Food Sci Biotechnol (2019) 28(2):599–607
https://doi.org/10.1007/s10068-018-0480-4

Inactivation effect of UV-C and mild heat treatment against *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on black pepper powder

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Received: 15 July 2018 / Revised: 19 September 2018 / Accepted: 21 September 2018 / Published online: 27 September 2018

Abstract In this study, UV-C and mild heat treatments were used alone or in combination for the inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on the black pepper powder. The treated pepper powder was analyzed microbiologically, and the physicochemical properties of the pepper powder were examined. UV-C combined with heat treatment at 60 °C most effectively inactivated the pathogens. However, heat treatment at 60 °C produced many injured cells and greatly reduced moisture content of the pepper powder. UV-C combined with heat treatment at 45 °C showed significantly ($p < 0.05$) similar inactivation results as that with heat treatment at 60 °C after 18 min. In addition, the combined treatment minimized the moisture loss and color change of the pepper powder compared to heat treatment at 60 °C. Thus, UV-C and heat treatment at 45 °C can effectively inactivate pathogens while maintaining the quality of black pepper powder.

Keywords UV-C · Mild heat · *Salmonella* Typhimurium · *Escherichia coli* O157:H7 · Black pepper powder

Introduction

Black pepper is one of the most widely used spices in the world. Because the pepper is grown in tropical regions with high temperature and humidity (Buckenhiiskes and Rendlen, 2004), high microbial levels of approximately 10^7 CFU/g have been reported occasionally in these crops (De Boer et al., 1985). Although black pepper is added as a condiment to foods in small quantities, the yeast, fungi, and pathogenic bacteria present in black pepper can cause food poisoning (Banerjee and Sarkar, 2003).

Black pepper grown in soils with animal manure can be infected with *Salmonella* Typhimurium and *Escherichia coli* O157:H7 (Islam et al., 2004). Contaminated pepper added to ready-to-eat food can cause food poisoning (Little et al., 2003). The Centers for Disease Control and Prevention (CDC) reported that large-scale infection by *Salmonella* species in the United States in November 2009 was due to consumption of salami products made with contaminated pepper (Julian et al., 2010).

There are various methods for sterilizing contaminated pepper powder. Fumigation with ethylene oxide is the oldest method for sterilizing spices (Leistritz, 1997). Although this method can certainly inhibit microbial growth, it has been prohibited in many countries because ethylene oxide is carcinogenic (Schweiggert et al., 2007). Steam treatment of spices at high temperatures for a short period is also an effective method for sterilization. Although steam treatment is performed safely without the use of chemicals, it can damage the sensory properties and cause color changes in pepper (Almela et al., 2002). In addition, the high water content produced after the steam treatment may promote microbial growth (Schweiggert et al., 2007). Recently, radiation treatment using gamma rays has been performed for the sterilization of black pepper. Radiation treatments for spices are legally performed in more than 51 countries (International Atomic Energy Agency, 2007), and many studies have proved its effectiveness. Radiation inactivates bacteria and fungi effectively without affecting the quality of the spices (Farag et al., 1995; Munasiri et al., 1987; Onyenekwe and

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Ogbadu, 1995; Sharma et al., 1989), but high doses of radiation cause oxidation and degradation of the color or the aroma of the spices (Hayashi, 1998). In addition, because of the consumers' negative perceptions of irradiation, this method is not widely used in industries.

UV-C is known as an effective method to inactivate microorganisms attached to food surfaces (Hamanaka et al., 2000). UV-C at a wavelength of 250–260 nm creates pyrimidine dimers in the DNA of microorganisms, thereby interfering with the transfer and replication of DNA and eventually resulting in cell death (Harm, 1980). UV-C has bactericidal effects against a wide range of microorganisms and viruses. moreover, these radiations produce no toxic residues and have low installation and processing costs (Chang et al., 1985). However, UV-C is used in combination with other sterilization methods because of its low penetration capacity into food (Bintsis et al., 2000). Heat treatment is the most basic intervention method for reducing the populations of foodborne pathogenic microorganisms (Blackburn et al., 1997). Many studies have shown that much higher temperatures are required for inactivating pathogenic microorganisms in low-humidity environments than for inactivating those in high-humidity environments (Nissen et al., 1996; Wilbey and Brennan, 2006). Heating at excessively high temperatures for the inactivation of microorganisms may cause changes in the sensory properties and loss of volatile flavor components. The mild heat treatment does not have sterilization effect when used alone, but it is used in combination with other antimicrobial treatments to produce a synergistic effect. Recent studies have used combinations of UV-C and mild heat treatments for the sterilization of spices. Cheon et al. (2015) used a combination of UV-C and mild heat treatment to inactivate *S.* Typhimurium and *E. coli* O157:H7 inoculated in red pepper powder. Erdoğdu and Ekiz (2011) investigated the inactivation effect of UV-C and far-infrared radiation on yeast and fungi in cumin seeds.

To date, no study has investigated the inactivation effect of UV-C and mild heat treatment against microorganisms on contaminated black pepper powder. Therefore, this study was performed to investigate the inactivation effect of UV-C and mild heat treatment on *S.* Typhimurium and *E. coli* O157:H7 inoculated on black pepper powder, with minimal effects on the quality of black pepper powder.

Materials and methods

Bacterial strains

Salmonella Typhimurium (ATCC 19585) and *Escherichia* coli O157:H7 (ATCC 35150) were obtained from the Korean Collection for Type Cultures. All strains were mixed with 0.5 mL of tryptic soy broth (TSB; Difco, Franklin Lakes, USA) and 0.5 mL of 100% glycerol. All stock cultures were stored at − 80 °C and cultured in TSB before use.

Culture preparation

S. Typhimurium and *E. coli* O157:H7 were inoculated into 10 mL of TSB and incubated at 37 °C for 9 h. Then, 1 mL of the bacterial culture was inoculated again into 200 mL of TSB, followed by secondary culture for 18 h. The culture was centrifuged at 9000×*g* for 20 min at 4 °C. The supernatant was discarded and the pellet was resuspended in 0.85% physiological saline. (Washing step) The washing step was repeated three times. After the final centrifugation, the pellet was resuspended in 20 mL of 0.85% saline solution and used for inoculation.

Sample preparation and inoculation

Black pepper powder was purchased at a large-scale mart in Seoul (Seoul, South Korea). In order to inoculate on the pepper powder, 150 g of black pepper powder was put into a sterilized bag (Whirl–pak, 1930 cm; Nasco, Fort Atkinson, WI, USA). A total of 3 mL of the bacterial suspension was added to this powder and hand massaged for 2 min. The inoculated pepper powder was dried in a biosafety hood for 1 h. The pepper powder was finally inoculated with *S.* Typhimurium and *E. coli* O157:H7 at about 10^7 CFU/g, respectively.

UV-C and mild heat treatment

UV-C and mild heat treatments for inactivating the microorganisms inoculated on black pepper powder were carried out using two UV lamps (58 W; SANTO-UV, Incheon, Korea) and a roaster machine (MK-300; JC Company, China), which were installed in an incubator. The black pepper powder was placed on the roaster machine (diameter: 24 cm; depth: 3.5 cm) and then subjected to UV-C and mild heat treatment. The distance between the black pepper powder and the UV lamp was adjusted to 5 cm and the sample was continuously mixed using the roaster machine for uniform UV-C irradiation during the treatment. The wavelength of the UV-emitting lamp (length: 510 mm; lamp diameter: 19 mm) installed in the incubator was 253.7 nm and the irradiation dose was 2.32 W/cm^2. The UV-C and heat treatments were applied alone or in combination for 0, 6, 12, 18, 24, 30, 36, and 42 min. The incubator chamber was maintained at 45 °C and 60 °C during the treatment.

Microbial analysis and injured cell enumeration

After each treatment, 90 mL of sterilized 0.85% saline solution was added to 10 g of black pepper powder and homogenized with a stomacher (Laboratory Blender Stomacher 400; Seward, MO, USA) for 2 min. Then, 1 mL of the sample aliquots was serially diluted with 9 mL of 0.85% saline solution and then plated on each selective medium. Xylose-lysine-deoxycholate agar (Oxoid, Hampshire, UK) and sorbitol MacConkey agar (Oxoid, Hampshire, UK) were used as selective media for *S.* Typhimurium and *E. coli* O157:H7, respectively. After plating, the plate was incubated at 37 °C for 24–48 h and the number of colonies formed was counted. To determine the population of the injured cells, aliquots of the samples were plated on each selective medium and nutrient medium simultaneously after each treatment. Tryptone soy agar was used as the nutrient medium for the pathogens. The plate was incubated at 37 °C for 24–48 h and the number of colonies formed was counted. As per the study by Ukuku and Gevek (2010), the colony count was calculated as the population of injured cells in percentage by using the following formula:

$$[1 - (\text{counts on selective agar}/\text{counts on nonselective agar})] \times 100$$

Measurement of moisture content and color

The moisture content of the black pepper powder was measured using the OHAUS MB 45 Moisture Analyzer (MB45; OHAUS, NJ, USA) during the experiment to determine the quality change in the pepper powder caused by UV-C and heat treatment. After each treatment, 2 g of black pepper powder was subdivided into an aluminum dish and heated at 140 °C for 10 min to measure the moisture content. To measure the color change of pepper powder by UV-C and heat treatment, 2 g of black pepper powder was subdivided into a petri dish and then L*, a*, and b* values were measured using a colorimeter (CR-400 Chroma Meter; Konica Minolta Sensing, Inc., Japan).

Statistical analysis

All experiments were repeated three times with duplicate samples. The average number of microorganisms was converted to log CFU/g. Results were statistically analyzed using the analysis of variance of the IBM SPSS statistics program (version 23, IBM Corp., USA) and the mean values were separated using Duncan's multiple range test. A $p < 0.05$ was used to determine the significant differences in the treatment.

Results and discussions

Inactivation effect of UV-C and mild heat treatment on black pepper powder

Inactivation effect of UV-C and mild heat treatment against *S.* Typhimurium on black pepper powder is shown in Fig. 1. The initial concentration of *S.* Typhimurium inoculated on black pepper powder was measured as 7.14 ± 0.11 log CFU/g.

In case of black pepper powder treated with UV-C alone, *S.* Typhimurium was reduced by 2.91 log CFU/g for 6 min and 3.79 log CFU/g for 42 min. Heat treatment at 45 °C for 6 min decreased *S.* Typhimurium by about 2.90 log CFU/g. The 45 °C heat treatment showed a somewhat higher reduction effect than the UV-C treatment during 18–36 min, but there was no significant difference during entire time. When combined with UV-C and 45 °C heat treatment (UV + 45 °C heat treatment), reduced populations of *S.* Typhimurium on black pepper powder by up to 6.37 log CFU/g. Heat treatment at 60 °C inactivated 3.84 log CFU/g of *S.* Typhimurium for 6 min and decreased *S.* Typhimurium up to 6.27 log CFU/g for 42 min. When UV-C and 60 °C heat treatment (UV + 60 °C heat treatment) was combined and treated for more than 30 min, the more 7 log CFU/g reduction occurred.

When combined with UV-C, inactivation efficiency was increased. And the higher the treatment temperature, the more reduction effect was observed. So UV + 60 °C heat treatment showed the highest reduction effect among the treatment. When the UV-C treatment was applied to the 45 °C heat treatment, the inactivation effect was greatly increased. UV + 45 °C heat treatment showed significantly ($p < 0.05$) similar inactivation results as that with heat treatment at 60 °C after 18 min.

Inactivation effect of UV-C and mild heat treatment against *E. coli* O157:H7 on black pepper powder is shown in Fig. 2. The initial concentration of *E. coli* O157:H7 inoculated on black pepper powder was measured as 7.06 ± 0.10 log CFU/g.

E. coli O157:H7 inoculated on black pepper powder was inactivated by 1.26 log CFU/g when irradiated with UV-C for 6 min and by 2.28 log CFU/g when irradiated with UV-C for 42 min. Heat treatment at 45 °C for 42 min reduced the concentration to 3.12 log CFU/g. UV + 45 °C heat treatment for 30 min showed a reduction of 3 log CFU/g against *E. coli* O157:H7. Heat treatment at 60 °C alone and UV + 60 °C heat treatment for 42 min reduced the amount of *E. coli* O157:H7 to 4.98 and 5.28 log CFU/g, respectively.

The reduction patterns of *E. coli* O157:H7 by the five treatments were similar to that of *S.* Typhimurium. The

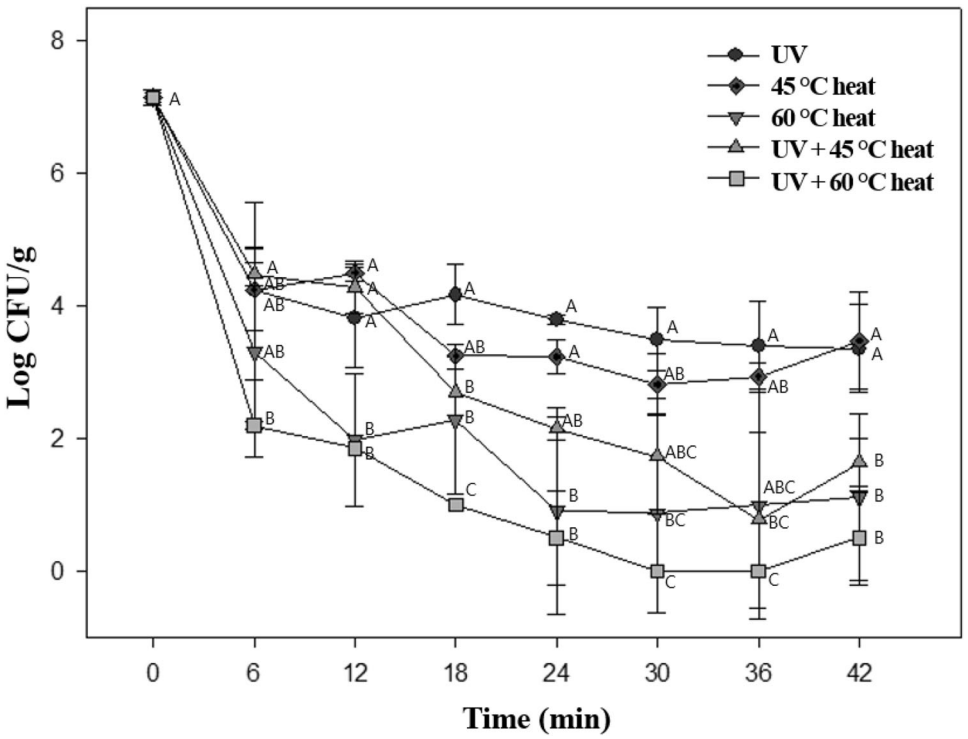

Fig. 1 Effect of UV-C and mild heat treatment against *S.* Typhimurium inoculated on black pepper powder. Means ± standard deviation obtained in three experiments, one of two experiments in duplicated (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time

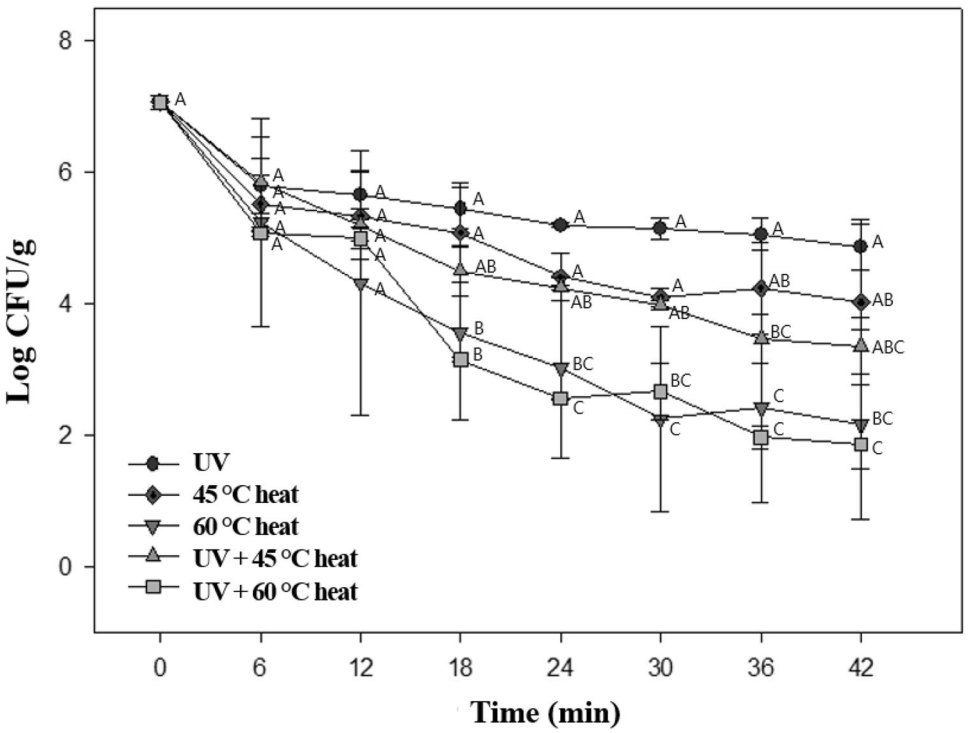

Fig. 2 Effect of UV-C and mild heat treatment against *E. coli* O157:H7 inoculated on black pepper powder. Means ± standard deviation obtained in three experiments, one of two experiments in duplicated (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time

order of the decreasing effect was as follows: UV + 60 °C heat treatment, 60 °C heat treatment, UV + 45 °C heat treatment, 45 °C heat treatment, and UV-C. Among the treatments, UV + 60 °C heat treatment showed the most effective reduction. however, there were no statistical differences in the reduction from 60 °C heat alone

treatment. It was confirmed that the inactivation effect was slightly increased when the UV-C treatment was applied to the 45 °C heat treatment. And The inactivation effect of UV + 45 °C heat treatment was significantly ($p < 0.05$) similar to the effect of heat treatment at 60 °C after 18 min.

Production of injured cell during UV-C and mild heat treatment on black pepper powder

The production of injured cells by UV-C and mild heat treatment against *S.* Typhimurium and *E. coli* O157:H7 on black pepper powder is shown in Fig. 3.

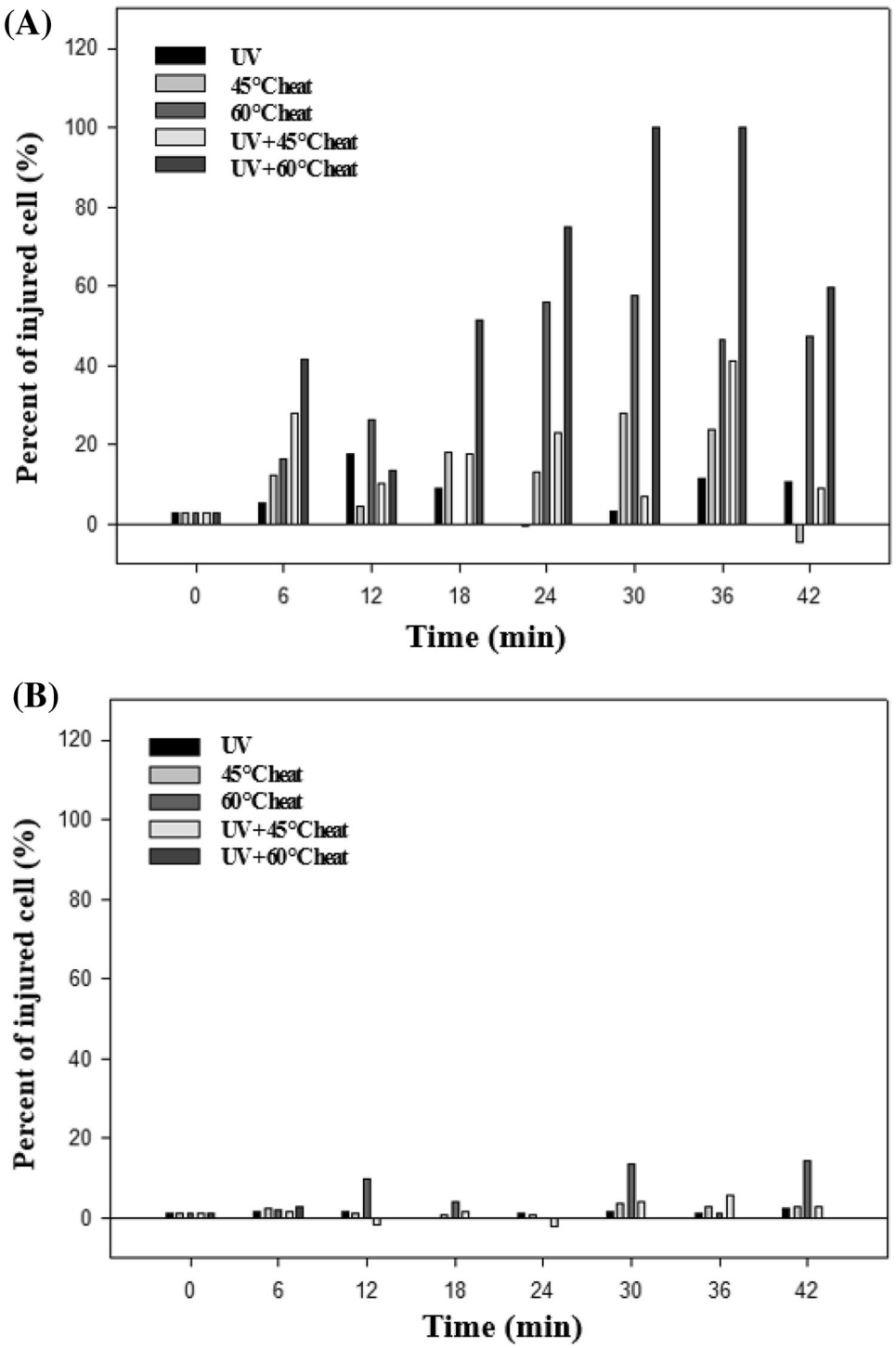

Fig. 3 Injured cell of *S.* Typhimurium (**A**) and *E. coli* O157:H7 (**B**) produced by UV-C and mild heat treatment

S. Typhimurium and *E. coli* O157:H7 on black pepper powder, which were not treated after the inoculation, showed 3.07% and 1.27% of injured cells, respectively. The production of injured cells on untreated black pepper powder is presumed to be due to the low water content of pepper. During the reduction treatment, a greater number of *S.* Typhimurium cells were injured than *E. coli* O157:H7. These findings suggest that the sensitivity to UV-C and heat treatments depends on the strain.

The UV-C treatment alone on black pepper powder resulted in 7.56% and 1.38% of injured *S.* Typhimurium and *E. coli* O157:H7 cells, on an average. The heat treatment at 45 °C resulted in 12.31% and 1.94% of injured cells of *S.* Typhimurium and *E. coli* O157:H7, respectively, and a maximum of 28.11% and 3.73% at the end of the treatment period, respectively. The UV + 45 °C heat treatment showed a similar effect as heat treatment at 45 °C. On the other hand, heat treatment at 60 °C resulted in an injury of 31.53% and 5.76% of *S.* Typhimurium and *E. coli* O157:H7 cells and up to 55.81% and 14.51% of injured cells, respectively. The higher the temperature, the greater the number of generated injured cells.

Although bacteria are damaged and killed by inactivation methods such as heat treatment and UV-C irradiation, the injured cells in the food environment can be revived (Back et al., 2012). The recovered bacteria can increase the possibility of food poisoning. Therefore, a microbial inactivation method, which forms a small injured cell with high inactivation effect, is considered as the most effective inactivation method.

Moisture contents change during UV-C and mild heat treatment of black pepper powder

The changes in the moisture content of black pepper powder during treatment are shown in Fig. 4. The initial moisture content of black pepper powder inoculated with *S.* Typhimurium and *E. coli* O157:H7 was 11.78 ± 0.03%.

Moisture content decreased continuously as treatment time increased and showed significant ($p < 0.05$) differences among the five different treatments. The moisture content of black pepper powder rapidly decreased in the order of UV + 60 °C heat treatment > heat treatment at 60 °C > UV + 45 °C heat treatment > heat treatment at 45 °C > UV-C. When UV-C treatment was carried out for more than 30 min, the moisture content of black pepper powder was reduced to less than 10%. Heat treatment at 45 °C and UV + 45 °C heat treatment decreased the moisture content to less than 10% when treated for 36 min and 24 min, respectively. The moisture content decreased to less than 10% by the 60 °C heat treatment and UV + 60 °C heat treatment for 18 min and 12 min, respectively.

When the treatment temperature was increased from 45 to 60 °C, the moisture content decreased rapidly. UV + 60 °C heat treatment resulted in the lowest moisture content ($p < 0.05$). These results suggest that the lower the

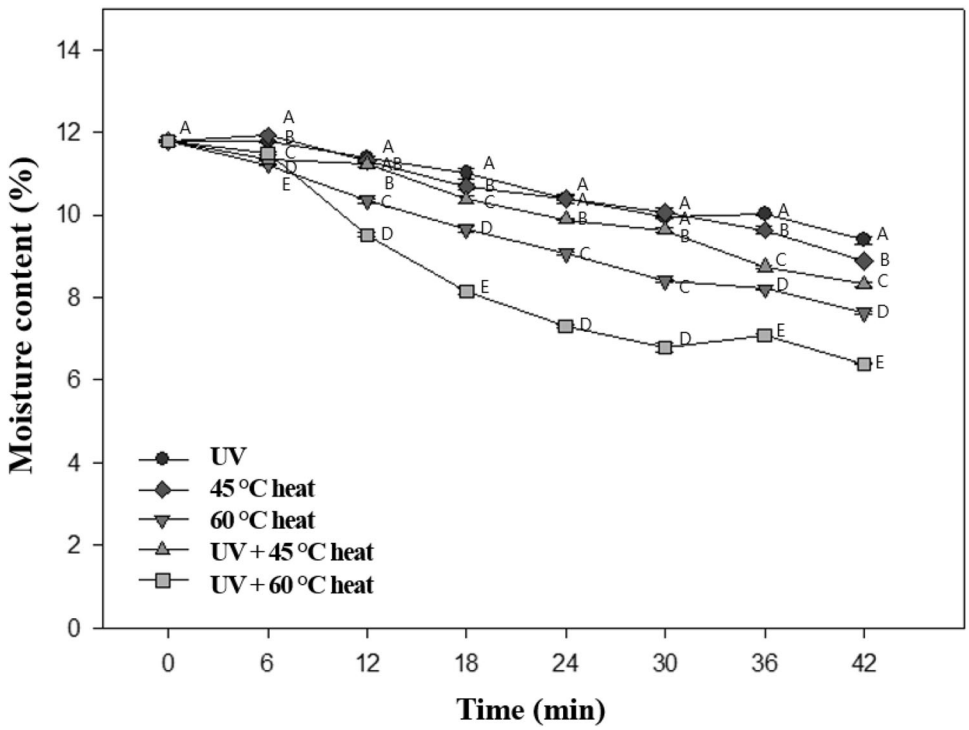

Fig. 4 Change of moisture content in black pepper powder by UV-C and mild heat treatment. Means ± standard deviation obtained in three experiments, one of two experiments in duplicated (n = 3). Different capital letters indicate significant differences ($p < 0.05$) among treatments for each time

Table 1 Change of color (L*, a*, b*) of black pepper powder by UV-C and mild heat treatment according to treatment time

Treatment	Time (min)							
	0	6	12	18	24	30	36	42
*L**								
UV	55.84 ± 0.19 A	55.65 ± 0.47 AB	56.04 ± 0.07 A	55.24 ± 0.11 AB	54.84 ± 0.02 B	52.69 ± 0.88 C	51.38 ± 0.02 D	51.92 ± 0.52 CD
45 °C heat	55.84 ± 0.19 A	56.05 ± 0.28 AB	55.49 ± 0.61 A	56.86 ± 0.25 AB	55.08 ± 0.40 A	55.85 ± 0.32 A	55.98 ± 0.01	55.85 ± 0.71 A
60 °C heat	55.84 ± 0.19 A	54.84 ± 0.49 BC	54.57 ± 0.20 C	54.80 ± 0.46 BC	55.63 ± 0.30 AB	55.93 ± 0.39 A	55.32 ± 0.30 ABC	55.59 ± 0.33 AB
UV + 45 °C heat	55.84 ± 0.19 AB	56.08 ± 0.45 A	55.90 ± 0.70 A	56.09 ± 0.44 A	55.89 ± 0.32 A	56.19 ± 0.12 A	55.64 ± 0.25 AB	54.79 ± 0.65 B
UV + 60 °C heat	55.84 ± 0.19 AB	55.38 ± 0.59 ABC	56.16 ± 0.18 A	55.71 ± 0.54 ABC	55.20 ± 0.57 ABC	54.75 ± 0.23 C	55.46 ± 0.40 ABC	55.14 ± 0.09 BC
*a**								
UV	2.02 ± 0.06 A	2.05 ± 0.08 AB	1.96 ± 0.01 A	2.05 ± 0.04 AB	2.13 ± 0.01 BC	2.21 ± 0.01 C	2.57 ± 0.00 D	2.58 ± 0.03 D
45 °C heat	2.02 ± 0.06 AB	2.10 ± 0.01 AB	2.15 ± 0.10 B	2.02 ± 0.04 AB	2.09 ± 0.06 AB	1.98 ± 0.01 B	1.96 ± 0.07 B	1.96 ± 0.00 B
60 °C heat	2.02 ± 0.06 AB	2.08 ± 0.02 BC	2.13 ± 0.04 C	2.13 ± 0.02 C	2.01 ± 0.01 A	2.07 ± 0.02 ABC	2.14 ± 0.04 C	2.10 ± 0.01 C
UV + 45 °C heat	2.02 ± 0.06 A	2.04 ± 0.03 AB	2.08 ± 0.01 ABC	2.10 ± 0.09 ABC	2.06 ± 0.01 AB	2.09 ± 0.03 ABC	2.19 ± 0.07 C	2.16 ± 0.01 BC
UV + 60 °C heat	2.02 ± 0.06 A	2.08 ± 0.04 AB	2.06 ± 0.00 A	2.12 ± 0.01 AB	2.05 ± 0.03 A	2.17 ± 0.08 B	2.12 ± 0.01 AB	2.17 ± 0.02 B
*b**								
UV	12.17 ± 0.04 ABC	12.57 ± 0.88 C	12.25 ± 0.08 ABC	12.31 ± 0.14 ABC	12.50 ± 0.08 BC	11.84 ± 0.02 ABC	11.62 ± 0.09 A	11.71 ± 0.21 AB
45 °C heat	12.17 ± 0.04 ABC	11.76 ± 0.31 A	12.74 ± 0.18 D	12.59 ± 0.06 CD	12.07 ± 0.16 AB	12.39 ± 0.25 BCD	12.23 ± 0.13 ABC	12.16 ± 0.28 ABC
60 °C heat	12.17 ± 0.04 A	12.42 ± 0.25 A	12.34 ± 0.04 A	12.20 ± 0.40 A	12.43 ± 0.08 A	12.50 ± 0.04 A	12.47 ± 0.18 A	12.19 ± 0.04 A
UV + 45 °C heat	12.17 ± 0.04 AB	12.39 ± 0.06 ABC	12.29 ± 0.30 ABC	12.39 ± 0.01 BC	12.19 ± 0.10 AB	12.53 ± 0.12 C	12.30 ± 0.01 ABC	12.08 ± 0.04 A
UV + 60 °C heat	12.17 ± 0.04 AB	12.03 ± 0.10 A	12.35 ± 0.06 BC	12.40 ± 0.27 BC	12.13 ± 0.02 AB	12.25 ± 0.00 ABC	12.23 ± 0.06 ABC	12.47 ± 0.13 C

Means ± 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

temperature of heat treatment, the lesser would be change in the quality of the black pepper powder.

Color changes during UV-C and mild heat treatment of black pepper powder

Table 1 shows the color changes of black pepper powder caused by UV-C and heat treatment. The L*, a*, and b* values of the pepper powder before treatment were 55.84 ± 0.19, 2.02 ± 0.06, and 12.17 ± 0.04, respectively. The L*, a*, and b* values of the black pepper powder treated with UV-C for 42 min were 51.92 ± 0.52, 2.58 ± 0.03, and 11.71 ± 0.21, respectively. The L* value was significantly ($p < 0.05$) decreased and the a* value was significantly ($p < 0.05$) increased when UV-C treatment was performed for more than 30 min. As the UV-C treatment time increased, the brightness decreased and the red color increased. The change of b* value by UV-C treatment was not significantly different ($p < 0.05$).

Other treatments besides UV-C treatment did not change the color of the black pepper powder. The L*, a*, and b* values were significantly increased or decreased by all treatments but did not show a trend during the treatment time. Therefore, the combination of UV-C and heat treatment did not significantly affect the quality of the black pepper powder.

In this study, we measured the inactivation effects of UV-C and mild heat (45 °C and 60 °C) treatments against *S.* Typhimurium and *E. coli* O157:H7 inoculated on black pepper powder. Higher treatment temperatures in combination with UV-C radiations increased the effects of inactivation. *S.* Typhimurium and *E. coli* O157:H7 inoculated on black pepper powder were most effectively inactivated by the combined treatment of UV-C and 60 °C heat treatment. However, greater numbers of *S.* Typhimurium and *E. coli* O157:H7 cells were injured at higher temperatures. The moisture content—one of the factors determining the quality of black pepper powder—decreased rapidly with increasing temperatures. The inactivation effect of UV + 45 °C heat treatment showed significantly ($p < 0.05$) similar to the 60 °C heat treatment after 18 min. Except for UV-C, all other treatments resulted in no significant color changes in the black pepper powder. Therefore, it was concluded that UV + 45 °C heat treatment is the most effective inactivation method for the sterilization of black pepper powder as it produces fewer injured cells and results in minimal moisture content loss and color changes in the black pepper powder.

Acknowledgements This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant Number 317030).

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