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Evaluation of Non-Thermal Decontamination Processes to Have the Equivalence of Thermal Process in Raw Ground Chicken

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Abstract The present study was aimed at examining the antibacterial effects of non-thermal decontamination processes, which are equivalent to thermal treatment, to ensure microbiological safety of raw ground chicken. *Escherichia coli* or *Salmonella* were inoculated into 25 g of raw ground chicken samples. The raw ground chicken samples were non-treated or treated with high hydrostatic pressure (HHP) at 500 MPa (1–7 min), light-emitting diode (LED) irradiation at 405 nm wavelength (30–120 min), and heat at 70°C, 90°C (1–60 min), and 121°C (1–15 min). *E. coli* and *Salmonella* cell counts were enumerated after treatments. Moreover, the color parameters of treated raw ground chicken were analyzed. HHP treatment reduced *E. coli* and *Salmonella* cell counts by more than 5 Log CFU/g and more than 6 Log CFU/g after 7 min and 1 min, respectively; these effects were equivalent to those of thermal treatment. However, LED irradiation reduced *Salmonella* cell counts by only 0.9 Log CFU/g after 90 min of treatment, and it did not reduce *E. coli* cell counts for 90 min. Compared with those of the non-treated samples, the ΔE (total color difference) values of the samples treated with HHP were high, whereas the ΔE values of the samples treated with LED irradiation were low (1.93–2.98). These results indicate that despite color change by HHP treatment, HHP treatment at 500 MPa could be used as a non-thermal decontamination process equivalent to thermal treatment.

Keywords heat-equivalent non-thermal technology, microbial quality, high hydrostatic pressure, ultraviolet light-emitting diode

Introduction

The most common pathogens contaminating poultry meat products are *Escherichia coli* and *Salmonella*. The chicken carcasses have been observed to have high contamination rates for *E. coli* (77.5%) and *Salmonella* (85%) (Yulistiani and

Praseptianga, 2019). *E. coli* are a gram-negative, facultatively anaerobic bacillus of the Enterobacteriaceae family (Lenhard-Vidal et al., 2011). *Salmonella* are rod-shaped, gram-negative bacteria that are one of the most prevalent causes of foodborne illness globally (Ha et al., 2020). These pathogens may contaminate meat and meat products through hands of workers in slaughterhouses and during processing. Thus, thermal processing of poultry meat products is performed before its use in food.

Thermal processing enhances the shelf life of food products and ensures microbiological safety, but it may alter the structural-chemical composition, modify heat-labile components, affect the functional properties of food products, and change meat color. When consumed raw ready-to-eat meat such as *Yukhoe*, the meat color is the important index for consumer's acceptance. Thus, minimizing change in meat color is a requirement for a non-thermal decontamination process.

High hydrostatic pressure (HHP) processing is a non-destructive food preservation technology that efficiently eliminates food spoilage microorganisms without using chemicals (Kim et al., 2016). According to Garriga et al. (2004), HHP has the potential to be useful in the meat industry. HHP destroys microorganisms regardless of product geometry (Kim et al., 2016), along with increases in the safety of raw meat products and shelf life (Kruk et al., 2014).

Ultraviolet light-emitting diodes (LED) are considered one of the most influential alternatives to UV lamps because of their numerous advantages. Although antibacterial efficacy of LED is not comparable to that of UV light, significant antibacterial effects by LED irradiation has recently been reported (Li et al., 2018). Previous studies have demonstrated significant microbial inactivation of LED on smoked salmon and papaya (Kim et al., 2017; Li et al., 2018). Furthermore, LED are cost-effective and induce material degradation to a low extent (Li et al., 2018). In addition, LED are environmentally friendly, and simple equipment is needed for LED lighting (Aoyagi et al., 2011).

Therefore, this study examined the antibacterial effect of non-thermal decontamination processes, which are equivalent to that of the thermal process, on raw ground chicken.

Materials and Methods

Detection of *E. coli* and *Salmonella* in raw ground chicken

To evaluate *E. coli* and *Salmonella* contamination levels in raw ground chickens in Korea, six raw ground chicken samples (1 kg) were purchased from grocery shop, and 25 g portions of raw ground chicken were transferred aseptically into sterile filter bags (3M, St. Paul, MN, USA). The filter bags were filled with 225 mL of 0.1% buffered peptone water (BPW; Becton Dickinson and Company, Sparks, MD, USA). The samples were macerated using a pummeler (BagMixer, Interscience, St. Nom, France) for 1 min. One milliliter of the homogenate was put into a Petrifilm™ *E. coli*/Coliform count plate (3M). The parafilm was used to seal the plate, which was then incubated for 24 h at 37°C. Typical blue colonies with obvious gas bubbles were carefully counted. In addition, for qualitative analysis of *E. coli*, 1 mL of the homogenate was added to *E. coli* broth (Becton Dickinson and Company) including Durham tube (Kisan Biotech, Seoul, Korea) and cultured for 24–48 h at 44.5°C. Following streaking, the aliquot in the gas-producing tube was then streaked onto eosin methylene blue agar (EMB; Becton Dickinson and Company) and incubated for 24–48 h at 37°C. Formation of typical colonies with luminescent metal color confirmed the presence of *E. coli*. One milliliter of the homogenate was spread out on the xylose lysine deoxycholate (XLD; Becton Dickinson and Company) agar, and the plate was sealed using parafilm before being incubated for 24 h at 37°C to count *Salmonella*. Colonies were carefully counted on XLD agar using typical black colonies. In addition, for qualitative analysis of *Salmonella*, the homogenate was incubated for 24 h at 37°C. Further, aliquots containing 100 µL were added to 10 mL Rappaport-Vassiliadis enrichment broth (Becton Dickinson and Company) and incubated for 24 h at 42°C.

The cultures were streaked onto XLD agar and then incubated for 24 h at 37°C. Formation of typical black colonies confirmed the presence of *Salmonella*. The colonies on EMB and XLD agar were identified by 16S rRNA sequencing following amplification of the primers 27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 1492R (5' TAC GGY TAC CTT GTT ACG ACT T 3'). The PCR was performed using an EF-Taq (Solgent, Daejeon, Korea), 20 ng genomic DNA was used as the template in a 30 µL reaction mixture. The following PCR conditions used: initial denaturation for 2 min at 95°C; 35 cycles of amplification (95°C for 1 min, 55°C for 1 min, and 72°C for 1 min), and ending with 72°C for 10 min. A multiscreen filter plate was used to purify the enhanced products (Millipore, Bedford, MA, USA). The extension product-containing DNA sample was mixed with Hi-Di™ formamide (Applied Biosystems, Foster City, CA, USA), and then incubated for 5 min at 95°C and for 5 min on ice before being examined with a DNA analyzer (Applied Biosystems).

Inoculum preparation

E. coli

E. coli strains (NCCP14037, NCCP14038, NCCP14039, NCCP15661, and ATCC43888) were cultured for 24 h in 10 mL tryptic soy broth (TSB; Becton Dickinson and Company) at 37°C. The aliquots of these cultures (100 µL) were transferred to 10 mL fresh TSB and sub-cultured at 37°C for 24 h. The subcultures were combined and centrifuged at 1,912×g for 15 min at 4°C. The cell pellets were washed twice with 1× phosphate buffered saline (PBS; pH 7.4; 1.5 g/L Na₂HPO₄·7H₂O, 0.2 g/L KH₂PO₄, 0.2 g/L KCl, and 8.0 g/L NaCl) after removing the supernatants to obtain inoculum containing 8–9 Log CFU/mL.

Salmonella

Salmonella strains (NCCP12231, NCCP12236, NCCP12243, NCCP14544, and NCCP10140) were cultured in 10 mL TSB at 37°C for 24 h. The aliquots of these cultures (100 µL) were transferred to 10 mL fresh TSB and sub-cultured at 37°C for 24 h. The subcultures were combined and centrifuged at 1,912×g for 15 min at 4°C. The cell pellets were washed twice with 1× PBS after removing the supernatants to obtain inoculum containing 8–9 Log CFU/mL.

Inoculation

To obtain 6–7 Log CFU/g on samples, aliquots containing 100 µL of *E. coli* or *Salmonella* inoculum were inoculated on 25 g raw ground chicken samples and left for 15 min to allow attachment of the bacterial cells. The inoculated raw ground chicken samples were aseptically transferred to polyethylene pouches and vacuum-packaged (Postech, Haman, Korea). The vacuum-packaged samples were stored at 4°C until further treatment.

Treatments

Thermal treatment

The sample pouches were placed on a water bath rack and immediately immersed in circulating water bath at 70°C and 90°C for 1, 15, 30, and 60 min and steam heated in an autoclave at 121°C for 1, 4, 7, and 15 min. After thermal treatment, the samples were aseptically transferred to filter bags containing 50 mL BPW and macerated with a pummeler. The homogenates were serially diluted with 9 mL BPW; 1 mL aliquots of the diluents were put into a Petrifilm™ *E. coli*/Coliform count plate for determination of *E. coli* cell counts, and 0.1 mL of the diluent was spread-plated onto XLD agar to enumerate *Salmonella*

cells. The parafilm was used to seal the plate, which then incubated for 24 h at 37°C. Followed by incubation, the bacterial colonies were carefully counted.

HHP treatment

The inoculated samples were loaded in a pressure chamber in an HP 300 high pressure machine (Hyperbaric, Burgos, Spain), and pressure was increased to 500 MPa within 3 min. The temperature of the pre-cooled samples increased up to 25°C owing to adiabatic heating during pressure build-up. The samples were pressurized at 500 MPa for 1, 3, 5, and 7 min. The pressure was immediately released to 0.1 MPa post treatment, and all samples were placed on ice until analysis. The samples were macerated with a pummeler after being aseptically transferred to filter bags containing 50 mL BPW. The homogenates were serially diluted with 9 mL BPW; 1 mL aliquots of the diluents were put into a Petrifilm™ *E. coli*/Coliform count plate for determination of *E. coli* cell counts, and 0.1 mL of the diluent was spread-plated onto XLD agar to count *Salmonella* cells. The plate was sealed using parafilm and incubated for 24 h at 37°C, and the bacterial colonies were carefully counted.

LED treatment

LED lamps (12 W, SWL-V2650, Sunwave, Suwon, Korea) with proportional integral derivative controller (ITC-100 VH, INKBIRD Tech, Shenzhen, China) were used. Five lamps were installed in a refrigerator (600 mm×600 mm). The raw ground chicken samples were placed at the distance of 50 mm from the lamps, and they were treated at 405 nm for 30, 60, 90, and 120 min. UV intensity was measured using a spectrometer (StellarNet BLK-C, Stellar Net, Tampa, FL, USA). An optical probe of a spectrometer was placed at distance of 50 mm from the lamps, and the light intensity was then integrated using an auto digitizer. UV intensity was calculated as 2.8, 5.6, 8.4, and 11.2 J/cm² under LED irradiation for 30, 60, 90, and 120 min, respectively. After LED irradiation, the samples were aseptically transferred to a filter bag containing 50 mL BPW and macerated using a pummeler. The homogenates were serially diluted with 9 mL BPW; 1 mL aliquots of the diluents were put into a Petrifilm™ *E. coli*/Coliform count plate for determination of *E. coli* cell counts, and 0.1 mL of the diluent was spread-plated onto XLD agar to count *Salmonella* cells. The plate was sealed with parafilm and incubated at 37°C for 24 h. Followed by incubation, the bacterial colonies were carefully counted.

Color analysis

To evaluate if HHP and LED treatment affect on the meat color. The lightness (L^*), redness (a^*), and yellowness (b^*) in the samples treated with HHP or LED were measured using a colorimeter (CR-10, Konica Minolta Sensing, Osaka, Japan). It had the illuminant of D65, observer angle of 2° and aperture diameter of 8 mm. The total color difference (ΔE) was estimated using the following equation to determine the overall color change compared with that of the control.

$$\Delta E = \sqrt{(L^* - L^*_{*0})^2 + (a^* - a^*_{*0})^2 + (b^* - b^*_{*0})^2}$$

where L^*_{*0} , a^*_{*0} , and b^*_{*0} represent the readings at 0 h, and L^* , a^* , and b^* represent the individual readings after the defined treatments.

Statistical analysis

The antibacterial effects and color parameters data were analyzed using the general linear model in SAS version 9.4 (SAS

Institute, Cary, NC, USA). The differences among the least square means were calculated using a paired *t*-test with at $\alpha=0.05$ significance level.

Results and Discussion

E. coli and *Salmonella* prevalence in raw ground chicken

E. coli were detected in all tested samples at 2.2 ± 0.5 Log CFU/g, whereas *Salmonella* were not detected in any of the samples. According to the Eyi and Arslan (2012), *E. coli* were detected in 87.5% of poultry samples at 3.3 Log CFU/g. Eyi and Arslan (2012) reported predominant contamination of carcasses of animals and birds with *E. coli* and suggested that contamination of meat and meat products with *E. coli* are likely during preparation and sale also. Although the prevalence of *Salmonella* in retail chicken in previous studies was reported as 53.3% in Vietnam (Van et al., 2007), 36.5% in Belgium (Uyttendaele et al., 1999), and 42.3% in Korea (Hyeon et al., 2011), the result from our study showed no positive samples for *Salmonella*. This difference might be caused by washing chicken carcasses with sodium hypochlorite in Korea. MFDS (2019) and Yoon et al. (2014) also showed very low prevalence of *Salmonella* in chicken. Collectively, these results suggest the importance of decontamination processes in foods containing raw chicken.

Antibacterial effects

Thermal treatment

In non-heated raw ground chicken samples, *E. coli* cell counts were 6.6 Log CFU/g, and these decreased to 6.2 Log CFU/g after 1 min, 0.6 Log CFU/g after 15 min, and limit of detection (LOD; 0.5 Log CFU/g) after 30 min at 70°C (Fig. 1A). *Salmonella* cell counts in non-heated raw ground chicken samples were 6.8 Log CFU/g, which also decreased to 6.3 Log CFU/g after 1 min, 0.8 Log CFU/g after 15 min, 0.6 Log CFU/g after 30 min, and LOD after 60 min at 70°C (Fig. 1B). These

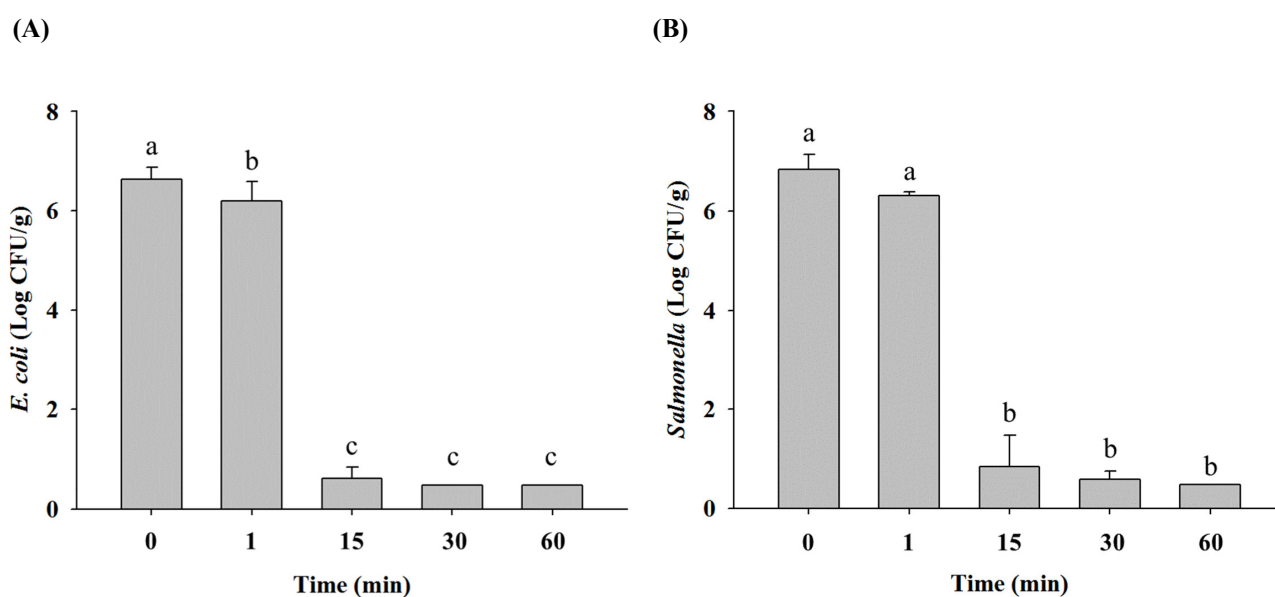


Fig. 1. *Escherichia coli* (A) and *Salmonella* cell counts (B) in raw ground chicken samples after thermal treatment at 70°C. Data shown are mean \pm SD. ^{a-c}Means with different letters are significantly different ($p<0.05$).

results showed that *E. coli* and *Salmonella* in raw ground chicken were destroyed within 30 min and 60 min of thermal treatment at 70°C, respectively. Thermal treatment at 90°C resulted in decrease in *E. coli* cell counts in raw ground chicken samples to 6.3 Log CFU/g after 1 min and LOD after 15 min compared with 6.6 Log CFU/g in untreated samples (Fig. 2A). Similarly, *Salmonella* cell counts significantly decreased to 6.2 Log CFU/g after 1 min and LOD after 15 min after treatment at 90°C from the initial concentration of 6.8 Log CFU/g (Fig. 2B). These findings showed that *E. coli* and *Salmonella* in raw ground chicken were destroyed within 15 min at 90°C. Moreover, both *E. coli* and *Salmonella* cell counts in raw ground chicken samples were estimated to reach LOD after 1 min when heated at 121°C (Fig. 3A and 3B). This shows that *E. coli* and *Salmonella* in raw ground chicken can be eliminated within 1 min by heating at 121°C.

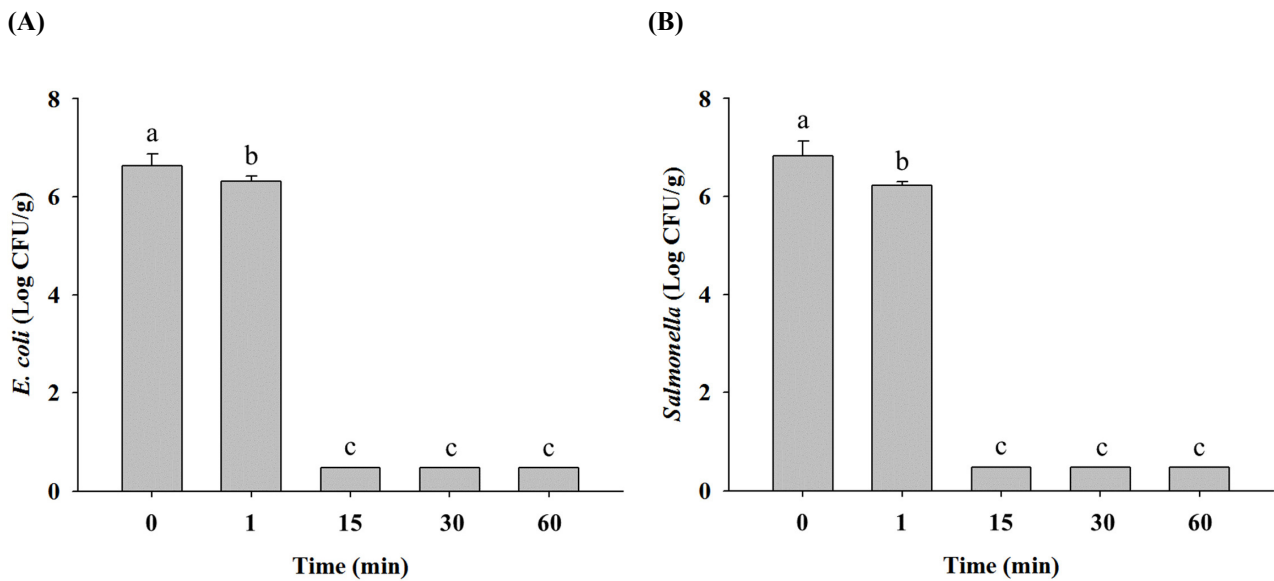


Fig. 2. *Escherichia coli* (A) and *Salmonella* cell counts (B) in raw ground chicken samples after thermal treatment at 90°C. Data shown are mean±SD. ^{a-c} Means with different letters are significantly different (p<0.05).

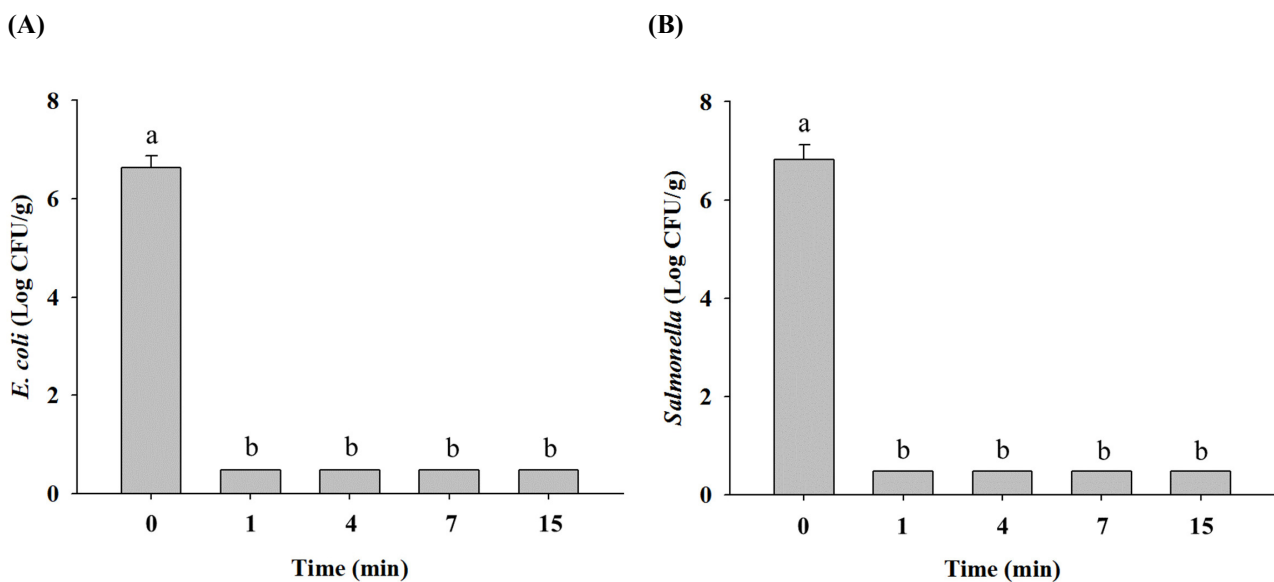


Fig. 3. *Escherichia coli* (A) and *Salmonella* cell counts (B) in raw ground chicken samples after thermal treatment at 121°C. Data shown are mean±SD. ^{a,b} Means with different letters are significantly different (p<0.05).

HHP treatment

HHP treatment reduced *E. coli* cell counts in raw ground chicken samples to 4.8 Log CFU/g after 1 min, 3.4 Log CFU/g after 3 min, 2.7 Log CFU/g after 5 min, and 0.9 Log CFU/g after 7 min from the initial concentration of 6.7 Log CFU/g (Fig. 4A). *Salmonella* cell counts decreased to 0.8 Log CFU/g after 1 min and LOD after 3 min of HHP treatment from the initial concentration of 6.5 Log CFU/g (Fig. 4B). This shows that *Salmonella* are more sensitive to HHP than *E. coli*. HHP treatment causes many changes in microbial cell membranes, resulting in lysis, nuclear material alteration, osmotic changes, and other modifications, finally leading to pathogen eradication (Mackey et al., 1994). Jung et al. (2012) and Kruk et al. (2011) reported that HHP of 450–600 MPa almost completely eliminated *E. coli* and *Salmonella*. These results indicate that 7-min HHP treatment at 500 MPa can reduce both *E. coli* and *Salmonella* cell counts, equivalent to thermal treatment for 60 min at 70°C, 15 min at 90°C, and 1 min at 121°C ($p < 0.05$).

LED irradiation

E. coli cell counts in raw ground chicken samples before and after LED irradiation at 405 nm was not significantly different (Fig. 5A). LED irradiation for 90 min reduced *Salmonella* cell counts in raw ground chicken only by 0.9 Log CFU/g ($p < 0.05$), but it did not reduce *E. coli* cell counts for 90 min (Fig. 5B). According to Kim et al. (2017), *Salmonella* cell counts in fresh-cut papaya were reduced by 1–1.2 Log CFU/cm² when exposed to 405 nm LED for 48 h. Li et al. (2018) suggested that LED irradiation at 405 nm for 8 h reduced a 0.5-Log CFU/cm² of *Salmonella* cell counts in ready-to-eat fresh salmon. These results indicate that LED irradiation has no significant effect on microbial reduction compared with thermal treatment, and *Salmonella* are more sensitive to LED irradiation than *E. coli*.

Color of raw ground chicken after treatments

HHP treatment

The parameters of chicken color (L^* , a^* , and b^*) are shown in Table 1. The L^* values were high ($p < 0.05$) in the HHP-

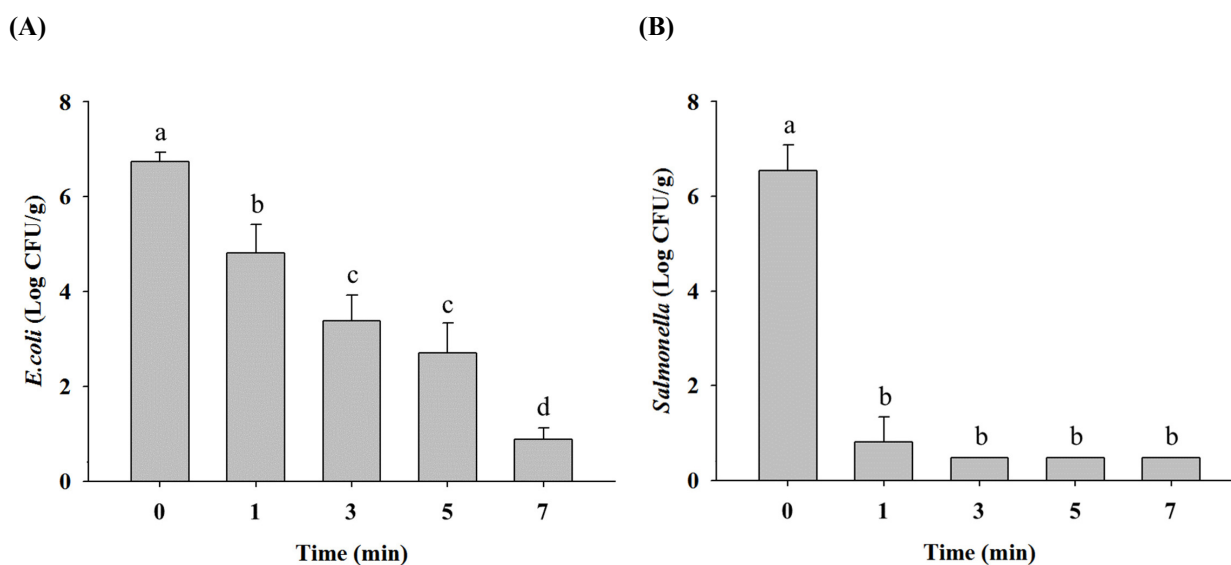


Fig. 4. *Escherichia coli* (A) and *Salmonella* cell counts (B) in raw ground chicken samples after high hydrostatic pressure at 500 MPa. Data shown are mean \pm SD. ^{a-d} Means with different letters are significantly different ($p < 0.05$).

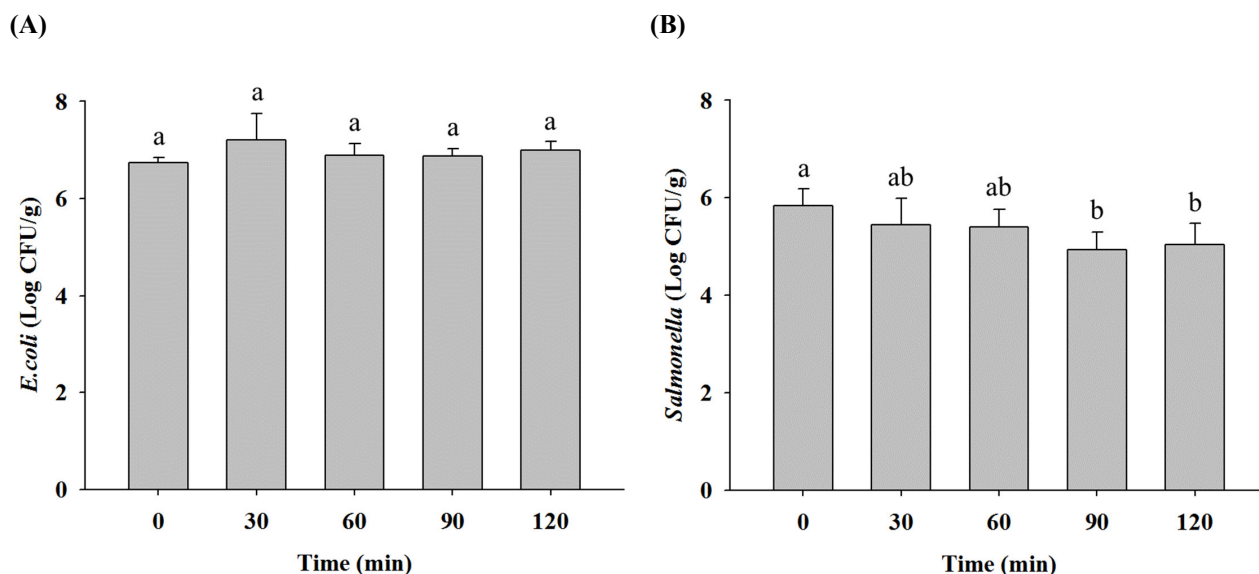


Fig. 5. *Escherichia coli* (A) and *Salmonella* cell counts (B) in raw ground chicken samples after light-emitting diode at 405 nm. Data shown are mean \pm SD. ^{a,b} Means with different letters are significantly different ($p < 0.05$).

Table 1. The color parameters (mean \pm SD) of the raw ground chicken samples after high hydrostatic pressure treatment at 500 MPa

Property	HHP treatment (min)				
	0	1	3	5	7
L*	44.63 \pm 2.15 ^b	52.90 \pm 1.51 ^a	52.77 \pm 0.75 ^a	54.47 \pm 2.50 ^a	55.43 \pm 0.76 ^a
a*	27.73 \pm 2.42 ^a	22.50 \pm 2.36 ^b	23.70 \pm 1.44 ^b	21.17 \pm 2.81 ^b	20.10 \pm 1.41 ^b
b*	18.27 \pm 2.63 ^a	20.27 \pm 0.21 ^a	20.13 \pm 1.04 ^a	19.93 \pm 0.93 ^a	20.43 \pm 0.51 ^a
ΔE	-	10.31 \pm 2.51	10.00 \pm 3.23	12.48 \pm 2.81	13.54 \pm 1.97

^{a,b} Means with different superscript letters in a row are significantly different ($p < 0.05$). HHP, high hydrostatic pressure; ΔE , total color difference.

treated raw ground chicken samples. The L* value represents the light-dark spectrum with a range from 0 (black) to 100 (white), which is closely related to the browning level of the samples (Pathare et al., 2013). The a* values were low ($p < 0.05$) in the HHP-treated raw ground chicken samples. Wang et al. (2018) reported a* value as the most important color parameter for fresh meat, which is defined as the red-green spectrum with a range of -60 (green) to +60 (red). The alterations in L* and a* values following HHP treatment were caused by the oxidation of ferrous myoglobin to metmyoglobin, which represents the effect of pressure on globin denaturation and structural rearrangement (Carlez et al., 1995; Fraqueza et al., 2019; Szerman et al., 2011). The b* value is defined as the blue-yellow spectrum with a range from -60 (blue) to +60 (yellow) (Wang et al., 2018). In our study, the b* values were not significantly different among the samples. The total color difference (ΔE) represented the magnitude of the overall color difference between the non-HHP and HHP-treated samples. The ΔE values in HHP-treated raw ground chicken samples ranged between 10.00–13.54 (Table 1). According to the study by Kim et al. (2016), ΔE values >12 indicates a significant color difference. Thus, the HHP-treated raw ground chicken samples showed a significant color difference from the non-HHP-treated samples (Table 1).

LED irradiation

The L* values decreased in the LED-irradiated raw ground chicken samples, whereas the a* values increased ($p < 0.05$;

Table 2. The color parameters (mean±SD) of the raw chicken samples after light emitting diode irradiation at 405 nm

Property	405 nm LED irradiation (min)				
	0	30	60	90	120
L*	40.50±0.69 ^a	39.20±0.26 ^b	39.87±0.42 ^{ab}	39.93±0.76 ^{ab}	39.97±0.75 ^{ab}
a*	23.90±0.40 ^c	26.07±0.67 ^a	25.27±0.80 ^{ab}	24.73±0.67 ^{bc}	25.13±0.12 ^{ab}
b*	19.87±0.96 ^b	21.20±0.46 ^a	21.83±0.15 ^a	21.30±0.53 ^a	22.07±1.10 ^a
ΔE	-	2.98±0.86	2.62±1.26	1.93±1.60	2.90±0.91

^{a-c} Means with different superscript letters in a row are significantly different ($p < 0.05$). LED, light-emitting diode; ΔE , total color difference.

Table 2). Our findings were comparable to those reported by Chun et al. (2010) that a* values increased in the chicken breast after UV irradiation. The b* values increased ($p < 0.05$) in the LED-irradiated raw ground chicken samples (Table 2). The ΔE values in LED-irradiated raw ground chicken samples were 1.93–2.98 (Table 2). Francis and Clydesdale (1975) suggested that color difference is not clearly distinguished by human eyes when ΔE values < 3 . Thus, the results indicate that LED irradiation slightly changed the color of raw ground chicken compared with that of the non-irradiated samples.

Conclusion

In this study, HHP treatment at 500 MPa can destroy more than 5 Log CFU/g of *E. coli* cell counts for 7 min and more than 6 Log CFU/g of *Salmonella* cell counts for 1 min; these effects are equivalent to thermal treatment of raw ground chicken at 70°C for 60 min, 90°C for 15 min, and 121°C for 1 min. However, LED irradiation at 405 nm does not have the significant antibacterial effects as much as by the thermal treatment. Collectively, although HHP treatment causes color change, HHP treatment at 500 MPa for more than 7 min can be used as a non-thermal decontamination process, equivalent to thermal treatment, to improve the microbiological safety of raw ground chicken.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Park E. Data curation: Park E, Park S. Formal analysis: Park E, Park S. Methodology: Park E, Park S. Software: Park S, Park E. Validation: Hwang JH, Jung AH. Investigation: Park E, Park S, Hwang JH, Jung AH. Writing - original draft: Park E, Yoon Y. Writing - review & editing: Park E, Park S, Hwang JH, Jung AH, Park SH, Yoon Y.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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