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Inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black pepper powder using UV-C, UV-A and TiO_2 coating

Mi-Jung Park[1] · Jin-Hee Kim[1] · Se-Wook Oh[1]

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Abstract This study was conducted to measure the inactivation characteristics of UVs and TiO_2 against *Salmonella*. Typhimurium and *Escherichia coli* O157:H7 on black pepper powder. The sample was irradiated by UV-A and UV-C combined with TiO_2 coating. After treatment, microbial and physicochemical analysis was carried out. Among various sterilization conditions, the largest number of pathogen in black pepper powder was inactivated by UV-A and UV-C combined with TiO_2 coating. The microbial count of black pepper powder treated simultaneously with UV-A and UV-C was less than that of black pepper powder treated with alone. The inactivation effect of UV-A and UV-C was increased when TiO_2 coating was combined. Moisture content was decreased with increasing treatment time, but color did not change. In this study, it was indicated that the combined treatment of UV-C, UV-A and TiO_2 coating was effective for reducing *S*. Typhimurium and *E. coli* O157:H7 on black pepper powder.

Keywords UV-C · UV-A · TiO_2 coating · Black pepper powder · Inactivation effect

✉ Se-Wook Oh
swoh@kookmin.ac.kr

Mi-Jung Park
qlorvv@nate.com

Jin-Hee Kim
Jinhi325003@naver.com

[1] Department of Food and Nutrition, Kookmin University, Seoul 02727, Republic of Korea

Introduction

In recent years, consumer's interest in natural flavor and taste has increased, and research on non-heat treatment technology to ensure food quality and safety has been actively conducted (Knorr et al., 2011; Ojha et al., 2015). Titanium dioxide (TiO_2) is a photocatalyst that is used to make an antimicrobial surface and has proved to be effective for sterilizing a wide range of microorganisms, including endospores (Foster et al., 2011). TiO_2 does not produce byproducts, can be used repeatedly, and has the advantages of being stable and non-toxic under UV irradiation. (Yemmireddy and Hung, 2015). TiO_2 has also been approved by the American Food and Drug Administration for use in human food, drugs, cosmetics, and food contact materials (Chawengkijwanich and Hayata, 2008). The mechanism of action of TiO_2 is due to the production of reactive oxygen species(ROS) with strong oxidizing power when UV light is irradiated at wavelengths of less than 385 nm (Maness et al., 1999). When microorganisms are exposed to $\cdot O_2$ and ·OH produced by photocatalyst, oxidative damage occurs in cell walls, cell membranes, DNA, and RNA (Blake et al., 1999). The oxidation of unsaturated phospholipids destroys the cell membrane, and the increase of ion permeability induces cell death by inducing oxidation of the internal cellular components (Alrousan et al., 2009).

Ultraviolet rays have a antimicrobial effect against many kinds of microorganisms including viruses (Guerrero Beltr·n and Barbosa C·novas, 2004) and are used to sterilize air, water, and surfaces of food or cooking utensils (Gayán et al., 2011). UV-C is a light with a wavelength of 200–270 nm, which is readily absorbed by DNA, leading to the production of a large amount of pyrimidine dimers. The pyrimidine dimers formed by the absorption of UV-C

into DNA kills cells by interfering with DNA transfer and replication, making UV-C effective in sterilizing microorganisms. (Chevremont et al., 2012). UV-C does not create chemical residues, has low cost maintenance, and does not change quality because it is dry and cold processes (Guerrero-Beltr·n and Barbosa-C·novas, 2004). UV-A has a wavelength of 320–400 nm that induces the formation of active substances such as peroxide, which has a inactivation effect on the survival of microorganisms (Oppezzo and Pizarro, 2001). UV-A causes cell membrane damage due to long wavelengths, and shows lethal effects against microorganisms (Bintsis et al., 2000). Recent studies have shown that the antimicrobial effect of combined treatment with UV-C and UV-A is more effective than the effect of using UV-C or UV-A alone (Chevremont et al., 2012).

Black pepper is one of the most important and widely used spices in the world, and about 315,000–320,000 tons of the pepper are produced in 26 countries (Peter, 2006). However, Boer et al. found that black pepper showed a high level of microbial contamination above 10^7 CFU/g (De Boer et al., 1985). Black pepper is used as a spice to enhance taste and aroma in various dishes. Therefore, if spices such as black pepper are contaminated with bacterial pathogens, pathogens can easily enter food and cause food poisoning (McKee, 1995). *Salmonella* Typhimurium and *Escherichia coli* O157:H7 are the most frequently reported pathogens causing food poisoning and life-threatening diseases (Tarr, 1995; Zweifel and Stephan, 2012).

Salmonella Typhimurium causes salmonellosis (Alley et al., 2002) and *E. coli* O157:H7 cause diarrhea and hemolytic uremic syndrome disease after infection (Besser et al., 1993). Animal manure is a common carrier of pathogens such as *S.* Typhimurium and *E. coli* O157:H7, and black pepper grown on the ground can be infected with pathogens due to animal manure (Islam et al., 2004). It was reported that the cause of a large-scale salmonella infection in the United States from July 2009 to April 2010 was contaminated pepper added to salami products (Julian et al., 2009).

The inactivation effects of TiO_2 coating, UV-A, and UV-C against *S.* Typhimurium and *E. coli* O157:H7 on black pepper powder have not been previously reported. Therefore, this study proposed treatment combined with TiO_2 coating, UV-A, and UV-C to inhibit *S.* Typhimurium and *E. coli* O157:H7 growth on black pepper powder without heating.

Materials and methods

Bacterial strains of *S.* Typhimurium and *E. coli* O157:H7

Three strains of *Salmonella* Typhimurium (ATCC 6994, 14028, 19585) and three strains of *Escherichia coli* O157:H7 (ATCC 43894, 43895, 35150) were obtained from Korean Collection for Type Cultures. All strains were stored at − 80 °C in stock form. Stocks were prepared by mixing 0.5 mL of bacteria culture and 0.5 mL of 100% glycerol. The strains were cultured in tryptic soy broth (TSB; Difco Co., Franklin Lakes, NJ, USA) before use.

Bacteria culture preparation

Three strains of *S.* Typhimurium and three strains of *E. coli* O157:H7 were each added to 10 mL of TSB and incubated at 37 °C for 9 h. After incubation, 0.1 mL of the culture broth was inoculated into 10 mL of another TSB and cultured at 37 °C for 18 h again. The culture broth was centrifuged at 9000×*g* for 20 min at 4 °C, and the resulting pellet was resuspended in 5 mL of 0.85% saline (8.5 g/L sodium chloride; Sigma–Aldrich Corp., St. Louis, Mo., USA) (washing step). The washing step was repeated two more times. In the process, cocktails of bacterial cultures were performed between the same strains. Finally, the pellet was resuspended using 30 mL of 0.85% saline and used for experiment.

Sample preparation and inoculation

Black pepper powder (BPP) was obtained a bulk packaged in a pouch from local market and used for the experiment. The BPP was filtered with a 35-mesh sieve, and the size of the pepper powder was less than 35 mesh. The pepper powder of 150 g was placed in a sterile bag (Whirl–pak; 19.30 cm, Nasco, Fort Atkinson, WI, USA), and was added 5 mL of the bacterial culture for inoculation. And then, BPP in the bag was massaged by hand for 2 min and was dried in a biosafety hood for 1 h. Finally, about 10^5–10^6 CFU/g of *S.* Typhimurium and *E. coli* O157:H7 was inoculated on BPP.

System combined with UV-A, UV-C, and roaster-coated TiO_2 and treatment

All treatments were performed in a biosafety hood built-in air circulation system to prevent heat accumulation and microbial contamination. A roaster machine (MK-300, Miko Electrical Co., Ltd., Foshan, Guangdong PR, China), G40T10 Germicidal UV-C lamp (253.7 nm, 40 W,

SANKYO DENKI Co., Ltd., Kanagawa, Japan), and F40T10 Blacklight UV-A lamp (352 nm, 40 W, SANKYO DENKI Co., Ltd.) were installed in the biosafety hood.

To make a TiO_2 paste, TiO_2 powder (Aeroxide® TiO2 P25; 21 nm, Defussa Co., Germany) of 5 g was added to 45 mL of polycrylic (MinWax® Water based polyacrylic; MinWax co., USA) and stirred for 1 h. After stirring, the solution was sonicated for 1 h. 5 mL of the prepared TiO_2 paste was coated once on Glad plastic wrap (Glad Food Plastic Wrap, width 30 cm, length 43.4 cm, United States) using a brush, and the TiO_2-coated plastic wrap was attached to top surface of the roaster machine for TiO_2-coating treatment.

The BPP was treated by UV-A (length 119.8 cm, lamp diameter 32 mm) and UV-C (length 119.8 cm, lamp diameter 32 mm) on the roaster machine (diameter 24 cm, depth 3.5 cm). The samples were placed 6 cm away from the UV lamp and the roaster machine continuously mixed the samples. The wavelengths of the UV-A and the UV-C installed in the biosafety hood were 352 and 253.7 nm, respectively, and the irradiation dose was 1.11 W/cm^2. UV-A and UV-C were treated alone or in combination for 0, 30, 60, 90, 120, 150, and 180 min. Two UV lamps were used for each treatment. One UV-A lamp and one UV-C lamp were used when combining UV-A and UV-C.

Microbial analysis and injured cell enumeration

Treated BPP of 10 g and 90 mL of sterilized 0.85% saline put into a sterile bag and was homogenized for 2 min by a stomacher (Laboratory Blender Stomacher 400; Seward, MO, USA). The sample of 1 mL was serially diluted with 0.85% physiological saline, and then plated on each selective medium. To detect for *S.* Typhimurium and *E. coli* O157:H7, Xylose-Lysine-Deoxycholate agar (XLD agar; Difco, Becton–Dickinson Co., Sparks, MD) and Sorbitol MacConkey agar (SMac agar; Oxoid, Hampshire, UK) were used, respectively. The homogenized sample solution was incubated on selective agar at 37 °C for 24–48 h and the colonies formed on the plates were counted.

The overlay (OV) method was used to measure the population of injured cells caused by UV-A, UV-C, and TiO_2 coatings (Lee and Kang, 2001). Tryptone soya agar (TSA; Oxoid, Hampshire, UK) was used as a nutrient medium to recover injured *S.* Typhimurium and *E. coli* O157:H7. The diluted sample suspension was plated on TSA, and the plate was cultured at 37 °C for 2 h. Then, 7–8 mL of XLD agar and SMac agar were poured on the plate (Kang and Siragusa, 1999). When the agar was hard, the plate was incubated at 37 °C for 24–48 h, and then and the colonies formed were counted. The colony number was calculated by Ukuku in terms of % population of injured cells via the following formula (Ukuku and Geveke, 2010):

$$[1 - \text{colonies on selective agar/counts on agar in OV method}] \times 100$$

Moisture measurement and color measurement

OHAUS MB 45 Moisture Analyzers (MB 45; OHAUS, NJ, USA) were used to measure the moisture content of BPP. Moisture content of BPP were measured at 0, 30, 60, 90, 120, 150, and 180 min during treatment. When each treatment was end, 2 g of BPP was put on an aluminum dish and was measured the moisture content by heating at 140 °C for 10 min.

A colorimeter (CR-400 Chroma Meter; Konica Minolta Sensing, Inc., Japan) was used to determine the effect of UV-A, UV-C and TiO_2 coatings on the color of BPP. To measure the color change of BPP, L*, a* and b* values were measured using 2 g of BPP before and after treatment.

Statistical analysis

All experiments were repeated three times to calculate average number of microorganisms and results were converted to log CFU/g. IBM SPSS statistics program (version 23, IBM Corp., USA) statistically performed the analysis of variance on calculated results. Duncan's multiple range test separated the mean values of results. This study concluded that there was a significant difference between the samples when p was less than 0.05.

Results and discussion

Antimicrobial effect of UV-A, UV-C and TiO_2 coating on BPP

The inactivation effect of UV-A, UV-C and TiO_2 coating against *S.* Typhimurium on BPP is shown in Fig. 1. In Fig. 1(A) is a group treated without TiO_2, and (B) is a group treated with TiO_2. The initial concentration of *S.* Typhimurium on BPP was 5.42 ± 0.11 log CFU/g.

UV-A alone (UA) and UV-C alone (UC) inhibited 1.04 and 0.93 log CFU/g of *S.* Typhimurium, respectively. When UV-A and UV-C were applied in combination (UAC), 1.74 log CFU/g of *S.* Typhimurium was inactivated. The combined treatment of TiO_2 and UV-A (TUA) and the combined treatment of TiO_2 and UV-C (TUC) reduced about 1.65 and 1.08 log CFU/g of *S.* Typhimurium, respectively. When TiO_2, UV-A and UV-C were

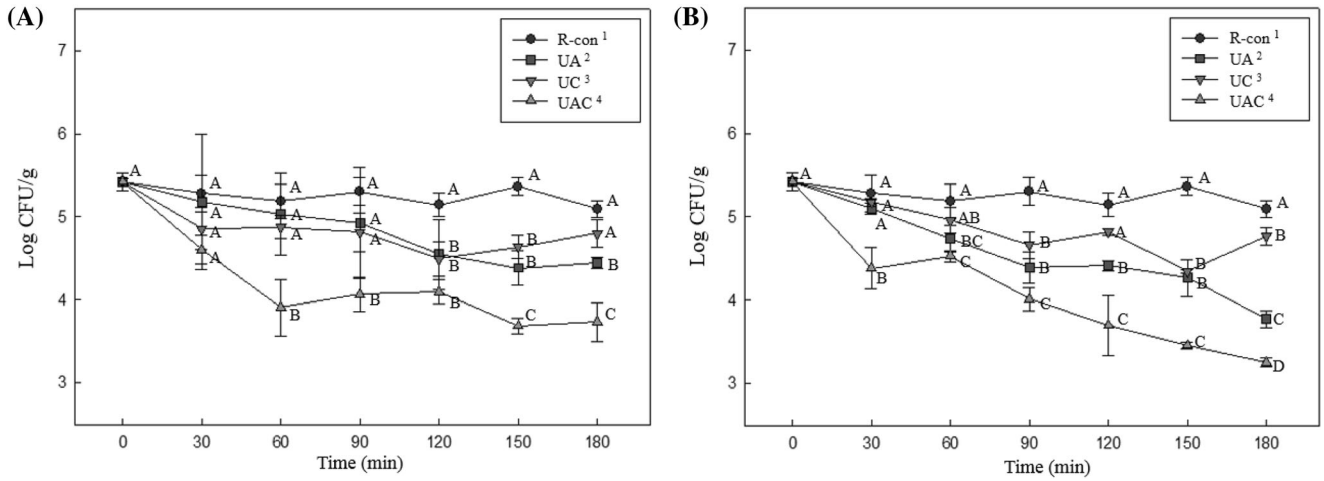

Fig. 1 Inactivation effect of UV-A, UV-C, and TiO_2 coating against *S.* Typhimurium inoculated on black pepper powder for 0, 30, 60, 90, 120, 150, and 180 min. Mean ± standard deviation obtained in two experiments, one of two experiments in duplicated (n = 3). The treatment involving TiO_2 coating is (**A**), and the treatment excluding TiO_2 coating is (**B**). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time. [1]R-con means control group that was treated using only a roaster machine. [2]UA means a group that was treated by UV-A alone. [3]UC means a group that was treated by UV-C alone. [4]UAC means a group that was treated by UV-A and UV-C simultaneously

treated together (TUAC) for 180 min, 2.17 log CFU/g of *S.* Typhimurium was reduced. The inactivation effect of UA was significantly ($p < 0.05$) higher than that of UC when treated for 150 min, and UAC was significantly ($p < 0.05$) higher than UA and UC after 60 min of treatment. When TUA or TUC was applied for more than 90 min, *S.* Typhimurium on BPP was significantly ($p < 0.05$) different from the control group (R-con) that was treated using only a roaster machine. When TUAC was treated for 30 min, the inactivation effect was significantly ($p < 0.05$) higher than that of TUA and TUC, and TUAC reduced most *S.* Typhimurium on BPP.

TUA showed a higher inactivation effect than UA against *S.* Typhimurium on BPP, and showed a significant ($p < 0.05$) difference between 90 and 180 min. When treated for 30 min, TUC reduced significantly ($p < 0.05$) more *S.* Typhimurium than UC, but there was no significant ($p > 0.05$) difference between the inactivation effects of the two treatments thereafter. *Salmonella* Typhimurium was significantly ($p < 0.05$) more inactivated on BPP treated by TUAC and UAC than R-con, and TUAC reduced significantly ($p < 0.05$) more *S.* Typhimurium than UAC after 150 min.

Salmonella Typhimurium was more inactivated by the treatment combined with UV-A and UV-C than treatment using UV-A and UV-C alone. In addition, TiO_2 coatings significantly ($p < 0.05$) increased the inactivation effects of UV-A and UV-C against *S.* Typhimurium on BPP.

The inactivation effect of UV-A, UV-C, and TiO_2 coating against *E. coli* O157:H7 on BPP is shown in Fig. 2. In Fig. 2(A) is a group treated without TiO_2, and (B) is a group treated with TiO_2. The initial concentration of *E. coli* O157:H7 on BPP was 6.64 ± 0.10 log CFU/g.

UA and UC inhibited *E. coli* O157:H7 by 1.13 and 1.54 log CFU/g, respectively. UAC inactivated 1.46 log CFU/g of *E. coli* O157:H7 for 180 min. For 180 min, TUA and TUC reduced *E. coli* O157:H7 by 1.46 and 1.80 log CFU/g, respectively. When TUAC was applied for 180 min, *E. coli* O157:H7 on BPP was inactivated by 1.74 log CFU/g. TUA inhibited significantly ($p < 0.05$) more *E. coli* O157:H7 than UA for 120 min. A number of *E. coli* O157:H7 was significantly ($p < 0.05$) reduced by UC and TUC compared with R-con, and *E. coli* O157:H7 was more inactivated by TUC than UC at 180 min. There was no significant ($p > 0.05$) difference between R-con, UAC, and TUAC until 30 min. However, TUAC showed a similar or significantly ($p < 0.05$) better inactivation effect than UCA after 60 min.

UC showed a bactericidal effect against *E. coli* O157:H7 similar to that of TUC. The BPP treated by UAC had significantly ($p < 0.05$) fewer *E. coli* O157:H7 than the pepper powder of R-con, and TUAC inactivated significantly ($p < 0.05$) more *E. coli* O157:H7 than the R-con from 60 to 180 min. The inactivation effect of UV-A and UV-C against *E. coli* O157:H7 was enhanced by the TiO_2 coating.

The inactivation effect of UA, UC and UAC against *S.* Typhimurium was increased with 0.67, 0.48, and 0.03 log CFU/g for 180 min by TiO_2 coating, respectively. The inactivation effect of UA, UC, and UAC against *E. coli* O157:H7 was increased with 0.33, 0.25, and 0.27 log CFU/g, when also treated with TiO_2 coating for 180 min. According to the results, it was confirmed that the

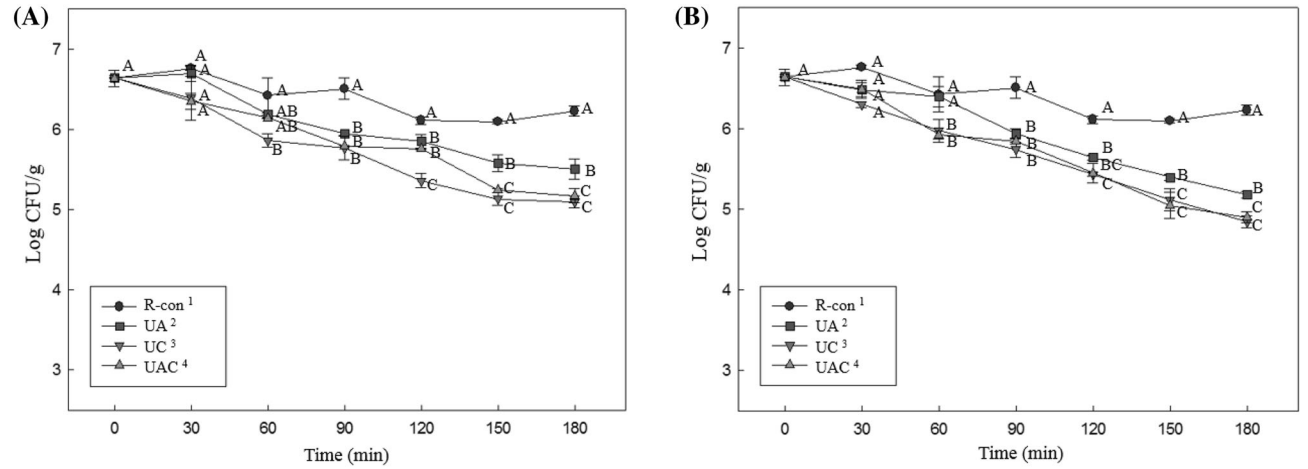

Fig. 2 Inactivation effect of UV-A, UV-C, and TiO_2 coating against *E. coli* O157:H7 inoculated on black pepper powder for 0, 30, 60, 90, 120, 150, and 180 min. Mean ± standard deviation obtained in two experiments, one of two experiments in duplicated (n = 3). The treatment involving TiO_2 coating is (**A**), and the treatment excluding TiO_2 coating is (**B**). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time. [1]R-con means control group that was treated using only a roaster machine. [2]UA means a group that was treated by UV-A alone. [3]UC means a group that was treated by UV-C alone. [4]UAC means a group that was treated by UV-A and UV-C simultaneously

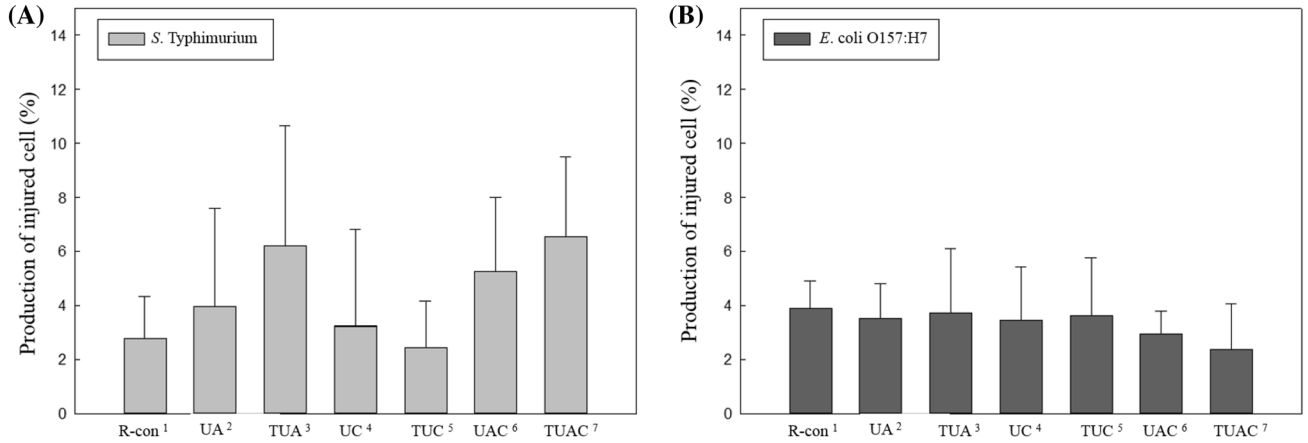

Fig. 3 Average number of injured cell of *S.* Typhimurium (**A**) and *E. coli* O157:H7 (**B**) produced by UV-A, UV-C, and TiO_2 coating during treatment. Mean ± standard deviation obtained in two experiments, one of two experiments in duplicated (n = 3). [1]R-con means control group that was treated using only a roaster machine. [2]UA means a group that was treated by UV-A alone. [3]TUA means a group that was treated by UV-C and TiO_2. [4]UC means a group that was treated by UV-C alone. [5]TUC means a group that was treated by UV-C and TiO_2. [6]UAC means a group that was treated by UV-A and UV-C simultaneously. [7]TUAC means a group that was simultaneously treated by UV-A, UV-C, and TiO_2

inactivation effect of UV combined with TiO_2 coating was increased compared with UV alone treatment.

Production of injured cell by UV-A, UV-C irradiation and TiO_2 coating

The number of injured *S.* Typhimurium and *E. coli* O157:H7 cells on BPP after treatment with UV-A, UV-C and TiO_2 coatings is shown in Fig. 3. In Fig. 3(A) is the number of injured *S.* Typhimurium, and (B) is the number of injured *E. coli* O157:H7. Immediately after inoculation, 4.70% and 2.46% of the *S.* Typhimurium and *E. coli* O157:H7 cells were injured, respectively. On average, *S.* Typhimurium and *E. coli* O157:H7 resulted in 2.77% and 3.89% injured cells, respectively, when the samples were continuously mixed using the roaster machine without any treatment (R-con).

UA and TUA produced an average of 3.95% and 6.20% injured cells, respectively, for *S.* Typhimurium on BPP. In the pepper powder treated with UC and TUC, the average number of injured *S.* Typhimurium was 3.24% and 2.43%. In the UAC- and TUAC-treated BPP, there was an average of 5.26% and 6.55% of injured *S.* Typhimurium cells. In the pepper treated with UA, UC, and UAC, the average

number of injured *E. coli* O157:H7 was 3.52%, 3.45%, and 2.95%, respectively. When treated with TUA, TUC, and TUAC, 3.74%, 3.63%, and 2.38% of *E. coli* O157:H7 were injured on average.

All treatments except for the TUC treatment formed injured cells of *S.* Typhimurium more than R-con on average. On the other hand, the injured *E. coli* O157:H7 produced by UA, TUA, UC, TUC, UAC, and TUAC was on average less than that produced by R-con. However, on both *S.* Typhimurium and *E. coli* O157:H7, there was no significant ($p > 0.05$) difference between productions of the injured cells depending on the treatments. This result shows that all treatments including UV-A, UV-C and TiO_2 coating do not affect production of the injured cell on BPP.

Moisture changes of BPP during UV-A and UV-C irradiation combined with TiO_2 coating

The changes in the moisture content of BPP depending on the length of treatments using UV-A, UV-C, and TiO_2 coatings are shown in Fig. 4. The initial moisture content of the pepper powder inoculated with *S.* Typhimurium and *E. coli* O157:H7 was 12.90 ± 0.08%. The moisture content of BPP treated by R-con for 180 min decreased by 2.61%.

When UA and TUA were applied for 180 min, the moisture content of BPP decreased by 3.81% and 3.69%, respectively. UC and TUC decreased the moisture content of BPP by 3.20% and 3.11%, respectively, for 180 min. The moisture content of BPP treated with UAC and TUAC for 180 min decreased by 3.66% and 3.60%, respectively. The moisture content of BPP treated by UA was significantly ($p < 0.05$) lower than that of R-con after 60 min, and TUA significantly ($p < 0.05$) decreased the moisture content to below that of R-con after 90 min. UC and TUC were significantly ($p < 0.05$) different from R-con after 90 min. Compared with R-con, UAC and TUAC had significantly ($p < 0.05$) lower moisture content after 30 min. UA, TUA, UC, TUC, UAC, and TUAC treatments for 180 min reduced the moisture content by 1.21%, 1.09%, 0.59%, 0.50%, 1.06%, and 1.00%, respectively.

The UC and TUC treatments for 180 min had significantly ($p < 0.05$) fewer differences with R-con, compared with other treatments. UCA- and TUCA-treated BPP had higher moisture content than UA- and TUA-treated BPP. These results show that UV-C is more effective than UV-A in maintaining the moisture content of BPP. In addition, it was confirmed that the treatment combined with TiO_2 coating kept a higher moisture content than the treatment without TiO_2 coating. These results indicate that TiO_2 coating helps preserve the moisture content of BPP.

Color changes of BPP during UV-A and UV-C irradiation combined with TiO_2 coating

Table 1 shows the effects of UV-A and UV-C treatments combined with TiO_2 coating on the color of BPP. The L*,

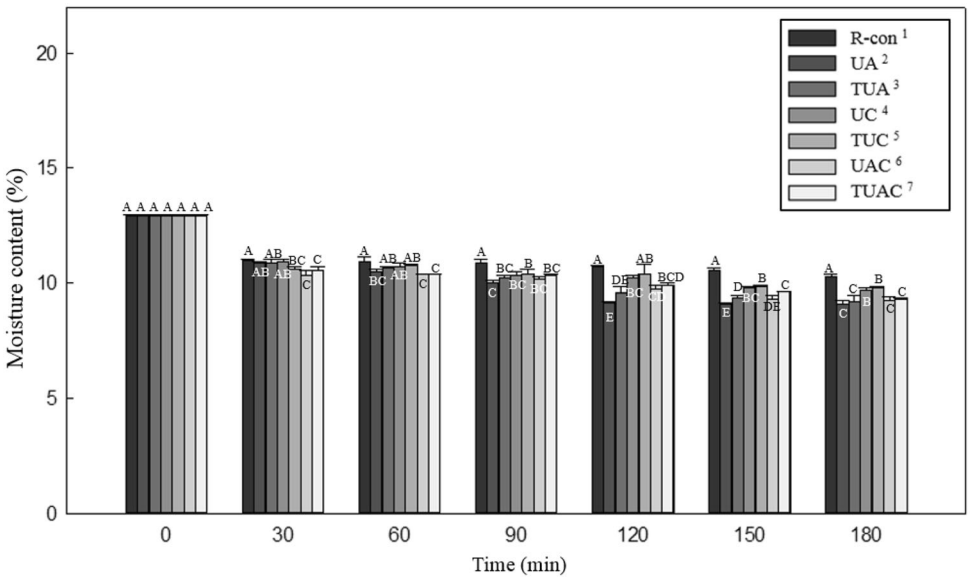

Fig. 4 Change of moisture content by UV-A, UV-C, and TiO_2 coating on black pepper powder according to treatment time. Mean ± standard deviation obtained in two experiments, one of two experiments in duplicated (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time. [1]R-con means control group that was treated using only a roaster machine. [2]UA means a group that was treated by UV-A alone. [3]TUA means a group that was treated by UV-C and TiO_2. [4]UC means a group that was treated by UV-C alone. [5]TUC means a group that was treated by UV-C and TiO_2. [6]UAC means a group that was treated by UV-A and UV-C simultaneously. [7]TUAC means a group that was simultaneously treated by UV-A, UV-C, and TiO_2

Table 1 Change of color (L*, a*, b*) of BPP by UV-A, UV-C, and TiO_2 coating according to treatment time[1]

Color value	Time (min)	R-con[2]	UA[3]	TUA[4]	UC[5]	TUC[6]	UAC[7]	TUAC[8]
L	0	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}	53.55 ± 0.36^{Aa}
	30	51.13 ± 0.97^{CDEa}	52.57 ± 0.32^{Bb}	52.23 ± 0.32^{Bb}	51.66 ± 0.51^{Cab}	62.19 ± 0.57^{Bb}	51.00 ± 0.32^{Ca}	50.99 ± 0.32^{Ca}
	60	50.65 ± 0.65^{Dea}	52.08 ± 0.04^{BCb}	51.99 ± 0.40^{Bb}	50.96 ± 0.42^{Da}	51.99 ± 0.60^{BCb}	51.920 ± 0.55^{Cab}	50.43 ± 0.47^{Ca}
	90	50.06 ± 0.78^{Ea}	50.67 ± 0.84^{Eabc}	51.66 ± 0.06^{Bcde}	52.50 ± 0.49^{Be}	50.39 ± 0.34^{Dab}	51.20 ± 0.55^{Ccde}	51.02 ± 0.13^{Cbcd}
	120	52.50 ± 0.40^{Aba}	51.51 ± 0.43^{CDbc}	52.21 ± 0.51^{Bab}	51.85 ± 0.25^{Bcabc}	51.36 ± 0.44^{Cbc}	51.51 ± 0.27^{Cbc}	51.03 ± 0.84^{Cc}
	150	52.19 ± 0.74^{Bcab}	50.86 ± 0.39^{Dec}	51.26 ± 0.50^{Cbc}	51.58 ± 0.23^{Cdabc}	52.00 ± 0.27^{Bcab}	50.95 ± 0.76^{Cc}	52.44 ± 0.44^{Ba}
	180	51.31 ± 0.22^{CDc}	48.72 ± 0.39^{Fe}	53.48 ± 0.42^{Aa}	52.41 ± 0.28^{Bb}	55.20 ± 0.57^{Dd}	52.44 ± 0.37^{Bb}	53.59 ± 0.22^{BB}
a	0	2.16 ± 0.05^{Aa}	2.16 ± 0.05^{Aa}	2.16 ± 0.05^{Ba}	2.16 ± 0.05^{Aa}	2.16 ± 0.05^{Aa}	2.16 ± 0.05^{Aa}	2.16 ± 0.05^{Ba}
	30	1.97 ± 0.14^{BCA}	2.09 ± 0.07^{Abab}	2.13 ± 0.02^{Bb}	2.07 ± 0.05^{Abab}	2.09 ± 0.03^{Bcab}	2.10 ± 0.06^{Cb}	2.07 ± 0.04^{Cab}
	60	2.07 ± 0.04^{Cabc}	2.11 ± 0.01^{Abab}	1.94 ± 0.03^{Cc}	2.19 ± 0.16^{Ca}	2.19 ± 0.05^{Aba}	2.079 ± 0.06^{Bcab}	2.00 ± 0.02^{Dbc}
	90	1.93 ± 0.06^{Ca}	2.12 ± 0.02^{Bc}	2.10 ± 0.03^{Cc}	2.05 ± 0.08^{Abc}	1.96 ± 0.09^{Dab}	2.03 ± 0.03^{Bcabc}	2.07 ± 0.02^{Cc}
	120	1.99 ± 0.07^{Aba}	2.17 ± 0.07^{Bc}	2.17 ± 0.02^{Ac}	2.13 ± 0.05^{Cc}	2.02 ± 0.02^{Cab}	2.09 ± 0.04^{BCbc}	2.12 ± 0.01^{BCc}
	150	1.95 ± 0.01^{ABa}	2.22 ± 0.04^{Bcd}	2.10 ± 0.04^{Cb}	2.19 ± 0.04^{BCc}	2.06 ± 0.01^{ABCb}	2.19 ± 0.02^{Bc}	2.26 ± 0.02^{Ad}
	180	2.15 ± 0.03^{Cab}	2.22 ± 0.04^{Cc}	2.15 ± 0.06^{Aab}	2.18 ± 0.01^{Abc}	2.19 ± 0.04^{Dbc}	2.12 ± 0.01^{Aa}	2.16 ± 0.01^{Bab}
b	0	10.84 ± 0.11^{Aa}	10.84 ± 0.11^{Aba}	10.84 ± 0.11^{Aa}	10.84 ± 0.11^{Aa}	10.84 ± 0.11^{ABa}	10.84 ± 0.11^{ABa}	10.84 ± 0.11^{Aa}
	30	10.21 ± 0.39^{Bcab}	10.49 ± 0.11^{Bbc}	10.65 ± 0.08^{Ac}	10.66 ± 0.13^{Ac}	10.44 ± 0.30^{BCc}	9.96 ± 0.20^{Ba}	10.24 ± 0.19^{Dabc}
	60	9.86 ± 0.28^{Aba}	10.55 ± 0.06^{Bbc}	10.32 ± 0.09^{Bb}	10.15 ± 0.30^{Aab}	10.73 ± 0.14^{Ac}	10.19 ± 0.20^{Bcab}	9.84 ± 0.15^{Ea}
	90	9.89 ± 0.38^{Cab}	10.19 ± 0.38^{Bbc}	10.25 ± 0.04^{Abc}	10.75 ± 0.11^{Ad}	9.64 ± 0.17^{Da}	10.24 ± 0.32^{Cbc}	10.42 ± 0.11^{Dcd}
	120	10.65 ± 0.22^{BCb}	10.10 ± 0.43^{Abc}	11.17 ± 0.17^{Aa}	10.22 ± 0.05^{Abc}	10.37 ± 0.14^{CDbc}	10.28 ± 0.11^{BCbc}	10.46 ± 0.34^{Cbc}
	150	10.45 ± 0.10^{BCbc}	10.26 ± 0.07^{Aab}	10.06 ± 0.28^{Aa}	10.42 ± 0.14^{Abc}	10.66 ± 0.08^{Cc}	10.34 ± 0.22^{Aabc}	12.28 ± 0.24^{Ad}
	180	9.88 ± 0.10^{Ac}	9.11 ± 0.31^{Aa}	$11,129 \pm 0.10^{Ad}$	10.73 ± 0.02^{Ac}	9.65 ± 0.10^{Ab}	10.76 ± 0.05^{Abc}	10.83 ± 0.08^{Bc}

[1]Mean ± standard deviation obtained in two experiments, one of two experiments in duplicated (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among each time for treatments and different small letters indicate significant differences ($p < 0.05$) among each treatment for times

[2]R-con means control group that was treated using only a roaster machine

[3]UA means a group that was treated by UV-A alone

[4]TUA means a group that was treated by UV-C and TiO_2

[5]UC means a group that was treated by UV-C alone

[6]TUC means a group that was treated by UV-C and TiO_2

[7]UAC means a group that was treated by UV-A and UV-C simultaneously

[8]TUAC means a group that was simultaneously treated by UV-A, UV-C, and TiO_2

a*, and b* values of untreated BPP were 53.55 ± 0.36, 2.16 ± 0.05, and 10.84 ± 0.11, respectively. In the case of R-con, L*, a*, and b* values both significantly ($p < 0.05$) decreased and increased during treatment. The L*, a*, and b* values of BPP increased or decreased by 3.49, 0.22, and 0.96, respectively. However, because there is no significant ($p > 0.05$) trend depending on treatment time or treatment group, the change of color values by R-con is considered to be within the standard deviation between the samples.

When UA treatment was performed for 180 min, the L* value and the b* value decreased by 4.83 and 1.73, respectively, which were significantly ($p < 0.05$) different from those of R-con. This results indicated that the brightness of BPP is decreased, and the degree of blue of the pepper powder is increased by treatment. After TUC, the BPP was bluer than the R-con pepper powder at 90 and 180 min. The blue color of BPP treated by TUAC increased at 30 min but decreased again after 60 min. During the treatment, the blue color of BPP seemed to be increased by TUC and TUAC. However, since the value increased and decreased irregularly, it remains unclear whether the color of BPP is affected by TUC and TUAC. Therefore, we concluded that all treatments except UA did not affect the color of BPP. The combined treatment of UV-A, UV-C, and TiO_2 coating was considered to be suitable for BPP as a treatment to inactivate food poisoning bacteria.

Ishibashi proved that $\cdot O_2$ is generated in air and TIO_2 interface by UV light using chemiluminescence method with high sensitivity (Ishibashi et al., 2000). This study tried to inactivate pathogens by directly contacting the black pepper powder inoculated pathogens with TIO_2 coated on the roaster machine during UV irradiation. The treatments were used alone or combined to confirm increasing inactivation effects. Combined treatment of UV-A and UV-C was effective for inactivating *S.* Typhimurium. The TiO_2 coating increased the inactivation effect of UV-A and UV-C against *S.* Typhimurium and *E. coli* O157:H7. There was no significant ($p > 0.05$) difference between productions of the injured *S.* Typhimurium and *E. coli* O157:H7 depending on the treatments. UV-A, UV-C and TiO_2 coating do not affect production of the injured cell on BPP. The moisture content of BPP was decreased more by UV-A than by UV-C. UV-A treatment alone decreased the brightness and increased the degree of blue on BPP for 180 min, but the other treatments did not affect the color of the BPP. Therefore, the combined treatment of UV-A, UV-C, and TiO_2 coating maintains the quality of BPP and can be used effectively to sterilize BPP without heating.

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