INTRODUCTION

Listeria monocytogenes is a gram-positive, psychrotrophic, and facultative anaerobic pathogen commonly found in poultry farms (Esteban et al., 2008), raw chicken (Gram and Melchior, 1996), and ready-to-eat products (Cox et al., 1989; Bohaychuk et al., 2006; Cabedo et al., 2008). Although Salmonella spp., Campylobacter spp., and Escherichia coli have been recognized as main pathogens present in chicken carcasses, L. monocytogenes received the greatest concern primarily due to its ability to grow under refrigerated temperatures and cause listeriosis with an approximately 20 to 30% fatality rate (Rouquette and Berche, 1996; Jalali and Abedi, 2008). The outbreak of listeriosis can be caused by improper handling of chicken and cross-contamination during skinning, cutting, and boning of broiler carcasses (Alain and Stéphane, 2008). Once contaminated, the pathogen causes problems continuously during processing (especially postthermal processing), transportation, and extended storage (Maragkoudakis et al., 2009; Hoelzer et al., 2012).

To control the pathogen, chemical treatments with chlorine, ethanol, organic acids, ozone, and hydrogen peroxide have been used, among which chlorine is the most widely used in the food industry because of advantages with regard to economics and convenience (Kim et al., 2006; Choi et al., 2009; Chun et al., 2010). Generally, chlorine is added at 45 to 50 ppm during poultry chilling to prevent pathogen growth and cross-contamination (Mead and Thomas, 1973; Izat et al., 1989; James et al., 2006). However, Bautista et al. (1997) found that 50 ppm chlorine could not eliminate L. monocytogenes more than 1 log cfu/g in chicken carcasses. Although chlorine at higher concentrations effectively reduces pathogens, lower levels of the chemical...
are preferred due to health concerns for employees in plants and consumers using the products (Ko et al., 2005; Conner et al., 2001).

Physical intervention methods include UV irradiation, high pressure, pulsed electric fields, and microwaves, among which UV irradiation is one of the most effective means maintaining sensory qualities such as color and flavor, nutrients, and pH (Chun et al., 2009; Begum et al., 2009). In fresh poultry, UV light (200–300 nm) has been reported to have germicidal and lethal properties on pathogens in meat and poultry skins (Stermer et al., 1987; Wallner-Pendleton et al., 1994; Sumner et al., 1996).

Predictive microbiology has been used for many purposes such as identifying growth or survival conditions for pathogens, assessing the risk of human exposure, and developing mathematical models to predict pathogen growths (Ross et al., 2000). To determine both shelf life and food safety improvements, mathematical models have been developed to predict L. monocytogenes growth as a function of important environmental factors such as sodium chloride, pH, and water activity (Buchanan and Phillips, 1990; Cole et al., 1990; McChure et al., 1997; Cheroutre-Vialette et al., 1998; Le Marc et al., 2002; Carrasco et al., 2006; Jin et al., 2006). It is generally accepted that microbial growth can be affected by postharvest processing, chlorine washing, and packaging (Beuchat and Brackett, 1991; Delaquís et al., 2002).

Therefore, the objective of this study was to examine both single and combined effects of chlorine and UV on L. monocytogenes growth in chicken breast as well as to develop predictive models as a function of chlorine concentration, UV, and storage temperature.

**MATERIALS AND METHODS**

**L. monocytogenes Strains and Preparation of the Cocktail**

Three strains of L. monocytogenes (serotype 4c, ATCC 19116; serotype 4e, ATCC 19118 isolated from chicken; and serotype 1/2a, ATCC 19111 isolated from poultry) were combined in a cocktail for this study. The cocktail was used to determine the growth characteristics of L. monocytogenes and to develop predictive models for its growth on chicken breast. Each strain was transferred from a stock culture at −70°C in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) containing 15% glycerol to 10 mL of TSB and incubated at 30°C for 24 h. One hundred microliters of cell suspension of each strain was then separately transferred to 10 mL of TSB and incubated at 30°C for 18 h. One milliliter of the cell suspension from each strain was combined and the resulting cocktail was harvested by centrifugation at 4.651 × g for 10 min at 4°C. The cell pellet was resuspended and diluted in 0.1% peptone water (PW) to approximately 6 log cfu/mL.

**Preparation of Chicken Samples and L. monocytogenes Inoculation**

We purchased the wrapped cut of raw skinless chicken breast meats from local market in Ansung, Korea. The samples (approximately 9 kg in total) in an ice box were transported from the market to the laboratory in 30 min and then were immediately cut into 10-g pieces where 100 µL of culture suspension was inoculated using a spot-inoculation method to obtain a final concentration of 4 log cfu/g. After inoculation, these samples were placed in a clean bench at room temperature (20 ± 2°C) for 1 h for the inoculum to absorb.

**Chlorine and UV Treatments**

Following inoculation, chicken breast was completely immersed in sterile distilled water (control) or chlorine (50, 100, and 200 mg/kg) for 5 min at room temperature (20 ± 2°C). Chlorine in the form of sodium hypochlorite (NaOCl, 12%, Shimadzu Co., Kyoto, Japan) was prepared using sterile distilled water due to its popularity in various food processing plants. After 10 min draining, the chlorine-treated samples were irradiated with UV in a fixed dose of 300 mW-s/cm², using a bench-scale collimated beam UV reactor that was equipped with 10-, 15-, and 30-W low-pressure UV lamps (Sankyo UV Co., Ltd., Seoul, Korea) emitting monochromatic UV radiation at 260 nm. The UV irradiation dose, having a constant intensity after 30-min warming, was adjusted accurately by exposing the meat to light for required times. After the exposure, the applied UV dose was calculated with the time (seconds) multiplied by irradiance (W/cm²). Finally, those samples were placed individually into stomaching bags (Nasco Whirl-Pak, Janesville, WI).

**Synergistic Effect**

To assess any synergistic effects, the efficacy of L. monocytogenes inactivation in chicken breast was compared after chlorine and UV treatments either singly or jointly using the procedure described by Koivunen and Heinonen-Tanski (2005). The combined application was carried out by chlorine dip as a primary disinfectant and UV exposure as a secondary disinfectant, from which synergistic effects were calculated using the following equation:

\[
\text{synergistic effect value} = A - (B + C),
\]

where A is the reduction of L. monocytogenes population from chlorine/UV combination, and B and C are the reductions from chlorine and UV alone.

**Microbial Analysis**

Breast samples (10 g) were diluted with 90 mL of sterile 0.1% PW, homogenized in a stomacher (Bag
Mixing N0 is the log of the initial cell number, T is the incubation time, and C is the difference between the initial and final cell numbers. Measured values were used to calculate lag time (lag) and maximum specific growth rate (µmax).

To evaluate the effect of temperature, chlorine, and UV on the growth of *L. monocytogenes* in chicken breast, a secondary model for SGR and LT based on the reparameterized Gompertz equation was developed using PROC GLM in SAS version 9.2 to fit the polynomial model. The following equation was used:

\[
Y = N_0 + C \times \exp\left\{ \exp\left[ (2.718 \times \mu_{\text{max}}/C) \times (\text{lag} - T) + 1 \right] \right\},
\]

where *Y* is the log of total cell number after incubation, *N0* is the log of the initial cell number, *T* is the incubation time, and *C* is the difference between the initial and final cell numbers. Measured values were used to calculate lag time (lag) and maximum specific growth rate (µmax).

To evaluate the effect of temperature, chlorine, and UV on the growth of *L. monocytogenes* in chicken breast, a secondary model for SGR and LT based on the reparameterized Gompertz model was developed using PROC GLM in SAS version 9.2 to fit the polynomial model. The following equation was used:

\[
Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_1 x_2 + b_4 x_1^2 + b_5 x_2^2,
\]

where *Y* is the predicted value (SGR or LT), with the following values of storage temperature (*x1*), concentration of chlorine (*x2*), intercept (*b0*), linear coefficients (*b1* and *b2*), interaction coefficient (*b3*), and squared coefficients (*b4* and *b5*; Gibson et al., 1988).

**Predictive Model**

Resulting data were applied to a primary model based on the reparameterized Gompertz equation (Zwietering et al., 1990) to estimate values for primary parameters, including specific growth rates (SGR: log cfu/h) and lag times (LT: h), using GraphPad Software (Prism, version 4.0, San Diego, CA). The reparameterized Gompertz equation was used as follows:

\[
Y = b_{0} + b_{1} x_{1} + b_{2} x_{2} + b_{3} x_{1} x_{2} + b_{4} x_{1}^{2} + b_{5} x_{2}^{2},
\]

where *Y* is the log of total cell number after incubation, *N0* is the log of the initial cell number, *T* is the incubation time, and *C* is the difference between the initial and final cell numbers. Measured values were used to calculate lag time (lag) and maximum specific growth rate (µmax).

Quality Measurement (Color and Texture)

After chlorine and UV treatments, the color of meat surface was measured for *L* (lightness), *a* (redness), and *b* (yellowness), using a Color Difference Meter (UltraScan PRO, HunterLab, Reston, VA). The mean of 6 measurements was recorded for each sample. For texture analysis, the core of each sample (1.5 cm in diameter and 1 cm thick) was compressed twice to 35% of its original thickness using a Stable Micro Systems Texture Analyzer (TA-XT Express, Stable Micro Systems Ltd., Surrey, UK). A probe (SMSP/35) was moved to the sample at a speed of 1.0 mm/s with a trigger force of 5.0 g. Texture was analyzed using Exponent Lite Express (version 4, 0, 8, 0 for the XT Express Lite) and the following parameters were recorded: hardness (kg) for maximum force required to compress the sample, cohesiveness (%) for the extent to which the sample could be deformed before rupture, and springiness (cm) for the ability of the sample to recover its original form after the deforming force. Color and textural properties of chicken breasts were analyzed initially and after 7 d of storage at 4°C.

Statistical Analysis

The experiment was repeated twice and *L. monocytogenes* numbers counted on duplication for each treatment were averaged as cfu per gram, and those average values were used in statistical analysis. A Statistical Analysis System software version 9.2 (SAS Institute, Cary, NC) was used to perform ANOVA of the effects. Average values and significance (*P < 0.05*) were analyzed using Duncan’s multiple range test.

**RESULTS AND DISCUSSION**

Single and Combined Effects of Chlorine and UV Against the Growth of *L. monocytogenes* in Chicken Breast

A step-wise reduction (from 3.85 to 3.63 log cfu/g) of *L. monocytogenes* population was observed in chicken...
breast when increased chlorine concentration from 0 to 200 mg/kg, with the best reduction seen at 200 mg/kg (Table 1). The pathogen population was further reduced to even lower levels (3.05–3.24 log cfu/g) when UV at 300 mW·s/cm² was additionally exposed after the chlorine dip, regardless of chlorine concentration.

Goncalves et al. (2005) reported that L. monocytogenes population was reduced by approximately 0.12 log most probable number /g when 45 ppm chlorine was used. Tsai et al. (1992) also stated that 40 ppm chlorine reduced L. monocytogenes in poultry by 37 to 50% in 3 to 5 min. In comparison of UV and chlorine treatments, combined treatments over the single treatment of either chlorine or UV had a greater inhibitory effect on L. monocytogenes growth. With UV treatment at 300 mW·s/cm², L. monocytogenes population was reduced by approximately 0.27 log cfu/g. Chun et al. (2009) reported that UV-C (254 nm) irradiation at 8,000 mW/cm² reduced L. monocytogenes in ready-to-eat sliced ham by 2.74 log cfu/g. In chicken breast, Chun et al. (2010) showed that UV-C (254 nm) irradiation at 5,000 mW/cm² reduced L. monocytogenes by 1.29 log cfu/g. Lyon et al. (2007) also indicated that approximately 2 log cfu/g reduction of L. monocytogenes was observed on broiler breast fillets in UV at 1,000 µW/cm² for 5 min. Compared with our result of UV treatment, the study of Lyon et al. (2007) had greater effect on reduction of L. monocytogenes. Generally, the various food structures and intrinsic factors of foods such as protein, food additives, antioxidants, and preservatives protect attached bacterial cells from the action of disinfectants (Gram and Melchiorson, 1996). Also, the survival state and attached time of bacteria on food surfaces during transportation and storage of products could affect the reduction effect of bacteria through the control methods. In our study, the combination of chlorine at 50 to 200 mg/kg and UV at 300 mW·s/cm² reduced L. monocytogenes numbers by approximately 0.61 to 0.80 log cfu/g, with a maximum reduction at 200 mg/kg of chlorine and the greatest synergistic effect at 100 mg/kg of chlorine.

The synergistic effect from the combined treatment is expected by 2 different intervention mechanisms: 1) chlorine-driven cell damage as a nonselective oxidant and 2) UV-driven DNA damage (Sastry et al., 2000). Sodium hypochloride (NaOCl) is converted to un-ionized hypochlorous acid (HOCl) in a hydrolysis reaction (NaOCl + H₂O → HOCl + NaOH⁻), which is a more effective antimicrobial component than its dissociated ion of hypochlorite (OCl⁻) at >pH 9.0 (Gavin and Weddig, 1995; Fukuzaki, 2006). Moreover, UV irradiation disrupts hydrogen bonds between adenine and guanine, blocks DNA transcription and replication, and eventually causes cell death (Sastry et al., 2000; Unlurturk et al., 2008). Sastry et al. (2000) stated that several factors can affect the sensitivity of pathogens to UV such as surface topography and transmissivity of foods, power and wavelength of UV, and bacterial species. Previously, our research team also found that ethanol and UV combination achieved a greater reduction on pathogens than any individual treatment (Ha and Ha, 2010). Based on the synergistic effects, the combination of chlorine and UV appears to provide a viable method to reduce chlorine concentration with no reducing the effect of L. monocytogenes inhibition in chicken.

**Development and Validation of the Predictive Growth Model**

The reparameterized Gompertz equation (Zwietering et al., 1990) was used to fit the growth data and to obtain the SGR (log cfu/h) and LT (h) of L. monocytogenes at each of 3 chlorine levels (50, 100, and 200 mg/kg) with/without UV at 300 mW·s/cm² at 3 different storage temperatures (4, 10, and 15°C). The SGR and LT values (Table 2) fit very well (R² > 0.91) for all experimental combinations. The observed SGR values were 0.008 to 0.056 at 4°C, 0.037 to 0.062 at 10°C, and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Population (log cfu/g)</th>
<th>Synergistic effect value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (mg/kg)</td>
<td>UV (mW·s/cm²)</td>
<td>0 (control)</td>
</tr>
<tr>
<td>0 (control)</td>
<td>3.85 ± 0.14a</td>
<td>3.82 ± 0.08a</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>3.58 ± 0.05b</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>3.24 ± 0.01c</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>3.11 ± 0.15c</td>
</tr>
</tbody>
</table>

*Means within a column with no common superscripts are different (P < 0.05).
1Values are mean ± SD.
2Synergistic effect values = (reduction achieved with the chlorine treatment and the UV treatment) – (reduction achieved by chlorine + UV treatment).
3Treated with sterile distilled water.
increased chlorine concentrations with UV. Sheen et al. (2011) determined both SGR and LT of *L. monocytogenes* after exposing ready-to-eat ham to 0, 25, and 50 ppm chlorine at 4, 8, and 16°C. Results indicated that lag times at 16°C were 1.2, 2.6, and 4.0 d at 0, 25, and 50 ppm chlorine, respectively. In our study, the best *L. monocytogenes* inhibition was shown from the chlorine/UV combination although temperature, chlorine, and UV exerted positive effects on the inhibition.

Nonlinear regression analysis using a polynomial model was applied to the SGR and LT values obtained from primary modeling. The equation developed to model the effect of temperature ($x_1$) and chlorine ($x_2$) with UV radiation at 300 mW·s/cm$^2$ on the SGR of *L. monocytogenes* was as follows:

$$Y = -0.0186 + 0.0077 \times x_1 + 0.00001 \times x_2 - 0.00005 \times x_1 x_2 - 0.00002 \times x_1^2 - 0.0000001 \times x_2^2.$$  

The equation developed for LT was as follows:

$$Y = 53.41 - 1.774 \times x_1 + 0.021 \times x_2 - 0.0007 \times x_1 x_2 - 0.115 \times x_1^2 - 0.0002 \times x_2^2.$$  

Figure 1A and 1B show a comparison of observed and predicted SGR and LT values of *Listeria monocytogenes* growth on chicken breast. The $R^2$ values for SGR and LT in the secondary model were 0.988 and 0.984, respectively, indicating a better model for the data. As we know, the inhibitory effect of temperature against *L. monocytogenes* was the highest according to the result of Giffel and Zwietering (1999).

Four additional experimental conditions were selected to validate the secondary model. The observed and predicted values are compared in Figure 1. Most plotted points were close to the line, indicating that the predicted values were similar to the observed values. The MSE, $B_0$, and $A_0$ were calculated to evaluate the reliability of the predictive model developed in our study for *L. monocytogenes*. Generally, the lower the MSE value, the better the predictive model (Adair et al., 1989). The $B_0$ indicates the average bias of predictions to verify the performance of the predictive model.

### Table 2. Specific growth rate and lag time values of *Listeria monocytogenes* on chicken breast meat under different experimental conditions

<table>
<thead>
<tr>
<th>Chlorine (mg/kg)</th>
<th>UV (mW·s/cm$^2$)</th>
<th>Specific growth rate (log cfu/h)</th>
<th>Lag time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4°C</td>
<td>10°C</td>
</tr>
<tr>
<td>0</td>
<td>0 (control)</td>
<td>0.056</td>
<td>0.062</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0.051</td>
<td>0.061</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0.038</td>
<td>0.058</td>
</tr>
<tr>
<td>200</td>
<td>0</td>
<td>0.036</td>
<td>0.048</td>
</tr>
<tr>
<td>0</td>
<td>300</td>
<td>0.010</td>
<td>0.060</td>
</tr>
<tr>
<td>50</td>
<td>300</td>
<td>0.010</td>
<td>0.060</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>0.009</td>
<td>0.049</td>
</tr>
<tr>
<td>200</td>
<td>300</td>
<td>0.008</td>
<td>0.037</td>
</tr>
</tbody>
</table>

1Treated with sterile distilled water.
and $A_f$ indicates the average estimation accuracy of the predictive model. Based on the model-dependent data, $MSE$, $B_f$, and $A_f$ for SGR were 0.00001, 1.02, and 1.12, respectively. When $A_f = B_f = 1$, the model fits perfectly. When $A_f = 0$, the model fits perfectly.

In additional experimental data, $MSE$, $B_f$, and $A_f$ for SGR were 0.0003, 0.96, and 1.11, respectively. Values are mean ± SD. Within a column at each storage day are not different ($P > 0.05$).

### Table 3. Color and textural properties of chicken breast meat treated with chlorine and UV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorine (mg/kg)</th>
<th>UV (mW·s/cm²)</th>
<th>L*²</th>
<th>a*²</th>
<th>b*²</th>
<th>Hardness (kg)</th>
<th>Cohesiveness (%)</th>
<th>Springiness (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0 0 (control³)</td>
<td>66.0 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>9.8 ± 0.5</td>
<td>2.64 ± 1.1</td>
<td>0.58 ± 0.15</td>
<td>0.75 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 300</td>
<td>65.6 ± 0.5</td>
<td>2.9 ± 0.5</td>
<td>9.4 ± 1.6</td>
<td>2.64 ± 0.4</td>
<td>0.63 ± 0.06</td>
<td>0.82 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 300</td>
<td>66.2 ± 0.5</td>
<td>2.7 ± 0.8</td>
<td>10.8 ± 0.6</td>
<td>2.55 ± 0.2</td>
<td>0.62 ± 0.04</td>
<td>0.81 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 300</td>
<td>65.6 ± 1.4</td>
<td>2.4 ± 0.0</td>
<td>10.3 ± 1.6</td>
<td>2.51 ± 0.2</td>
<td>0.63 ± 0.02</td>
<td>0.78 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 0 0 (control³)</td>
<td>62.2 ± 0.4</td>
<td>4.6 ± 0.1</td>
<td>11.5 ± 2.2</td>
<td>1.97 ± 0.1</td>
<td>0.60 ± 0.12</td>
<td>0.82 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 300</td>
<td>62.1 ± 0.2</td>
<td>4.5 ± 0.9</td>
<td>12.6 ± 0.1</td>
<td>1.96 ± 0.3</td>
<td>0.62 ± 0.06</td>
<td>0.84 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 300</td>
<td>63.7 ± 0.4</td>
<td>4.4 ± 0.1</td>
<td>13.1 ± 0.7</td>
<td>1.91 ± 0.1</td>
<td>0.63 ± 0.14</td>
<td>0.80 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 300</td>
<td>63.9 ± 0.0</td>
<td>4.8 ± 0.1</td>
<td>11.2 ± 0.1</td>
<td>1.97 ± 1.0</td>
<td>0.61 ± 0.09</td>
<td>0.77 ± 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Values are mean ± SD. Means within a column at each storage day are not different ($P > 0.05$).
2$L^*$ = lightness; $a^*$ = redness; $b^*$ = yellowness.
3Treated with sterile distilled water.

Effect of Chlorine and UV on the Quality of Chicken Breast Meat

Table 3 shows the results of color and texture analyses on chicken breasts. On the initial day, no significant differences ($P > 0.05$) were seen in color and texture values. After 7 d of storage at 4°C, all treated samples were darker and more red and yellow than the initial samples. Petracci and Fletcher (2002) and Lyon et al. (2007) found that chicken breast fillets became more yellow ($b^*$ value) and less red ($a^*$ value) after 7 d of storage at 4°C. However, $L^*$, $a^*$, and $b^*$ values in our study were not significantly different ($P > 0.05$) between the treated and control samples regardless of treatment and storage. In texture analysis, no significant differences were found for hardness, cohesiveness, and springiness between the treated and control samples ($P > 0.05$), although hardness on 7 d samples was numerically lower. Overall, chlorine and UV treatments did not affect both color and textural properties. Dixon and Pooley (1961) reported that meat flavor could change after chlorine exposure at 200 ppm for 10 min. High concentration of chlorine in the chilling water results in a strong chlorine odor and an unacceptable appearance (Teotia and Miller, 1975; Izat et al., 1989). Our analysis for quality showed that the combined treatment (chlorine at 50–200 mg/kg and UV at 300 mW·s/cm²) in chicken breast does not change the meat color or texture after 7 d of storage.

We investigated the inhibitory effect of chlorine/UV treatment on $L. monocytogenes$ in chicken breast and developed a predictive growth model as a function of chlorine concentration, storage temperature, and fixed UV radiation. We also analyzed the color and texture properties of chicken breasts treated with chlorine and UV. The combined treatment with chlorine at 100 mg/kg and UV at 300 mW·s/cm² significantly reduced $L. monocytogenes$ growth over the single or nontreated control with no negative effect on visual and textural quality. Also, our secondary model was in good agreement with the validation, and it can be used to predict $L. monocytogenes$ growth on chicken breast.

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