

Changes in the Microbiological Characteristics of Korean Native Cattle (Hanwoo) Beef Exposed to Ultraviolet (UV) Irradiation Prior to Refrigeration

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Abstract

The effects of ultraviolet (UV) radiation were investigated with regards to the microbial growth inhibitory effect on the shelf life of Korean native cattle (Hanwoo) beef prior to refrigerated storage. The Hanwoo samples were exposed to UV radiation (4.5 mW/cm²) for 0, 5, 10, 15, and 20 min. The UV-irradiated beef that was exposed for 20 min showed significantly reduced mesophilic and psychrotrophic bacterial populations to the extent of approximately 3 log cycles, as compared to that of non-irradiated beef. About 2.5 Log CFU/g of mesophilic bacteria were different compared with UV-irradiated and non-irradiated meat. UV irradiation showed the most significant growth inhibition effects on mesophilic and psychrotrophic bacteria. Coliform and Gram-negative bacteria were also reduced by 1 log cycle. The population of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 decreased significantly to 53.33, 39.68, and 45.76% after 10 min of UV irradiation. They decreased significantly to 84.64, 80.76, and 84.12%, respectively, after 20 min of UV irradiation. The results show that UV irradiation time and the inhibitory effect were proportional. These results verified that UV radiation prior to refrigeration can effectively reduce the number of pathogenic bacteria on the surface of meat and improve the meat's microbial safety.

Keywords: ultraviolet (UV) irradiation, Korean native cattle (Hanwoo) beef, pathogenic bacteria

Introduction

Fresh meat such as Hanwoo beef is a highly perishable product due to its biological composition. Shelf life in fresh meat is influenced by various interrelated factors such as temperature, oxygen level, endogenous enzymes, moisture, and most significantly, microorganisms that contaminate the meat products immediately after the animals are slaughtered (Zhou *et al.*, 2010). With the increased consumer demand for high meat quality, safety, and extended shelf life, a proper non-thermal preservation method is required for fresh meat during the production and distribution process. However, fresh meat is difficult to store for long periods because it contains many nutrients such as sugars, free amino acids, and the volatile metabolites which lead to easy deterioration by microorganisms (Ercolini *et al.*, 2009; Joo *et al.*, 1998). Approximately 25

different kinds of microorganism species engage in meat spoilage through meat surface attachment and glycolocalyx formation (Chung *et al.*, 1989; Costerson *et al.*, 1981; Firstenberg-Eden, 1981). Typical pathogenic bacteria are *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Bacillus cereus*. In particular, *E. coli* O157:H7 and *L. monocytogenes* are considered serious pathogenic microorganisms in the worldwide meat market (Sofos, 2008). Historically, freezing has been considered the best way to prevent deterioration, but freezing meat causes issues that result from protein degradation (Barbut, 2002; Winger and Fennema, 1976) which decreases the beef's quality and flavor. Refrigeration at 4°C is considered the best way to store fresh meat without causing protein degradation. Compared to frozen meat, chilled (refrigerated) meat is able to maintain a high meat quality, but also has a shorter shelf life due to a lower level of microbiological safety (Kang *et al.*, 1997). Some pathogens, such as *Listeria*, have been reported to grow even in cold temperatures. Besides temperature control mechanisms such as freezing and refrigeration (Stevens *et al.*, 1990; Winger and Fen-

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nema, 1976) as ways to extend the safe storage period of meat, improved packaging methods (Brewer *et al.*, 1992), acid treatments (Podolak *et al.*, 1996) and synthetic preservatives (Unda *et al.*, 1990) have been studied. However, environmental, economical, and technical concerns have arisen with all of these methods. The UV radiation method has recently been evaluated as a possible means of extending the shelf life of meat (Devine *et al.*, 2001; Thayer, 1994).

UV light has a shorter wavelength region than visible light (400 nm) and a longer wavelength region than X-rays (100 nm). It is well known that UV light can have sterilization and disinfection effects without requiring chemical or heat treatments. Typically, UV-C between 220-300 nm is used for sterilization, which is approved by the FDA as a means of controlling food spoilage microorganisms (US Food, 2007). UV disinfection methods for food irradiation do not affect food quality or make food resistant to bacteria. Moreover, it is a very simple and safe process without residual components (Ryeong, 1996). UV irradiation, as it is used to improve storage periods, has been studied in onions (Lu *et al.*, 1987; Perez-Gregorio *et al.*, 2011), sweet potatoes (Chan *et al.*, 2010), apples (Wilson *et al.*, 1997), apple cider (Assatarakul *et al.*, 2012; Harrington and Claude, 1968), and whole fish (Huang and Toledo, 1982; Sucre *et al.*, 2012). UV has also been used for drinking water treatment (Lyon, 2012). In this study, we investigated the microbial growth inhibitory effect of UV irradiation as it can be used to improve the safety and storage of Hanwoo beef.

Materials and Methods

Materials

The female Hanwoo beef were obtained from Boseong-Gun, South Korea in the form of a four year old beef cow weighing approximately 301 kg. About 1 cm thick slices of beef muscle (*Longissimus dorsi*) were cut 5 h after slaughter for use in the experiment.

Methods

UV radiation

A UV lamp (Germicidal lamp G10T8, Sankyo Denki Co., Ltd. Korea) was equipped with a wavelength of 254 nm and an output of 10 W. Each bank of lights contained 10 lamps and was mounted either above or below the device. UV radiation intensity was measured using a UVX digital radiometer (UVP Inc., USA). The UV radiation

was applied at 4.5 mW/cm² for 0, 5, 10, 15, and 20 min. The beef irradiated with UV was put in a polystyrene foam tray and packed with polyvinylchloride wrap, then stored at 4±1°C. The microbiological samples were investigated via UV irradiation after a nine day storage period.

Microbiological changes in the number of mesophilic, psychrotropic, coliform, and gram-negative bacteria during refrigerated storage

To measure the number of mesophilic bacteria, a 20 g parcel of beef was placed in a sterile stomacher filter bag with 180 mL of 0.1% peptone solution (1:9), and blended for 2 min using a stomacher blender (Lab blender 400, Dongkok Inc, Korea) at room temperature. Dilutions of 10¹ up to 10⁸ were made in 0.1% peptone solution, and the diluted bacteria solution was inoculated in a plate count agar medium (PCA, Difco, USA). The colony was counted after a 48 h incubation at 37°C and reported as the log of colony-forming units (CFUs) (Kim *et al.*, 1992). The measurement of psychrotrophic bacteria followed the same method as that which was used for mesophilic bacteria. The colony was counted after a seven day incubation period at 4°C. The diluted bacteria solution from the mesophilic bacteria count was inoculated in violet red bile agar (VRBA) to measure the coliform bacteria. The colony was counted after a 48 h incubation at 37°C. The diluted bacteria solution was inoculated into a PCA medium which was then mixed with 0.1% crystal violet and 2,3,5-triphenyltetrazolium chloride to measure the Gram-negative bacteria. The colony was counted after a 48 h incubation at 20°C (Junillon and Flandrois, 2014).

The inhibitory effects on the growth of pathogens after UV irradiation

Inhibitory effect on the growth of pathogens in the plate

Three typical pathogens were tested to investigate the pathogen growth inhibitory effects of UV. They were cultured at 37°C for three days and then sub-cultured every 24 h. The experiments were conducted at an initial concentration of bacteria between 8.00-9.73 Log CFU/mL (18 h incubation). The three pathogens were *L. monocytogenes* ATCC 19113, *S. Typhimurium* ATCC 19430, and *E. coli* O157:H7 ATCC 43890. Tryptic phosphate broth and agar medium (Difco) were used for *L. monocytogenes*. Tryptic soy broth and agar medium (Difco) were used for *S. Typhimurium* and *E. coli* O157:H7. After growth on the

agar medium, each strain was treated with UV radiation for different times (0, 5, 10, 30, and 60 min), and the inhibitory effect was measured by enumeration after a 24 h incubation at 37°C (Sumner *et al.*, 1996).

Inhibitory effect on the growth of pathogens in the beef

Hanwoo sample was cut into 5×5×0.6 cm pieces and placed in a petri dish. *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 were diluted with a 0.1% peptone solution to 2-3 Log CFU/mL. The beef pieces were immersed in the pathogen-diluted solution for 15 min, and then dehydrated for 10 min. UV radiation was applied to each beef piece for different times (10, 20, 30, 40, and 60 min) to identify the inhibitory effect. Then the pieces were placed in a sterile stomacher filter bag with 180 mL of 0.1% peptone solution (1:9) and blended for 2 min using a stomacher blender. A different agar medium was used for the selective isolation of different pathogens. Oxford *Listeria* selective agar (Merck, USA) medium was used to inoculate *L. monocytogenes*. MacConkey's agar (Difco) medium was used to inoculate the *S. Typhimurium*, *E. coli* O157:H7 was inoculated into the MacConkey's sorbitol agar (Difco) medium. The inhibitory effect was measured by bacterial colony counting after a 24 h incubation at 37°C.

Statistical analysis

Measurements were replicated (n=2) and analyzed by an analysis of variance (ANOVA) using a GLM (general linear model) procedure in SAS (2003). Results were shown as mean values with their standard error bars. The statistical significance of the differences between the averages in treatments were accessed by Duncan's multiple range tests. Differences were considered significant for *p*-values lower than 0.05.

Results and Discussion

Microbiological examination during refrigeration

Mesophilic bacteria

Fig. 1 shows the changes in the number of mesophilic bacteria after UV irradiation during storage. Values of 3.41, 3.21, 3.15, and 3.09 Log CFU/g mesophilic bacteria were observed after 5, 10, 15, and 20 min of UV treatment, respectively, before storage. At the beginning of the storage process, the mesophilic bacteria population of the non-irradiated Hanwoo beef (3.51 Log CFU/g) was not

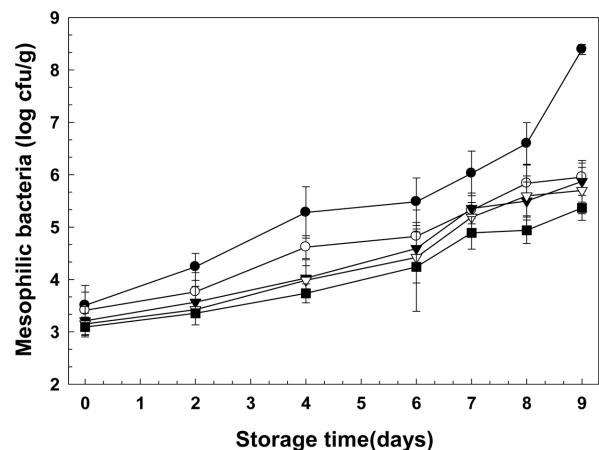


Fig. 1. Changes in mesophilic bacteria of Korean native beef treated after UV irradiation for various times during storage at 4°C. ●-● Control, ○-○ 5 min, ▼-▼ 10 min, ▽-▽ 15 min, ■-■ 20 min. Error bars show the standard error of the mean.

significantly different from that of the UV-treated beef samples ($p < 0.05$). However, the number of mesophilic bacteria in the non-irradiated Hanwoo beef meat was significantly different from that of the other irradiated meats with storage time ($p < 0.05$). There was an increase of 4.88 Log CFU/g during the time in refrigeration. The mesophilic bacteria in the non-irradiated meat were 8.39 Log CFU/g, and that of 20 min irradiated meat was 5.36 Log CFU/g after nine days of storage. The non-irradiated meat had already reached decay between 7 and 8 d of storage; when the bacteria reached a level of 6.5-7.0 Log CFU/g the meat was considered to be significantly decayed (Dogbevi *et al.*, 1999; Ehiba *et al.* 1987). Meat irradiated for 5, 10, 15, and 20 min did not reach the decay stage (6.5-7.0 Log CFU/g) until nine days of storage (5.95, 5.87, 5.70, and 5.36 Log CFU/g, respectively). Mesophilic bacteria generally has a growth temperature range of 10 to 50°C (*Salmonellae*: 10-45°C; *Clostridium perfringens*: 15-50°C; and *Staphylococci*: 10-45°C). However, some species such as the *Enterobacteria* can grow slowly below a temperature of 10°C (Barnes, 1976). Although refrigeration may have had the effect of slowing the mesophilic bacteria growth, the bacteria eventually dominated the beef's surface (Bahmani *et al.*, 2011), which was catastrophic to the quality of meat. In this study, we observed about 2.5 Log CFU/g of mesophilic bacteria were different compared with UV-irradiated and non-irradiated meat. This leads to the conclusion that UV treatment should prevent the growth of mesophilic bacteria during cold storage.

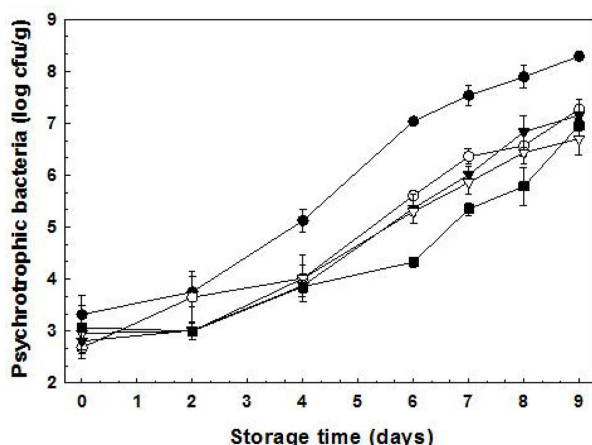


Fig. 2. Changes in psychrotrophic bacteria of Korean native beef treated with UV irradiation for various times during storage at 4°C. ●-● Control, ○-○ 5 min, ▼-▼ 10 min, ▽-▽ 15 min, ■-■ 20 min. Error bars show the standard error of the mean.

Psychrotrophic bacteria

The changes in the number of psychrotrophic bacteria following UV irradiation during storage can be seen in Fig. 2. Psychrotrophic bacteria are typical spoilage organisms of cold-stored food products due to the bacteria's ability to grow at low temperatures (Samelis, 2006). The bacterial population in the UV-irradiated and non-irradiated meats was not significantly different until after two days of storage ($p > 0.05$). However, the psychrotrophic bacteria population in the non-irradiated meat was 5.13 Log CFU/g after four days of storage, and the 5, 10, 15, and 20 min irradiated meat values were 4.02, 3.87, 4.01, and 3.85 Log CFU/g, respectively. The 20 min irradiated meat (4.33 Log CFU/g) showed about a 3 log cycle reduction after six days of storage as compared with that of non-irradiated meat (7.04 Log CFU/g), indicating that the UV radiation effectively inhibited the psychrotrophic bacteria growth. A similar effect was observed for *L. monocytogenes* (psychrotrophic bacteria) which was treated at 5 kg/m² UV irradiation, which reduced the population to 4.89 Log CFU/g from 6.18 Log CFU/g for the non-irradiated meat stored at 4°C for 6 d. (Chun *et al.*, 2010). UV radiation causes DNA damage and mutation to microorganisms and eventually leads to cell death (Sastri *et al.*, 2000; Unluturk *et al.*, 2008). In addition, the number of psychrotrophic bacteria was about 1-1.5 log cycle higher than mesophilic bacteria with a nine day incubation due to the optimum growth temperature of refrigeration for psychrotrophic bacteria.

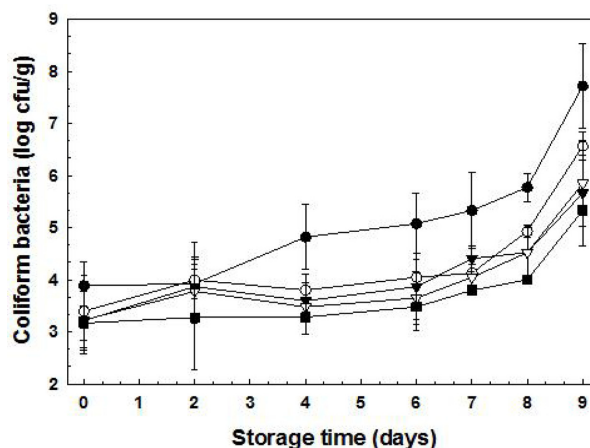


Fig. 3. Changes in coliform bacteria of Korean native beef treated after UV irradiation for a variety of times during storage at 4°C. ●-● Control, ○-○ 5 min, ▼-▼ 10 min, ▽-▽ 15 min, ■-■ 20 min. Error bars show the standard error of the mean.

Coliform bacteria

Fig. 3 shows the changes in the number of coliform bacteria following UV irradiation during storage. All Hanwoo beef samples showed a modest growth of coliform bacteria with eight days of storage, and then increased rapidly between days eight and nine of storage. According to Allende and Artes (2003), coliform bacteria growth significantly increased with three days of storage at 5°C after 8.14 kJ/m² UV-C radiations; their study focused on lettuce at 40% less UV radiation and 5 min treatment. Therefore, UV radiation doses are an important factor to control coliform bacteria growth rate. The coliform bacteria population in the non-irradiated meats was 4.82 Log CFU/g after four days of storage, and the 5, 10, 15, and 20 min irradiated meats were 3.80, 3.60, 3.48, and 3.28 Log CFU/g, respectively. In particular, the non-irradiated and 20 min irradiated meat showed a difference of more than a 2 log cycle after nine days of storage. The UV irradiation time and the degree of growth inhibition for coliform bacteria were proportional. According to the Sastri *et al.* (2000), UV irradiation on pathogenic microorganisms lead to the structural damage of DNA which causes cross-linking between adjacent pyrimidine bases. Simultaneously, hydrogen bonds of the purine bases are impaired to the opposite strand formation, then DNA transcription and replication are blocked and eventually cell death occurs (Unluturk *et al.*, 2008).

Gram-negative bacteria

Fig. 4 shows the changes in the number of Gram-nega-

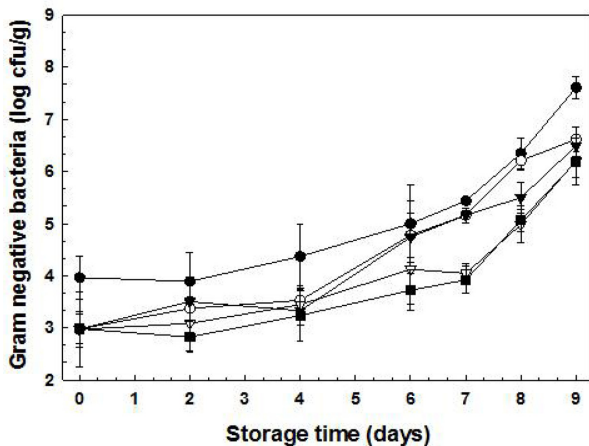


Fig. 4. Changes in Gram-negative bacteria of Korean native beef treated with UV irradiation for various times during storage at 4°C. ●-● Control, ○-○ 5 min, ▼-▼ 10 min, ▽-▽ 15 min, ■-■ 20 min. Error bars show the standard error of the mean.

Gram-negative bacteria following UV irradiation during storage. Overall, the growth of Gram-negative bacteria increased slowly for seven days of storage, and then increased rapidly. The Gram-negative bacteria in the non-irradiated meat was 3.97 Log CFU/g, whereas in the 5, 10, 15, and 20 min irradiated meats, those values were 2.98, 2.98, 2.83, and 2.96 Log CFU/g, respectively. There was more than a 1 log cycle interval between the non-irradiated meats and the 15 and 20 min irradiated meats for the 9 d storage. UV irradiation in Gram-negative bacteria distorts the DNA helix structure in the cells of the bacteria, preventing cell replication and the cross-linking of aromatic amino acids at their C-C double bonds. As a result, cell membrane depolarization and abnormal ionic flow occur due to protein denaturation, which leads to cell death (Moseley, 1990).

Inhibitory effects on pathogen growth after UV irradiation

The UV irradiation disinfection was examined using major pathogens in meats such as *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7. Table 1 shows the inhibited pathogen growth according to the degree of UV irradiation. The initial concentration of bacteria was about 8.00 Log CFU/mL *L. monocytogenes*, 9.73 Log CFU/mL *S. Typhimurium*, and 9.04 Log CFU/mL *E. coli* O157:H7, respectively. *L. monocytogenes*, with 5 and 10 min UV-irradiation, was significantly reduced to 2.15 and 1.23 Log CFU/mL while 30 and 60 min UV-irradiation were not detected. UV-irradiated *S. Typhimurium* and *E. coli* O157:H7 was showed to have similar

Table 1. Effect of UV irradiation (4.5 mW/cm²) time on viability of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 inoculated on agar plates

| UV irradiation time (min) | Concentration (Log CFU/plate) | | % Reduction | |
|---------------------------|-------------------------------|-----------|-------------|------|
| | Initial | Final | | |
| <i>L. monocytogenes</i> | 5 | 8.00±0.40 | 2.15±0.11 | >99% |
| | 10 | 8.00±0.40 | 1.62±0.08 | >99% |
| | 30 | 8.00±0.40 | ND | >99% |
| | 60 | 8.00±0.40 | ND | >99% |
| <i>S. Typhimurium</i> | 5 | 9.73±0.49 | 2.32±0.12 | >99% |
| | 10 | 9.73±0.49 | 1.95±0.09 | >99% |
| | 30 | 9.73±0.49 | 1.30±0.07 | >99% |
| | 60 | 9.73±0.49 | ND | >99% |
| <i>E. coli</i> O157:H7 | 5 | 9.04±0.45 | 2.75±0.14 | >99% |
| | 10 | 9.04±0.45 | 2.46±0.12 | >99% |
| | 30 | 9.04±0.45 | 1.60±0.08 | >99% |
| | 60 | 9.04±0.45 | ND | >99% |

Values are mean±S.D; ND: Not Detected.

Table 2. Effect of UV irradiation (4.5 mW/cm²) time on viability of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 inoculated with Hanwoo beef

| UV irradiation time (min) | Concentration (Log CFU/plate) | | % Reduction | |
|---------------------------|-------------------------------|-----------|-------------|-------|
| | Initial | Final | | |
| <i>L. monocytogenes</i> | 10 | 3.13±0.16 | 2.80±0.14 | 53.33 |
| | 20 | 3.13±0.16 | 2.32±0.12 | 84.64 |
| | 30 | 3.13±0.16 | 2.94±0.15 | 93.46 |
| | 40 | 3.13±0.16 | 1.66±0.08 | 96.63 |
| | 60 | 3.13±0.16 | 1.20±0.06 | 98.80 |
| <i>S. Typhimurium</i> | 10 | 2.57±0.13 | 2.36±0.12 | 39.68 |
| | 20 | 2.57±0.13 | 1.86±0.09 | 80.76 |
| | 30 | 2.57±0.13 | 1.28±0.06 | 94.84 |
| | 40 | 2.57±0.13 | 1.08±0.05 | 96.78 |
| | 60 | 2.57±0.13 | 1.00±0.05 | 97.32 |
| <i>E. coli</i> O157:H7 | 10 | 2.92±0.15 | 2.65±0.13 | 45.76 |
| | 20 | 2.92±0.15 | 2.11±0.11 | 84.12 |
| | 30 | 2.92±0.15 | 1.71±0.09 | 93.86 |
| | 40 | 2.92±0.15 | 1.49±0.07 | 96.24 |
| | 60 | 2.92±0.15 | 1.15±0.06 | 98.35 |

Values are mean±S.D.

effects. Above 99.99% of the bacteria were extinguished after UV irradiation (4.5 mW/cm² for 5, 10, 30, and 60 min). These inhibitory effects (>99.99% extinction) of UV irradiation were also reported by Sumner *et al.* (1996) with *S. Typhimurium* and Wong *et al.* (1998) with *E. coli* and *S. Senftenberg*. The study of Sumner *et al.* (1996) was verified that the UV was effectively destroying *S. Typhimurium* on agar plates and poultry skin. UV-irradiated agar plates showed almost complete elimination (99.9%) of *S. Typh-*

imurium at 2.0 W.s/cm² while 80.5% reduction was seen on the poultry skin surface post-irradiation. According to the Wong *et al.* (1998), in agar plate, above 5 Log CFU/g of *E. coli* reduction was observed at 100 μ W/cm² and > 7 Log CFU/g of *S. Senftenberg* was reduced by 80 μ W/cm².

Major pathogens such as *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 were inoculated on the surface of Hanwoo beef (see Table 2). The initial concentrations of *L. monocytogenes*, *S. Typhimurium* and *E. coli* O157:H7 were 3.13, 2.57, and 2.92 Log CFU/g, respectively. The population of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 decreased significantly to 53.33, 39.68, and 45.76% after 10 min of UV irradiation. They decreased significantly to 84.64, 80.76, and 84.12%, respectively, after 20 min of UV irradiation. In addition, all pathogens decreased >90% with 30 min of UV irradiation at 4.5 mW/cm². These results show that UV irradiation inhibited the growth of pathogenic microorganisms during storage. According to the Park *et al.* (2014), UV irradiation (260 nm) on experimentally contaminated dried filefish (*Stephanolepis cirrhifer*) fillet surfaces significantly ($p < 0.05$) reduced (above 95%) the major food spoilage molds (*Aspergillus niger*, *Penicillium citrinum*, and *Cladosporium cladosporioides*). The similar effects were observed by Guan *et al.* (2012). *E. coli* O157:H7 was significantly reduced (>84% on mushroom cap surfaces and >87% on surface of mushroom) by 3.15 kJ/m² UV-C treatment to button mushrooms. Interestingly, the reduction of pathogens inoculated on the agar plate was significantly higher than that of the inoculated pathogens that was directly tested on the Hanwoo beef (see Table 1 and 2). This anomaly might be explained by the uneven beef sample surface which may have allowed pathogens to suppress direct contact from the UV irradiation. Therefore, in order to use the UV irradiation method on a commercial scale, the meat intended for irradiation must be manipulated in such a way where the surface area that is irradiated is maximized.

Conclusions

This experiment was conducted to measure the effects of UV irradiation on improving the storage shelf life of Korean native beef by inhibiting the growth of pathogenic microorganisms. UV irradiation showed the most significant growth inhibition effects on mesophilic and psychrotrophic bacteria. The coliform and Gram-negative bacteria populations also significantly decreased by more than 1 log cycle during storage. The results show that UV

irradiation time and the inhibitory effect were proportional. Moreover, UV irradiation for 5 min killed 99.99% of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7 inoculated at 8.00-9.73 Log CFU/mL in the plate. UV irradiation for 20 min killed over 80% of the pathogenic bacteria inoculated in Hanwoo beef at 2.00-3.00 Log CFU/mL. These results verify that UV irradiation can effectively reduce the number of pathogenic bacteria on the surface of meat and improve microbial safety during cold storage.

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