RESPONSE SURFACE MODELING OF LISTERIA MONOCYTOGENES INACTIVATION ON LETTUCE TREATED WITH ELECTROLYZED OXIDIZING WATER

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ABSTRACT

Electrolyzed oxidizing water has been estimated that it has strong bactericidal activity and has been widely used as a disinfectant for inactivating microbial organisms. The combined effects of temperature (15–35°C), chlorine concentration of electrolyzed oxidizing water (30–70 ppm) and treatment time (1–5 min) on the reduction of Listeria monocytogenes in lettuce were investigated. Reductions of 1.39–2.79 log10 cfu/g were observed in different combinations of the three factors. Also, a quadratic equation for L. monocytogenes inactivation kinetic was developed by multiple regression analysis using response surface methodology. The predicted values were shown to be significantly in good agreement with experimental values because the adjusted determination coefficient ($R^2_{Adj}$) was 0.9578 and the level of significance was $P < 0.0001$. Besides, average mean deviation (E%), bias factor (Bf) and accuracy factor (Af), which are validation indicators of the model were 0.0218, 1.0003 and 1.0220, respectively. Thus, predicted model showed a good correlation between the experimental and predicted values, indicating success at providing reliable predictions of L. monocytogenes growth in lettuce.

PRACTICAL APPLICATIONS

Electrolyzed oxidizing water is an important sanitizer, and nowadays it has been widely used in food industry. Lettuce is regarded as a “healthier”

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food, which is one of the most popular vegetables consumed, whereas many outbreaks caused by \textit{L. monocytogenes} have been reported until now. According to the model developed in this study, inactivation of \textit{L. monocytogenes} in lettuce treated with EO water could be predicted by inputting a certain group of environmental factors.

**INTRODUCTION**

Fresh fruit and vegetables are an essential component of a healthy diet (Abadias \textit{et al.} 2008), which can decrease the risk of cardiovascular diseases and cancer (Allende \textit{et al.} 2006). As a result, the demand for fresh fruit and vegetables has continued to grow (Liu \textit{et al.} 2000). However, the foodborne illnesses caused by consumption of fresh produce have significantly increased (Mannoud and Linton 2008). As lettuce is one of the most popular vegetables, a number of foodborne disease outbreaks with consuming lettuce have been reported previously (Beuchat 1990; Brackett 1999; Corbo \textit{et al.} 2006). Most outbreaks were resulted from bacteria, especially \textit{Escherichia coli} O157:H7, \textit{Listeria monocytogenes} and \textit{Salmonella} spp. (Ukuku and Fett 2004; Ukuku \textit{et al.} 2004). Among them, \textit{L. monocytogenes} was regarded as an important opportunistic human foodborne because \textit{L. monocytogenes} can cause serious diseases, such as septicemia, meningitis, meningoencephalitis in immunocompromised individuals, newborns and the elderly, and abortion and stillbirth in pregnant women (Vazquez-Boland \textit{et al.} 2001).

Washing produce with sanitizing solutions is the only step in the fresh-cut produce production chain where a reduction in spoilage microorganisms and potential pathogens can be achieved (Allende \textit{et al.} 2004, 2008). So far, several sanitizers such as ozone (Yuk \textit{et al.} 2007), nisin (Economou \textit{et al.} 2009), some organic acids such as acetic, citric and lactic acid (George \textit{et al.} 1996; Sommers \textit{et al.} 2003), diacetate, lactate (Mbandi and Shelef 2002), trisodium phosphate solution (Somers \textit{et al.} 1994) and electrolyzed water (Izumi 1999; Ozer and Demirci 2006) have been investigated to eliminate pathogens from food. Compared with the traditional disinfectants, electrolyzed water has the potential to be more cost-effective and it is also less dangerous and less expensive (Ozer and Demirci 2006). Up to now, EO water has been widely used as a sanitizing agent in both research and practice. There are a large number of published literatures with regard to using EO water to decontaminate microflora or kinds of pathogens in lettuce (Izumi 1999; Park \textit{et al.} 2001), which reported that EO water has strong antimicrobial effects on many foodborne pathogens including \textit{L. monocytogenes}. However, few works have been done to determine the combined effects of chlorine concentration of EO water, temperature and treatment time on inactivation of \textit{L. monocytogenes}. This
study aims to develop a response surface model to describe the reduction kinetic of *L. monocytogenes* as a function of those factors.

In this research, response surface methodology (RSM) is used to analyze experimental data and develop predictive model, which is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response (Ju *et al.* 2007). RSM is preferred in the work because relatively few experimental combinations of the variables are adequate to estimate potentially complex response function.

The objectives of the present study were: (1) to evaluate the antimicrobial capability of EO water with different chlorine concentrations on inactivation of artificially inoculated lettuce with *L. monocytogenes* under diverse conditions; and (2) to establish predictive model to describe the effect of chlorine concentration of EO water, temperature and treatment time on the survival of *L. monocytogenes* in inoculated lettuce.

**MATERIALS AND METHODS**

**Cultures and Cell Suspension**

Three strains of *Listeria monocytogenes* used in this study were Scott A and ATCC 19116, ATCC 19118, which obtained from the Department of Food Science, University of Georgia (Griffin, GA). Stock cultures were stored at −70°C in tryptic soy broth (TSB; Difco, Sparks, MD) with a 0.6% yeast extract (YE; Difco) containing 20% glycerol. Each bacterial strain was individually cultured in 10 mL TSBYE, and then incubated at 35°C for 24 h. Each strain was transferred three times to TSB by loop inoculate at successive 24 h intervals. Following the incubation, the cells were harvested by centrifugation for 15 min at 3,500 × g and washed twice with sterile 0.1% (w/v) buffered peptone water (Difco), equal volumes of cell suspensions of three strains were combined to make the inoculum containing approximately equal populations of cells of each strain of *L. monocytogenes* (10⁹ colony-forming units [cfu]/mL).

**Inoculation of Lettuce**

Lettuce (*Lactuca Sativa var. capita*) was purchased from a local supermarket in Chunchon, Korea, and then immediately transported to laboratory and stored in 4°C refrigerator as soon as possible. Discolored, wilted and damaged portions were discarded and the remaining leaves were weighed every 10 g for one group and every group of leaves was inoculated by pipetting 0.1 mL of ~10⁹ cfu/mL *L. monocytogenes* suspension on to the surface of each leave, and then left the leaves in the clean bench for three hours until
completely dry. This procedure resulted in initial pathogen inoculum levels of approximately 7.0 \log_{10} \text{ cfu/g} of lettuce.

**Preparation of Treatment Solutions**

The electrolyzed oxidizing water was produced by EO generator (A2-1000, Korean E & S Fist Inc, Seoul, Korea). A continuous supply of softened tap water and 12% sodium chloride solution at room temperature was pumped into this equipment. The amperage of EO generator was set at 8, 12 and 16 A, respectively. When stable amperage was reached after 15 min, EO water with different chlorine concentration (30, 50 and 70 ppm), pH values (2.3–2.7) and oxidation-reduction potential of (1,110 mV–1,200 mV) were collected. A pH/ORP meter (YK-2001PH, Lutron, Taipei, Taiwan) was employed to determine the pH and ORP of the EO water. Free chlorine concentrations were determined by chlorine meter (RC-3F, KRK, Kuki City, Japan). The solutions were placed into a water bath to make sure to keep the preconcerted treatment temperatures (15, 25 and 35°C). Considering the electrolyzed water is not stable enough, immediate experiments were done as soon as experimental environments set.

**Methodology and Design of Experiments**

Inoculated lettuce samples (10 g) were immersed in 100 mL EO water solutions under different preconcerted conditions. Sequentially, all samples were aseptically drained on absorbent paper for drying and then immediately placed in a stomacher bag (Nasco Whirl-Pak, Janesville, WI). Each 10-g sample of treated lettuce was mixed with 90 mL of 0.1% sterile peptone water and then homogenised for 2 min in a stomacher (Lab-blender 400, Seward, London, UK). After homogenization, 1-mL aliquots of the sample were serially diluted in 9 mL of sterile buffered peptone water and 0.1 mL of sample suspensions was spread-plated onto Oxford Medium Base (Difco) supplemented with 10 mL rehydrated modified Oxford Antimicrobial supplement (Difco).

Three factors in this study including temperature, chlorine concentration and treatment time were chosen as main variables and designed as X₁, X₂ and X₃, respectively. The low, middle and high levers of each variable were designated as −1, 0 and +1, respectively, and are shown in Table 1. The variables were coded according to the equation (Eq. 1) as follows (Gao and Ju 2007):

$$x_i = \frac{(X_i - X_0)}{\Delta X}$$  \hspace{1cm} (1)

where $x_i$ is the (dimensionless) coded value of the variable $X_i$, $X_0$ is the value of $X_i$ at the centre point, and $\Delta X$ is the step change. Table 2 shows the actual design of experiments.
Model Development and Validation

Polynomial regression equation was developed to describe the effects of treatments on the *L. monocytogenes* inoculated lettuce. Hence, the general form of the quadratic polynomial model regression equation employed in this study is shown in Eq. (2).

\[ Y = B_0 + \sum_{i=1}^{n} B_i x_i + \sum_{i<j}^{n} B_{ij} x_i x_j + \sum_{i=1}^{n} B_{ii} x_i^2 \]  

(2)

* x₁ = (X₁ - 25)/10; x₂ = (X₂ - 250)/20; x₃ = (X₃ - 23)/2.

**TABLE 1.**
CODE AND LEVEL OF VARIABLES CHOSEN FOR THE TRIALS

<table>
<thead>
<tr>
<th>Factor</th>
<th>Symbols</th>
<th>Level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (C)</td>
<td>x₁</td>
<td>15 25 35</td>
</tr>
<tr>
<td>Chlorine concentration (ppm)</td>
<td>x₂</td>
<td>10 30 50</td>
</tr>
<tr>
<td>Treatment time (min)</td>
<td>x₃</td>
<td>1 3 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factor Symbols Level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coded Uncoded</td>
</tr>
<tr>
<td>x₁ X₁ 15 25 35</td>
</tr>
<tr>
<td>x₂ X₂ 10 30 50</td>
</tr>
<tr>
<td>x₃ X₃ 1 3 5</td>
</tr>
</tbody>
</table>

**TABLE 2.**
BOX–BEHNKEN RESPONSE SURFACE DESIGN ARRANGEMENT AND LOG REDUCTION OF *LISTERIA MONOCYTOGENES* IN LETTUCE

<table>
<thead>
<tr>
<th>Trial</th>
<th>Temperature (C)</th>
<th>Chlorine concentration (ppm)</th>
<th>Treatment time (min)</th>
<th>Log reduction* (log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−1</td>
<td>−1</td>
<td>0</td>
<td>1.39</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>−1</td>
<td>0</td>
<td>1.77</td>
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<td>0</td>
<td>2.01</td>
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<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2.79</td>
</tr>
<tr>
<td>5</td>
<td>−1</td>
<td>0</td>
<td>−1</td>
<td>1.88</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>−1</td>
<td>2.17</td>
</tr>
<tr>
<td>7</td>
<td>−1</td>
<td>0</td>
<td>1</td>
<td>2.04</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2.58</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>−1</td>
<td>−1</td>
<td>1.52</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1</td>
<td>−1</td>
<td>2.53</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>−1</td>
<td>1</td>
<td>1.71</td>
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<tr>
<td>12</td>
<td>0</td>
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<td>1</td>
<td>2.73</td>
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<td>0</td>
<td>2.21</td>
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<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.25</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.18</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.27</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.31</td>
</tr>
</tbody>
</table>

* The initial population of *L. monocytogenes* inoculated on lettuce is 6.67 log₁₀ CFU/g.

**Model Development and Validation**

Polynomial regression equation was developed to describe the effects of treatments on the *L. monocytogenes* inoculated lettuce. Hence, the general form of the quadratic polynomial model regression equation employed in this study is shown in Eq. (2).
where \( Y \) is predicted response. It can be observed that, in the present study, three variables are involved; hence, \( n \) takes the value 3. Thus, by substituting the value 3 for \( n \), Eq. (2) becomes (Gao and Ju 2007):

\[
Y = B_0 + B_{11}x_1 + B_{22}x_2 + B_{33}x_3 + B_{12}x_1x_2 + B_{13}x_1x_3
+ B_{23}x_2x_3 + B_{11}x_1^2 + B_{22}x_2^2 + B_{33}x_3^2
\]

(3)

where \( x_1, x_2 \) and \( x_3 \) are input variables (viz., temperature, chlorine concentration and treatment time); \( B_0 \) is a constant; \( B_1, B_2 \) and \( B_3 \) are linear coefficients; \( B_{12}, B_{13}, B_{23} \) are cross-product coefficients; \( B_{11}, B_{22}, B_{33} \) are quadratic coefficients; and \( Y \) is predicted response.

The criterion used to characterize the fitting efficiency of the data to the model was the multiple correlation coefficients (\( R^2 \)) and their average mean deviation (Patras et al. 2009) (\( E\% \)):

\[
E\% = \frac{1}{n_e} \sum_{i=1}^{n} \left( \frac{\mu_{\text{observed}} - \mu_{\text{predicted}}}{\mu_{\text{observed}}} \right) \times 100
\]

(4)

The mathematical predictive model assessments were carried out by calculating the model performance indices, bias factor (\( B_f \)) and accuracy factor (\( A_f \)) (Baranyi et al. 1999; Ross 1996).

\[
B_f = 10^{\left( \frac{\sum_{i=1}^{n} \log(\mu_{\text{observed}}/\mu_{\text{predicted}}) }{n} \right)}
\]

(5)

\[
A_f = 10^{\left( \frac{\sum_{i=1}^{n} \log(\mu_{\text{predicted}}/\mu_{\text{observed}}) }{n} \right)}
\]

(6)

where \( n \) is the number of observations; \( \mu_{\text{predicted}} \) is the predicted values obtained from the developed polynomial model; and \( \mu_{\text{observed}} \) is the observed values obtained from the experiment.

**Statistical Analysis**

Bacteria numbers were expressed as log colony forming units per gram (log\(_{10} \) cfu/g) for statistic analysis. Design Expert package (Version 7.1.6, Stat Ease Inc., Minneapolis, MN, 2008) was used for regression analysis of the data obtained and to estimate the coefficients of the regression equation. The fit of the regression model attained was checked by the adjusted coefficient of determination (\( R^2_{\text{Adj}} \)). The statistical significance of the model was determined by the application of Fischer’s \( F \)-test.
RESULTS AND DISCUSSION

The experimental data under different conditions containing combinations of temperature, chlorine concentration of EO water and treatment time using Box–Behnken response surface design were summarized in Table 2. Reductions of $1.39–2.79 \log_{10} \text{cfu/g}$ of *L. monocytogenes* in lettuce were observed in different trials of experiments, and results showed $2.24 \pm 0.02$ log reductions from the experiment at the central point (temperature: $25^\circ\text{C}$; chlorine concentration: $50 \text{ ppm}$; treatment time: $3 \text{ min}$). Similar results from Guentzel *et al.* (2008), who found nearly $2.53$ log reductions for *L. monocytogenes* in lettuce treated with $50 \text{ ppm}$ EO water for $10 \text{ min}$ were reported. However, Fabrizio and Cutter (2005) found approximately $1.5$ log reductions for *L. monocytogenes* in meat at $50 \text{ ppm}$ EO water for $15 \text{ min}$, whereas only $0.92$ log reductions for *L. monocytogenes* were observed in salmon fillets treated with more than $50 \text{ ppm}$ EO water for $3 \text{ min}$ in the study of Ozer and Demirci (2006).

Regression Model Development for the Reductions of *L. monocytogenes*

Table 3 is a summary of the analysis of variance (ANOVA). Results showed that $R^2$ and $R^2_{\text{Adj}}$ was $0.9815$ and $0.9578$, respectively. High $R^2$ value demonstrated a better prediction by the model. The value of the $R^2_{\text{Adj}}$ indicated a high degree of correlation between the observed and predicted values, which suggested that only $4.22\%$ of the total variation can not be explained by the current model.

Statistical testing of the model was done in the form of ANOVA, which was required to test the significance and adequacy of the model. $F$ value

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>Prob* &gt; $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>2.50</td>
<td>9</td>
<td>0.28</td>
<td>41.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0.047</td>
<td>7</td>
<td>6.71E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>0.037</td>
<td>3</td>
<td>0.012</td>
<td>4.74</td>
<td>0.0835</td>
</tr>
<tr>
<td>Pure error</td>
<td>0.010</td>
<td>4</td>
<td>2.58E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>2.54</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R^2 = 0.9815\dagger$, $R^2_{\text{Adj}} = 0.9578\ddagger$

* Probability value.
\dagger Correlation coefficient.
\ddagger Adjusted determination coefficient.

df, degree of freedom.
and probability value ($P < 0.0001$) indicated that the differences between different treatments are highly significant and also the model is highly significant. Moreover, the model showed statistically insignificant lack of fit by reason of the lower calculated $F$-value (4.74) compared with the tabulated $F$ value ($F_{0.05 \ [9, 3]} = 8.81$) and the greater probability value ($P = 0.0835 > 0.05$). As a result, the model was proved to be fitting for prediction absolutely within the range of variables employed.

The coefficient values were calculated and tested for their significance using Design Expert software and are listed in Table 4. Results showed that only the linear coefficients ($x_1$, $x_2$, $x_3$), two quadratic term coefficients ($x_1^2$, $x_2^2$) and one intercept product coefficient ($x_1x_2$) were significant with the very small $P$ values ($P < 0.05$). Therefore, for the coefficients of the secondary model, only significant ones could be used and the secondary polynomial equation was established as follows:

$$Y = 2.24 + 0.25x_1 + 0.46x_2 + 0.12x_3 - 0.10x_1^2 - 0.15x_2^2 + 0.10x_1x_2 \quad (7)$$

### Analysis of Response Surface Diagram and Its Contour Diagram

The graphical representations of the regression Eq. (7) entitled the response surfaces and the contour diagrams are shown in Figs. 1–3. The response surface diagram in Fig. 1A is a function of chlorine concentration and temperature at fixed treatment time of 3 min (0 level). Reduction of *L. monocytogenes* population in lettuce significantly increased as chlorine concentration and temperature increased. In Fig. 1B, 2.09 log reductions could be

### Table 4.

**TEST OF SIGNIFICANCE FOR REGRESSION COEFFICIENTS**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient estimate</th>
<th>df</th>
<th>Standard error</th>
<th>$F$ value</th>
<th>Prob* &gt; $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.24</td>
<td>1</td>
<td>0.037</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$x_1$</td>
<td>0.25</td>
<td>1</td>
<td>0.029</td>
<td>8.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_2$</td>
<td>0.46</td>
<td>1</td>
<td>0.029</td>
<td>15.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$x_3$</td>
<td>0.12</td>
<td>1</td>
<td>0.029</td>
<td>4.14</td>
<td>0.0043</td>
</tr>
<tr>
<td>$x_1^2$</td>
<td>−0.10</td>
<td>1</td>
<td>0.040</td>
<td>−2.62</td>
<td>0.0346</td>
</tr>
<tr>
<td>$x_2^2$</td>
<td>−0.15</td>
<td>1</td>
<td>0.040</td>
<td>−3.74</td>
<td>0.0072</td>
</tr>
<tr>
<td>$x_3^2$</td>
<td>0.028</td>
<td>1</td>
<td>0.040</td>
<td>0.70</td>
<td>0.5058</td>
</tr>
<tr>
<td>$x_1x_2$</td>
<td>0.100</td>
<td>1</td>
<td>0.041</td>
<td>2.44</td>
<td>0.0447</td>
</tr>
<tr>
<td>$x_1x_3$</td>
<td>0.063</td>
<td>1</td>
<td>0.041</td>
<td>1.53</td>
<td>0.1709</td>
</tr>
<tr>
<td>$x_2x_3$</td>
<td>2.500E-03</td>
<td>1</td>
<td>0.041</td>
<td>0.061</td>
<td>0.9530</td>
</tr>
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</table>

* Probability value.

df, degree of freedom.
FIG. 1. RESPONSE SURFACE DIAGRAM (A) AND ITS CONTOUR DIAGRAM (B) SHOWING EFFECTS OF CHLORINE CONCENTRATION AND TEMPERATURE AND THEIR MUTUAL INTERACTIONS (TREATMENT TIME IS A CONSTANT OF 3 MIN) ON REDUCTION OF L. MONOCYTOGENES IN LETTUCE
FIG. 2. RESPONSE SURFACE DIAGRAM (A) AND ITS CONTOUR DIAGRAM (B) SHOWING EFFECTS OF TREATMENT TIME AND TEMPERATURE AND THEIR MUTUAL INTERACTIONS (CHLORINE CONCENTRATION IS A CONSTANT OF 50 PPM) ON REDUCTION OF *L. MONOCYTOGENES* IN LETTUCE
FIG. 3. RESPONSE SURFACE DIAGRAM (A) AND ITS CONTOUR DIAGRAM (B) SHOWING EFFECTS OF CHLORINE CONCENTRATION AND TREATMENT TIME AND THEIR MUTUAL INTERACTIONS (TEMPERATURE IS A CONSTANT OF 25°C) ON REDUCTION OF *L. MONOCYTOGENES* IN LETTUCE
gained no matter what temperature is fixed in the range of 15–35°C; whereas for chlorine concentration, it must be fixed at least 40 ppm. Therefore, it can be deduced that chlorine concentration plays a more important role in reduction of *L. monocytogenes* than temperature.

Figure 2A presents the three-dimensional response surface diagram at various treatment time and temperature at fixed chlorine concentration of 50 ppm (0 level). It was observed obviously that the contours were very sparse in Fig. 2B, indicating that the effects of treatment time and temperature and their mutual interactions on reduction of *L. monocytogenes* were less than those of chlorine concentration and temperature shown in Fig. 1.

Figure 3A shows the changes of log reduction of *L. monocytogenes* with chlorine concentration and treatment time at fixed temperature of 25°C (0 level). Reduction of *L. monocytogenes* in lettuce increased observably as chlorine concentration and treatment time increased. According to Fig. 3B, the effect of treatment time on the inactivation of *L. monocytogenes* in lettuce was not as prominent as that of chlorine concentration.

As a whole, chlorine concentration and temperature play a more important role in reducing the population of *L. monocytogenes* than treatment time. Similar conclusion has been published by Ozer and Demirci (2006). They demonstrated that the treatment time in acidic and alkaline electrolyzed water has less effect than temperature.

**Validation**

Validation is an important step, which can assess the capacity of developed model (Bang *et al.* 2008). Figure 4 shows the scatter plot diagram of observed value versus predicted value. The correlation coefficient (*R*²) was 0.9818, which indicates that the model provided a good prediction.

Table 5 showed E%, Bf, and Af values of mathematical and statistical indices for developed model. E% was used to measure the model prediction accuracy, which assesses how close the predicted values are to the observed values. E% value was 0.0218, indicating that predicted values were in close agreement with those of experimental values. Besides, bias factor (Bf) and accuracy factor (Af) has been used as valuable tools for the validation of the performance of predictive models (Dalgaard and Jorgensen 1998; te-Giffel and Zwietering 1999). Bf is a measurement of the extent of under- or overprediction by the model giving the structural deviations (te-Giffel and Zwietering 1999), whereas Af can provide indication of the average accuracy of estimates. As shown in Table 5, the value of Bf for the secondary polynomial equation was 1.0003. It indicates that the predicted value is a lower level of confidence than observed ones, which tend to be fail-dangerous. Similar results have been reported by Farber *et al.* (1996), Duffy *et al.* (1994). They reported that bias
factors of more than 1 were calculated for most foods. However, fail-safe predictions were given in the publication of Grau and Vanderlinde (1993). Ross (1996) proposed that, for models describing pathogen growth kinetics, $B_f$ in the range $0.9–1.05$ could be considered good for models describing a pathogen growth rate, whereas the range of $0.7–0.9$ or $1.06–1.15$ was considered acceptable, and less than $0.7$ or $>1.15$ was considered unacceptable. According to this standard, the $B_f$ value in this study was in the good range. On top of that, other standards of content $B_f$ value have been recommended in previous publications. Armas et al. (1996) reported that if $B_f$ value was in the range of $0.6–3.99$, it could be accredited, whereas Dalgaard (2000) advised the range of $0.8–1.3$ for $B_f$ value as a successful validation of seafood spoilage model.

Considering that $B_f$ value can not supply the designation of the mean accuracy, as a result, $A_f$ value was also calculated. Lebert et al. (2000) have
shown that $A_f$ value of 1 demonstrates a perfect consistency between all predicted and observed values. As listed in Table 5, $A_f$ value obtained from this study was 1.0220, indicating that on average the predictions differ from observations by merely 2.2%. Ross et al. (2000) reported that acceptable $A_f$ value could be determined by considering the effect of the number of environmental parameters in a kinetic model. Consequently, the $A_f$ value of our model was in the suggested range, which indicates that the established secondary model could provide highly reliable prediction of *L. monocytogenes* in the future.

**CONCLUSIONS**

In summary, the electrolyzed oxidizing (EO) water exhibited strong antimicrobial effect on reduction of *L. monocytogenes* in lettuce. Reduction of 1.39–2.79 $\log_{10}$ cfu/g of *L. monocytogenes* in lettuce was observed under different conditions containing combinations of temperature, chlorine concentration of EO water and treatment time. Among the three preconcerted factors in this study, it has been demonstrated that chlorine concentration and temperature play a more important role in reducing the population of *L. monocytogenes* than treatment time. A quadratic equation for *L. monocytogenes* inactivation kinetic in lettuce was established and it has been validated using validation indicators such as average mean deviation (E%), bias factor (Bf) and accuracy factor ($A_f$). Results showed that the developed model has ability to provide valuable insight on the reduction of *L. monocytogenes* treated with EO water under different condition in lettuce.

**REFERENCES**


