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Short communication

# Efficacy of UV-C irradiation for inactivation of food-borne pathogens on sliced cheese packaged with different types and thicknesses of plastic films

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ABSTRACT

In this study, the efficacy of using UV-C light to inactivate sliced cheese inoculated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* and, packaged with 0.07 mm films of polyethylene terephthalate (PET), polyvinylchloride (PVC), polypropylene (PP), and polyethylene (PE) was investigated. The results show that compared with PET and PVC, PP and PE films showed significantly reduced levels of the three pathogens compared to inoculated but non-treated controls. Therefore, PP and PE films of different thicknesses (0.07 mm, 0.10 mm, and 0.13 mm) were then evaluated for pathogen reduction of inoculated sliced cheese samples. Compared with 0.10 and 0.13 mm, 0.07 mm thick PP and PE films did not show statistically significant reductions compared to non-packaged treated samples. Moreover, there were no statistically significant differences between the efficacy of PP and PE films. These results suggest that adjusted PP or PE film packaging in conjunction with UV-C radiation can be applied to control foodborne pathogens in the dairy industry.



## 1. Introduction

Non-thermal decontamination technologies are increasingly being applied in food processing as a practical alternative to thermal processing (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Non thermal processing can preserve food products from hazardous microorganisms while maintaining the nutritional and sensory characteristics of foods, which are often changed when thermal treatments are applied (Butz and Tauscher, 2002). There is a growing interest in the use of ultraviolet radiation as an alternate and inexpensive method for food preservation to reduce the number of microorganisms on food surfaces (Allende and Artes, 2003; Bintsis et al., 2000; Fonseca and Rushing, 2006; Guerrero-Beltrán and Barbosa-Cánovas, 2004; Shama, 2006; Yaun et al., 2004).

UV light at wavelengths shorter than 280 nm (termed UV-C) is considered germicidal on most types of microorganisms (Bintsis et al., 2000; Morgan, 1989; Sizer and Balasubramaniam, 1999). The highest germicidal effect is obtained between 250 and 270 nm, but it may decrease as the wavelength is increased (Bachmann, 1975). For this reason, a wavelength of 254 nm has been used for disinfection of surfaces, water, and some food products. UV-C radiation can be absorbed by nucleic acids and proteins, which can cause photo-damage and conformational changes, and subsequently interrupt vital metabolic functions such as DNA replication, transcription, and translation (Buma et al., 2003, 1995; Karentz et al., 1991; Lao and Glazer, 1996). More specifically, a cross-linking between adjacent thymine and cytosine (pyrimidine nucleoside bases) in the same DNA strand is caused by UV-C radiation. Due to this mutation, formation of the hydrogen bonds to the purine bases on the complimentary strand is impaired. Finally, DNA transcription and replication are inhibited, thereby rendering the microorganism unable to reproduce and eventually leading to cell death (Bintsis et al., 2000; Guerrero-Beltrán and Barbosa-Cánovas, 2004; Shama, 2006). UV-C radiation is a U.S. Food and Drug Administration (FDA) approved technology that can be used to inactivate pathogenic bacteria in liquid foods and water, and food contact surfaces (U.S. FDA, 2000).

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Post-processing contamination is the most frequent contributing factor to foodborne illness outbreaks. Generally recognized after-processing control points for access of pathogens to food products include human handling, transport containers, processing line, pumps or tanks, and sorting, packaging, cutting, and further processing equipment (Beuchat and Ryu, 1997; Reij and Den Aantrekker, 2004; Zottola and Smith, 1991). Thus, protective measures are needed to inactivate hazardous microorganisms on food surfaces after the packaging step. Currently, petrochemical-based plastics such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polypropylene (PP), and polyethylene (PE) have been increasingly used as packaging materials for foodstuffs. This popularity is due to their ready availability, low cost, light weight, and good mechanical performance such as tensile and tear strength, good barrier to oxygen, carbon dioxide, anhydride and aromatic compounds, heat sealability, and so on (Lange and Wyser, 2003; Marsh and Bugusu, 2007; Siracusa et al., 2008). However, to date, there is a paucity of information in the literature on the effect of UV-C radiation to eliminate or control growth of foodborne pathogens on food surfaces packaged with plastic films.

In this study, we evaluated the transmission efficiency of UV-C radiation through various types of plastic packaging film, and also investigated its efficacy for inactivation of foodborne pathogens, including *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*, on sliced cheese packaged with different types and thicknesses of plastic films.

## 2. Materials and methods

### 2.1. Bacterial strains

All bacterial strains, namely, *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, and ATCC 700408), and *L. monocytogenes* (ATCC 7644, ATCC 19114, and ATCC 19115) were obtained from the Bacterial Culture Collection at Seoul National University (Seoul, Korea) and used for all experiments. Stock cultures were stored at −80 °C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol. Working cultures were streaked onto Tryptic Soy Agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

### 2.2. Culture preparation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000 × g for 20 min at 4 °C and washed three times with Buffered Peptone Water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately $10^7$ ~ $10^8$ CFU/ml. Mixed culture cocktails were prepared by blending together equal volumes of each test strain.

### 2.3. Sample preparation

Sliced cheddar cheese was purchased from a local grocery store (Seoul, Korea). Sliced cheese (25 g) was placed on aluminum foil in a biosafety hood for inoculation, and cut into square pieces approximately 5by 5 cm. Then, 0.1 ml of previously described culture cocktail was applied onto the surface of each cheese piece by depositing droplets at 15 to 20 locations with a micropipette. This inoculation level was much higher than would normally be encountered in commerce. A high-inoculum concentration was used to make enumeration of surviving bacteria easier. The samples were air-dried for 2 h in the hood with the fan running at room temperature (22 ± 2 °C).

### 2.4. UV-C treatment system

The UV-C radiation device consisted of one bank of 5 germicidal emitting lamps (G6T5, Sankyodenki, Japan) located in the ceiling of the radiation vessel (Fig. 1). The UV-C lamps and treatment area were enclosed in an incubator to reflect UV-C radiation effectively and maintain room temperature (25 °C). The distance between lamps and tray was 10 cm and treatment time was 1 min. The tray was 50 cm long and 50 cm wide for UV-C treatments; the lamps had a 0.01 mm filament size and 1 cm spacing, and the light intensity at the sample location was 3.04 mW/cm$^2$. Prior to use, the UV lamps were allowed to stabilize by turning them on for at least 15 min.

### 2.5. Plastic packaging films and UV light transmission analysis

Four types of food packaging films (Polyethylene terephthalate; PET, Polyvinyl chloride; PVC, Polypropylene; PP, and Polyethylene; PE) were used and the thickness of each of the films was 0.07 mm. These films are widely used in food packaging applications. Each film was purchased from a local store (Seoul, Korea). Specular light transmission properties of packaging films were analyzed using a UV spectrophotometer (UV-2450, Shimadzu CO., Tokyo, Japan) in the range of 200–500 nm.

### 2.6. UV-C light treatment on film packaged media

For preliminary experiments performed on surfaces of a microbiological medium, the cocktail suspension was 10-fold serially diluted two times with 0.2% sterile peptone water (PW) resulting in a final concentration of approximately $10^5$–$10^6$ CFU/ml. One-tenth ml of culture suspension was spread-plated onto non-selective medium (TSA). After inoculation, the medium was dried for approximately 30 min prior to treatment. Each inoculated and film-covered sample of medium was treated with UV-C radiation for 1and 5 min at 25 °C. Following UV treatment, treated medium samples were immediately incubated at 37 °C for 24 h.

### 2.7. UV-C light inactivation of pathogens on film packaged cheese

To evaluate UV light inactivation of pathogenic bacteria on packaged cheese, inoculated samples were vacuum-sealed with each film using a vacuum packager (AZ-450-E, INTRISE CO., Ansan, Korea). Packaged samples were treated for 1 min at 25 °C to evaluate quantitatively the efficacy of UV light.

### 2.8. Effect of film thickness on UV-C light inactivation of pathogens

Packaging materials which allow penetration of UV light (PP and PE films) were tested at different film thicknesses. Before treatment, the inoculated samples were packaged with UV-C transparent films at thicknesses of 0.07, 0.10, and 0.13 mm. Then, packaged cheese samples were treated as described previously.

### 2.9. Bacterial enumeration

For enumeration of pathogens, 25 g treated samples were immediately transferred into sterile stomacher bags (Lab Plas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). One ml aliquots of homogenized samples were tenfold serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (Difco), Xylose Lysine Desoxycholate agar (Difco) and Oxford Agar Base (Difco) with antimicrobic supplement

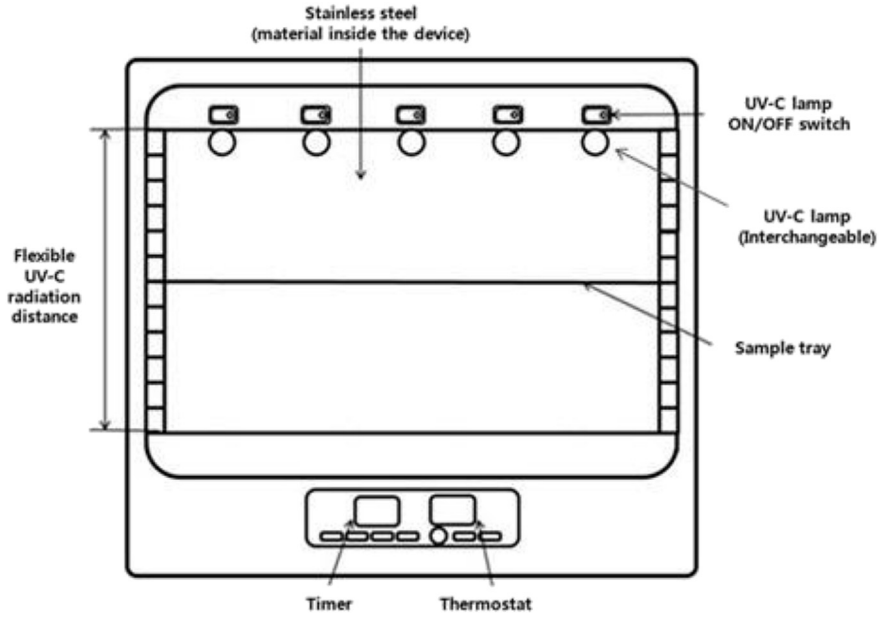


**Fig. 1.** Schematic diagram of UV-C radiation device at Seoul National University (Seoul, Korea).

(Bacto™ Oxford Antimicrobic Supplement, Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium and, *L. monocytogenes* respectively. All agar media were incubated at 37 °C for 24 h and colonies were counted.

### 2.10. Statistical analysis

All experiments were repeated three times with duplicate samples. Data were analyzed by analysis of variance (ANOVA) and Duncan's multiple range test of the Statistical Analysis System (SAS Institute, Cary, NC, USA). A P value of <0.05 was used to indicate significant differences.

## 3. Results

### 3.1. UV-C light transmittance

Fig. 2 shows the transmittance profiles for PET, PVC, PP, and PE films. The transmission of light in the UV-C range (200–280 nm) through PET and PVC was minimal. However, PP and PE permitted ample transmission of UV-C light. At 254 nm, the wavelength range which is used for disinfection of food products, 80.4% and 59.6% of UV-C was transmitted by PP and PE, respectively. These results show that UV-C radiation penetrates effectively through PP and PE film.

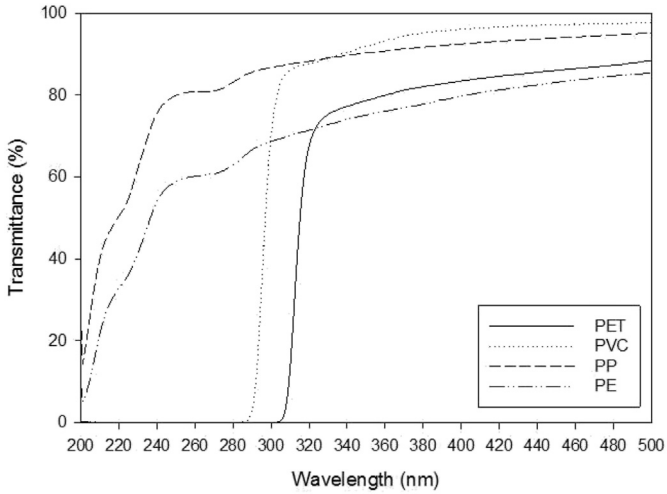


**Fig. 2.** Percent transmittance versus wavelength for PET, PVC, PP and PE films.

### 3.2. Inactivation of pathogen microorganisms in various film packaging

Fig. 3 visually shows inactivation of pathogens spread-plated onto TSA then covered with each type of film and treated with UV-C radiation for 1 or 5 min. Differences between levels of microbial reduction obtained on PP or PE-covered agar versus non-packaged agar were not observed at whole treatment time intervals. However, pathogen colonies on PET or PVC covered medium samples did not begin to show reduction until 5 min of UV-C treatment compared with those of the untreated control (Fig. 3).

The survival of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on surfaces of sliced cheese is shown in Table 1. UV-C treatment significantly ($P < 0.05$) decreased populations of *E. coli* O157:H7 with 3.36 and 3.12 log reductions after 1 min exposure of PP and PE packaged cheese slices, respectively, and there was no significant ($P > 0.05$) difference compared with non-packaged treated samples. However, significant ($P > 0.05$) reductions were not observed in PET and PVC packaged cheese slices. The patterns of inactivation of *S.* Typhimurium, and *L. monocytogenes* depending on packaging film were similar to the results of *E. coli* O157:H7 (Table 1).

### 3.3. Effect of PP and PE film thickness on pathogen inactivation

Table 2 shows populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* corresponding to each thickness of PP and PE packaging film. In both the PP and PE film experiments, thicker films were ineffective in reducing pathogen numbers. When inoculated cheese slices were packaged in 0.10 or 0.13 mm thick PP or PE films, the UV-C reduction levels of *E. coli* O157:H7, *S.*

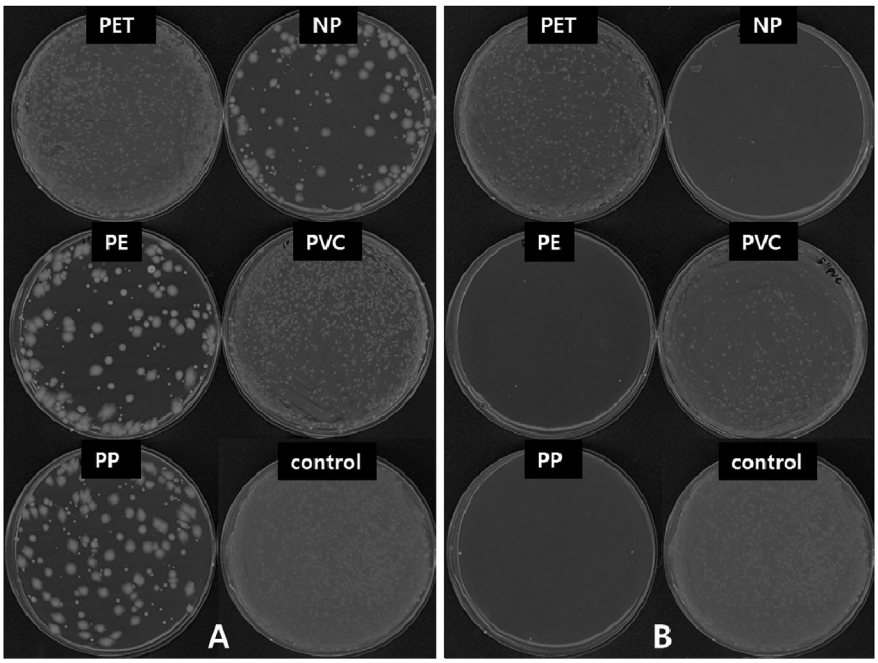


**Fig. 3.** Visual inactivation of pathogens spread-plated onto TSA then covered with each type of film, then treated with UV-C radiation for 1 (A) or 5 min (B). PET; polyethylene terephthalate, PVC; polyvinyl chloride, PE; polyethylene, PP; polypropylene, and NP; non-packaged.

**Table 1**
Populations ($log_{10}$ CFU/g)[a] of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on sliced cheese packaged with different films (PET, PVC, PE, and PP)[b] and treated with 3.04 mW/cm$^2$ of UV-C for 1 min.

| Film type | Population ($log_{10}$ CFU/g) | | |
|---|---|---|---|
| | *E. coli* O157:H7 | *S.* Typhimurium | *L. monocytogenes* |
| Control | 7.20 ± 0.07 A | 7.40 ± 0.08 A | 6.63 ± 0.31 A |
| Non-packaged | 3.75 ± 0.32 B | 3.83 ± 0.46 B | 3.44 ± 0.23 B |
| PET | 7.16 ± 0.06 A | 7.37 ± 0.01 A | 6.61 ± 0.34 A |
| PVC | 7.17 ± 0.15 A | 7.37 ± 0.11 A | 6.51 ± 0.32 A |
| PE | 4.08 ± 0.25 B | 4.14 ± 0.42 B | 3.78 ± 0.37 B |
| PP | 3.84 ± 0.42 B | 4.01 ± 0.50 B | 3.48 ± 0.26 B |

[a] Data represent means ± standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different ($P < 0.05$).
[b] PET; polyethylene terephthalate, PVC; polyvinyl chloride, PE; polyethylene, and PP; polypropylene.

**Table 2**
Populations ($log_{10}$ CFU/g) of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on sliced cheese packaged with different thicknesses (0.07 mm, 0.10 mm, and 0.13 mm) of PP or PE[b] films and treated with 3.04 mW/cm$^2$ of UV-C for 1 min.

| Pathogen | Film thickness | Population ($log_{10}$ CFU/g)[a] | |
|---|---|---|---|
| | | PP | PE |
| *E. coli* O157:H7 | Control | 7.07 ± 0.13 Aa | 7.18 ± 0.07 Aa |
| | Non-packaged | 3.69 ± 0.23 Da | 3.72 ± 0.29 Da |
| | 0.07 mm | 3.87 ± 0.21 C Da | 4.00 ± 0.26 C Da |
| | 0.10 mm | 4.16 ± 0.26 BCa | 4.32 ± 0.17 BCa |
| | 0.13 mm | 4.50 ± 0.10 Ba | 4.62 ± 0.10 Ba |
| *S.* Typhimurium | Control | 7.48 ± 0.04 Aa | 7.43 ± 0.12 Aa |
| | Non-packaged | 3.69 ± 0.24 Da | 3.70 ± 0.25 Ba |
| | 0.07 mm | 3.88 ± 0.09 Da | 3.91 ± 0.45 Ba |
| | 0.10 mm | 4.19 ± 0.23 Ca | 4.39 ± 0.14 Ca |
| | 0.13 mm | 4.56 ± 0.13 Ba | 4.63 ± 0.08 Ca |
| *L. monocytogenes* | Control | 6.31 ± 0.25 Aa | 6.49 ± 0.19 Aa |
| | Non-packaged | 3.45 ± 0.25 Ca | 3.48 ± 0.31 Ca |
| | 0.07 mm | 3.49 ± 0.29 Ca | 3.61 ± 0.30 Ca |
| | 0.10 mm | 3.90 ± 0.19 BCa | 3.87 ± 0.17 BCa |
| | 0.13 mm | 4.11 ± 0.23 Ba | 4.27 ± 0.21 Ba |

[a] Data represent means ± standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different ($P < 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P < 0.05$).
[b] PP; polypropylene, PE; polyethylene.

Typhimurium, and *L. monocytogenes* were slightly decreased compared with those of the non-packaged treated samples or those packaged with 0.07 mm films. The use of 0.13 mm films afforded the lowest level of reduction for the three pathogens, and no significant differences ($P > 0.05$) were observed between non-packaging and 0.07 mm thick PP or PE films. There were no statistically significant differences between the two UV-C-transparent film types (PP and PE).

## 4. Discussion

*E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* have been categorized as high risk microorganisms to the cheese industry (Kousta et al., 2010; Zottola and Smith, 1991), and several studies have shown that the main bacterial sources of cheese contamination can survive outside the product, such as on equipment and storage facilities (Greenwood et al., 1991; Jacquet et al., 1993; Kousta et al., 2010; Linnan et al., 1988; McLauchlin et al., 1990; Temelli et al., 2006). Although foodborne pathogens are inactivated during the pasteurization step, risks can arise from post-processing contamination. Possible sources of post-pasteurization contamination include the product transferring, cutting, and packaging steps in cheese manufacturing, due to the possibility of introducing foodborne pathogens from utensils and through worker handling (Silva et al., 2003). Thus, cheese products without an antimicrobial intervention step after packaging are at risk.

Gamma irradiation is well known for controlling post-processing bacterial contaminations. Lately, several studies have evaluated the effect of gamma irradiation on packaged foodstuffs to reduce levels of foodborne pathogens (Clardy et al., 2002; Mishra

et al., 2006). Foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium, *Staphylococcus aureus*, and *Campylobacter jejuni* were reported to be inactivated by gamma irradiation after packaging in plastic films (Clavero et al., 1994; Thayer and Boyd, 1992; Thayer et al., 1992; Tsiotsias et al., 2002). These studies showed gamma irradiation to be effective for controlling pathogens after packaging. However, not all foods are suitable for gamma irradiation treatment and another major limitation of irradiation processing of food is its negative perception by consumers, due to a perceived association with radioactivity. Furthermore, the high cost of gamma irradiation treatment is a reason why UV-C light treatment could be more attractive to commercial companies. UV-C irradiation is a dry and cold process that can be a simple and effective method requiring very low maintenance and operating cost (Bachmann, 1975; Morgan, 1989).

To date, however, there have been no published reports evaluating the decontaminating effects of UV-C light on foodstuffs packaged in various plastic films. In this study, therefore, we investigated the effect of UV-C light transmittance of representative plastic packaging films and efficacy of UV-C light for controlling pathogens on packaged inoculated cheese samples. The transmission of UV light through plastic film packaging is an important parameter for eliminating or controlling growth of foodborne pathogens on food surfaces. The primary wavelength range of interest in packaging applications for inactivating pathogenic bacteria is UV-C (200–280 nm). In the present study, the transmission spectra of plastic packaging films were collected within the wavelength region of 200–500 nm. PET and PVC films allowed almost no penetration of UV-C light; however, PP and PE afforded ample transmission of UV-C light (Fig. 2). The reason for this is that PET and PVC have UV absorbing properties based on their chemical structures. These materials block the transmission of most wavelengths in the UV range below 370–380 nm (Auras et al., 2004; Raviv and Antignus, 2004). The UV-C penetration capacities of films agree with corresponding results of pathogen reduction. The greater transmission of UV-C light through PP versus PE films was reflected in the reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in packaged inoculated cheese (Table 1), although data showed that the inactivation levels of pathogen between PP- and PE-covered cheese samples were not significantly ($P > 0.05$) different. Also, the degree of reduction decreased significantly ($P < 0.05$) with increasing thickness of packaging film (from 0.07 mm to 0.13 mm) (Table 2). Therefore, by selecting an appropriate packaging film, more than 3 $\log_{10}$ CFU/g reductions could be achieved by UV-C irradiation in sliced cheese products. Additionally, no gross alterations in sensory attributes (color and flavor) of PP- or PE-packaged cheese products were detected after 3.04 mW/cm$^2$ of UV-C treatment for 1 min.

In conclusion, the results of the present study indicate that 0.07 mm thick PP or PE packaging films could be effectively used on sliced cheese products for inactivating major foodborne pathogens, including *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, by UV-C light. The thickness of film used to package commercial sliced cheese products is commonly less than 0.04 mm. Therefore, the proposed film type and thickness (less than 0.07 mm) can be applied widely to develop decontaminating interventions for protecting not only sliced cheese products but also various packaged foodstuffs from post-processing bacterial contaminations.

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