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Prediction of Growth of *Escherichia coli* O157 : H7 in Lettuce Treated with Alkaline Electrolyzed Water at Different Temperatures

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ABSTRACT - This study was conducted to develop a model for describing the effect of storage temperature (4, 10, 15, 20, 25, 30 and 35°C) on the growth of *Escherichia coli* O157 : H7 in ready-to-eat (RTE) lettuce treated with or without (control) alkaline electrolyzed water (AIEW). The growth curves were well fitted with the Gompertz equation, which was used to determine the specific growth rate (SGR) and lag time (LT) of *E. coli* O157 : H7 ($R^2 = 0.994$). Results showed that the obtained SGR and LT were dependent on the storage temperature. The growth rate increased with increasing temperature from 4 to 35°C. The square root models were used to evaluate the effect of storage temperature on the growth of *E. coli* O157 : H7 in lettuce samples treated without or with AIEW. The coefficient of determination (R^2), adjusted determination coefficient (R^2_{Adj}), and mean square error (MSE) were employed to validate the established models. It showed that R^2 and R^2_{Adj} were close to 1 (> 0.93), and MSE calculated from models of untreated and treated lettuce were 0.031 and 0.025, respectively. The results demonstrated that the overall predictions of the growth of *E. coli* O157 : H7 agreed with the observed data.

Key words: predictive model, alkaline electrolyzed water, *Escherichia coli* O157 : H7, lettuce, validation

Sales of ready-to-eat (RTE) fruits and vegetables have grown rapidly in the last decades as a result that nutritionists emphasize the importance of fruit and vegetables in healthy diets, and researchers and governmental campaigns around the world are likely to recommend the consumption of at least five servings of fruit and vegetables per day[1)]. However, the incidence of foodborne outbreaks due to the consumption of contaminated fresh produce has increased[2)]. In recent years, lettuce has been involved in some outbreaks, most of which were caused by pathogenic bacteria, including *Escherichia coli* O157 : H7, *Listeria monocytogenes* and *Salmonella*[3)]. In 1995, two outbreaks of *E. coli* O157 : H7 infections were epidemiologically associated with the consumption of leaf lettuce; one of them involving at least 40 persons in Montana[4)] and another affecting 30 persons in Maine[5)].

Washing is known as the best way to eliminate pathogens in RTE foods. In recent, the use of several sanitizers has been investigated to control bacterial growth in fresh produce[2)]. Among them, the electrolyzed water produced by the electrolysis of an aqueous sodium chloride solution in an anode cell, has been widely used in food industry. Especially a strong antimicrobial effect of acidic electrolyzed water against pathogenic bacteria has been reported in lettuce[6,7)], and the alkaline electrolyzed water (AIEW) with a high pH (approximately 11.6) has been reported to possess antioxidative effect on highly unsaturated fats and oils. Few people focused on its sanitizing effect[6,8)].

Predictive microbiology is always employed to predict the microbial behaviour in foods over time as a function of different factors[9,10)]. Primary models describe growth or survival kinetics over time under various conditions, while secondary models describe the effect of environmental factors on the growth kinetic parameters such as the specific growth rate (SGR), the lag time (LT), and the maximum population density (MPD) obtained from the primary model[11)]. Predictive models are regarded as valuable tools in planning Hazard Analysis Critical Control Point (HACCP) programs, making decisions, and regulating plans and policies for the food industry, as they provide the first estimates of expected changes in microbial populations when exposed to a specific set of conditions[9,11)]. In general, temperature is the one of the most essential environmental factors, which affects

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microbial growth in food. This factor constantly changes during processing, storage, and distribution of food products[12]. Predictive models are useful tools for assessing and controlling food safety, particularly when the models are able to cope with dynamic conditions such as changing temperatures.

The purposes of this study were to examine the bactericidal effect of AIEW against *E. coli* O157 : H7 in lettuce, furthermore, develop primary and secondary models for *E. coli* O157 : H7 growth kinetic in RTE lettuce treated with or without AIEW, and to compare the SGR and LT obtained at different storage temperatures.

Materials and Methods

Sample preparation

RTE iceberg lettuce (*Lactuca sativa* var. capita) samples were purchased from a local supermarket in Chuncheon, Korea. The outer leaves and core were removed aseptically, and wilted portions were discarded. The remaining fresh leaves were cut into 3 by 3 cm pieces using a sterile knife. The sliced lettuce of each sample (10 g) was put into stomacher bag (Nasco Whirl-Pak, Janesville, WI) and stored at 5°C.

Bacterial strains

Two strains of *E. coli* O157 : H7 were used: 932 (human feces isolate) and 933 (ground beef isolate). All strains were maintained at -70°C in tryptic soy broth (TSB, Difco, Sparks, MD, U.S.A.) with a 0.6% yeast extract (YE, Difco, USA) containing 20% glycerol. The strains were transferred 10 μL of the stock culture into 10 mL of TSBYE, and then incubated at 35°C for 24 h. When the cultures reached to the late stationary phase, the cells were harvested by centrifugation for 5 min at 5,000 × g and washed twice in sterile 0.1% (w/v) buffered peptone water (Difco, USA), subsequently, adjusted with final inoculum level of 7 log CFU/mL.

Inoculation

Inoculum solution (2 L) was prepared by transferring 1 mL mixed strain cocktails (7.0 log CFU/mL) of each bacterial species into 2 L of sterile distilled water, in which the pieces of sliced lettuce were immersed for 3 min, and then let them dry completely in the clean bench on absorbent paper. The inoculum levels of *E. coli* O157 : H7 were approximately 3.0 log CFU/g on lettuce. The inoculated samples without AIEW treatment were used as a positive control.

Treatment with alkaline electrolyzed water and storage of samples under constant temperature

Alkaline electrolyzed water (AIEW) was produced from 0.1% NaCl solution using a flow-type electrolysis generator A2 (EN'S & ST'S, Seoul, Korea) set at 16 A, and collected from the cathode compartment. After treatment of inoculated sliced lettuce samples (100 g each) with 2 L of the AIEW for 3 min, neutralizing agent (0.85% NaCl containing 0.5% $Na_2S_2O_3$) was used to stop the effect of AIEW on lettuce, and then all treated lettuce were transferred into new stomacher bag. At the same time, the untreated lettuce was used as control. All samples were marked carefully before storage at different storage temperatures (4, 10, 15, 20, 25, 30, and 35°C) until they reached the stationary phase. Sampling was generally carried out at different intervals according to the storage temperatures; lower temperatures had longer sampling intervals, while shorter intervals were chosen for higher temperatures. Each experiment was performed in duplicate with three replicates.

Model development and validation

Bacterial numbers (CFU/g) were transformed into log10 for statistical analysis. The growth parameters were estimated by fitting the data to the modified Gompertz equation (Eq. 1) using GraphPad prism software (version 4, GraphPad Software, Inc., San Diego, CA, U.S.A.).

$$y = A\exp[-\exp\{\mu e/A(\lambda - t) + 1\}] \qquad \text{Eq. (1)}$$

where A is the difference between initial and final cell numbers, μ is specific growth rate, t is time and λ is lag time.

The SGR values were analyzed employing SPSS 16.0 package program (Statistical package for the social science, Chicago, IL, USA) to develop square root equation as a secondary model using the equation described below[13,14]:

$$\sqrt{k} = b\,(T - T_0) \qquad \text{Eq. (2)}$$

where k is the growth rate, b is the regression constant, T is the temperature (°C), and T_0 is the minimum temperature for growth. The goodness-of-fit of the models was evaluated by the coefficients of determination (R^2) and the adjusted determination coefficient (R^2_{Adj}). The performance of the predictive equations was validated by the mean square error (MSE) as follows:

$$MSE = \Sigma(\mu_{observed} - \mu_{predicted})^2/n \qquad \text{Eq. (3)}$$

where n = the number of observations; $\mu_{predicted}$ = the predicted specific growth rate; $\mu_{observed}$ = the observed specific growth rate.

Results and Discussion

Bactericidal effect of alkaline electrolyzed water

The results indicated that the antimicrobial effect of AIEW was not so effective, showing that only 0.5-0.6 log reductions were observed in lettuce inoculated with *E. coli* O157 : H7 compared to untreated samples (Fig. 1). Similar achievement has been reported in previous research[6], in which approximately 0.55-0.69 log reductions of *E. coli* O157 : H7 were observed in lettuce treated with AIEW at

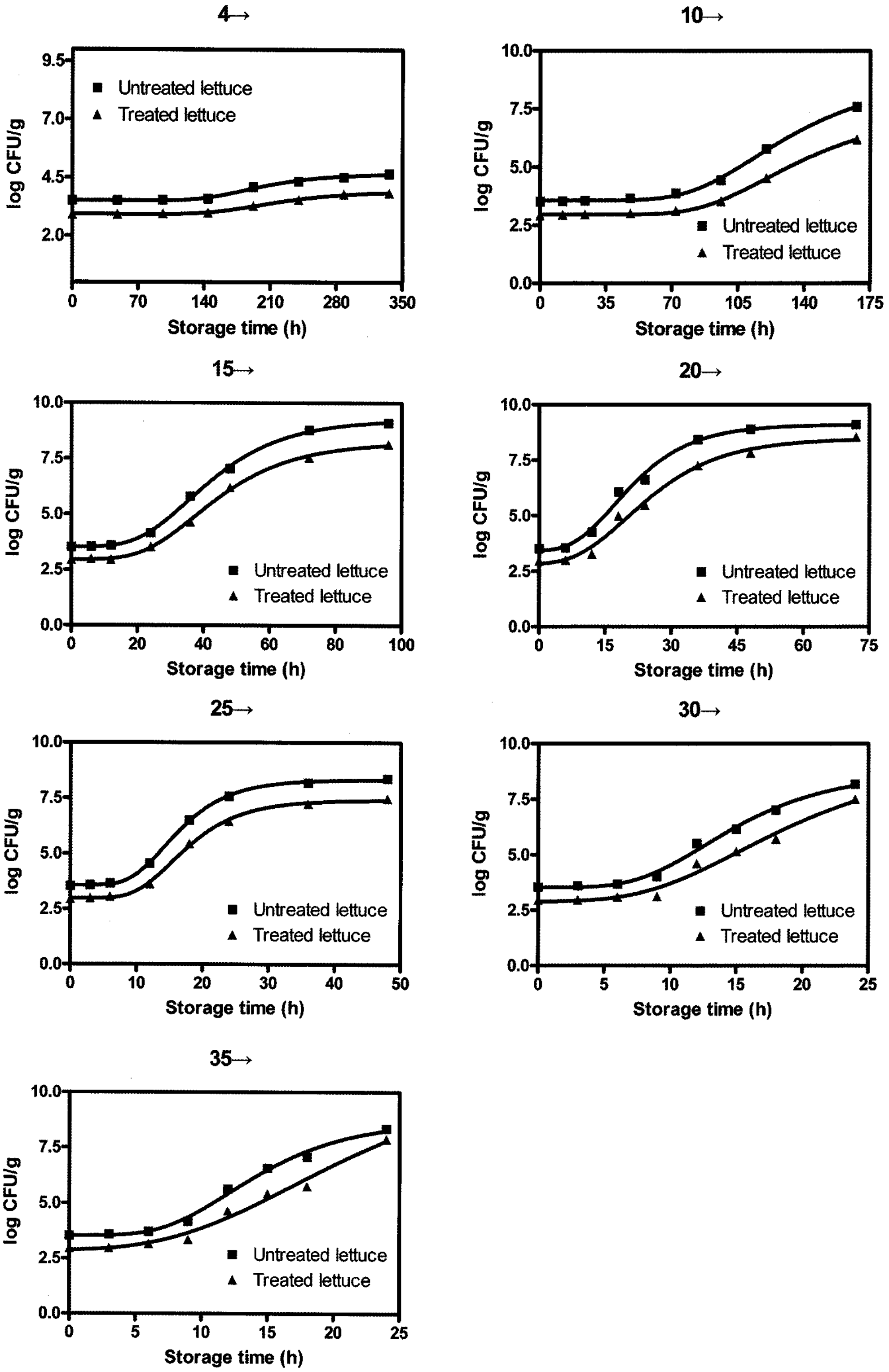

Fig. 1. The observed growth of *E. coli* O157 : H7 on untreated lettuce and treated lettuce at different storage temperatures: 4, 10, 15, 20, 25, 30, and 35°C. The growth curves were obtained using the modified Gompertz model.

20°C. Another previous work[15] showed 0.92 log reduction in their literature, which was also not a satisfied benefit for inactivation of foodborne pathogens population. Until now, few publications were reported on the effective application of AIEW as a sanitizing agent. The results obtained from this study could demonstrate that AIEW was not a good sanitizer as acidic electrolyzed water. Consequently, AIEW may act as a surfactant, and the hydrophobicity of the fruit

Table 1. Specific growth rate (SGR) and lag time (LT) of *E. coli* O157:H7 inoculated on the lettuce treated with or without alkaline electrolyzed water.

Temperature (°C)	SGR [1,2] (log CFU/h)		LT [1,2] (h)	
	Untreated lettuce	Treated lettuce	Untreated lettuce	Treated lettuce
4	0.007 ± 0.002^{a}A	0.009 ± 0.001^{a}A	141.6 ± 3.33^{f}B	134.7 ± 3.79^{f}A
10	0.043 ± 0.007^{b}A	0.051 ± 0.007^{b}B	83.73 ± 4.14^{e}B	77.87 ± 3.63^{e}A
15	0.120 ± 0.012^{c}A	0.134 ± 0.014^{c}B	21.20 ± 1.73^{d}A	19.87 ± 2.25^{d}A
20	0.179 ± 0.011^{d}A	0.222 ± 0.015^{d}B	9.684 ± 1.54^{c}B	8.974 ± 1.34^{c}A
25	0.286 ± 0.024^{e}A	0.329 ± 0.019^{e}B	8.260 ± 1.10^{b}B	7.711 ± 1.11^{b}A
30	0.304 ± 0.017^{ef}A	0.358 ± 0.011^{ef}B	7.810 ± 0.88^{ab}B	7.341 ± 1.09^{ab}A
35	0.306 ± 0.021^{f}A	0.377 ± 0.018^{f}B	7.356 ± 0.97^{a}B	6.985 ± 1.20^{a}A

[1]Within the same row, values not preceded by the same capital letter are significantly different ($p < 0.05$).
[2]Within the same column, values not followed by the same small letter (superscript) are significantly different ($p < 0.05$).

and vegetables surface, therefore, would be decreased when washed with such a solution[6].

Growth kinetics of *E. coli* O157 : H7

All the experimental data obtained from each medium were fitted into the modified Gompertz model. The modified Gompertz model is widely used to describe microbial growth kinetics[16]. The growth curves of *E. coli* O157 : H7 on untreated lettuce and treated lettuce at different storage temperatures are shown in Fig. 1. The modified Gompertz fitted well all growth curves in general goodness-of-fit. The curves obtained from GraphPad prism Software at 4, 10, 15, 20, 25, 30, and 35°C showed a high correlation coefficient ($R^2 = 0.994$). It can be observed obviously from Fig. 1, there were almost no growth at 4°C, and also the growth of *E. coli* O157 : H7 was not observed in nearly 70 hours at 10°C in both untreated lettuce and treated lettuce with AIEW. With increasing temperature, the growth of *E. coli* O157 : H7 increased, especially as the temperature was fixed at 35°C, it could reach to the stationary phase within 25 hours. Similar results were reported by previous publication[17]. They found that the higher the storage temperature, the faster the bacteria grow. There was almost no growth in nearly 50 hours at 5 and 10°C, while the populations of *E. coli* O157 : H7 could reach to the stationary phase within 35, 20 and 15 hours when at 15, 20 and 25°C, respectively.

Primary modeling of *E. coli* O157 : H7 growth on untreated and treated lettuce

Table 1 shows the parameters for SGR and LT on untreated and treated lettuce samples obtained by fitting the Gompertz function (Eq. 1) to the growth curves. As shown in Table 1, when storage temperature increased, the lag time decreased and specific growth rate increased. Compared to the SGR and LT obtained from untreated lettuce and treated lettuce samples, significant differences were observed ($p < 0.05$). The greater SGR value and lower LT value were observed in treated lettuce samples than in untreated lettuce. As neutralizing agent was used after treated with AIEW, there was no AIEW residue on the lettuce samples. It meant that the AIEW can not inactivate *E. coli* O157 : H7 any more during storage. The results might be due to the adhered properties of bacteria. Sanitizer treatment reduced bacteria counts. After that, once the remaining bacteria adapted to the new environment, they grew more rapidly. In addition, the SGR values obtained from different storage temperatures were differed significantly from both samples of untreated lettuce and treated lettuce, and there was also a significant difference among the LT values, with the exception of 15°C.

Secondary modeling of *E. coli* O157 : H7 growth and validation

The SGR value obtained from the Gompertz equation were used to develop the secondary model to describe the relations between specific growth rate and storage temperature using mathematical software. Square root analysis was employed to determine the SGR of *E. coli* O157 : H7 in untreated lettuce and treated lettuce (Fig. 2). There was a linear relationship between $\sqrt{SGR}$ and storage temperature with a high correlation coefficient of linearity ($R^2 > 0.92$, Table 2). High R^2 values may result from a small number of degrees of freedom[16]. In such a situation, a very good matching of a model to the data is not surprising[18]. Furthermore, it could be observed that the SGR of untreated lettuce showed a slightly larger slope than the one of treated lettuce.

The square root models established for SGR of *E. coli* O157 : H7 on untreated lettuce and treated lettuce samples were presented in Table 2. It provided a good illustration of the relationship between bacteria growth rate and temperature[19], showing high R^2 and low mean standard error. The adjusted

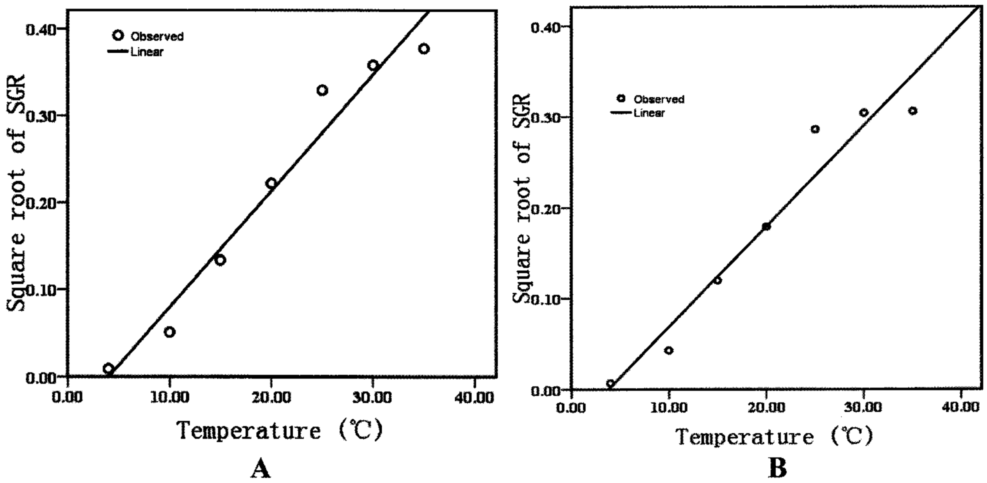

Fig. 2. The relationship between the square root of specific growth rate and storage temperature of *E. coli* O157 : H7 in untreated lettuce (A), treated lettuce (B).

Table 2. The square root model of *E. coli* O157:H7 on untreated lettuce and treated lettuce.

	Square root equation	R^2	R_{adj}^2	Standard error	MSE[1]
Untreated lettuce	SGR[3] = $(0.013T - 0.054)^2$	0.963	0.956	0.032	0.031
Treated lettuce	SGR = $(0.011T - 0.042)^2$	0.946	0.936	0.032	0.025

[1]Mean square error
[2]Specific growth rate

determination coefficient (R^2_{Adj}) is the correlation measure which tests the goodness-of-fit of the regression equation[20]. As shown in Table 2, the values of the R^2_{Adj} (0.956, 0.936) indicated a high degree of correlation between the observed and predicted values, which suggested that only 4.4% and 6.4% of the total variation can not be explained by the current models, respectively. The mean square errors for the two models were also obtained in Table 2. A report proposed that the lower MSE indicates the more adequacy of the model to describe the data[21]. In the results, MSE showed highly lower values (0.25, 0.31), which demonstrated that the predicted SGR showed a good prediction with our models.

요 약

본 연구는 오염된 양상치를 알카리전해수로 세척한 처리구와 비처리구 에 오염된 *E. coli* O157 : H7균이 다양한 온도 (4, 10, 15, 20, 25, 30, 35°C) 에 저장할 경우 이균의 specific growth rate (SGR) 과 lag time (LT) 생육변수에 미치는 영향을 조사하기 위한 모델을 개발하기 위하여 수행되었다. *E. coli* O157 : H7의 specific growth rate (SGR) 과 lag time (LT) 를 결정하기 위해 생육도를 Gompertz 식을 사용하여 fitting한 결과, R^2값이 0.994로 나타났다. 실험값으로부터 얻은 SGR과 LT는 저장온도에 의존하는 것으로 나타났으며 4°C에서 35°C까지 온도가 증가할수록 성장속도가 증가하는 것으로 나타났다. AIEW 처리구 또는 비처리구의 양상치에서 *E. coli* O157 : H7의 성장 kinetics에 대한 저장 온도의 효과를 평가하기 위해 SRG에 대한 두개의 모델을 개발하였다. 유도된 2개의 모델 검증은 R^2, R^2_{Adj} (adjusted determination coefficient) 및 MSE (mean square error) 를 적용하였으며, 그 결과 R^2, R^2_{Adj}가 1 (> 0.93) 에 근접하였으며, 알카리 전해수 처리구 및 비처리구 양상치 모델의 MSE는 각각 0.031, 0.025로 나타났다. 따라서, 본 연구에서 개발된 모델의 생육변수는 실험치에서 얻은 *E. coli* O157 : H7의 생육변수 결과와 매우 유사한 것으로 나타났다.

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