Journal of Food Protection, Vol. 70, No. 11, 2007, Pages 2638–2641

Research Note

Determination of 5-Log Reduction Times for Food Pathogens in Acidified Cucumbers during Storage at 10 and 25°C†‡

FRED BREIDT, JR.,* JANET HAYES, AND ROGER F. MCFEETERS

U.S. Department of Agriculture, Agricultural Research Service and North Carolina Agricultural Research Service, North Carolina State University, Raleigh, North Carolina 27695-7624, USA

MS 07-100: Received 21 February 2007/Accepted 13 June 2007

ABSTRACT

Outbreaks of acid-resistant foodborne pathogens in acid foods with pH values below 4.0, including apple cider and orange juice, have raised concerns about the safety of acidified vegetable products. For acidified vegetable products with pH values between 3.3 and 4.6, previous research has demonstrated that thermal treatments are needed to achieve a 5-log reduction in the numbers of *Escherichia coli* O157:H7, *Listeria monocytogenes,* or *Salmonella enterica.* For some acidified vegetable products with a pH of 3.3 or below, heat processing can result in unacceptable product quality. The purpose of this study was to determine the holding times needed to achieve a 5-log reduction in *E. coli* O157:H7, *L. monocytogenes,* and *S. enterica* strains in acidified vegetable products with acetic acid as the primary acidulant, a pH of 3.3 or below, and a minimum equilibrated temperature of 10°C. We found *E. coli* O157:H7 to be the most acid-resistant microorganism for the conditions tested, with a predicted time to achieve a 5-log reduction in cell numbers at 10°C of 5.7 days, compared with 2.1 days (51 h) for *Salmonella* or 0.5 days (11.2 h) for *Listeria.* At 25°C, the *E. coli* O157:H7 population achieved a 5-log reduction in 1.4 days (34.3 h).

Acidified foods are defined as low-acid food products to which acid or acid food ingredients have been added. The final pH value for acidified foods must be at or below 4.6 for all ingredients. In the United States, regulations governing the safe manufacture of acidified vegetables were promulgated in 1979 (21 CFR part 114). The purpose of regulating these products, which have an excellent safety record, was to prevent botulism due to improper acidification. It has been shown that spore outgrowth and toxin production by *Clostridium botulinum* will not occur if the pH is maintained below 4.6 *(9, 11).* Recent outbreaks of disease caused by vegetative cells of acid-resistant food pathogens in some acid foods have caused concern about the safety of acidified vegetable products by the U.S. Food and Drug Administration and the acidified vegetable industry.

Outbreaks of disease from *Escherichia coli* O157:H7 and *Salmonella enterica* strains have occurred in apple cider and orange juice *(5, 6),* which typically have pH values between 3.5 and 4.0. In response to these outbreaks, the juice hazard analysis critical control point regulation, 21 CFR part 120, was promulgated in 2001. This regulation mandates a 5-log reduction in acid-resistant bacterial pathogens, including *E. coli* O157:H7, which may be present in juices. Because *E. coli* O157:H7 has been shown to be more heat and acid resistant than other bacterial food pathogens in juices *(10),* most research on the safety of juice products has focused on this organism.

The U.S. Food and Drug Administration has raised concerns about the safety of acidified vegetable products that are not heat processed. The minimum times and temperatures for heat processing acidified cucumbers to ensure safety have been determined *(3).* However, acidified vegetable products with a pH of 3.3 or below may contain enough acid to ensure a 5-log reduction in bacterial pathogens without a heat treatment. These products typically contain 400 mM or greater acetic acid. Some of these products cannot be heat treated and maintain desirable sensory properties. Safety concerns by U.S. Food and Drug Administration and the acidified vegetable industry that *E. coli* O157:H7 could survive in acidified foods that did not receive a heat process are addressed by this study. In this article, we show that in a representative acidified vegetable product (cucumbers) with a pH at or below 3.3, a 5-log reduction in viable cell counts for acid-resistant pathogens occurred without a heat treatment within 6 days. We have shown that manufacturers of acidified foods with pH values at or below 3.3, with acetic acid as the primary acidulent, can therefore safely produce these products without heat treatment.

Temperature has been shown to influence acid killing of bacterial pathogens under conditions typical of acidified vegetables *(1).* Refrigerated products were not considered in this study, however, and they are exempted from the

* Author for correspondence. Tel: 919-513-0186; Fax: 919-513-0180; E-mail: breidt@ncsu.edu.

† Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

‡ Paper FSR07-03 of the journal series of the Department of Food Science, North Carolina State University, Raleigh.

acidified food regulations (21 CFR part 114). We therefore chose the most permissive conditions for survival of bacterial pathogens that are representative of nonpasteurized acidified vegetable products that have a pH at or below 3.3: 10°C, 400 mM acetic acid, and an ionic strength of 0.342 (equivalent to 2% NaCl, typical of many acidified vegetable products). Higher temperatures were not investigated because previous reports have shown that the acid sensitivity of *E. coli* increases with temperature *(1)*.

MATERIALS AND METHODS

Preparation of pickle brines. Size 2B cucumbers were obtained a local supplier. Approximately 9 cucumbers (789 ± 2 g) were packed in 571 ± 2 ml of cover solution in 1.36-liter (46-oz) glass jars to equilibrate at 2% sodium chloride, 0.1% calcium chloride, and pH 3.3. Vinegar (20% acetic acid) was used to achieve the target pH. After filling and sealing, the jars were heat processed so that the cold point of each jar was held at 74.4°C for 15 min. The jars were then cooled with cold tap water to a temperature of ≤40°C and allowed to reach ambient temperature of approximately 25°C in air. The metal jar lids had rubber septa (approximately 15 mm in diameter) inserted to allow aseptic sampling of the cover liquid without opening the jars or introducing contamination. The jars were stored at 25°C for at least 10 days to allow the equilibration of water-soluble components (e.g., acids, salt, sugars) between the cover solution and the cucumbers. Prior to use, the concentrations of organic acids and the pH of the brines in the jars were measured as described below. In some jars, pH values were adjusted aseptically by the addition of a small volume of HCl or NaOH. Jars were equilibrated at the indicated incubation temperatures (10 or 25°C) for a minimum of 48 h prior to inoculation, to allow temperature equilibration (data not shown), and then held at the incubation temperature for the duration of the experiment.

Preparation and handling of bacterial cells. Bacteria used in this experiment consisted of cocktails of five strains for each of three different bacterial species (Table 1). For a given experiment, five strains of a single species were used. Bacteria were grown statically at 37°C for 16 h in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) plus 1% glucose to induce acid resistance *(4)*. Each of the five cultures was grown separately. Cells were harvested by centrifugation, concentrated by resuspending in 1/100 the original volume with sterile saline, and combined into a cocktail with approximately equal concentrations of each bacterial culture. The cell cocktails were added to the jars through the septum to give an initial total cell count of approximately 10^8 CFU/ml in each jar. Jar contents were mixed by manual inversion prior to sampling with approximately 1 ml of brine. At indicated time intervals, samples were removed and serially diluted with 10-fold dilutions prior to plating with a spiral plater (Spiral Biotech, Inc., Norwood, Mass.) on a neutral pH nonselective agar medium, tryptic soy agar (Difco, Becton Dickinson) plus 1% glucose. After 24 to 48 h of incubation at 37°C, colonies were counted with an automated spiral plate counter (Q-Count, Spiral Biotech). The lower detection limit was between 10^2 and 10^3 CFU/ml by this method.

Biochemical analysis. Samples were withdrawn with a 1-ml syringe through the rubber septum in the jar lids for high-performance liquid chromatographic (HPLC) analysis to determine the concentrations of organic acids and the equilibrated pH. The pH was determined with an IQ240 pH meter (IQ Scientific Instruments, San Diego, Calif.). Organic acid concentrations were measured with a Thermo Separation Products HPLC (ThermoQuest, Inc., San Jose, Calif.) system consisting of a P2000 pump, an SCM100 solvent degasser, an AS3000 autosampler, and a UV6000 diode array detector (ThermoQuest). A Bio-Rad HPX-87H column, 300 by 7.8 mm (Bio-Rad Laboratories, Hercules, Calif.), was used to resolve malic, lactic, and acetic acids. The operating conditions of the system included a sample tray at 6°C, a column at 75°C, and 0.03 N H_2SO_4 eluent at a 1-ml/min flow rate. The UV6000 detector was set to 210 nm at a rate of 1 Hz for data collection. ChromQuest version 4.1 chromatography software was used to control the system and analyze the data, utilizing the peak heights for quantitative integration.

TABLE 1. *Bacterial strains*[a]

Strain ID	Strain name	Previous ID	Origin
B0195	*Listeria monocytogenes*	SRCC 529	Pepperoni
B0196	*L. monocytogenes*	SRCC 1791	Yogurt
B0197	*L. monocytogenes*	SRCC 1506	Ice cream
B0198	*L. monocytogenes*	SRCC 1838	Cabbage
B0199	*L. monocytogenes*	SRCC 2075	Diced coleslaw
B0200	*Escherichia coli* O157:H7	ATCC 43888	Human feces
B0201	*E. coli* O157:H7	SRCC 1675	Apple cider outbreak
B0202	*E. coli* O157:H7	SRCC 1486	Salami outbreak
B0203	*E. coli* O157:H7	SRCC 2061	Ground beef
B0204	*E. coli* O157:H7	SRCC 1941	Pork
B0206	*Salmonella* Branderup	SRCC 1093	10% salted yolk
B0207	*Salmonella* Cerro	SRCC 400	Cheese powder
B0208	*Salmonella* Enteritidis	SRCC 1434	Ice cream
B0209	*Salmonella* Newport	SRCC 551	Broccoli with cheese
B0210	*Salmonella* Typhimurium	SRCC 1846	Liquid egg

[a] SRCC strains obtained from Silliker, Inc., Chicago, Ill.; ATCC, American Type Culture Collection, Manassas, Va.; ID, identification.

Modeling and statistical analysis. Survival curves of CFU per milliliter versus time were generated and typically showed nonlinear killing kinetics. For this reason, a Weibull model was used for curve fitting to determine the 5-log reduction times as described by van Boekel *(12)*:

$$\text{Predicted log survivors} = N_0 - [1/\text{Ln}(10)](\tau/\alpha)^{1/\beta}$$

Parameters of the model include the initial cell numbers (N_0) and two shape parameters (α and β). The predicted survivor curve was plotted as log survivors versus time (τ). With parameters α and β, the 5-log reduction times were calculated:

$$\text{Predicted 5-log reduction time} = \alpha\,[-\text{Ln}(10^{-5})]^{\beta}$$

Statistical analysis of the predicted 5-log reduction time versus temperature data was carried out by the NLIN procedure of the SAS program (SAS Institute Inc., Cary, N.C.) to determine the standard errors of the fitted data and the upper standard error for the 5-log reduction estimate *(3)*. We added five times the standard error to the predicted 5-log reduction time, as was done previously for thermal treatments, to ensure at least a 5-log reduction of these pathogens *(3)*. For each bacterial species tested, the data

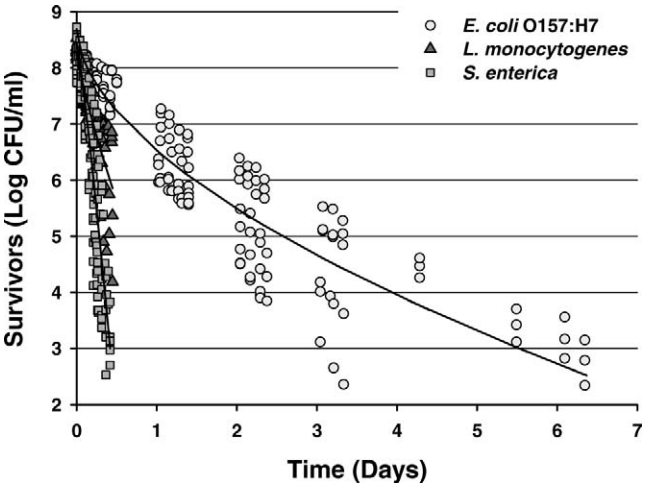

FIGURE 1. *The survival of* E. coli *O157:H7,* S. enterica, *and* L. monocytogenes *strains in acidified pickle jars at 10°C. The data for* E. coli *O157:H7 (circles),* S. enterica *(triangles), and* L. monocytogenes *(squares) show the log of the viable cell count from seven or more replicate experiments, each with a cocktail of five strains of a given species. The solid lines represent the predicted survival curves from the Weibull model.*

from seven or more replicate experiments were used to determine the model parameters.

RESULTS

The initial measured pH for the brined cucumbers varied from 3.31 to 3.14, but all jars were adjusted to pH 3.3 ± 0.05 pH units prior to use. The acetic acid concentration for the brine samples used in these experiments varied between 395 and 445 mM (data not shown). Lactic, propionic, or butyric acid was not detected in the jars before or after the experiments, indicating that no fermentation or spoilage of the pasteurized brined cucumbers had occurred.

Predicted 5-log reduction times were based on the Weibull model for the *E. coli, Salmonella,* and *Listeria* strains at 10°C, which are shown in Figure 1. The 5-log reduction times (Table 2) for *Listeria* and *Salmonella* were 11.2 and 51.3 h, respectively. For *Salmonella,* a 2-log reduction was found within the first 10 h of incubation; however, at 24 h, the cell counts were not detectable (data not shown). For the model, only the recorded viable cell counts were used, resulting in a conservative estimate (by extrapolation) of the 5-log reduction time for *Salmonella.* The predicted 5-log reduction time for *E. coli* O157:H7 was found to be 137.9 h, or 5.75 days. To be consistent with previously reported data *(3),* the 5-log reduction times reported (Table 2) include the addition of five times the standard error. These data show that *E. coli* O157:H7 was much more resistant to the acid conditions at 10°C than *Listeria* or *Salmonella,* requiring more than twice the time needed for a 5-log reduction.

The predicted 5-log reduction time for the survival of *E. coli* O157:H7 at 25°C is shown in Figure 2. By a Weibull model, to be consistent with the 10°C data (above), we determined a 5-log reduction time of 34.3 h. The predicted value included five times the standard error (1.3 h). Data for *Salmonella* and *Listeria* were not included, but we have found that the 5-log reduction times for these organisms are significantly less than for *E. coli* at 25°C (data not shown).

TABLE 2. *The 5-log reduction times of* E. coli *O157:H7,* Salmonella, *and* Listeria *and parameters for Weibull curves*

Parameter[a]	Estimate	SE of the estimate	Predicted 5-log reduction time[b]
E. coli data			
Alpha	8.693	0.157	
Beta	0.567	0.015	
Tstar	105.4 h	6.5 h	137.9 h (5.75 days)
Listeria data			
Alpha	8.448	0.189	
Beta	1.173	0.125	
Tstar	9.3 h	0.4 h	11.2 h
Salmonella data			
Alpha	8.292	0.188	
Beta	0.823	0.175	
Tstar	25.8 h	5.1 h	51.3 h

[a] Parameters for the Weibull model include alpha and beta, parameters controlling the shape of the nonlinear killing curve. Tstar is the time in hours for the predicted 5-log reduction in bacterial cell numbers.

[b] The recommended 5-log reduction time, based on the Tstar estimate plus five times the standard error, as described *(3).*

DISCUSSION

Some acidified vegetable products may have undesirable sensory properties (e.g., softening, off-flavors) when exposed to heat. In addition, some of these vegetable products may be distributed in institutional-size packages (plastic containers or pouches) that cannot be thermally processed. The U.S. Food and Drug Administration expressed safety concerns about the survival of acid-resistant patho-

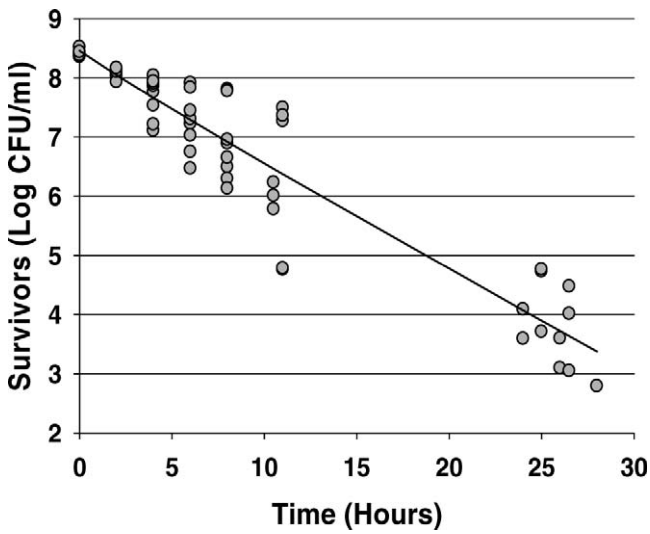

FIGURE 2. *The survival of* E. coli *O157:H7 in acidified pickle jars at 25°C. The data for* E. coli *O157:H7 (circles) show the log of the viable cell count for nine independent replications with a five strain cocktail. The solid line represents the predicted survival curve from the Weibull model.*

gens in these products because of outbreaks of disease in some acid foods, such as apple cider and orange juice. In the United States, some banana pepper or jalapeno pepper ring products may be produced with little or no heat treatment, but they typically have pH values below 3.3 and 400 mM or greater acetic acid. Our objective was to determine if these acidic and low pH conditions were sufficient to ensure a 5-log reduction of *E. coli* O157:H7 and other acid-resistant pathogens. However, peppers have been found to contain components with antimicrobial activity *(7, 8).* We have observed *(2)* that acidified banana and sweet peppers are more resistant to microbial spoilage than cucumbers, and we have no evidence for natural antimicrobial activity in brined cucumbers. Therefore, to test the most permissive conditions for survival of acid-tolerant pathogens, cucumbers were used to investigate the die-off of acid-tolerant pathogens. We found that a 5-log reduction in bacterial pathogen numbers (CFU per milliliter) required 5.7 days at a temperature of 10°C (50°F) or greater and required 1.4 days at 25°C (77°F) with acetic acid as the primary acidulant.

The data obtained in this study have shown that a 5-log reduction in the cell numbers of acid-resistant pathogens, including *E. coli* O157:H7, *Salmonella,* and *Listeria,* can be achieved in the absence of a heat treatment for cucumbers equilibrated with acetic acid to give a pH of 3.3. The study was carried out with acidified cucumbers under the most permissive conditions for the survival of the pathogens that would be typical of acidified vegetable products with a pH of 3.3 or below. The variance observed among the replications of the survival curves may reflect the variance in the brine pH and acid concentration that resulted in individual jars of cucumbers, but further research will be needed to determine this. Although there have been no known outbreaks of acid-resistant pathogens in acidified vegetable products, fruit juices with pH values (3.5 to 4.0) similar to some acidified vegetable products have had outbreaks of disease resulting from *E. coli* O157:H7 contamination *(5, 6).* The results reported in the present study may be used to help maintain the long history of safe production of acidified vegetable products.

ACKNOWLEDGMENTS

We thank Dr. Jason Osborne for help with statistical analysis and Ms. Sandra L. Parker for excellent secretarial assistance. This investigation was supported in part by a research grant from Pickle Packers International, Inc., Washington, D.C.

REFERENCES

1. Breidt, F., Jr., J. S. Hayes, and R. F. McFeeters. 2004. Independent effects of acetic acid and pH on survival of *Escherichia coli* in simulated acidified pickle products. *J. Food Prot.* 67:12–18.
2. Breidt, F., Jr., J. S. Hayes, and R. F. McFeeters. Unpublished data.
3. Breidt, F., Jr., J. S. Hayes, J. A. Osborne, and R. F. McFeeters. 2005. Determination of 5-log pathogen reduction times for heat-processed acidified foods. *J. Food Prot.* 68:305–310.
4. Buchanan, R. L., and S. G. Edelson. 1999. pH-dependent stationary phase acid resistance response of enterohemorrhagic *Escherichia coli* in the presence of various acidulants. *J. Food Prot.* 62:211–218.
5. Centers for Disease Control and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. *Morb. Mortal. Wkly. Rep.* 45: 975.
6. Centers for Disease Control and Prevention. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice—United States and Canada, June 1999. *Morb. Mortal. Wkly. Rep.* 48. 27:582–585.
7. Cichewicz, R. H., and P. A. Thorpe. 1996. The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in Mayan medicine. *J. Ethnopharmacol.* 52:61–70.
8. De Marino, S., N. Borbone, F. Gala, F. Zollo, G. Fico, R. Pagiotti, and M. Iorizzi. 2006. New constituents of sweet *Capsicum annum* L. fruits and evaluation of their biological activity. *J. Agric. Food Chem.* 54:7508–7516.
9. Ito, K. A., J. K. Chen, P. A. Lerke, M. L. Seeger, and J. A. Unverferth. 1976. Effect of acid and salt concentration in fresh-pack pickles on the growth of *Clostridium botulinum* spores. *Appl. Environ. Microbiol.* 32:121–124.
10. Mazzotta, A. S. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* in fruit juices. *J. Food Prot.* 64:315–320.
11. Townsend, C. T., L. Yee, and W. A. Mercer. 1954. Inhibition of the growth of *Clostridium botulinum* by acidification. *Food Res.* 19:536–542.
12. van Boekel, M.A.J.S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol.* 74:139–159.