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Prediction of Microbial Growth in Fresh-Cut Vegetables Treated with Acidic Electrolyzed Water during Storage under Various Temperature Conditions

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ABSTRACT

Effects of storage temperature (1, 5, and 10°C) on growth of microbial populations (total aerobic bacteria, coliform bacteria, *Bacillus cereus,* and psychrotrophic bacteria) on acidic electrolyzed water (AcEW)-treated fresh-cut lettuce and cabbage were determined. A modified Gompertz function was used to describe the kinetics of microbial growth. Growth data were analyzed using regression analysis to generate "best-fit" modified Gompertz equations, which were subsequently used to calculate lag time, exponential growth rate, and generation time. The data indicated that the growth kinetics of each bacterium were dependent on storage temperature, except at 1°C storage. At 1°C storage, no increases were observed in bacterial populations. Treatment of vegetables with AcEW produced a decrease in initial microbial populations. However, subsequent growth rates were higher than on nontreated vegetables. The recovery time required by the reduced microbial population to reach the initial (treated with tap water [TW]) population was also determined in this study, with the recovery time of the microbial population at 10°C being <3 days. The benefits of reducing the initial microbial populations on fresh-cut vegetables were greatly affected by storage temperature. Results from this study could be used to predict microbial quality of fresh-cut lettuce and cabbage throughout their distribution.

The presence of numerous genera of spoilage bacteria, yeasts, and molds and an occasional pathogen on fresh produce has been recognized for many years. In particular, fresh-cut vegetables are highly susceptible to microbial spoilage, because processing by slicers, shredders, and peelers may lead to microbial contamination *(16),* and inner tissues are exposed to microbial contamination after processing *(7).* Bolin et al. *(6)* reported that the initial microbial load of shredded lettuce influenced the storage life of the product. Chlorination and ozonation have long been widely used for the disinfection of foods. However, washing, together with chlorine or ozone treatment, cannot completely remove or inactivate microorganisms on fresh produce *(3, 5, 8, 26, 33).*

There have been reports on the antimicrobial and antiviral activities of acidic electrolyzed water (AcEW) produced by the electrolysis of a dilute aqueous sodium chloride solution using an instrument in which an anode and cathode are separated by a membrane to form two compartments *(19, 27).* Recently, the efficacy of AcEW as a disinfectant for fruits and vegetables has been reported *(20–22).* However, AcEW treatment also cannot completely remove or inactivate microorganisms on fresh produce *(24).* Therefore, microorganisms on fresh produce will continue to multiply for a few days. As microorganism populations increase, quality of fresh produce decreases. Moreover, the risk of outbreaks of human gastroenteritis also increases.

Predictive microbiology has emerged as a discipline in its own right in recent years, and the usefulness of this approach is becoming widely accepted, resulting in considerable international interest being shown, particularly in Western Europe, the United States, Canada, and Australia. This approach is late in Japan. In the past 10 years, many microbiologists have worked on growth kinetics models for various pathogens *(4, 9, 10, 25, 28–30, 36, 37).* Most of these studies were carried out using selected growth media in which factors such as pH, NaCl contents, and temperature were controlled. The characteristics of each microorganism have been revealed. However, few studies have examined the behavior of microorganisms in foods *(14, 31, 34).* Prediction of the shelf life and risk assessment of foods is very important information for retailers and consumers, and a prediction model of microbial growth is especially valuable for fresh-cut vegetables, which are easily damaged and eaten raw.

The objectives of this study were to examine microbial growth on fresh-cut vegetables during storage at various temperatures and to develop a growth model for the prediction of shelf life. Moreover, we examined the effect of reducing initial microbial populations using AcEW on microbial growth during storage. From these studies, we proposed a model for the prediction of the shelf life of fresh-cut vegetables under various storage temperatures.

MATERIALS AND METHODS

Plant materials. Lettuce and cabbage were purchased at a local supermarket in Sapporo, Hokkaido, Japan. These vegetables were harvested before 2 days of sale at retail establishments. The

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storage temperature was kept below 10°C during distribution. The outer leaves were removed and discarded. Lettuce leaves were then cut into approximately 5- by 5-cm squares, and cabbage leaves were shredded to a width of 2 to 3 mm.

Electrolyzed water treatment. A batch-type electrolysis apparatus, Super Oxeed Labo (Model JED-020, AOI Engineering, Shizuoka, Japan), was used to prepare the electrolyzed water. It was prepared by electrolysis of 0.1% sodium chloride solution at 9 to 12 direct current volts for 10 min at room temperature. AcEW was prepared in the anode side of an electrolytic cell, and alkaline electrolyzed water (AlEW) was prepared in the cathode side. Samples of lettuce or cabbage (500 g) were washed by soaking in AlEW (10 liters) for 1 min, then were decontaminated by soaking in AcEW (10 liters) for 1 min. As a control, samples of lettuce or cabbage (500 g) were washed by soaking in tap water (TW; 10 liters) for 2 min. Water adhering to the lettuce or cabbage was removed by centrifugation (3,000 rpm, 20 s). The pH of the tested solution was measured with a pH meter (HM-11P, TOA Electronics Ltd., Tokyo, Japan). Oxidation-reduction potential (ORP) was measured with an ORP meter (RM-10P, TOA). Initial concentrations of the available chlorine in the AcEW and TW used in this study were determined with chlorine test kits (Hach Co., Loveland, Colo.).

Storage of treated fresh-cut vegetables. A 50-g sample of each fresh-cut vegetable was enclosed in a package (effective area 700 cm^2) of polyethylene film (thickness 40 μm). The air was removed from each package by vacuum, then 300 ml of flashed air was added and the opening closed by heat sealing. Each air-enriched package was stored at 1, 5, and 10°C for 5 days. Microbial populations were determined before storage, and after storage, these populations were determined for 1, 3, and 5 days. Sample packages were prepared for every assay date with all temperature conditions.

Microbiological analysis. To enumerate the microorganisms on fresh-cut vegetables, a sample of each vegetable (25 g) was combined with 225 ml of sterile 0.85% sodium chloride solution in a sterile polyethylene bag and was pummeled with a stomacher for 2 min at high speed. The wash fluid was then serially diluted. All microbiological media used in this study were purchased from Merck (Darmstadt, Germany). Total aerobic bacterial counts were determined by pouring 1 ml of diluted sample into plate count agar. Plates were incubated at 35°C for 48 h, and colonies were counted. Coliform counts were determined by pouring 1 ml of diluted sample into violet red bile agar. Plates were incubated at 35°C for 24 h, and colonies were counted. *Bacillus cereus* counts were quantified by direct plating 0.1 ml of diluted sample onto the surface of a manitol-egg yolk-polymyxin agar plate. Plates were incubated at 35°C for 24 to 48 h, and colonies were counted. Psychrotrophic bacterial counts were quantified by direct plating 0.1 ml of diluted sample onto the surface of a plate count agar plate. Plates were incubated at 7°C for 10 days, and colonies were counted. All pour and spread plates used for quantitative analysis were carried out in duplicate at each relevant dilution.

A sample of each vegetable (25 g) was used in duplicate on each day of analysis. Three independent replications of each experiment were conducted. Significant differences in plate count data were established by the least significant difference test at the 5% level of significance.

Curve fitting and prediction of shelf life. The first stage involved fitting the bacterial growth curve with the modified Gompertz *(15)* sigmoid curve to calculate growth curve parameters such as growth rate and lag times. At each temperature, bacterial count data, which were the mean values obtained from three replications, were modeled as a function of time. Based on the curve fits, estimates of *B, M, C,* and *A* were obtained from the following relationships:

$$L(t) = A + C \exp\{-\exp[-B(t - M)]\}$$

where $L(t) = \log_{10}$ count at time t; A = asymptotic log count as t decreases indefinitely; C = asymptotic amount of growth that occurs as t increases indefinitely; B = relative growth rate at M; and M = time at which absolute growth rate is at a maximum. These parameters were used to derive growth rate, lag time, and generation time as follows:

$$\text{growth rate } (\log_{10}\text{count/d}) = BC/e$$

$$\text{lag time (d)} = M - 1/B$$

$$\text{generation time (d)} = \log_{10} 2e/BC$$

The shelf life of fresh-cut vegetables stored at various temperatures was determined from obtained growth curve equations and parameters. Shelf life was defined as the period that aerobic bacterial populations on fresh-cut vegetables increased by 10^5 CFU/g in this study. This aerobic bacterial population is a standard during distribution in Japan. Moreover, the period needed for microbial growth on cut vegetables treated with AcEW to reach initial (just after being treated with TW) microbial populations was determined from obtained curve equations.

In this study, curve fitting and kinetic parameterization were performed using KaleidaGraph software version 3.5.1 (Synergy Software, Reading, Pa.).

RESULTS

Disinfectant effect of AcEW on fresh-cut vegetables. The pH values for AcEW, AlEW, and TW were 2.5 ± 0.1, 11.3 ± 0.1, and 7.0 ± 0.1, respectively. Although the ORP of AcEW was high, such as 1,140 ± 7 mV, AlEW showed a very low ORP, such as −870 ± 10 mV. The ORP of TW was 416 ± 15 mV. The available chlorine concentration of AcEW and TW was 40.3 ± 1.5 ppm and 0.3 ± 0.1 ppm, respectively. Washing with AlEW for 1 min, then decontaminating with AcEW for 1 min, reduced total aerobic bacteria, coliform bacteria, *B. cereus,* and psychrotrophic bacteria on lettuce by 1.7, 1.6, 1.0, and 1.1 logs CFU/g, respectively. In cabbage, total aerobic bacteria, coliform bacteria, *B. cereus,* and psychrotrophic bacteria were reduced by 1.5, 1.5, 1.5, and 1.0 logs CFU/g, respectively. These results are in agreement with those of our previous experiment *(23).* By contrast, treatment with TW did not reduce bacterial populations on lettuce or cabbage. Accordingly, treatment with AlEW and AcEW could reduce initial microbial populations on fresh-cut vegetables prior to storage.

Microbial growth during storage. Changes in bacterial populations on lettuce during storage at three temperatures are shown in Figure 1A through 1D for aerobic bacteria, coliform bacteria, *B. cereus,* and psychrotrophic bacteria, respectively. No bacterial populations increased during storage at 1°C for 5 days. However, all populations increased during storage at 5 and 10°C. There were significant differences ($P < 0.05$) in all bacterial populations, except for psychrotrophic bacteria, on lettuce treated with AcEW among storage temperatures after storage the third

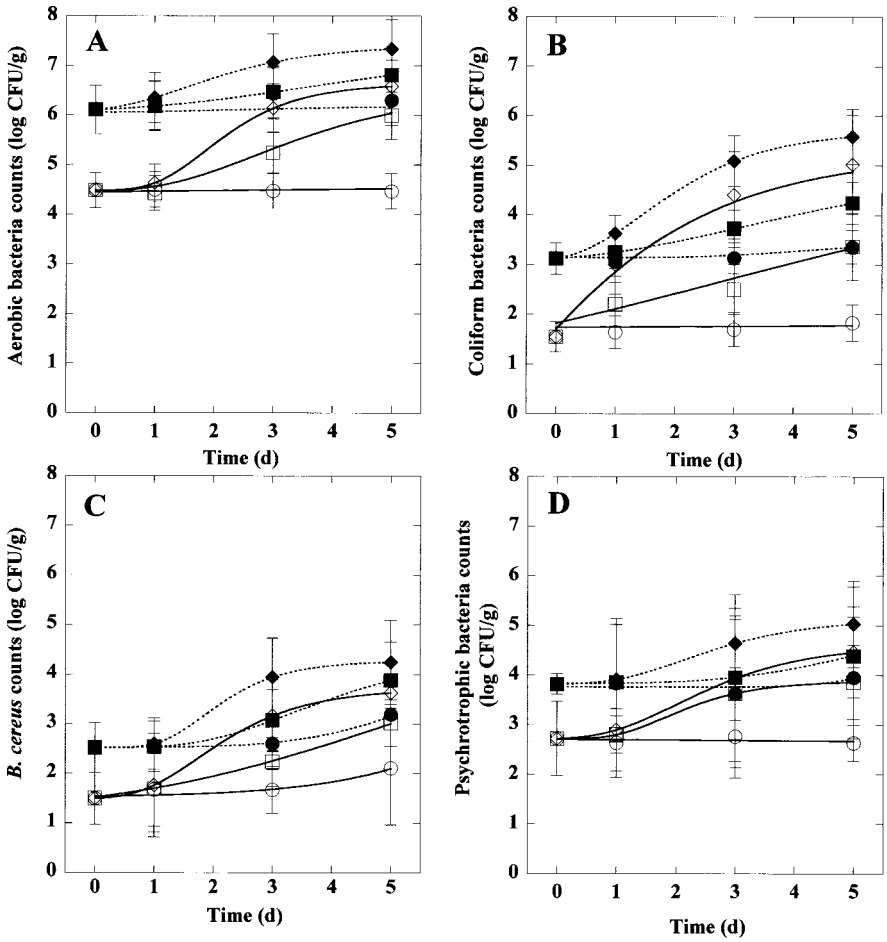

FIGURE 1. *Changes in populations of (A) aerobic bacteria, (B) coliform bacteria, (C)* Bacillus cereus, *and (D) psychrotrophic bacteria in the lettuce treated with acidic electrolyzed water (AcEW) and tap water (TW) during storage at 1, 5, and 10°C for 5 days. Results are mean ± standard deviation,* n = 3. ○, *AcEW 1°C;* □, *AcEW 5°C;* ◇, *AcEW 10°C;* ●, *TW 1°C;* ■, *TW 5°C;* ◆, *TW 10°C.*

day. In lettuce treated with TW, there were significant differences ($P < 0.05$) in aerobic bacterial and coliform bacterial populations among temperatures after the third day. There were no differences in *B. cereus* populations between 5 and 10°C. All bacteria examined in this study grew more quickly on lettuce treated with AcEW than on lettuce treated with TW. In other words, when initial microbial populations were low, growth rates quickened. This trend was particularly evident for aerobic and psychrotrophic bacteria. However, after 5 days' storage, microbial populations on lettuce treated with AcEW did not exceed those on lettuce treated with TW at the same storage temperature. Figure 2A through 2D shows the growth of aerobic bacteria, coliform bacteria, *B. cereus,* and psychrotrophic bacteria on cabbage treated with AcEW and TW, respectively. Microbial growth on cabbage was similar to that on lettuce. After 5 days' storage, there were significant differences ($P < 0.05$) in all bacterial populations on cabbage with each treatment among storage temperatures. Microbial growth on fresh vegetables was dependent on storage temperature.

Shelf life was defined as the period that aerobic bacterial populations of fresh-cut vegetables increased by 10^5 CFU/g in this study. Shelf life of lettuce treated with AcEW was 2.29 and 1.51 days at 5 and 10°C, respectively. Treatment with TW did not reduce aerobic bacterial counts of lettuce $<10^5$ CFU/g. The shelf life of cabbage treated with AcEW was 4.36 and 2.72 days at 5 and 10°C, respectively. The shelf life of cabbage treated with TW was 1.67 and 0.91 days at 5 and 10°C, respectively. The shelf life of neither vegetable was determined at 1°C.

Calculated Gompertz parameters. Every growth curve, except for storage at 1°C, was fitted to the modified sigmoid Gompertz function (see "Materials and Methods"). Tables 1 and 2 list the values for time to maximum growth rate, lag time, and generation time calculated from the parameters *B, M, C,* and *A* obtained by fitting the Gompertz function to the data. As storage temperature increases, lag time decreases and growth rates quicken. Growth rates of all bacteria, except for *B. cereus,* on lettuce treated with AcEW were higher than on lettuce treated with TW. Generation times for aerobic bacteria, coliform bacteria, and psychrotrophic bacteria treated with AcEW were shorter than when treated with TW. For cabbage treated with AcEW, growth rates of all bacteria, except for psychrotrophic bacteria, were higher than when treated with TW. Generation times of aerobic bacteria, coliform bacteria, and *B. cereus* treated with AcEW on cabbage were shorter than when treated with TW. Lag time depended on storage temperature, and as storage temperatures increased, lag times of all bacteria used in this study decreased. Moreover, lag times for coliform bacteria, *B. cereus,* and psychrotrophic bacteria on both cut vegetables treated with AcEW were shorter than when treated with TW.

Recovery of reduced microbial populations after treatment with AcEW. Microbial populations reduced by

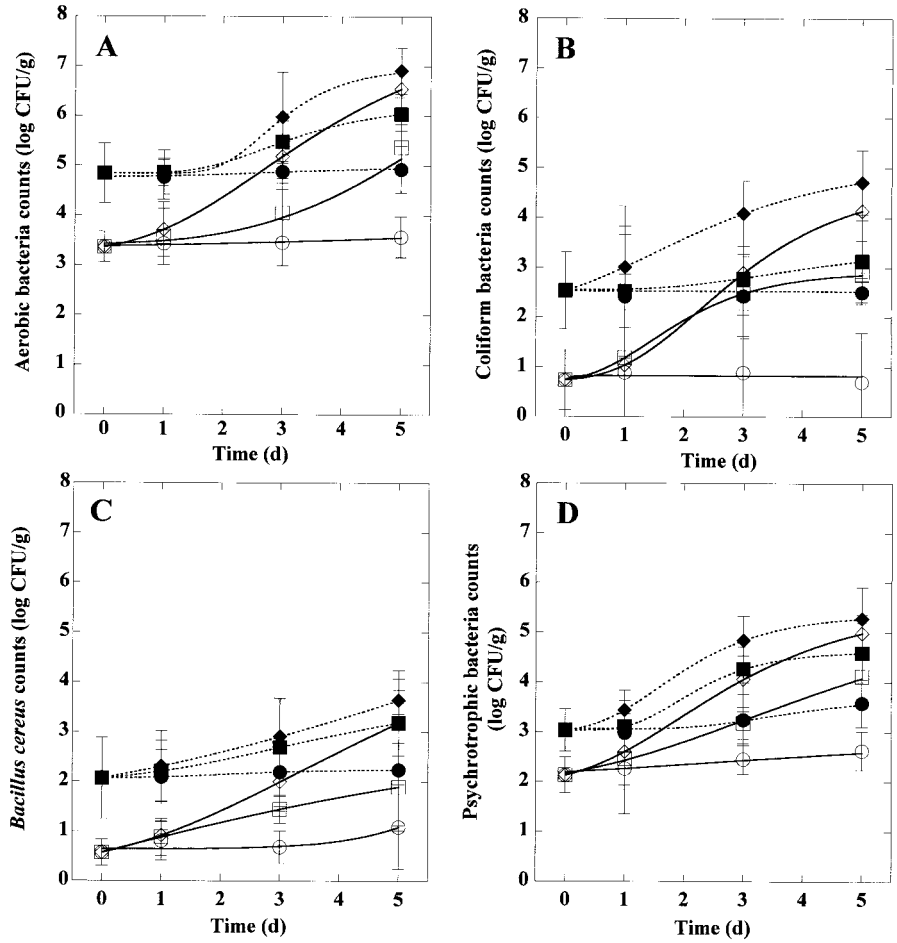

FIGURE 2. *Changes in populations of (A) aerobic bacteria, (B) coliform bacteria, (C)* Bacillus cereus, *and (D) psychrotrophic bacteria in the cabbage treated with acidic electrolyzed water (AcEW) and tap water (TW) during storage at 1, 5, and 10°C for 5 days. Results are mean ± standard deviation,* n = 3. ○, *AcEW 1°C;* □, *AcEW 5°C;* ◇, *AcEW 10°C;* ●, *TW 1°C;* ■, *TW 5°C;* ◆, *TW 10°C.*

AcEW also grew again during storage. The period needed for the reduced populations to reach the initial (just after treated with TW) microbial population was determined from the obtained curve equations (Table 3). This period is referred to as the recovery time in this study. Since no bacteria grew on cut vegetables at 1°C storage, recovery time was not determined at that temperature. Although aerobic bacteria on lettuce needed 5 days to reach initial populations at 5°C storage, other bacteria on lettuce needed only about 4 days. When stored at 10°C, recovery time of all bacteria on lettuce was within 3 days. Coliform bacteria, in particular, grew quickly and reached initial populations in 1.3 days at 10°C. Recovery time of all bacteria, except for *B. cereus* stored at 5°C, was shorter for cabbage than for lettuce. Recovery time of aerobic bacteria, coliform bacteria, and psychrotrophic bacteria on cabbage stored at 5°C was 4.2, 3.2, and 2.6 days, respectively. However, *B. cereus* populations needed 5.9 days to reach the initial population at 5°C, which was longer than for lettuce. By contrast, recovery times of all bacteria on cabbage stored at 10°C were <3 days, similar to those on lettuce.

DISCUSSION

AlEW has a high pH (>11) and a very low ORP (below −800 mV). When lettuce or cabbage was washed in AlEW for 1 min and then decontaminated with AcEW for 1 min, the disinfectant effect on aerobic and coliform bacteria was larger than that for the treatment of lettuce and cabbage by soaking in AcEW for 10 min *(23).* AlEW is considered to act like a dilute sodium hydroxide aqueous solution. Thus, it would act as a surface-active agent against the surface of lettuce or cabbage, and microorganisms on the surface of the vegetable could be easily decontaminated using AcEW. Therefore, cut lettuce and cabbage could be well decontaminated within a short time, such as within the 2-min treatment used in this study.

Aerobic bacterial populations can be regarded as an index of microbial contamination. Generally, an aerobic bacterial population $>10^7$ CFU/g on foods is considered to represent the initial stage of spoilage. Thus, after storing at 10°C for 5 days, lettuce and cabbage treated with TW would start to spoil.

Coliform bacterial populations represent the cleanliness of the vegetable. Because there was not much lag time of coliform bacteria at 5 and 10°C, management of storage temperature rather than reduction of initial populations is important in controlling coliform populations. Coliform is a group of bacteria that is defined as all aerobic and facultative aerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation. Mesophilic and psychrotrophic gram-negative rods are the predominant microorganisms on fresh-cut vegetables *(2, 18).* It is considered that vegetables may be suited for the growth of coliform bacteria. Therefore, coliform, including mesophilic gram-negative rods, grows more rapidly than other bacteria. Moreover, since reduction of coliform pop-

TABLE 1. *Growth conditions and calculated kinetic parameters from Gompertz equations of aerobic bacteria, coliform bacteria,* Bacillus cereus, *and psychrotrophic bacteria in or on the cut lettuce*[a]

Bacterium	Treatment	Storage temperature (°C)	Growth rate (log count/d)	Lag time (d)	Generation time (d)
Aerobic bacteria	AcEW	1	NG	NG	NG
	AcEW	5	0.46	1.11	0.59
	AcEW	10	0.92	0.99	0.30
	TW	1	NG	NG	NG
	TW	5	0.18	0.83	1.51
	TW	10	0.41	0.34	0.66
Coliform bacteria	AcEW	1	NG	NG	NG
	AcEW	5	0.62	0.03	0.44
	AcEW	10	1.44	0.01	0.19
	TW	1	0.09	2.50	3.11
	TW	5	0.28	0.58	0.98
	TW	10	0.86	0.35	0.32
B. cereus	AcEW	1	NG	NG	NG
	AcEW	5	0.43	1.06	0.62
	AcEW	10	0.82	0.74	0.33
	TW	1	NG	NG	NG
	TW	5	0.45	1.79	0.60
	TW	10	0.87	1.10	0.31
Psychrotrophic bacteria	AcEW	1	NG	NG	NG
	AcEW	5	0.52	0.99	0.52
	AcEW	10	0.58	0.82	0.46
	TW	1	NG	NG	NG
	TW	5	0.30	3.13	0.89
	TW	10	0.42	0.95	0.65

[a] AcEW, acidic electrolyzed water; TW, tap water; NG, no growth.

ulations decreased competitive coliform bacteria, residual coliform could easily grow. Accordingly, growth rate of coliform bacteria could be rapid on fresh vegetables.

B. cereus, some strains of which are pathogenic, is one of the organisms responsible for the spoilage of food. It has been reported that psychrotrophic strains of *B. cereus* grow rapidly at 8°C *(12, 17, 35).* Because cut vegetables are often exposed to this temperature during distribution, *B. cereus* grows rapidly during storage and distribution. Thus, both from a public health and an economic point of view, monitoring and control of *B. cereus* are very important for cut vegetables. Our results showed that *B. cereus* grew rapidly at 10°C; therefore, psychrotrophic strains of *B. cereus* are believed to exist on cut vegetables.

Psychrotrophic bacteria are mainly of the genus *Pseudomonas*; they grow at low temperatures and cause deterioration of foods. Psychrotrophic bacteria as well as other bacteria grow rapidly at 10°C. Accordingly, storage temperatures of fresh-cut vegetables should be kept below 5°C to protect the growth of microorganisms connected with spoilage and pathogenic illness.

Growth curves of each bacteria examined in this study were fitted to a Gompertz function. There have been many reports of the fitting of bacterial growth to Gompertz curves *(4, 9, 10, 25, 28–30, 36, 37).* However, many of them were performed on specific bacteria in controlled environments, such as modified nutrient broth or agar. There have been few reports on the fitting of microbial growth on foods to the Gompertz sigmoid curve *(14, 31, 34).* From this study, it is apparent that microbial growth on cut lettuce and cabbage could be fitted to the Gompertz curve. Parameters obtained from the Gompertz function could be used to calculate lag time and growth rate. Therefore, increases in bacterial populations on cut vegetables will be predicted on the basis of storage temperature, storage period, and initial population. If the history of temperature during distribution is monitored using a time-temperature integrator label, any potential health concerns would be indicated.

Initial microbial populations on cut vegetables affected growth rate and lag time. In vegetables treated with AcEW, the initial microbial population was reduced by 1 to 2 logs CFU/g. However, almost all bacteria treated with AcEW in this study showed a higher growth rate and shorter lag time than did those treated with TW. This trend was particularly apparent when stored at 10°C. There would be plenty of room for microbial growth on cut vegetables treated with AcEW, because of the reduction in initial microbial populations. Therefore, the microbial growth rate would be faster and the lag time would be shorter at higher temperatures such as those used in this study. Accordingly, beneficial effects of treatment with AcEW should not be overestimated, and treated vegetables should be managed at an appropriate temperature below 5°C. Recovery time to initial microbial population was adopted as an index of bacterial

TABLE 2. *Growth conditions and calculated kinetic parameters from Gompertz equations of aerobic bacteria, coliform bacteria,* Bacillus cereus, *and psychotrophic bacteria in or on the cut cabbage*[a]

Bacterium	Treatment	Storage temperature (°C)	Growth rate (log count/d)	Lag time (d)	Generation time (d)
Aerobic bacteria	AcEW	1	NG	NG	NG
	AcEW	5	0.76	2.33	0.35
	AcEW	10	0.82	0.66	0.33
	TW	1	NG	NG	NG
	TW	5	0.44	1.57	0.61
	TW	10	0.58	0.77	0.47
Coliform bacteria	AcEW	1	NG	NG	NG
	AcEW	5	0.79	0.39	0.34
	AcEW	10	1.04	0.88	0.26
	TW	1	NG	NG	NG
	TW	5	0.19	1.69	1.45
	TW	10	0.58	−0.33	0.47
B. cereus	AcEW	1	NG	NG	NG
	AcEW	5	0.31	0.41	0.87
	AcEW	10	0.61	0.23	0.44
	TW	1	0.06	0.76	4.57
	TW	5	0.27	0.45	1.00
	TW	10	0.43	0.22	0.64
Psychrotrophic bacteria	AcEW	1	0.08	2.91	3.20
	AcEW	5	0.45	0.22	0.61
	AcEW	10	0.77	0.09	0.35
	TW	1	0.19	2.13	1.40
	TW	5	0.72	1.08	0.38
	TW	10	0.83	0.50	0.33

[a] AcEW, acidic electrolyzed water; TW, tap water; NG, no growth.

reduction in this study, although the maximum period of population reduction was only 3 to 4 days at 5 or 10°C. Therefore, after 3 or 4 days, the storage temperature of vegetables should be carefully monitored.

In this study, although the storage period was 5 days, additional information on microbial growth could be extrapolated from Gompertz functions. However, after 5 days of storage at 5 and 10°C, the appearance of both cut vegetables had deteriorated, with the browning of vegetables stored at 10°C being particularly noticeable. Since the appearance of cut vegetables determines the product's value, browning leads to a loss of value regardless of microbial contamination. Therefore, in practical terms, it would be enough to predict the microbial growth for 5 days at 5 or 10°C.

TABLE 3. *Recovery time (days) that bacterial populations in or on the cut vegetables reduced by acidic electrolyzed water need to reach the initial (treated with tap water) bacterial populations*

Vegetable	Storage temperature (°C)	Recovery time (d)[a] Aerobic bacteria	Coliform bacteria	*Bacillus cereus*	Psychrotrophic bacteria
Lettuce	1	ND[b]	ND	ND	ND
	5	5.3 ± 0.3	4.2 ± 0.5	3.8 ± 0.5	4.2 ± 0.3
	10	2.9 ± 0.5	1.3 ± 0.2	2.0 ± 0.3	2.7 ± 0.3
Cabbage	1	ND	ND	ND	ND
	5	4.2 ± 0.5	3.2 ± 0.5	5.9 ± 0.3	2.6 ± 0.1
	10	2.6 ± 0.3	2.6 ± 0.5	3.1 ± 0.4	1.7 ± 0.2

[a] Mean value ± standard deviation of three replications.
[b] ND, not determined.

The risk of outbreaks of food poisoning could be assessed by predicting microbial growth during storage. There have been a number of reports on the relationship between microbial populations and the probability of infection *(13, 18, 32).* Rose and Gebra *(32)* reported that the probability of *Salmonella* infection was about 50% when $>10^3$ cells were ingested. Another report specified that the probability of *Shigella flexneri* infection was >50% when $>10^5$ cells were ingested *(11). B. cereus* may cause food poisoning with an infective dose as low as 10^3 to 10^4 CFU/g *(1).* In this way, the maximum number of ingested pathogens necessary to produce clinical symptoms in humans varies according to the bacteria. However, if the number of pathogens ingested is $<10^3$ cells, risk of infection remains relatively low. Therefore, on cut vegetables, pathogen populations in this study—both coliform bacteria and *B. cereus*—should be kept below 10^3 CFU/g until consumption. To control this level, cut vegetables should be decontaminated and stored at <5°C. Although the time limit for storage at 5°C is 5 days, vegetables can be kept safely for longer periods at 1°C.

In Japan, although there is no legal standard for the maximum acceptable microbial contamination of fresh-cut vegetables, an independent industry standard for total aerobic bacterial counts during distribution stands at $<10^5$ CFU/g. Because washing with TW is not enough to bring microbial counts below this standard, an effective decontamination process is indispensable for the cut vegetable industry. However, effective decontamination methods have not been established. AcEW treatment would be an effective method. Even on decontaminated vegetables, microbial populations will increase rapidly if kept at an inappropriate temperature for several days. Moreover, the growth rate on decontaminated vegetables would be higher than on undecontaminated vegetables. Therefore, temperature management after the reduction of microbial populations is very important. Control of microbial populations on cut vegetables requires both decontamination and storage at low temperatures (i.e., <5°C or, if possible, 1°C). However, storage at this temperature is difficult to manage using present distribution systems. Low temperature storage should not be relied on as the sole preservative technique, because of the potential for abuse by distributors, retailers, and consumers. Moreover, the risk of foodborne illness when fresh vegetables are stored for longer periods would be high, because pathogens have an opportunity to reach higher populations. Any vegetable may present a public health hazard if pathogenic microorganisms are present.

Microbial populations on cut vegetables did not increase when stored at 1°C for 5 days. Moreover, as the appearance of cut vegetables did not change, freshness would be ensured for 5 days or more. The best conditions for distribution of fresh-cut vegetables are at 1°C, according to this study. However, storage at 1°C would increase cooling costs greatly, due to the need for ventilation cooling. Other cooling methods, such as packing with crushed ice in an insulated container, need to be examined in the future. The combination of decontamination with AcEW and storage at 1°C would provide a useful system for the safe distribution of fresh-cut vegetables.

REFERENCES

1. Andersson, A., U. Rönner, and P. E. Granum. 1995. What problem does the food industry have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringers?* Int. J. Food Microbiol. 28:145–155.
2. Babic, I., A. E. Watada, and J. G. Buta. 1997. Growth of *Listeria monocytogenes* restricted by native microorganisms and other properties of fresh-cut spinach. J. Food Prot. 60:912–917.
3. Barth, M. M., C. Zhou, J. Mercier, and F. A. Payne. 1995. Ozone storage effects on anthocyanin content and fungal growth in blackberries. J. Food Sci. 60:1286–1288.
4. Benedict, R. C., T. Partridge, D. Wells, and R. L. Buchanan. 1993. *Bacillus cereus:* aerobic growth kinetics. J. Food Prot. 56:211–214.
5. Beuchat, L. R. 1992. Surface disinfection of raw produce. Dairy Food Environ. Sanit. 12:6–9.
6. Bolin, H. R., A. E. Stafford, A. D. King, Jr., and C. C. Huxsoll. 1977. Factors affecting the storage stability of shredded lettuce. J. Food Sci. 42:1319–1321.
7. Brackett, R. E. 1987. Microbiological consequences of minimally processed fruits and vegetables. J. Food Qual. 10:195–206.
8. Brackett, R. E. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfections. J. Food Prot. 55:808–814.
9. Buchanan, R. L., and J. G. Phillips. 1990. Response surface model for predicting the effects of temperature pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes.* J. Food Prot. 53:370–376.
10. Buchanan, R. L., H. G. Stahl, and R. C. Whiting. 1989. Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes.* J. Food Prot. 52:844–851.
11. Buchanan, R. L., and R. C. Whiting. 1996. Risk assessment and predictive microbiology. J. Food Prot. 59(Suppl.):31–36.
12. Christiansson, A., A. S. Naidu, I. Nilsson, T. Wadstorm, and H. E. Pettersson. 1989. Toxin production by *Bacillus cereus* dairy isolates in milk at low temperatures. Appl. Environ. Microbiol. 55:2595–2600.
13. D' Aoust, J.-Y. 1985. Infective dose of *Salmonella typhimurium* in cheddar cheese. Am. J. Epidemiol. 122:717–720.
14. Gibson, A. M., N. Bratchell, and T. A. Roberts. 1987. The effect of sodium chloride and temperature on the rate and extent of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62:479–490.
15. Gibson, A. M., N. Bratchell, and T. A. Roberts. 1988. Predicting microbial growth of *Salmonella* in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6:155–178.
16. Grag, N., J. J. Churey, and D. F. Splittstoesser. 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. J. Food Prot. 53:701–703.
17. Griffiths, M. W. 1990. Toxin production by psychrotrophic species of *Bacillus* spp. present in milk. J. Food Prot. 53:790–792.
18. Haas, C. N. 1983. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. Am. J. Epidemiol. 118:573–582.
19. Hotta, K., K. Kawaguchi, F. Saitoh, N. Saitoh, K. Suzuki, K. Ochi, and T. Nakayama. 1994. Antimicrobial activity of electrolyzed NaCl solutions: effect on the growth of *Streptomyces* spp. Actinomycetologica 8:51–56.
20. Izumi, H. 1999. Electrolyzed water as a disinfectant for fresh-cut vegetables. J. Food Sci. 64:536–539.
21. Kim, C., Y. C. Hung, and R. E. Brackett. 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food related pathogens. J. Food Prot. 63:19–24.
22. Koseki, S., and K. Itoh. 2000. Effect of acidic electrolyzed water on the microbial counts in shredded vegetables. J. Jpn. Soc. Food Sci. Technol. 47:722–726. (In Japanese.)
23. Koseki, S., and K. Itoh. 2000. Effect of acidic electrolyzed water on the microbial counts in shredded vegetables (Part II)—pretreatment effect of alkaline electrolyzed water. J. Jpn. Soc. Food Sci. Technol. 47:907–913. (In Japanese.)
24. Koseki, S., K. Yshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. J. Food Prot. 64:652–658.
25. McClure, P. J., M. B. Cole, and K. W. Davies. 1994. An example of the stages on the development of a predictive mathematical model for microbial growth: the effects of NaCl, pH and temperature on the growth of *Aeromonas hydrophila.* Int. J. Food Microbiol. 23: 359–375.
26. Nguyen-the, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. 34: 371–401.
27. Okuda, R., H. Sasazaki, M. Kanehira, T. Okabe, S. Abe, A. Tagami, Y. Iwamatsu, Y. Miya, and Y. Shimizu. 1994. Electron microscopical study of bactericidal actions of high oxidation potential water. Jpn. J. Conservative Dent. 37:755–765. (In Japanese.)
28. Palumbo, S. A., A. C. Williams, R. L. Buchanan, and J. G. Phillips. 1991. Model for the aerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. 54:429–435.
29. Palumbo, S. A., A. C. Williams, R. L. Buchanan, and J. G. Phillips. 1992. Model for the anaerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. 55:260–265.
30. Ratokowsky, D. A., R. K. Lowry, T. A. McMeekin, A. N. Stokes,

and R. E. Chandler. 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154: 1222–1226.
31. Riva, M., L. Franzetti, and A. Galli. 2001. Microbial quality and shelf life modeling of ready-to-eat cicorino. J. Food Prot. 64:228–234.
32. Rose, J. B., and C. P. Gebra. 1991. Use of risk assessment for development of microbial standards. Water Sci. Technol. 24:29–34.
33. Spotts, R. A., and L. A. Cervantes. 1992. Effect of ozonated water on postharvest pathogens of pear in laboratory and packing house tests. Plant Dis. 76:256–259.
34. Stecchini, M. L., I. Sarais, and P. Giavedoni. 1993. Effect of essential oils *Aeromonas hydrophia* in a culture medium and in cooked pork. J. Food Prot. 56:406–409.
35. van Netten, P., A. van de Moosdijk, P. van Hoensel, D. A. A. Mossel, and I. Perales. 1990. Psychrotrophic strains of *Bacillus cereus* producing enterotoxin. J. Appl. Bacteriol. 69:73–79.
36. Zaika, L. L., A. H. Kim, and L. Ford. 1991. Effect of sodium nitrite on growth of *Shigella flexneri.* J. Food Prot. 54:424–428.
37. Zaika, L. L., J. G. Phillips, and R. L. Buchanan. 1992. Model for aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride, and sodium nitrite concentrations. J. Food Prot. 55:509–513.