosage at room temperature, 4.5 cm below the UVA-LEDs. The sample was mixed continuously with a magnetic stirrer (HY-HS11; Hanyang Science, Seoul, Republic of Korea). UVA-LED dosage were calculated by multiplying UVA-LED intensity by the irradiation time.

2.5. Bacterial cell enumeration

For bacterial enumeration, 1 ml of treated sample was obtained each dosage. After then, 1 ml of sample were 10-fold serially diluted in 9 ml of 0.2% PW and 0.1 ml of sample or diluents were spread-plated onto selective media. Sorbitol MacConkey agar (SMAC) (Difco) and Xylose lysine deoxycholate (XLD) agar (Difco) were used as selective media for *E. coli* O157:H7*, S.* Typhimurium, respectively. All plates were incubated at 37℃ for 24 h before counting the number of colonies.

2.6. Analyzing inactivation mechanism

2.6.1. Reactive oxygen species (ROS) measurement

To measure amount of ROS following treatment, DCFH-DiOxyQ (dichlorodihydrofluorescein) fluorogenic probe which is a specific ROS/RNS probe that is based on similar chemistry to the popular 2', 7'-dichlorodihydrofluorescein diacetate, of OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence) was used. The DCFH-DiOxyQ probe is first primed with a quench removal reagent, and subsequently stabilized in the highly reactive DCFH form. In this reactive state, ROS and RNS species can react with DCFH, which is rapidly oxidized to the highly fluorescent 2', 7'-dichlorodihydrofluorescein (DCF). Fluorescence intensity is proportional to the total ROS/RNS levels within the sample.

After treatment, 50 µL of treated sample solution was transferred to 96-well plate and add 50 µL of catalyst of the assay kit to each well. Incubate 5 minutes at room temperature, add 100 µL of DCFH solution to each well. Cover the plate wells to protect them from light and incubate at room temperature for 15-45 minutes. After that, read the fluorescence with a spectrophotometer at 480 nm excitation and 530 nm emission.

2.6.2. PI uptake

To identify cell membrane damage, the fluorescent dye propidium iodine (PI; Sigma-Aldrich, USA) was used to identify the viability of cell after treatment.

PI, which can bind to nucleic acids (DNA and RNA) and emits fluorescence, does not pass through the undamaged cell membrane. Using this, the degree of viability loss can be identified by fluorescent value generated by PI binding with nucleic acids through the PI uptake assay. Following treatments, treated samples were incubated with PI solution at a final concentration of 2.9 μM for 15 min at 37 °C. After incubation, cells were collected by centrifugation at 10,000 g for 10 min and washed twice with PBS, and fluorescence was measured with a spectrofluorophotometer (Spectramax M2e; Molecular Devices, CA, USA) at excitation/emission wavelengths of 535/617 nm for PI uptake assay.

2.6.3. Measurement of redox potential (ORP)

To identify the degree of oxidation, 5 ml of PBS was treated at 30 J/cm^2, for control and UVA treatment. And the mixture of 4.718 ml of PBS and 0.282 ml of riboflavin was treated at 30 J/cm^2 for UVA-riboflavin treatment and riboflavin treatment. The sample was transferred to 15 ml falcon tube following treatment, and the ORP was measured by using pH meter (Seven multi 8603, Mettler Toledo, Greifensee, Switzerland). The temperature was constantly kept at room temperature because ORP is affected by temperature.

2.6.4. Transmission electron microscopy

To identifying the morphological differences in *E. coli* O157:H7 between each treatment, transmission electron microscopy (TEM) analysis was performed. BPW containing *E. coli* O157:H7 was treated with UVA only treatment, riboflavin treatment, and UVA and riboflavin combination treatment and then centrifuged at 10,000 x g for 10 min. The cells were fixed at 4 °C for 2 to 4 h in modified Karnovsky's fixative containing 2 % paraformaldehyde and 2 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2). After primary fixation, each sample was centrifuged and rinsed three times with 0.05 M sodium cacodylate buffer (pH 7.2) at 4 °C for 5 min. Cells were then fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.2) at 4 °C for 2 h, briefly washed three times with distilled water at room temperature, and then stained overnight with 0.5% uranyl acetate at 4 °C. After staining, each sample was rinsed three times with distilled water. Samples were dehydrated at room temperature through a graded ethanol series (10 min each) of 30%, 50%, 70%, 80%, and 90% and three times at 100%. The transition was performed two times with 100% propylene oxide at room temperature for 15 min. Then first, the cells were infiltrated for 1 h with a 1:1 solution of propylene oxide and Spurr's resin. And cells were infiltrated for 1 h with a 1:2 solution of propylene oxide and

Spurr's resin. Following this, samples were placed into Spurr's resin overnight. The cells were then immersed in Spurr's resin for 3 h as a final step of infiltration and were polymerized at 70 °C for overnight. Observations were carried out by using a transmission electron microscope (Libra 120; Carl Zeiss, Oberkochen, Germany).

2.7. Statistical analysis

All experiments were replicated three times. The data were analyzed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (version 9.4, SAS Institute, Cary, NC), and mean values were separated using Duncan's multiple-range test. Significant differences were determined at a significance level of $p = 0.05$.

III. RESULTS

3.1. Emission spectrum of UVA-LED

All UVA-LED used in this study yielded similar voltage at each rated current for 6.5 V. Spectral intensity of the UVA-LEDs on PCBs was measured with a spectrometer; actual peak wavelengths was 398 nm.

3.2. Inactivation of foodborne pathogen in various conditions

3.2.1. Effect of inactivation in phosphate buffer solution (PBS)

The survival of *E. coli* O157:H7, *S.* Typhimurium in PBS following dose of 10, 20, 30 J/cm^2 of UVA, riboflavin, and UVA - riboflavin combination treatment is shown in Fig. 1. In the case of *E. coli* O157:H7, it was reduced by 1.22 log CFU/ml following 30 J/cm^2 of UVA treatment, 0.36 log CFU/ml following 30 J/cm^2 of riboflavin treatment, 6.36 log CFU/ml following 30 J/cm^2 of combination treatment, respectively. For *S.* Typhimurium, it was reduced by 2.52 log CFU/ml following 30 J/cm^2 of UVA treatment, 0.97 log CFU/ml following 30 J/cm^2 of riboflavin treatment, 4.45 log CFU/ml following 30 J/cm^2 of UVA - riboflavin treatment, respectively. There was further inactivation with UVA–riboflavin treatment compared to UVA

treatment, about 5 log CFU/ml and 2 log CFU/ml for *E. coli* O157:H7 and *S.* Typhimurium, respectively. These results indicate that the higher UV dosage increase the inactivation effect of UVA treatment and UVA-riboflavin treatment significantly ($p>0.05$), but not significantly ($p<0.05$) increasing with riboflavin treatment.

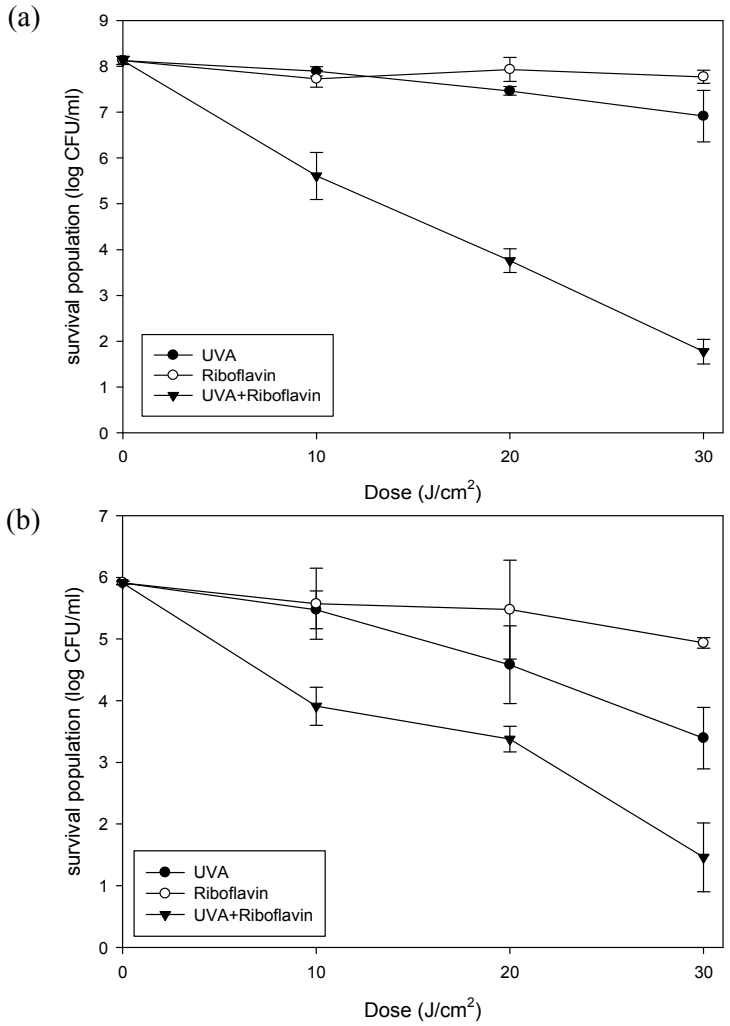

Fig. 1. Survivals of (a) *E. coli* O157:H7 (b) *S.* Typhimurium in PBS, after treatment with riboflavin and UVA irradiation, combination of UVA and riboflavin with dosages of 10, 20, and 30 J/cm^2. The error bars indicate standard deviations.

3.2.2. Comparison of effect of inactivation between different concentration of riboflavin

The inactivation efficacy of different initial concentration of riboflavin in PBS when treated with UVA is shown in Figure 2. In the case of *E. coli* O157:H7, the inactivation was significantly ($p>0.05$) increased from between 0.005 μmol/L and 0.05 μmol/L of riboflavin, from 0.79 log CFU/ml to 1.68 log CFU/ml. For *S.* Typhimurium, the inactivation was not increased until 0.5 μmol/L, but increased significantly ($p>0.05$) from between 5 μmol/L and 50 μmol/L of riboflavin, from 2.49 log CFU/ml to 4.34 log CFU/ml. This result indicates that the further inactivation of inactivation at *E. coli* O157:H7 was observed from the between 0.005 μmol/L and 0.05 μmol/L. but the further inactivation of inactivation at *S.* Typhimurium was not observed until 5 μmol/L.

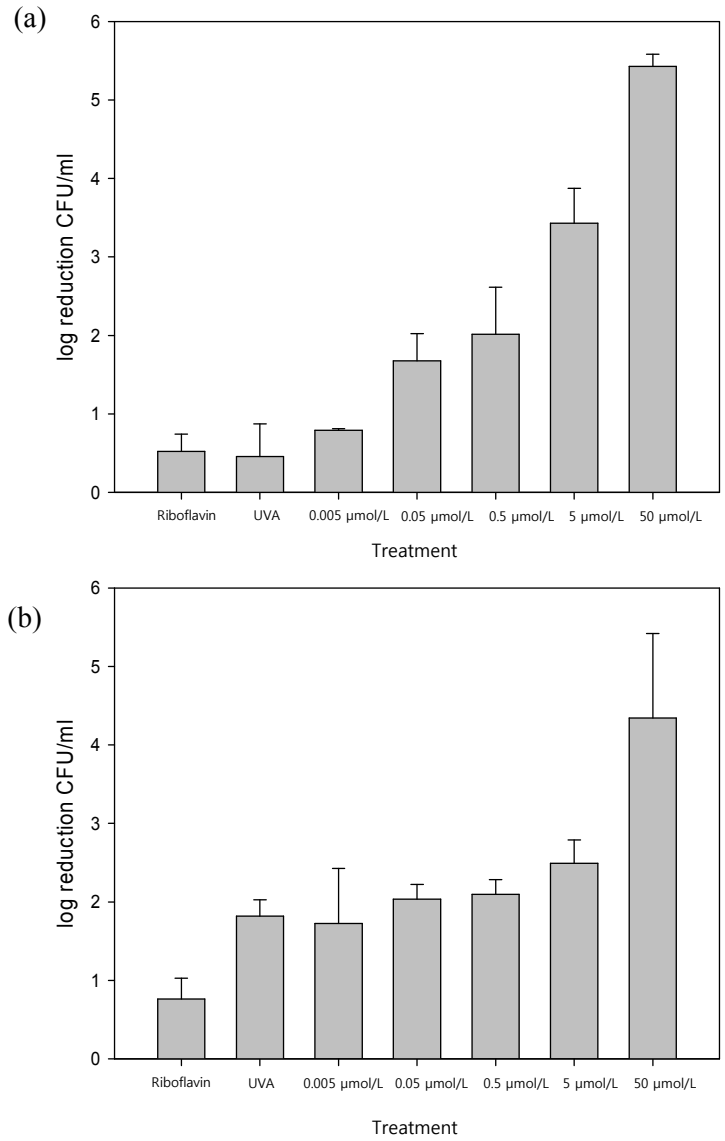

Fig 2. Log reduction of (a) *E. coli* O157:H7 and (b) *S.* Typhimurium at different riboflavin concentration, after treatment with combination of riboflavin and UVA irradiation with dosages of 30 J/cm^2. The error bars indicate standard deviations.

3.2.3. Comparison of effect of inactivation between different pH

The effect of different initial pH of PBS is shown in Table 1. In the case of *E. coli* O157:H7, there was no inactivation observed in riboflavin treatment at all pH. And no significant ($p < 0.05$) additional inactivation observed in UVA treatment between all pH. However, at the UVA-riboflavin treatment, there was further inactivation according to pH. *E. coli* O157:H7 was reduced from 5.49 log CFU/ml (pH 6) to 7.01 log CFU/ml (pH 3) at 30 J/cm^2 treatment. For *S.* Typhimurium, there was no inactivation in riboflavin treatment at all pH. And no significant ($p < 0.05$) additional inactivation observed in UVA treatment. And same as *E. coli* O157:H7, there was further inactivation in UVA-riboflavin treatment, from 3.95 log CFU/ml (pH 6) to 5.28 log CFU/ml (pH 5). However, there was no increased inactivation since pH 5. This result indicates that the range of pH 3-6 was not a factor causing additional inactivation. But the inactivation tendency increased depending on decreased pH in UVA-riboflavin treatment.

(a)

E. coli O157:H7	Dose (J/cm^2)	Log reduction[a] (log_{10} CFU/ml)			
		pH 3	pH 4	pH 5	pH 6
UVA	10	0.42±0.14Ade	0.43±0.09Aef	0.37±0.25Ad	0.61±0.26Ade
	20	0.87±0.03ABde	1±0.17Acd	0.76±0.05Acd	0.8±0.16Acd
	30	1.61±0.47Ac	1.39±0.35Ac	1.03±0.22Ac	1.47±0.35Abc
Riboflavin	10	0.13±0.10ABe	-0.06 ±0.05Bg	0.31±0.18Ad	0.18±0.25ABde
	20	0.14±0.19Ae	-0.12 ±0.22Ag	0.19±0.51Ad	-0.18±0.40Ae
	30	0.18±0.08Ae	0.04±0.14Afg	0.24±0.05Ad	0.20±0.17Ade
UVA + Riboflavin	10	0.63±0.21ABde	0.62±0.05ABde	0.96±0.32Ac	0.39±0.09Bde
	20	2.6±0.18Ab	2.37±0.39Ab	2.75±0.47Ab	2.23±0.46Ab
	30	7.01±0.81Aa	6.37±0.33ABa	5.37±0.37Ca	5.49±0.26BCa

(b)

S. Typhimurium	Dose (J/cm^2)	Log reduction[a] (log_{10} CFU/ml)			
		pH 3	pH 4	pH 5	pH 6
UVA	10	0.79±0.34Ae	0.89±0.16Ade	0.81±0.25Aef	0.55±0.11Ae
	20	1.53±0.44Ad	1.96±0.79Acd	1.58±0.11Ade	1.19±0.13Ad
	30	2.74±0.04Ac	2.57±0.34Ac	2.64±0.37Ac	2.21±0.32Abc
Riboflavin	10	0.23±0.23Af	-0.04±0.20Ae	-0.07±0.28Ag	0.04±0.09Aef
	20	-0.08±0.16ABf	0.04±0.18ABe	0.39±0.26Afg	-0.14±0.37Bf
	30	-0.10±0.20Bf	-0.02±0.15Be	0.54±0.26Afg	0.15±0.19Bef
UVA + Riboflavin	10	2.51±0.24Ac	2.21±0.35ABc	1.75±0.27Bd	1.98±0.28ABc
	20	3.53±0.24Bb	3.89±0.45ABb	4.33±0.51Ab	2.78±0.11Cb
	30	4.97±0.15Ba	5.5±0.38Aa	5.28±0.21ABa	3.95±0.21Ca

Table 1. The log reduction of *E. coli* O157:H7, *S.* Typhimurium in PBS at pH 3, 4, 5, 6 after treatment with UVA – LED, riboflavin, and combination treatment. [a] Data represent means ± standard deviations from three replications. Values followed by same uppercase letters within columns per each dose and lowercase within rows per each pH are not significantly different.

3.2.4. Comparison of effect of inactivation between different water activity (A_w)

The effect of different initial A_w in D.W. is shown Table 2. In the case of *E. coli* O157:H7, there was no inactivation observed in riboflavin treatment at all A_w. In UVA treatment, there was no additional inactivation according to A_w. However, the effect of inactivation decreased depends on A_w, from 7.51 log CFU/ml (A_w = 0.975) to 3.22 log CFU/ml (A_w = 0.942) at 30 J/cm^2 treatment in UVA-riboflavin treatment. For *S.* Typhimurium, there was no inactivation in riboflavin treatment at all A_w. In UVA treatment, inactivation efficacy was not significantly increased ($p < 0.05$) at all A_w. And same as *E. coli* O157:H7, the effect of inactivation decreased depending on decreased A_w, from 4.93 log CFU/ml (A_w = 0.975) to 3.43 log CFU/ml (A_w = 0.942) at 30 J/cm^2 treatment in UVA – riboflavin treatment.

(a)

E. coli O157:H7	Dose (J/cm^2)	Log reduction[a] (log_{10} CFU/ml)		
		0.942	0.95	0.975
UVA	10	1.41±0.09Ac	1.14±0.21ABe	0.8±0.25Be
	20	2.43±0.38Ab	2.41±0.17Ac	1.67±0.17Bd
	30	3.41±0.54Aa	2.74±0.27Ac	3.03±0.22Ac
Riboflavin	10	0.22±0.27Ad	0.31±0.28Af	-0.06±0.23Af
	20	-0.09±0.23Ad	0.19±0.26Af	0.06±0.04Af
	30	-0.18±0.05Ad	-0.04±0.35Af	-0.24±0.11Af
UVA + Riboflavin	10	1.33±0.25Ac	1.6±0.09Ad	1.68±0.46Ad
	20	1.77±0.57Bbc	4.04±0.30Ab	4.74±0.66Ab
	30	3.22±0.71Ba	7.04±0.35Aa	7.51±0.00Aa

(b)

S. Typhimurium	Dose (J/cm^2)	Log reduction[a] (log_{10} CFU/ml)		
		0.942	0.95	0.975
UVA	10	1.74±0.19Ac	1.90±0.861Ac	2.09±0.30Ad
	20	2.68±0.86Ab	2.45±0.85Abc	2.48±0.22Acd
	30	3.35±0.43Aab	2.77±0.52Abc	3.38±0.71Abc
Riboflavin	10	-0.25±0.43Ad	-0.14±0.17Ad	0.24±0.40Ae
	20	0.14±0.27Ad	-0.10±0.30Ad	-0.04±0.23Ae
	30	-0.08±0.26Ad	-0.13±0.19Ad	-0.20±0.45Ae
UVA + Riboflavin	10	1.19±0.06Cc	1.78±0.33Bc	2.64±0.08Acd
	20	1.62±0.16Bc	3.83±0.28Aab	3.85±0.20Ab
	30	3.43±0.47Ba	5.2±0.35Aa	4.93±0.34Aa

Table 2. The log reduction of (a) *E. coli* O157:H7 (b) *S.* Typhimurium in PBS at A_w = 0.942, 0.95, 0.975 after treatment with UVA-LED, Riboflavin, and combination treatment. [a] Data represent means ± standard deviations from three replications. Values followed by same uppercase letters within columns per each dose and lowercase within rows per each pH are not significantly different.

3.3. Inactivation of foodborne pathogens in apple juice

To identify the inactivation in actual food sample, the apple juice was selected as sample and the results are shown in Fig 3. For *E. coli* O157:H7, the inactivation was not estimated in riboflavin treatment. But the inactivation in UVA treatment was 1.95 log CFU/ml at 30 J/cm^2, and the inactivation of UVA-riboflavin treatment was 3.40 log CFU/ml at 30 J/cm^2. For *S.* Typhimurium, there was no inactivation in riboflavin treatment, the inactivation in UVA treatment was 2.65 log CFU/ml, and the inactivation in UVA-riboflavin treatment was 4.12 log CFU/ml. This result shows that there was further inactivation at apple juice. For *E. coli* O157:H7, the inactivation in UVA treatment was increased in apple juice compared to PBS, but the difference was less than 1 log CFU/ml. however the inactivation of UVA-riboflavin treatment was decreased compared to PBS, almost 3 log CFU/ml. For *S.* Typhimurium, there were no differences of inactivation in UVA treatment and UVA-riboflavin treatment between PBS and apple juice sample.

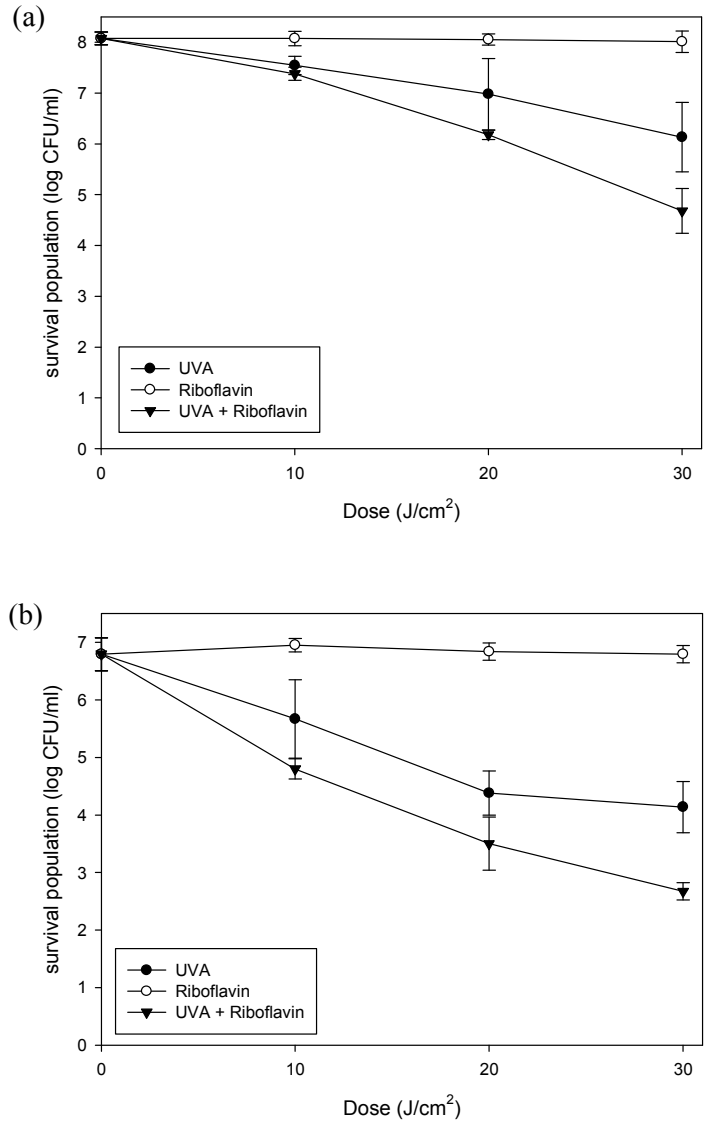

Fig 3. Survivals of (a) *E. coli* O157:H7 and (b) *S.* Typhimurium in apple juice, after treatment with riboflavin and UVA irradiation, combination of UVA and riboflavin with dosages of 10, 20, and 30 J/cm^2. The error bars indicate standard deviations

3.4. Measurement of inactivation mechanism in various conditions

3.4.1. ROS measurement

Since the ROS is the main mechanism of the UVA-riboflavin combination treatment, the amount of ROS in the treated sample was measured by using DCFH-DiOxyQ, which can detect the ROS in the sample. The normalizing data of measured ROS is shown in Fig 4. Fig 4a shows the ROS production at the PBS sample. It shows that the amount of ROS increased in UVA-riboflavin compared to other treatment. Fig 4b shows the result of measured ROS by concentration of riboflavin. The results indicate that ROS generation was not significantly ($p>0.05$) different until 0.5 μmol/L. But from the 5 μmol/L, the amount of ROS increased by almost twice compared to amount of 0.5 μmol/L. Fig 4c shows the ROS production according to pH. The tendency of ROS generation in UVA treatment and riboflavin treatment was not proportioned to change of pH. For UVA - riboflavin treatment, however, the ROS generation increased accordance of decreasing pH, which is consistent with the result of inactivation of *E. coli* O157:H7 and *S.* Typhimurium in Table 1. Fig 4d shows the ROS production by the change of A_w. The normalized amount of ROS at the $A_w = 0.950$ and $A_w = 0.975$ is about 24 % higher than at $A_w = 0.942$. And these difference

amount of ROS support the result that bacterial inactivation was lower at the $A_w = 0.942$ than $A_w = 0.950$ and $A_w = 0.975$

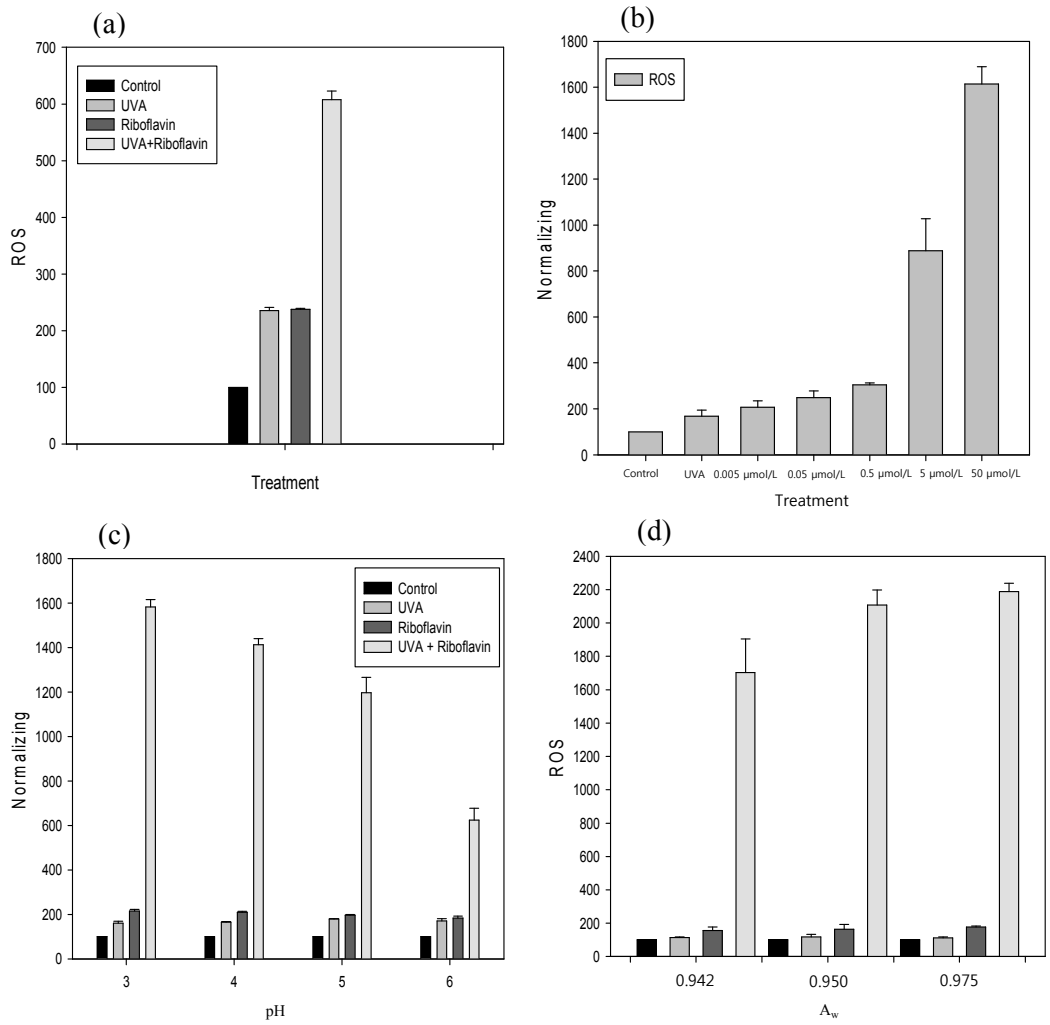

Fig 4. Normalizing of ROS generation at the (a) PBS (b) concentration of riboflavin (c) pH (d) A_w for 30 J/cm^2. The error bars indicate standard deviations.

3.4.2. PI uptake assessment

The PI uptake value depending on the change of pH and A_w is shown in Fig 5. Fig 5a shows the result at PBS that the cell membrane was intact at the riboflavin treatment, but membrane was damaged at UVA and UVA-riboflavin treatment. However, the PI uptake value was higher at the UVA-riboflavin treatment. Fig 5b shows the result accordance of pH. There was no difference in membrane damage by change of pH, and also no additional cell membrane damage in UVA treatment and riboflavin treatment. But in UVA-riboflavin treatment, the cell membrane was damaged and depends on decreasing of pH. This is consistent with the result that inactivation increased when pH is lower in UVA-riboflavin treatment. Fig 5c shows the result at change of A_w. Cell membrane damage was not shown in the riboflavin treatment but observed in the UVA treatment and UVA-riboflavin treatment. However, the cell membrane damage accordance of A_w was observed in UVA-riboflavin treatment. This is consistent with the result that bacterial inactivation increased when A_w is higher in UVA-riboflavin treatment.

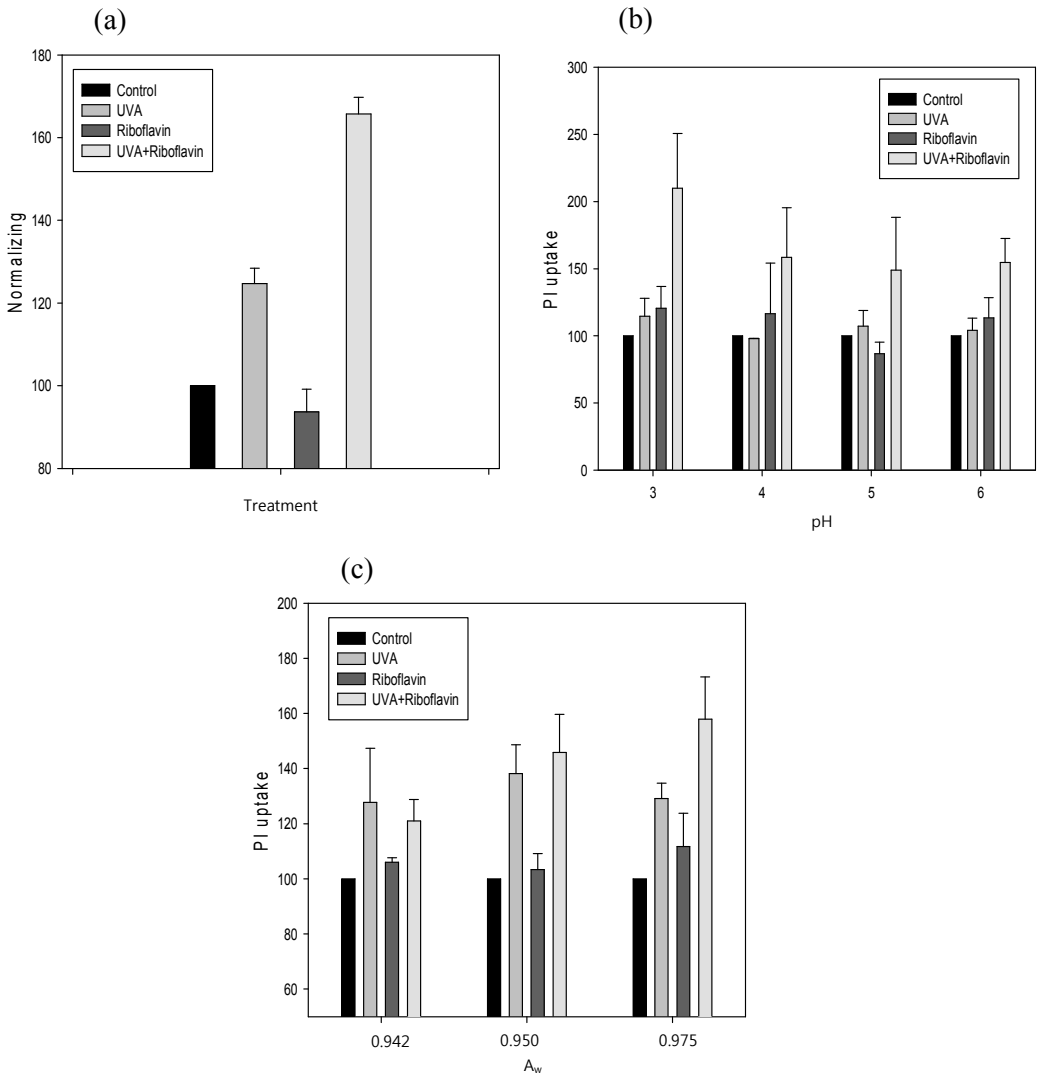

Fig 5. Normalizing of PI uptake at the different (a) PBS (b) pH (c) A_w for 30 J/cm^2. The error bars indicate standard deviations.

3.4.3. ORP measurement

The oxidative-inactivation potential (ORP) is shown in Fig 10. The ORP is defined as the ratio between oxidant and reductant. When oxidizing agent, e.g., ROS; increases, the ORP value increases and as the reducing agent, e.g., scavenger; increases, the ORP value decreases (18). The ORP value is shown in Fig 6. The ORP value of UVA single treatment was significantly ($p > 0.05$) increased by 45 mv, and the value of riboflavin treatment was slightly ($p > 0.05$) increased by 11 mv. For UVA-riboflavin combination treatment, the value of ORP was significantly ($p > 0.05$) increased by 83 mv, more than UVA treatment. This result indicated that sample treated with UVA and UVA-riboflavin generated ROS and increase the ORP value.

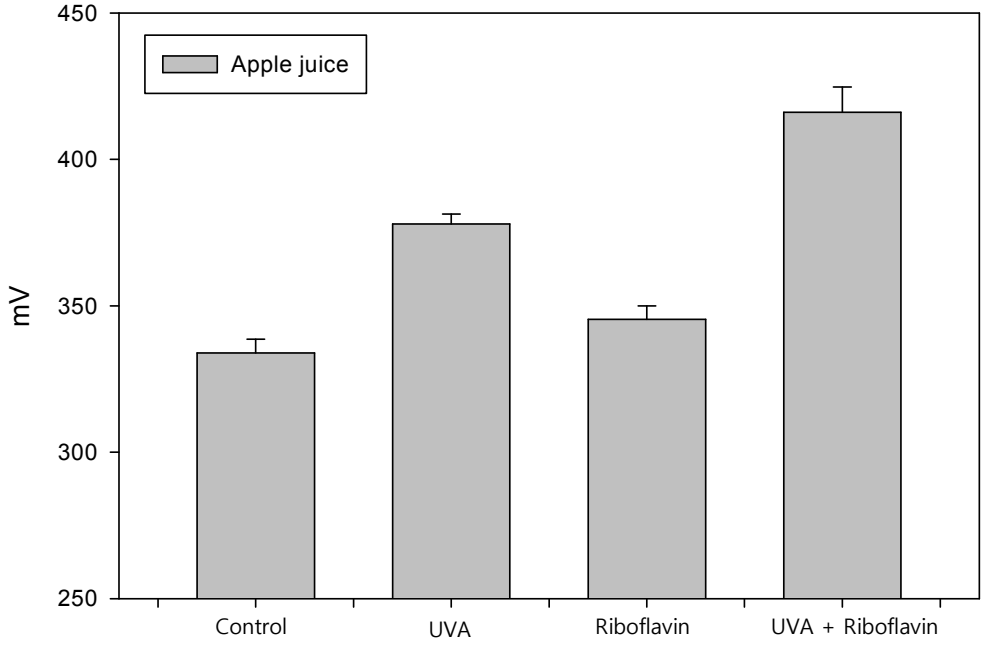

Fig 6. Normalizing of redox potential in the apple juice for 30 J/cm^2. The error bars indicate standard deviations

3.5. Transmission electron microscopy analysis

UVA and UVA – Riboflavin induced morphological cell damage was characterized by using TEM (micrographs are shown in Fig. 7). The appearance of control is shown in Fig. 7a. The appearance of riboflavin treated *E. coli* O157:H7 is shown in Fig. 7b. and the morphological differences between UVA treatment and UVA-riboflavin treatment cells are shown in the Fig. 7c, and Fig. 7d, respectively. The image of control and riboflavin treated sample shows that cell was undamaged and intact. Images of UVA treated cells shows a slight decomposition of cytoplasm, near the cell wall. In the case of UVA-riboflavin combination treatment, it is observed more clearly that the destruction of internal cellular substances, more than UVA single treatment.

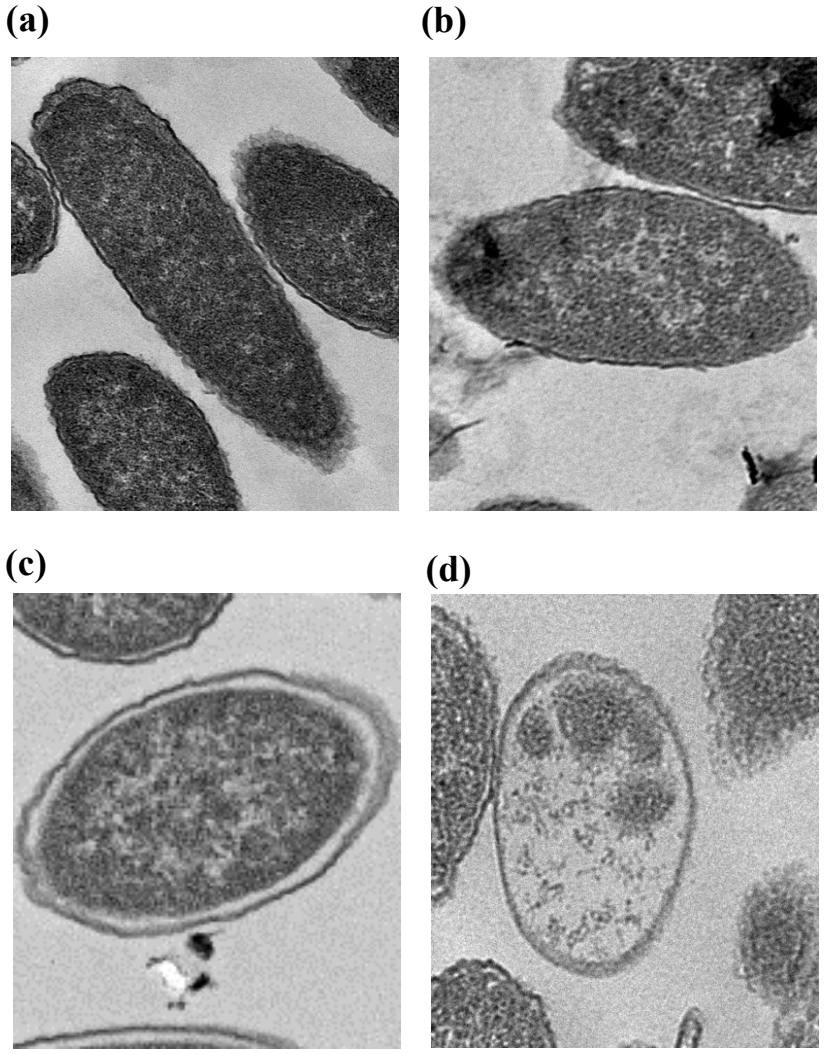

Fig 7. TEM Image analysis *E. coli* O157:H7, Show different morphological characteristics for (a) Control (b) Riboflavin (c) UVA (d) UVA + Riboflavin treatment

IV. DISCUSSION

Ultraviolet (UV) has long been used as an effective treatment to disinfect water and inactivate foodborne pathogens on many fresh foods and vegetable. Ultraviolet-A (UVA), which is a part of the UV, has a wavelength of 315 nm ~ 400 nm. Many studies have clarified the germicidal effect of UVA. It has been known that UVA inactivates the bacteria by generating ROS (e.g. H_2O_2, OH^-, O_2^-) inside the cell, by photosensitizing endogenous photosensitizer like flavin, e.g., FAM and FAD, porphyrin, NAD^+/$NADP^+$. Generated ROS inactivates the intracellular enzyme, damage membrane lipids and cellular proteins, and damage DNA by oxidative damage (3, 8, 14, 16).

Riboflavin, vitamin B2, is a yellowish compound present in most living systems and widely existed in many foods. And Riboflavin act as a photosensitizer, riboflavin can make ROS with presence of oxygen when it absorbed the specific wavelength of light e.g., maximum absorption at 225, 275, 370, and 450 nm (11, 19). Absorption of light by riboflavin results in the excitation of molecules from the ground state to the excited singlet state, and through the intersystem crossing, it transfers to the triplet excited state. After riboflavin reaches to the triplet excited state, riboflavin induces the generation of ROS via type 1 and type 2 photooxidation (17, 22, 23).

I evaluated the germicidal effect of UVA-LED, riboflavin, and UVA-LED and riboflavin combination treatment under various conditions. And the apple juice was selected to investigate the inactivation effect in food sample.

There was difference in effect of inactivation between UVA treatment and UVA-riboflavin treatment in PBS. Firstly, for *E. coli* O157:H7 and *S.* Typhimurium, there was additional inactivation when bacterial treated with UVA-riboflavin treatment compared to UVA treatment, 5.12 log CFU/ml and 1.93 log CFU/ml, respectively. It has been known that UVA radiation mainly generate ROS inside cell through endogenous photosensitizer (3, 8, 14). However, UVA-riboflavin can generate ROS not only inside the cell but also outside cell via inoculated exogenous riboflavin. As shown in Fig 4a and Fig 5a, The main mechanisms of UVA and UVA-riboflavin combination are both ROS, disturbing the ROS balance in cells, generating oxidative stress, and membrane lipid peroxidation and membrane damage, the degree of which is related to the unsaturation of membrane fatty acids (30). The difference is, ROS is generated inside cell via endogenous photosensitizer, but its amount is not sufficient to inactivate foodborne pathogens completely in UVA treatment. However, when the photosensitizer riboflavin was inoculated into sample, ROS is not only generated inside cell but also outside cell by inoculated riboflavin. So as exogenous ROS is increased, the cell

membrane damage and inactivation was increased at the UVA-riboflavin treatment compared to UVA treatment. Normalized PI uptake value of UVA-riboflavin treatment is 33% higher than that of UVA single treatment and normalized amount of ROS of UVA-riboflavin is 258 % higher than that of UVA treatment. So, it can be concluded that cell was damaged via both intracellular ROS and extracellular ROS at UVA-riboflavin treatment, compared to intracellular ROS only at UVA treatment. And secondly *E. coli* O157:H7 had a greater inactivation effect than *S.* Typhimurium. It is assumed that *S.* Typhimurium has more resistant to ROS than *E. coli* O157:H7, due to higher density of its cell membrane with higher content of phospholipid components such as phosphatidylethanolamine and phosphatidylglycerol, compared to that of *E. coli* O157:H7 (2).

And the experiment accordance of concentration of riboflavin indicates that inactivation increased as the concentration of riboflavin increased for both pathogens, however, the concentration which the inactivation started to increase is different between two pathogens. For *E. coli* O157:H7, the inactivation started to increase between 0.005 μmol/L and 0.05 μmol/L. For *S.* Typhimurium, the inactivation started to increase between 5 μmol/L and 50 μmol/L. *E. coli* O157:H7 tended to have a higher inactivation in UVA-riboflavin treatment at a relatively lower concentration of riboflavin than *S.*

Typhimurium. In other words, *E. coli* O157:H7 is more susceptible to concentration of riboflavin, amount of ROS, than *S.* Typhimurium. These results support the results of the PBS experiment that *S.* Typhimurium is more resistant to ROS than *E. coli* O157:H7 (2), due to higher density of *S.* Typhimurium cell membrane, *S.* Typhimurium is more resistant to ROS than *E. coli* O157:H7. Thereby, the inactivation at the *S.* Typhimurium was increased at the higher concentration of riboflavin than *E. coli* O157:H7

pH and A_w are intrinsic factors that have a significant impact on the survival of foodborne pathogens. In pH test, there was no additional inactivation in both pathogen when treated with UVA treatment as pH decreased. For the combination treatment, it was observed that as pH decreased, the inactivation increased. For *E. coli* O157:H7, the inactivation increased 1.52 log CFU/ml between pH 3 and pH 6, and for *S.* Typhimurium, the inactivation increased 1.02 log CFU/ml between pH 3 and pH 6. However, there was no further inactivation lower than pH 5 for *S.* Typhimurium. It is postulated that acidic pH is not sufficient to inactivate pathogens effectively but gives further inactivation when treated with UVA-riboflavin treatment. As a result of ROS assay and PI uptake value, the normalized amount of ROS kept increasing as pH decreased. And pH 3 was significantly higher than the amount of control compared to that of pH 6,

about 253%. And the normalized PI uptake value, was higher at pH 3 than pH 6 about 36%. It was confirmed that UVA-riboflavin treatment has higher inactivation efficacy at lower pH. These results can be explained by the results of (12, 33). According the results of (34), there is antioxidant enzyme such as SOD (superoxide dismutase), catalase, glutathione peroxidase, and glutathione reductase in living organisms. And each enzyme has all optimum pH, e.g., catalase is pH 7(26), SOD is pH 7.5(35), glutathione peroxidase is pH 9(15), glutathione reductase is pH 7.5(27). Lowering the pH of the medium resulted in decreasing of enzyme activity and decreasing ROS degradation in cell, so increase cell membrane damage. And according to studies of (12), rate constants for the uncatalyzed dismutation of O_2- depend strongly on the pH of the solution. Rates for dismutation of O_2- were 10^2 at pH 11 and 5×10^5 $M^{-1}s^{-1}$ at pH 7.0. It means that the formation of H_2O_2 from O_2- is faster at lower pH. So, it can be concluded that inactivation and ROS increased at the lower pH is because decreased antioxidant enzyme activity and increasing dismutation of O_2- at lower pH.

In A_w test, in the case of UVA treatment, there was no significant ($p < 0.05$) additional inactivation all A_w for *E. coli* O157:H7 and *S.* Typhimurium. For UVA-riboflavin treatment, it was shown that the inactivation at A_w = 0.942 was lower than that of A_w = 0.950 and A_w = 0.975. According to (29)

studies, radical adduct, such as $\cdot O_2^-$, H_2O_2, formation needed both the presence of dissolved oxygen as well as the photosensitizer riboflavin in the UVA irradiated sample. And following research of (6), as A_w decreases, the estimated critical dissolved oxygen concentrations decrease. Summarize it, when A_w decreases, the dissolved oxygen concentrations decrease too, and that result in decreasing formation of reactive radical in the sample, so the cell membrane damage and amount of ROS decreased.

To confirm this result, PI uptake test and ROS assay was taken. The normalized PI uptake value of the UVA-riboflavin treatment at the A_w = 0.975 and A_w = 0.950 had a value up to 30% and 20% greater than the value of the A_w = 0.942, respectively. And the normalized amount of ROS in UVA-riboflavin treatment at the A_w = 0.975 and A_w = 0.950 had a value up to about 24% greater than the value of the A_w = 0.942. This result indicates the relation that ROS formation and A_w are proportional.

In apple juice, for *E. coli* O157:H7, The UVA treatment increased about 1 log reduction compared to that of PBS. In the case of UVA-riboflavin treatment, about 3 log reduction was reduced compared to that of PBS. For *S.* Typhimurium, there was no further inactivation occurred in both the UVA treatment and the UVA-riboflavin treatment. There can be several reasons that inactivation decreases at the UVA treatment. According to the studies

(21), first, the absorbance band of the apple juice color may overlap with the UVA wavelength and thereby decrease the effective dose on the pathogens. Second, the antioxidant compound inside apple juice like carotenoids can quench ROS and block free radical-mediated reactions. Finally, the pigments and other particles suspended in the juice may reflect and scatter the UVA radiation. ORP value increases as it is oxidized by an oxidizing agent such as ROS (18). After treatment of apple juice sample, ORP value measurement was performed to measure the degree of oxidation caused by the generated ROS. As a result of UVA treatment, the degree of oxidation increased by 45 mv due to the riboflavin contained in the apple juice, there was slight ($p > 0.05$) differences between riboflavin treatment and control, and the UVA-riboflavin combination treatment was increased by 82 mv compared to the control, and it was confirmed that the oxidation was higher than that of UVA single treatment. Based on these results, it was confirmed that further inactivation caused by the combination treatment was also observed in apple juice.

Finally, the TEM showed the morphological differences between the treatment, there was no membrane and intracellular damage in control and riboflavin treated bacteria. And the outer cell membrane is slightly destroyed

in single UVA treatment sample. For UVA-riboflavin combination treatment, it was shown that most of the intracellular structure was damaged.

In this study, I demonstrated the UVA-riboflavin combination treatment has synergistic germicidal effect compared to UVA single treatment. That is, foodborne pathogens treated with UVA-riboflavin can be inactivated more effectively than UVA treatment at same dose. Through ROS measurement analysis, unlike UVA treatment that induce inactivation through ROS generated inside the cell, it was proved that ROS was generated not only inside the cell, but also outside the cell in case of UVA-riboflavin treatment, thereby damaging the cells internally and externally, showing a synergistic germicidal effect. I find that UVA-LED using riboflavin as safe photosensitizer can inactivate bacteria effectively and can be used at liquid food.

V. REFERENCES

1. Alegre, I., Abadias, M., Anguera, M., Usall, J., & Viñas, I. (2010). Fate of Escherichia coli O157: H7, Salmonella and Listeria innocua on minimally-processed peaches under different storage conditions. *Food microbiology, 27*(7), 862-868.
2. Alwi, N. A., & Ali, A. (2014). Inactivation of Escherichia coli O157, Listeria monocytogenes and Salmonella enterica sv. Typhimurium populations on fresh-cut bell pepper using gaseous ozone. *Food Control, 46*, 304-311.
3. Baier, J., Maisch, T., Maier, M., Engel, E., Landthaler, M., & Bäumler, W. (2006). Singlet oxygen generation by UVA light exposure of endogenous photosensitizers. *Biophysical journal, 91*(4), 1452-1459.
4. Besser, R. E., Lett, S. M., Weber, J. T., Doyle, M. P., Barrett, T. J., Wells, J. G., & Griffin, P. M. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157: H7 in fresh-pressed apple cider. *Jama, 269*(17), 2217-2220.
5. Bolton, J. R., & Linden, K. G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of environmental engineering, 129*(3), 209-215.
6. Bošnjak, M., & Mihaljeviæ, K. (2007). Oxygen solubility and oxygen transfer and uptake rates as functions of water activity.
7. Burnett, S., Gehm, E., Weissinger, W., & Beuchat, L. (2000). Survival of Salmonella in peanut butter and peanut butter spread. *Journal of applied microbiology, 89*(3), 472-477.
8. Cadet, J., & Douki, T. (2011). Oxidatively generated damage to DNA by UVA radiation in cells and human skin. *Journal of Investigative Dermatology, 131*(5), 1005-1007.
9. CDC, 2018. Multistate Outbreak of Salmonella Typhimurium Infections Linked to Dried Coconut: https://www.cdc.gov/salmonella/typhimurium-03-18/index.html
10. CDC, 2019. Multistate Outbreak of Salmonella Typhimurium Infections Linked to Peanut Butter, 2008-2009: https://www.cdc.gov/salmonella/2009/peanut-butter-2008-2009.html (last access on august 2020).
11. Choe, E., Huang, R., & Min, D. B. (2005). Chemical reactions and stability of riboflavin in foods. *Journal of food science, 70*(1), R28-R36.

12. Choe, E., & Min, D. B. (2006). Chemistry and reactions of reactive oxygen species in foods. *Critical reviews in food science and nutrition, 46*(1), 1-22.
13. Gibson, B. (1973). The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. *Journal of Applied Bacteriology, 36*(3), 365-376.
14. Graindorge, D., Martineau, S., Machon, C., Arnoux, P., Guitton, J., Francesconi, S., . . . Girard, P.-M. (2015). Singlet oxygen-mediated oxidation during UVA radiation alters the dynamic of genomic DNA replication. *PLoS One, 10*(10).
15. Guo, X., Yu, Y., Liu, X., Zhang, Y., Guan, T., Xie, G., & Wei, J. (2014). Heterologous expression and characterization of human cellular glutathione peroxidase mutants. *IUBMB life, 66*(3), 212-219.
16. 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.
17. Insińska Rak, M., & Sikorski, M. (2014). Riboflavin interactions with oxygen—A survey from the photochemical perspective. *Chemistry–A European Journal, 20*(47), 15280-15291.
18. Kohen, R., & Nyska, A. (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic pathology, 30*(6), 620-650.
19. Lee, Y.-H. (2009). *Effect of riboflavin and lumichrome degradation on the oxidative stability of salad dressing.* The Ohio State University,
20. Lewandowski, D., Schroeder, G., Sawczak, M., & Ossowski, T. (2015). Fluorescence properties of riboflavin-functionalized mesoporous silica SBA-15 and riboflavin solutions in presence of different metal and organic cations. *Journal of Physics and Chemistry of Solids, 85*, 56-61.
21. Lian, X., Tetsutani, K., Katayama, M., Nakano, M., Mawatari, K., Harada, N., . . . Kinouchi, Y. (2010). A new colored beverage disinfection system using UV-A light-emitting diodes. *Biocontrol science, 15*(1), 33-37.
22. Luksiene, Z., & Brovko, L. (2013). Antibacterial photosensitization-based treatment for food safety. *Food Engineering Reviews, 5*(4), 185-199.

23. Luksienė, Z., & Zukauskas, A. (2009). Prospects of photosensitization in control of pathogenic and harmful microorganisms. *Journal of applied microbiology, 107*(5), 1415-1424.
24. Oyarzabal, O. A., Nogueira, M. C., & Gombas, D. E. (2003). Survival of Escherichia coli O157: H7, Listeria monocytogenes, and Salmonella in juice concentrates. *Journal of food protection, 66*(9), 1595-1598.
25. Park, I.-K., & Kang, D.-H. (2013). Effect of electropermeabilization by ohmic heating for inactivation of Escherichia coli O157: H7, Salmonella enterica serovar Typhimurium, and Listeria monocytogenes in buffered peptone water and apple juice. *Appl. Environ. Microbiol., 79*(23), 7122-7129.
26. Pedrini, N., Juárez, M. P., Crespo, R., & de Alaniz, M. J. (2006). Clues on the role of Beauveria bassiana catalases in alkane degradation events. *Mycologia, 98*(4), 528-534.
27. Ramos-Martinez, J. I., Bartolomé, T. R., & Pernas, R. V. (1983). Purification and properties of glutathione reductase from hepatopancreas of Mytilus edulis L. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 75*(4), 689-692.
28. Schnellbaecher, A., Binder, D., Bellmaine, S., & Zimmer, A. (2019). Vitamins in cell culture media: Stability and stabilization strategies. *Biotechnology and bioengineering, 116*(6), 1537-1555.
29. Sel, S., Nass, N., Pötzsch, S., Trau, S., Simm, A., Kalinski, T., . . . Brömme, H.-J. (2014). UVA irradiation of riboflavin generates oxygen-dependent hydroxyl radicals. *Redox Report, 19*(2), 72-79.
30. Song, K., Mohseni, M., & Taghipour, F. (2019). Mechanisms investigation on bacterial inactivation through combinations of UV wavelengths. *Water research, 163*, 114875.
31. Strawn, L. K., & Danyluk, M. D. (2010). Fate of Escherichia coli O157: H7 and Salmonella spp. on fresh and frozen cut mangoes and papayas. *International journal of food microbiology, 138*(1-2), 78-84.
32. US Food and Drug Administration. 2019. Code of Federal Regulations, Title 21. Riboflavin, chapter I, part 184, subpart B. U.S. Food and Drug Administration, Silver Spring, MD. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1695
33. Vojdani, J. D., Beuchat, L. R., & Tauxe, R. V. (2008). Juice-associated outbreaks of human illness in the United States, 1995 through 2005. *Journal of food protection, 71*(2), 356-364.

34. Weydert, C. J., & Cullen, J. J. (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature protocols, 5*(1), 51.
35. Zhao, N., Feng, Z., Shao, M., Cao, J., Wang, F., & Liu, C. (2017). Stability profiles and therapeutic effect of Cu/Zn superoxide dismutase chemically coupled to O-Quaternary Chitosan derivatives against dextran sodium sulfate-induced Colitis. *International journal of molecular sciences, 18*(6), 1121.
36. Zhuang, R., Beuchat, L., & Angulo, F. (1995). Fate of Salmonella montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Appl. Environ. Microbiol., 61*(6), 2127-2131.

VI. 국문 초록

낮은 수소이온농도 (pH)와 수분활성도 (A_w)는 세균의 생존에 큰 악영향을 주지만, 그럼에도 불구하고 그러한 조건에서도 식품이 오염되어 식중독을 일으킨 사례가 수 차례 보고된 바 있다. 이러한 식중독균의 저감화를 위하여, 이번 실험에서는 UVA-LED 를 광원으로 하는 UVA 광선과 리보플라빈을 이용하여 활성산소종을 이용한 저감화 효과를 알아보았고, 이하 pH 와 A_w 의 변화에 따른 저감화 효과를 살펴보았다.

파장대가 398 nm 인 UVA-LED 세트를 실험에 사용하였으며, 사용한 리보플라빈의 경우 0.33 g/L 의 수용액으로 만들어 실험마다 샘플의 농도가 50 μmol/L 가 되도록 실험하였다. 빛의 양은 10 J/cm^2, 20 J/cm^2, 30 J/cm^2 으로 처리하였다. PBS 용액 실험 결과, *E. coli* O157:H7, *S.* Typhimurium 모두 UVA 광선과 리보플라빈의

조합처리가 UVA 광선 단일 처리에 비해 시너지 효과가 있음을 확인하였다. 리보플라빈의 농도를 낮춘 실험 결과, *E. coli* O157:H7 는 0.005 μmol/L 와 0.05 μmol/L 사이에서 저감화가 늘어나기 시작했고, *S.* Typhimurium 는 0.5 μmol/L 와 5 μmol/L 사이에서 저감화가 늘어난 것을 확인하였다. 이를 통해 *E. coli* O157:H7 는 *S.* Typhimurium 보다 활성산소종의 증가에 민감하다는 것을 밝혀냈다.

다음으로, 식품의 내재인자인 pH 와 수분활성도에 따른 저감화를 비교하였다. 그 결과, pH 가 낮을수록 UVA 광선 단일 처리는 추가적인 저감화가 없었고 UVA-리보플라빈 조합처리의 경우 시너지 효과를 확인할 수 있었다. 수분활성도의 경우, UVA 광선 단일 처리는 수분활성도가 낮을수록 저감화가 높아졌으며, UVA-리보플라빈 조합 처리는 저감화가 낮아지는 결과를 확인할 수 있었다. 실제 식품 처리결과, 사과주스에서 *E. coli* O157:H7 는

PBS 의 처리에 비해 UVA 광선 단일 처리는 저감화가 늘었으며 UVA-리보플라빈 조합 처리는 저감화가 감소하였다. *S.* Typhimurium 의 경우 PBS 에 비해 저감화가 차이가 없었다. 이를 확인하기 위해 PI, DCFH-DiOxyQ, redox potential 을 이용하여 세포막 손상과 활성산소종, 산화환원전위를 측정한 결과 저감화와 같은 경향을 보였다. 이 연구에서 모든 UVA-리보플라빈 조합 처리의 경우 UVA 단일 광선 처리보다 시너지 저감화 효과가 있다는 것을 밝혀냈다.

주요단어: UVA-LED, 리보플라빈, 비타민 B_2, pH, 수분활성도, 활성산소종