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Independent Effects of Acetic Acid and pH on Survival of *Escherichia coli* in Simulated Acidified Pickle Products†‡

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ABSTRACT

Our objective was to determine the effects of organic acids and pH on the rate at which selected strains of *Escherichia coli* O157:H7 die in acid solutions representative of acidified pickle products (pH < 4.6). We used gluconic acid/sodium gluconate (pK_a = 3.7) as a noninhibitory buffer to maintain pH at selected values in the absence of other organic acids. This was possible because we found that the inhibitory effects of this acid on *E. coli* strains at pH 3.1 were independent of acid concentration over a range of 2 to 200 mM. By this method, the lethal effects of acetic acid solutions (100 to 400 mM) at selected pH values between 3.1 and 4.1 were compared with the effects of pH alone (as determined using gluconate buffer). We found *D*-values were two- to fourfold lower with acetic acid compared with the effect of pH alone for simulated pickle brines in this pH range. Glutamic acid, an amino acid that is known to enhance acid resistance in *E. coli* and is a component of pickle brines, protected the *E. coli* strains from the specific effects of acetic acid.

Organic acids (OA) and their conjugate bases have widespread application for preventing food spoilage in fermented and acid or acidified foods *(5, 38, 41).* The U.S. Food and Drug Administration regulations, however, do not allow the use of preservatives (such as benzoate) as the primary barriers to the growth of microbial pathogens in acidified foods. For these food products, the Code of Federal Regulations (21 CFR part 114) states only that acid or acid ingredients must be added so that the pH is maintained at or below 4.6; a heat treatment must be included in the process, if necessary, to prevent the growth of microbial pathogens. These regulations were designed to control the growth and toxin production by *Clostridium botulinum.* The regulations do not take into account the amount or type of OA present in acidified foods. After recent outbreaks of *Escherichia coli* O157:H7 in apple cider and *Salmonella* in orange juice *(8, 9),* products that have pH values similar to many acidified pickle products, the Food and Drug Administration in 2001 proposed the requirement that all new process filings for acidified foods should include a heating or pasteurization step. Of primary concern was *E. coli* O157:H7 because of its low infectious dose and lethal sequelae that can result from infection *(13, 19). E. coli* and other food pathogens have been shown to have inducible acid resistance mechanisms *(7, 16, 40, 44, 45).* If only pH is considered, acid-resistant pathogens might, therefore, pose a potential threat to acidified foods. Because acidified food products have been produced safely for many years without heat treatments *(40),* it is likely that the OA present in these products has contributed to their excellent safety record, although quantitative measurements of the independent effects of OA and pH on the killing of pathogens in these products are lacking.

The effects of pH and selected OA on the growth and death of pathogenic bacteria in acid and acidified foods has been investigated in a variety of products, including apple cider *(21, 28, 36, 39, 46),* mayonnaise, dressings and condiments *(25, 34, 40, 45),* and fermented meats *(14, 15, 18, 32, 35).* A complicating factor in the study of acid inhibition of microorganisms is that the concentration of the undissociated (protonated) form of OA, which can diffuse across bacterial cell membranes, and pH are interdependent variables linked by the Henderson-Hasselbalch equation. OA (type, concentration) and pH can both independently affect the growth and death of bacterial cells *(30),* or they can interact. For example, reported pH values for the inhibition of growth of *E. coli* O157:H7 were over 1 pH unit apart, depending on whether acetic acid (MIC = pH 5.5) or HCl (MIC = pH 4.5) was used *(27).* In addition, the inhibitory effects of OA can be modulated by factors other than pH. Temperature is a primary factor influencing OA activity, with increasing temperature typically resulting in increasing effectiveness of OA *(4, 22, 33, 46).* Other factors affecting acid inhibition of microbial pathogens include: specific effects of the acid or acid anion on cellular enzymes or membranes, the internal pH values and buffering capacity of cells, proton pumping at the expense of cellular ATP, and facilitated transport of acid molecules *(1, 11, 22, 43).* A review by Shelef *(38)* cites the effects of lactic acid and lactate salts on the survival of bacteria, due to chelation of iron, and inhibition of lactate dehydrogenase.

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Interpretation of the reported results from acid inhibition studies is confounded by variations in the sensitivity of the bacterial strains tested and variation in experimental conditions. Experimental variables include pH, concentration and type of OA, ionic strength, and temperature. For example, Young and Foegeding *(48)* showed that for given pH values ranging from 4.7 to 6.0, the order of effectiveness of OA inhibiting the growth of *Listeria monocytogenes* in brain heart infusion broth on an equimolar basis was acetic > lactic > citric. However, with the same data, if the effectiveness was based on initial concentration of undissociated acid, the order was reversed. When Ostling and Lindgren *(29)* determined MICs for the inhibition of *L. monocytogenes* by lactic, acetic, and formic acids, they found (undissociated) lactic acid was the most inhibitory, with an MIC of less than 4 mM for aerobic growth and less than 1 mM for anaerobic growth. An MIC for the inhibition of growth of *Listeria innocua* was reported as 217 mM sodium lactate at pH 5.5 *(20).* This corresponded to a concentration of about 5 mM protonated lactic acid. More recently, Buchanan and Edelson *(6)* looked at the effects of citric, malic, lactic, and acetic acids on cell viability of *E. coli* O157:H7 at a fixed acid concentration of 0.5% (wt/vol). For nine strains, lactic acid was the most effective at reducing the viable cell population, and HCl was the least effective, with the order of acetic, citric, and malic acid sensitivity varying in a strain-dependent manner. In the same year, a study *(10)* that used growth/no-growth assay showed that the order of inhibition of a mixture of three *E. coli* O157:H7 strains by OA at selected pH values was acetic > citric > malic. In these experiments, acid was added until the desired pH value was reached. We focused our studies on acetic acid, which is the primary acidulant used in many acidified pickle products.

The objectives of this study were to determine the specific effects of acetic acid concentration and pH (independently and combined) on the survival of *E. coli* O157:H7 in conditions typical of commercial acidified pickle products. To determine the inhibitory effects of pH in the absence of acetic acid, we used sodium gluconate as a noninhibitory buffer to maintain a pH at values in the range of 3.1 to 4.1.

MATERIALS AND METHODS

Bacterial strains and growth media. *E. coli* B179 (O157:H7 strain 87-23, tox$^-$) was kindly provided by Dr. Alison O'Brien (Uniformed Services University, Bethesda, Md.), *E. coli* strains B185 (serotype O29:NM, ATCC 43892) and B194 (serotype O157:H7, ATCC 43895) and *E. coli* strain B187 (K12, ATCC 10798) were obtained from the U.S. Food Fermentation Laboratory Culture Collection (USDA-ARS, Raleigh, N.C.). Bacterial strains were grown overnight on tryptic soy broth or agar (Difco Laboratories, Detroit Mich.) supplemented with 10 g/liter glucose (Sigma Chemical Co., St. Louis, Mo.). For acid inhibition experiments, each culture was grown statically overnight for 15 h in tryptic soy broth–glucose at 37°C to induce acid resistance. Cells were harvested by centrifugation and resuspended in an equal volume of 8.5 g/liter NaCl (saline), and combined cultures were diluted 100-fold into a final volume of 20 ml of acid solution as described below. Cells were enumerated from the acid suspensions by plating on nonselective tryptic soy agar–glucose with a spiral plater (Model 4000, Spiral Biotech, Inc., Norwood, Mass.) and an automated plate reader (QCount, Spiral Biotech). The lower limit of detection for this method was 400 CFU/ml. Cucumber juice (CJ) broth was prepared with washed size 2B pickling cucumbers (~36 mm in diameter), which were blended to homogeneity. The slurry was mixed with an equal volume of rice hulls and pressed with a custom wine press (Fruit and Vegetable Processing Laboratory, North Carolina State University, Raleigh, N.C.) to express juice. The raw juice was then stored at −20°C. CJ medium was prepared from frozen juice by thawing and heating until the temperature reached 80 to 85°C. The juice was rapidly cooled and centrifuged at 23,500 × *g* for 20 min at 4°C in 250-ml bottles (Sorvall GSA rotor, Kendro Laboratory Products, Newton Conn.). The CJ medium was sterilized by filtration (#430624 bottle filters, Corning Inc., Acton, Mass.) and stored at 4°C. This medium was added to acid solutions where indicated below to simulate the typical brine conditions of fresh-packed, acidified (nonfermented) pickles.

TABLE 1. *Acid–pH conditions for inhibition experiments*

Initial pH[a]	Acetic concentration (mM)	Protonated acetic acid (mM)
3.1	0	0
3.7	0	0
3.7[b]	0	0
4.1	0	0
3.1	400	330
3.7	100	50
3.7[b]	200	110
4.1	100	30

[a] All solutions contained 20 mM gluconate as a buffer, which was required to maintain pH in the treatments with no acetic acid.
[b] Isomerose (250 g/liter; Isosweet 100 high fructose corn syrup; Staley Manufacturing, Decatur, Ill.) was added.

Acid inhibition experiments. Acid solutions were prepared with acetic and gluconic acid (Sigma), and the pH was adjusted with HCl to the indicated values. NaCl was added to all acid treatments to give a final resulting ionic strength of 0.37, which is equivalent to 20 g/liter NaCl (2% NaCl), the typical salt concentration in many acidified pickle products. Gluconic acid was added to 20 mM for acid killing experiments, except where otherwise indicated. The total ionic strength was determined by summing the acid anion contributions of both gluconic and acetic acids at the different acid and pH combinations (calculated using the Henderson-Hasselbalch equation), and adjusted to 0.37 by adding NaCl. Data from a previous experiment indicated that ionic strength, under acid conditions used in this report, can influence the survival of bacteria *(3).* Acid and pH conditions typical of acidified pickle products were prepared using gluconate as a buffer to control pH (in all treatments) as indicated in Table 1. Additional treatments included solutions of pH 3.1, 400 mM acetic acid with final ionic strength adjusted to 0.37 (with NaCl, as described above) containing one or more of the following: 60% (vol/vol) CJ, 55 mM glucose or fructose, and 1 mM glutamic acid or arginine.

Cells were added to the acid solutions immediately before dispensing 20 ml into sterile Vacutainer tubes (16 by 165 mm, #366433, BD Biosciences, San Jose, Calif.). Tubes were placed in a plastic rack in a heating/cooling water bath (RTE-211, Neslab Instruments Inc., Newington, N.H.) at 10, 20, or 30°C, as indi-

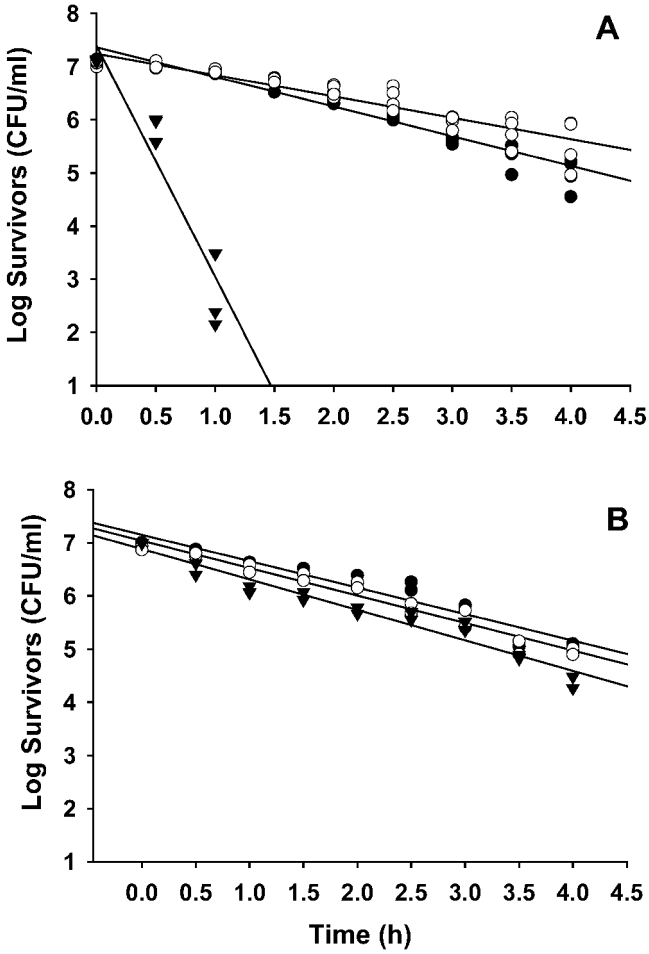

FIGURE 1. *Data for the survival of* E. coli *strains at 25°C in acetic (A) and gluconic (B) acids for three concentrations of acid: filled circles, 2 mM; open circles, 20 mM; triangles, 200 mM. The pH and ionic strength for all treatments was held at 3.1 and 0.37 (respectively) with NaCl to adjust ionic strength. Regression lines were determined from two or more independent trials.*

cated. Samples (1 ml) were removed aseptically at the indicated times with a 1-ml syringe through the septum top of the Vacutainer tube. Samples were diluted appropriately in sterile saline and plated on nonselective agar (tryptic soy agar–glucose) as described above. To prepare samples for biochemical analysis, cells were removed by centrifugation at 12,000 × *g* in a microcentrifuge (model 16KM, Fisher Scientific, Hampton, N.H.), and the supernatant was frozen at −20°C prior to high-performance liquid chromatography analysis.

Biochemical analysis. The acids (gluconic and acetic) and sugars (glucose and fructose) were separated with a Bio-Rad HPX-87H 30-cm acid column (Bio-Rad Laboratories, Hercules, Calif.). The chromatographic system included a P2000 pump, an AS300 autosampler, and an SCM 1000 vacuum membrane degasser (ThermoQuest, Inc., San Jose, Calif.). A Waters 410 Differential Refractometer was used for quantitation of the sugars and alcohols (Waters Associates, Milford, Mass.). A UV6000 diode array detector (ThermoQuest) with a 50-mm light path was used for quantitation of the acids. The eluent was 0.03 N sulfuric acid with a flow rate of 0.6 ml/min, and the column was held at 52°C. Sample vials were maintained at 8°C in the autosampler tray. The ChromQuest program (ThermoQuest) was used to control the equipment and analyze the data.

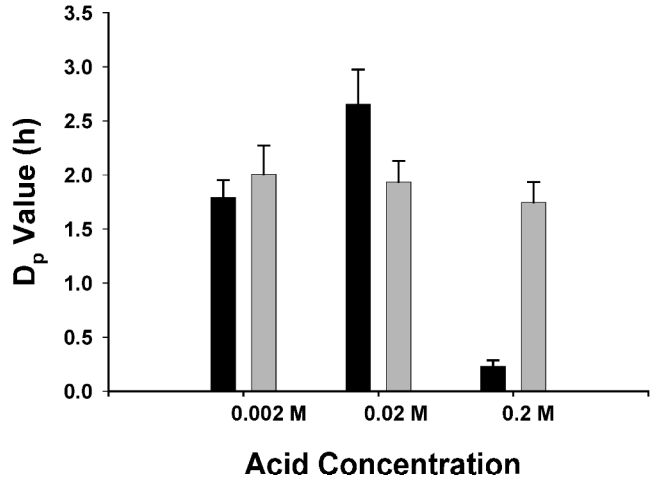

FIGURE 2. D_p *values (*D*-value for a population) for the survival of* E. coli *strains at 25°C in acetic (black bars) and gluconic (gray bars) acids at 2, 20, and 200 mM. The pH and ionic strength for all treatments was held at 3.1 and 0.37 (respectively) with NaCl to adjust ionic strength. The error bars indicate the upper 95% confidence intervals for two or more independent trials.*

Statistical analysis and death rate calculations. *D*-values were determined as the negative inverse of the slope of the regression line from linear survivor curves (log CFU/ml versus time data). Because a cocktail of *E. coli* strains was used for these values, we reported D_p-values, to denote the *D*-value for a population of bacterial strains *(2).* To determine the slope of the regression line and corresponding standard error, the LINEST function from Excel (Microsoft Office XP, Microsoft, Redmond, Wash.) was used. In simple linear regression, the least squares and maximum likelihood estimate of the slope are the same; therefore, the 95% upper confidence limits for the slope were calculated using the 95 percentile of the Student's *t* distribution. To determine the upper 95% confidence limit for D_p-values, the negative inverse of confidence limit for the slope was used. For nonlinear survivor curves, survival data was modeled with a Weibull model, and 5-log reduction times were calculated as described *(47).* Statistical inferences concerning the similarity of D_p-values were determined with the general linear models procedure of SAS (SAS Institute Inc., Cary, N.C.).

RESULTS

To determine the inhibitory effects of pH independent of OA, a noninhibitory buffer with a pK value around 4.0 or lower was needed. Gluconate was considered for this role because it is a polar molecule that might have difficulty traversing bacterial membranes, even in the protonated form. Survival curves for a cocktail of *E. coli* strains in the presence of acetic acid and gluconate (or gluconic acid) were generated at 25°C and pH 3.1 using a 100-fold range of concentrations (2 to 200 mM; Fig. 1). Because some acid was required to buffer pH, the lowest acid concentration used was 2 mM. We found that the D_p-values (*D*-value for a population of strains as described in *(2)*), determined for gluconate at each concentration were not significantly different ($P > 0.05$), ranging from 1.75 to 2.0 h (Fig. 2). However, the D_p-value for 200 mM acetic acid (0.23 h) was about 8- to 10-fold lower than that for 2 or 20 mM (which were 1.79 and 2.66 h, respectively; Fig. 2). Inter-

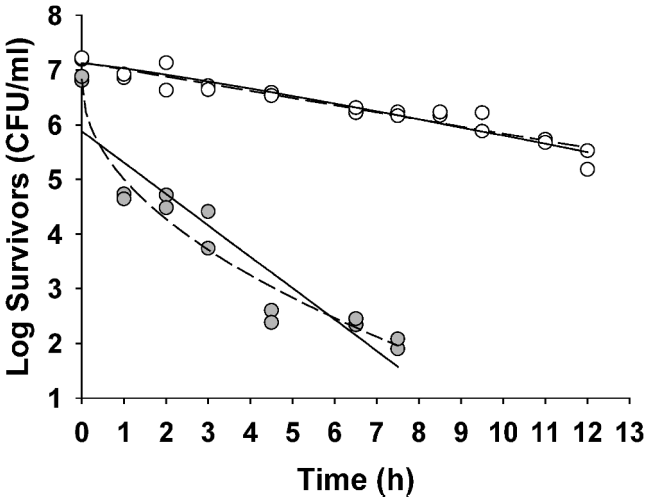

FIGURE 3. *Survival curves for* E. coli *strains in the presence (gray circles) or absence (open circles) of 400 mM acetic acid at 20°C. The pH and ionic strength for all treatments was held at 3.1 and 0.37 (respectively) with NaCl to adjust ionic strength. Survival data were modeled with a linear (solid line) or Weibull model (dashed line) from two independent trials.*

estingly, we consistently saw an increase in the D_p-value recorded for acetic acid at 20 mM when compared with 2 mM under the conditions of this experiment. The mechanism responsible for this increase in survival at the intermediate acetic acid concentration remains to be investigated. From these data, we concluded that the survival curves observed for gluconate were primarily influenced by pH because the D_p-values were independent of gluconate concentration. Results from high-performance liquid chromatography analysis showed no change in gluconate during these experiments (not shown). Gluconate was, therefore, used in subsequent experiments as a buffer at 20 mM to determine the effects of pH on the survival of *E. coli* at pH values between 3.1 and 4.1.

The effect of 400 mM acetic acid on the survival of the cocktail of *E. coli* strains at pH 3.1, 20°C, was determined with the use of gluconate as a buffer to control pH for a control treatment with no acetic acid (Fig. 3). This acetic acid concentration was chosen because it is typical of acidified pickle products with a pH of 3.1 *(26).* The treatment with acetic acid showed approximately fourfold lower 5-log reduction times when either a linear (7.9 versus 32.5 h) or Weibull model (8.7 versus 38.4 h) was used (Table 2). The difference in reduction times observed for these treatments, therefore, measured the effect of acetic acid in combination with pH versus the effect of pH alone.

The effect of acetic acid and pH in conditions typical of acidified pickle products on the survival of the cocktail of *E. coli* strains was tested at three temperatures: 10, 20, and 30°C. For all treatments (Table 1), gluconate was used as a buffer to control pH, and the ionic strength was held constant at 0.37, as described above. The D_p-values for the survival data are presented in Figure 4. In each case, with the exception of treatments giving D_p-values greater than 40 h, the survivor curves (not shown) were modeled by linear regression over a range of 2 or more log cycle reductions. For the acid and pH treatments shown in Figure 4, the D_p-values were between two- and fourfold lower when the treatments with acetic acid were compared with those without acetic acid. One exception was the pH 4.1 treatment at 20°C. For that treatment, the difference in D_p-values was only 1.6-fold lower with the addition of acetic acid. The data show that temperature played an important role in the survival of the *E. coli* strains, with the greatest survival at 10°C for all conditions tested. For three of the treatments, D_p-values were not obtained because either the cells died too quickly (the pH 3.7 treatment with acetic acid at 30°C) or too slowly (10°C, pH pH 4.1 treatments) for accurate measurements. The initial and final concentrations of gluconic and acetic acids were measured for all treatments, and no differences were found (not shown).

We used a simulated pickle brine containing CJ to determine the effect of pickle brine components, representative of a fresh pack (nonfermented, acidified) pickle product, on the survival of the cocktail of *E. coli* strains (Fig. 5). At 20°C, the addition of CJ resulted in an increase in

TABLE 2. *Modeling the effect of acetic acid on* Escherichia coli *O157:H7 at pH 3.1*

	Weibull model[a]			Linear model[b]	
Parameter	With acetic acid	Without acetic acid	Parameter	With acetic acid	Without acetic acid
Alpha	0.053	4.091	Slope	−0.574	−0.130
Beta	0.488	1.178	Intercept	5.876	7.137
N_0 (CFU/ml)	6.821	7.073	R^2	0.867	0.919
RSS	1.593	0.497	D_p-value (h)	1.743	7.673
RT_5 (h)	7.935	32.53	CI (h)	0.44	1.03
			RSS	4.757	0.511
			RT_5 (h)	8.715	38.37

[a] Weibull model data include alpha and beta parameters for *E. coli* strains in 0.4 M acetic acid at 20°C. The pH and ionic strength for all treatments was held at 3.1 and 0.37 (respectively) with NaCl to adjust ionic strength. N_0, the initial cell number; RSS, the residual sum of squared errors; RT_5, the predicted 5-log reduction time.

[b] Linear model data include slope and intercept parameters, the R^2 value for the regression, the predicted D_p-value, the upper 95% confidence interval (CI), the residual sum of the squared errors (RSS), and the predicted 5-log reduction time (RT_5) calculated as described in van Boekel *(45).*

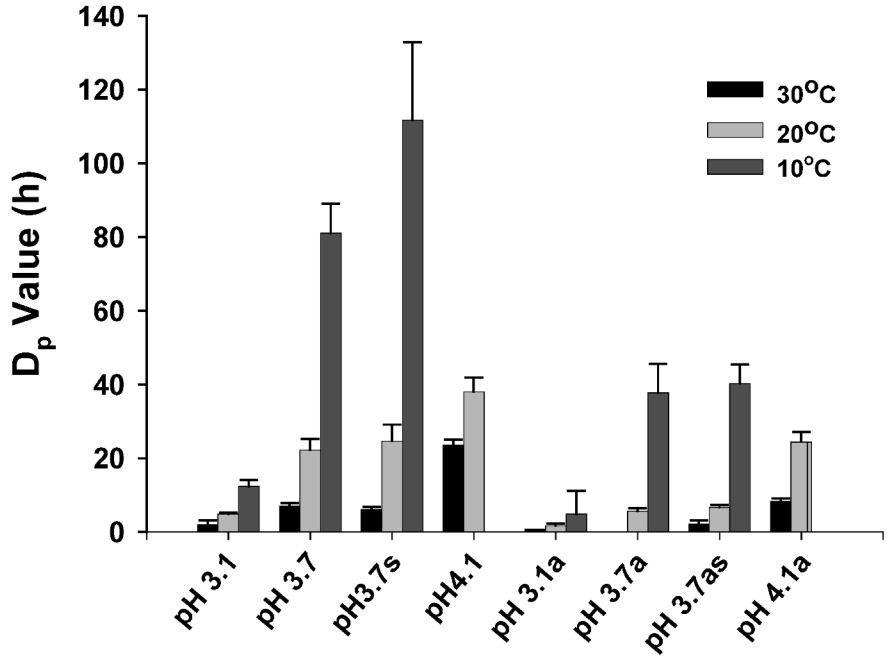

FIGURE 4. D_p *values (*D*-value for a population) for the survival of* E. coli *strains in selected pH and acid conditions specified in Table 2 at 10°C (dark gray bar), 20°C (light gray bar), and 30°C (black bar). Treatment pH values are indicated on the* x*-axis. s, Treatments contained 250 g/liter isomerose; a, treatments contained acetic acid corresponding to the values in Table 2. The error bars indicate the upper 95% confidence intervals for two or more independent trials.*

the survival of the cells (D_p = 5.88) in the presence of 400 mM acetic acid at pH 3.1 when compared with similarly treated cells with only acetic acid (D_p = 3.10). In an effort to determine which components of CJ might be responsible for the increased survival of *E. coli* with CJ, we added compounds known to be in CJ *(24),* including selected amino acids at 1 mM concentration, as well as the sugars glucose and fructose at 55 mM. Of these compounds, glutamic acid and arginine, which have previously been shown to influence the survival of bacteria in the presence of acid conditions *(23)* were both found to significantly increase the D_p-values ($P < 0.05$) observed for treatments with just acetic acid. The D_p-values for the glutamic acid and arginine treatments were 14.37 and 4.87, respectively, when compared with acetic acid alone (D_p = 3.10). However, combining the two amino acids did not result in any significant difference from the treatment with glutamic acid alone ($P > 0.05$). From these data, we concluded that amino acids present in CJ could be one of the factors that contributed to the increased survival of *E. coli* in CJ, whereas addition of sugars did not increase survival compared with the acetic acid treatment alone.

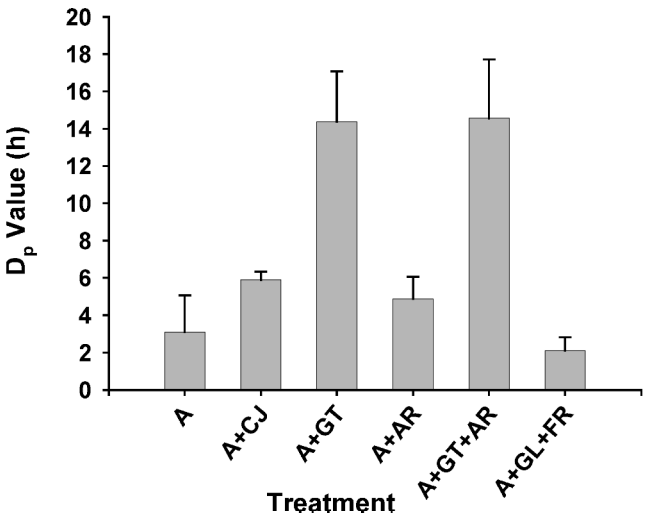

FIGURE 5. *Effect of cucumber juice (CJ) and selected components of CJ on the survival of* E. coli *strains* (D_p*-values,* D*-value for a population) at pH 3.1, 20°C. Acetic acid (A) was present in all treatments at 400 mM. Treatments include (singly or in combination as indicated): CJ (60% vol/vol), amino acids GT (glutamic acid, 1 mM) and AR (arginine, 1 mM), and sugars GL (glucose, 55 mM) and FR (fructose, 55 mM). The error bars indicate the upper 95% confidence intervals from two or more independent trials.*

DISCUSSION

Regulations (21 CFR part 114) were established in 1979 to ensure the safe production of acidified foods consumed in the United States. However at the time, no vegetative pathogenic organisms were known to survive at or below pH 4.6, which was required to prevent spore outgrowth and toxin production by *C. botulinum.* The range of acidified food products has continued to expand in the 20-plus years since the regulation was promulgated. A number of acidified foods are currently produced without heat processing and some might be at risk from acid-resistant food pathogens. The pH and OA conditions required to make these products microbiologically shelf stable must also have killed or at least prevented any growth of vegetative microorganisms. However, scientific data are lacking to define the role of acid independent of pH to determine conditions that will assure the destruction of acid-resistant pathogens in acidified foods without a heat treatment.

To address the safety concerns of the U.S. Food and Drug Administration and the acidified foods industry, we investigated the specific effects of pH independent of inhibitory OA with the use of gluconate buffer to control pH. We then investigated how the addition of acetic acid at a given pH alters survival of *E. coli.* Gluconate was selected because the survival of *E. coli* strains at pH 3.1 (Fig. 1)

was found to be independent of the concentration of this acid. Although gluconic acid has been investigated for use as an antimicrobial agent in meats *(17, 42),* it has not proven to be as effective as acetic or lactic acid. Our research indicates that the antimicrobial effects of gluconic acid (or its conjugate base, gluconate) solutions at pH values between 3 and 4 might be primarily a result of pH, rather than specific effects of the compound itself.

For most of the treatments, coefficients of determination (R^2) were greater than 0.9 over a 2-log cycle or more reduction in cell numbers. Only the pH 3.1 treatments with acetic acid had R^2 values lower than 0.9. A Weibull model *(31)* showed a better fit for these data because the residual sum of squares error term was lower when compared with the linear model (1.59 versus 4.76, or 0.50 and 0.51 for treatments with and without acid, respectively; Table 2). However, the linear model was chosen because it allowed a simple and direct comparison of the reduction times for all the data presented and simplified the statistical analysis of the data.

By using gluconate as a noninhibitory buffer, we were able to determine the inhibitory effects of pH and the combined effects of pH and acetic acid for a range of conditions typically found in acidified pickle products. The specific inhibitory effect of acetic acid was measured as the difference between the D_p-values determined for a given set of pH and temperature conditions with and without acetic acid. For each treatment, increasing temperature resulted in a reduced D_p-value. These results are in agreement with previous studies on the relationship between temperature and the inhibitory effects of OA on foodborne pathogens *(4, 21, 33, 46).* Although we expected to see a decrease in the D_p-values with the addition of acetic acid, we found gluconic acid did not significantly decrease D_p-values. The gluconate buffer might, therefore, have wider application as a noninhibitory buffer for similar experiments with other OA, although some specific effects of gluconate cannot be ruled out by one experiment.

The primary bactericidal actions of OA have long been assumed to be the acidification of cell cytoplasm facilitated by the diffusion of uncharged protonated acid across bacterial membranes. To test the theory that acetic acid acted as an uncoupler, allowing acidification of the cell cytoplasm, Diez-Gonzalez and Russell *(12)* compared the effects of acetic acid and the uncoupler carbonylcyanide-*m*-chlorophenylhydrazone. The acetic acid had very little or no effect on intracellular ATP levels, even at concentrations greater than 200 mM. An alternative explanation for the inhibitory effects of OA is the accumulation of acid anion in the interior of bacterial cells because of higher internal pH than the external environment *(37).* Depending on the internal to external pH difference, molar quantities of acid anion (which should not diffuse through bacterial membranes) might be predicted inside cells, greatly increasing the internal ionic strength *(37).* Additional specific effects of OA on bacterial cell survival could vary with the type and concentration of acid, and might include the inhibition of cellular metabolic enzymes such as lactate dehydrogenase, transport proteins, membrane function, and chelating metal ions *(1, 11, 12, 22, 38, 43).* The inhibitory activity of acetic acid on the survival of the cocktail of *E. coli* strains could be due to one or more of these effects.

We have demonstrated that acetic acid can significantly decrease the survival time for the *E. coli* strains tested at a given pH when compared with effects attributed to pH alone. This was true for a range of pH values between 3.1 and 4.1 with acetic acid concentrations between 100 and 400 mM, which are typical of acidified pickle products (unpublished data). Additional research will be needed to determine the survival of *E. coli* in specific acidified food products because many factors can affect survival of *E. coli* strains in foods.

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