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Survival Characteristics of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in Pickling Solutions at 5°C

ABSTRACT

There has been a surge of interest in pickling at retail food establishments. We investigated the time to a 5-log reduction of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in organic acid solutions containing 25 mM fumaric acid, 5 mM benzoic acid and 2% NaCl, pH 3.8, at 5°C. *E. coli* O157:H7 survived significantly longer than *L. monocytogenes* or *Salmonella* ($P < 0.05$). Mean decimal reduction values ranged from 10.52 h for *Salmonella* in malic acid solution to 56.40 h for *E. coli* O157:H7 in citric acid. Across acid solutions, pH 3.8, we noticed some significant differences in survival, e.g., acetic acid was significantly more lethal than citric acid ($P < 0.05$) to *E. coli* O157:H7. No pathogen reduction was observed at 5°C over four days in acid solutions that did not contain added fumaric and benzoic acid. A retailer who formulates a pickling brine with 25 mM fumaric acid, 5 mM benzoic acid and 2% NaCl, with a pH of 3.8 or below, using citric acid as the acidulant, would establish a holding period of at least 282 h at 5°C to achieve a 5-log pathogen reduction. Similarly, a holding time of at least 237 h would ensure a 5-log pathogen reduction in acetic acid-acidified brine.

INTRODUCTION

On-site manufacturing and preparation of food in retail food establishments has increased because of the demand for locally prepared and sourced foods. According to media reports, high-end restaurants across the United States have embraced pickling, combining seasonal fruits and vegetables with unique spices to create menu appeal *(35, 36)*. Some retail establishments are preparing traditional shelf-stable pickles, while others, wishing to avoid the quality changes associated with heat processing, are manufacturing refrigerated pickles. In 2004, Reina et al. estimated that about 25% of all pickle sales were refrigerated pickles *(29)*. Despite the appeal of these products, outbreaks of illness from *Escherichia coli* O157:H7 have been traced to pickled products manufactured without a heat step. An outbreak of *E. coli* O157 infection from the consumption of pickled napa cabbage, reported in 2012, affected 169 people in Japan. A subsequent outbreak in 2014, also in Japan, was linked to

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pickled cucumber and caused 510 people to be ill *(34)*. In the United Kingdom, also in 2014, 20 people became ill after eating slaw garnish contaminated with *E. coli* O157 *(11)*. Taguchi and colleagues examined the prevalence of Shiga toxin-producing *E. coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes* in 100 retail pickled vegetable products in Japan and detected *L. monocytogenes* in 12 samples *(34)*. A review of the steps used in manufacturing these pickles indicated that vegetables were cut, disinfected, and water-rinsed (in various orders), followed by holding at 10°C and packaging; no heat treatment was applied.

Lee *(22)* described the manufacture of a typical refrigerated pickle, an overnight or refrigerated dill, this way, "The refrigerated dill is essentially a non-heated, well-acidified, low-salt content, refrigerated green cucumber, containing one or more preservatives with spices and flavoring." In the absence of a heating step, refrigerated pickles rely on a combination of pH/acid and low-temperature to help protect public health *(23)*.

Breidt and colleagues determined the holding times needed to achieve a 5-log reduction of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella enterica* in acidified vegetable products at pH 3.3, with acetic acid as the primary acidulant, during storage at 10 and 25°C. Data showed that *E. coli* O157:H7 was much more resistant to acid conditions at 10°C than *Listeria* or *Salmonella*, with a predicted 5-log reduction time of 5.7 days for *E. coli* O157:H7, compared with 2.1 days for *Salmonella* and 0.5 day for *L. monocytogenes*. At 25°C, the *E. coli* O157:H7 population achieved a 5-log reduction in 1.4 days *(6)*. Subsequently, Lu and colleagues developed a novel refrigerated pickle brine containing 25 mM fumaric acid, 5 mM benzoic acid, 70 mM acetic acid and 2% sodium chloride, pH 3.8. In this brine, a 5-log reduction of *E. coli* O157:H7 was achieved in 8.83 days at 10°C *(25)*.

Retail food establishments must maintain potentially hazardous foods under refrigeration, at ≤ 5°C *(37)*. We investigated whether the work of Lu and colleagues *(25)* could be adapted to support pickling operations in retail food establishments. Our goal was to establish the time needed to achieve a 5-log reduction of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in organic acid pickling solutions at a maximum refrigeration temperature of 5°C. Because one hallmark of refrigerator pickles is the lack of a thermal process, we also briefly investigated the survival of typical native microbiota in the same organic acid solutions.

MATERIALS AND METHODS

Strain selection and maintenance

Strains of *E. coli* O157:H7, *S. enterica*, *L. monocytogenes* *(Table 1)* and native microbiota isolated from produce *(Table 2)* were used in this study. Bacterial identification of the native microbiota was achieved using matrix-assisted laser desorption ionization time-of-flight mass spectroscopy Biotyper 3.1 (MALDI-TOF MS; Bruker Daltonik; Bremen, Germany), with comparative reference spectra analysis performed using the MALDI MS Biotyper 3.1 software (Bruker Daltonik). Stock cultures of each strain of pathogen or microbiota were maintained in tryptic soy broth at pH 7.1 (TSB; Difco, Becton, Dickinson and company, Sparks, MD) containing 10% (v/v) glycerol (Fisher Scientific, Itasca, IL) and stored frozen at -20°C. Working cultures were prepared monthly by streaking for isolation from partially thawed stock cultures as follows: *L. monocytogenes* on Listeria Selective agar (LSA; Oxoid LTD, Basingstoke, Hampshire, England) with added Listeria Selective Supplement (Oxoid), *Salmonella* and *E. coli* O157:H7 on Levine's Eosin Methylene Blue agar (Difco) modified with added D-sorbitol (10 g/l; Fisher) and NaCl (5 g/l; Fisher) (mLEMB), and native microbiota on Tryptic Soy Agar (TSA; Difco). Working culture plates were incubated at 35°C for 24 h, *Salmonella* and *E. coli* O157:H7, or 48 h, *L. monocytogenes* and native microbiota, followed by storage at 4°C for ≤ 40 days. *E. coli* O157:H7 colonies appear creamy white on mLEMB agar, *Salmonella* colonies appear dark red to iridescent green on mLEMB agar, and *L. monocytogenes* colonies appear pale yellow or grey surrounded by a black halo on LSA. Periodically, strain identity was confirmed based on Gram reaction, cell and colony morphology, and biochemical identification (API 20E, bioMérieux, Durham, NC).

Inoculum preparation

For each trial, a single-pathogen cocktail was prepared: a 5-strain cocktail for *Salmonella* and *L. monocytogenes*, and a 6-strain cocktail for *E. coli* O157:H7. For the native microbiota, a cocktail of six strains was prepared. Inocula were prepared by the selection, for each strain, of a single colony from a working culture plate that was suspended in 9 ml of TSB with 1% added glucose (Fisher). Cells were statically incubated at 35°C for 24 h to allow acid to form *(15)* and to obtain stationary-phase cells (10^8–10^9 CFU/ml). Contents of all five or six tubes for a species were combined in one conical 50 ml centrifuge tube and harvested by centrifugation (4,500 g, 7 min, 21°C, Marathon 21K, Fisher). The supernatant was discarded and the pellet was suspended in 8-ml Butterfield's Phosphate Diluent (Nelson Jameson, Marshfield, WI) at 21°C and vortexed to obtain an inoculum cocktail (~ 10^9 CFU/ml). At least three independent trials were conducted for each pathogen/acid combination. We conducted one trial for each combination of native microbiota and acid solution.

Preparation of acid solutions

Sterilized TSB containing 25 mM fumaric acid, 5 mM benzoic acid and 2% NaCl was prepared as described by Lu et al. *(25)*. Concentrated acid was aseptically added to sterilized TSB to achieve a target pH of 3.8 for each acid solution. The resulting concentrations of acid in solution were as follows: 49 mM acetic, 16 mM DL-lactic, 19 mM

TABLE 1. Pathogen strains used in this study

Strain	ID[a]	Origin
Escherichia coli O157:H7	SRCC 1675	Apple cider-linked outbreak
Escherichia coli O157:H7	SRCC 1486	Salami-linked outbreak
Escherichia coli O157:H7	SRCC 2061	Ground beef
Escherichia coli O157:H7	SRCC 1941	Pork
Escherichia coli O157:H7[b]	ATCC 43895	Ground beef-linked outbreak
Escherichia coli O157:H7	ATCC 43888	Research strain
Salmonella Braenderup[c]	SRCC 1093	Eggs
Salmonella Cerro	SRCC 400	Cheese powder
Salmonella Enteritidis	SRCC 1434	Ice cream
Salmonella Newport	SRCC 551	Broccoli with cheese
Salmonella Typhimurium	SRCC 1846	Liquid egg
Listeria monocytogenes	SRCC 529	Pepperoni
Listeria monocytogenes	SRCC 1791	Yogurt
Listeria monocytogenes	SRCC 1506	Ice Cream
Listeria monocytogenes	SRCC 1838	Cabbage
Listeria monocytogenes	SRCC 2075	Diced Coleslaw

[a]ID = identification; SRCC strains obtained from Silliker, Inc., Chicago, IL; ATCC, American Type Culture Collection, Manassas, VA.
[b]Included in *E. coli* O157:H7 cocktail based on previously described acid tolerance *(38)*.
[c]*Salmonella enterica* serotype.

TABLE 2. Fresh produce isolates used in this study

Strain	ID[a]	Origin
Pseudomonas synxanth	P3	Green pepper
Most closely resembling *Pseudomonas synxanth*	O4	Diced onion
Pantoea ananatis	T2	Tomato
Erwinia sp.	S4	Strawberry
Erwinia sp.	A4	Apple
Leuconostoc citreum	CA1	Cut cantaloupe

[a]ID = lab assigned identification. Strain isolation and survival characteristics in Roerter *(20)*.

citric, and 13 mM malic. Hydrochloric acid (35 mM) was used as an acid control. All chemicals were reagent grade and purchased from Fisher Scientific. Sterilized, acidified broth was dispensed in 99 ml aliquots into sterile containers and frozen (-20°C) until use. Prior to a given experiment, acid solution was thawed at 4°C and the pH on a room-temperature aliquot affirmed to be 3.8.

Survival of inoculum in acid solutions

For each combination of inoculum/acid solution, 1 ml of inoculum, prepared as already described, was added to 99 ml of acid solution to achieve a starting concentration of ~10^8 CFU/ml and incubated at 5°C. At designated intervals ranging from up to 240 h for *E. coli* O157:H7-, 72 h for *L. monocytogenes*-, and 48 h for *Salmonella*-inoculated acids,

TABLE 3. Mean estimated decimal reduction time, D-value, for each pathogen/acid combination, pH 3.8, at 5°C

	Decimal Reduction Time in Hours[a]				
Pathogen	Acetic[b]	Lactic	Citric	Malic	HCl
E. coli O157:H7	47.37 (3.23) ^{B,a}	49.84 (2.73) ^{B,a}	56.40 (4.89) ^{A,a}	51.43 (4.07) ^{A,B,a}	50.13 (1.70) ^{B,a}
Salmonella	11.51 (1.52) ^{F,c}	10.75 (1.02) ^{F,c}	10.93 (0.74) ^{F,c}	10.52 (0.87) ^{F,c}	11.86 (1.62) ^{F,c}
L. monocytogenes	17.77 (1.49) ^{E,b}	24.87 (4.36) ^{C,D,b}	26.92 (3.97) ^{C,b}	22.92 (3.03) ^{D,b}	28.35 (5.28) ^{C,b}

[a]Mean (standard deviation) of the D-value (h) n = 3.

[b]Mean values within a row with different uppercase superscripts (A–F) are significantly different.

($P < 0.05$). Mean values within a column with different lowercase superscripts (a–c) are significantly different ($P < 0.05$).

solutions were briefly removed from incubation (5°C), and surviving inoculum cells were serially diluted and enumerated by spread-plating (0.1 ml) on TSA, followed by incubation (35°C) for 24 h (*E. coli* O157:H7 and *Salmonella*) or 48 h (*L. monocytogenes* and native microbiota). Enumeration was performed every 12 h for *Salmonella*- and every 24 h for *Listeria*-inoculated acids, and every 24 h for the first 96 h and every 48 h thereafter for *E. coli* O157:H7-inoculated acids. In the first trial for each pathogen/acid combination, the pH of the solution was checked at every sampling point. If no significant change in pH was found, in future trials the pH was checked at time zero and at the last sampling point for each pathogen/acid combination.

Mathematical modeling and statistical analysis

Log surviving cells/ml was plotted against incubation time. When the data were graphically displayed, non-linearity was observed, so the Weibull model was used for data handling, as previously described *(7, 15)*. At least three data points were included in each analysis, and experiments were designed so at least a 5-log reduction was observed for each pathogen. The analysis of variance was conducted using the SAS statistical software package (SAS Institute, Inc., Cary, NC) as a randomized complete block (RCB) design, with treatments represented as a 3*5 factorial. Trial was the block for the experiment. Mean separation tests were conducted using Fisher Least Significant Difference (LSD) and converted into letter groupings, seen as superscripts (A–F or a–c) in *Table 3 (32)* with $\alpha = 0.05$.

RESULTS

Survival of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* was noted in each of the five acid solutions but to varying degrees *(Table 3)*. The average pH of inoculated acid solutions was 3.77 (n = 175) and changed little during storage; average change was 0.07 units (n = 11).

A preliminary study was conducted over four days to evaluate the survival pattern of each pathogen in acid solution (pH 4.0) without added fumaric and benzoic acids. No reduction of *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* was observed in broth acidified with acetic, lactic, citric, or HCl on storage at 5°C (data not shown). Subsequent trials therefore included 25 mM fumaric acid, 5 mM benzoic acid and 2% NaCl in broth acidified with either acetic, lactic, citric, or malic acid. Acidification with HCl served as an acid control.

Pathogen D-values in acid solutions (pH 3.8) with added salt and antimicrobial compounds at 5°C ranged from 10.52 h for *Salmonella* in malic acid solution to 56.40 h for *E. coli* O157:H7 in citric acid solution *(Table 3)*. Based on the calculated mean decimal reduction times, achieving a 5-log reduction for *E. coli* O157:H7 would require from 9.87 days in an acetic acid solution to 11.75 days with citric acid.

Across three independent trials for each pathogen, the average reduction in *E. coli* O157:H7 counts ranged from 3.27 log CFU/ml in citric acid to 4.70 log CFU/ml in acetic acid over 240 h; the average reduction in *Salmonella* ranged from 4.13 log CFU/ml in HCl to 4.84 log CFU/ml in malic acid over 48 h; and the average reduction in *L. monocytogenes* ranged from 3.11 log CFU/ml in HCl to 4.69 log CFU/ml in acetic acid over 72 h. Additional trials were conducted with extended time periods until a 5-log reduction was achieved for each pathogen/acid combination.

There was a significant difference ($P < 0.05$) between the effectiveness of the various acids against survival of *E. coli* O157:H7 and *L. monocytogenes* *(Table 3)*. At pH 3.8 and 5°C, acetic acid was significantly more lethal than the other acids when *L. monocytogenes* was challenged. Conversely, when *E. coli* O157:H7 was studied, citric acid was significantly less lethal than the other acids studied ($P < 0.05$) *(Fig. 1)*. For *Salmonella*, there was no significant difference in the effect of the various acids on survival ($P > 0.05$).

In all cases, there was a significant difference ($P < 0.05$) among pathogens in their ability to survive in organic acid solution with added antimicrobials, at pH 3.8 and upon storage at 5°C *(Table 3)*. The survival of each pathogen in a citric acid solution, pH 3.8, and 5°C, is shown in *Fig. 2*. For

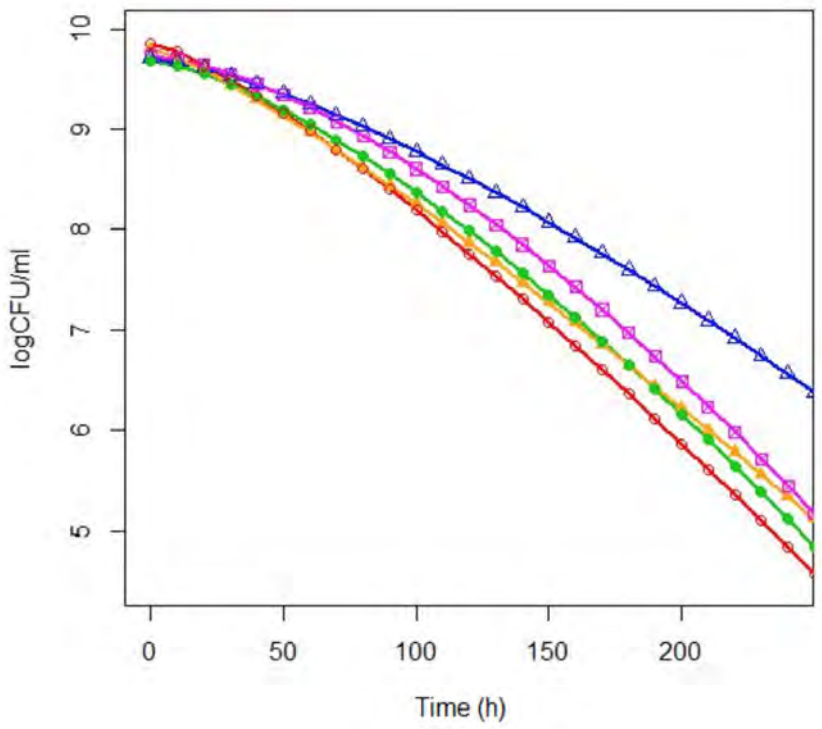

Figure 1. Average survival of *E. coli* O157:H7 at 5°C in acid solutions of pH 3.8; acetic (open circles – red), lactic (closed circles – green), citric (open triangles – blue), malic (closed triangles – orange), and HCl (open squares – purple); (n = 3)

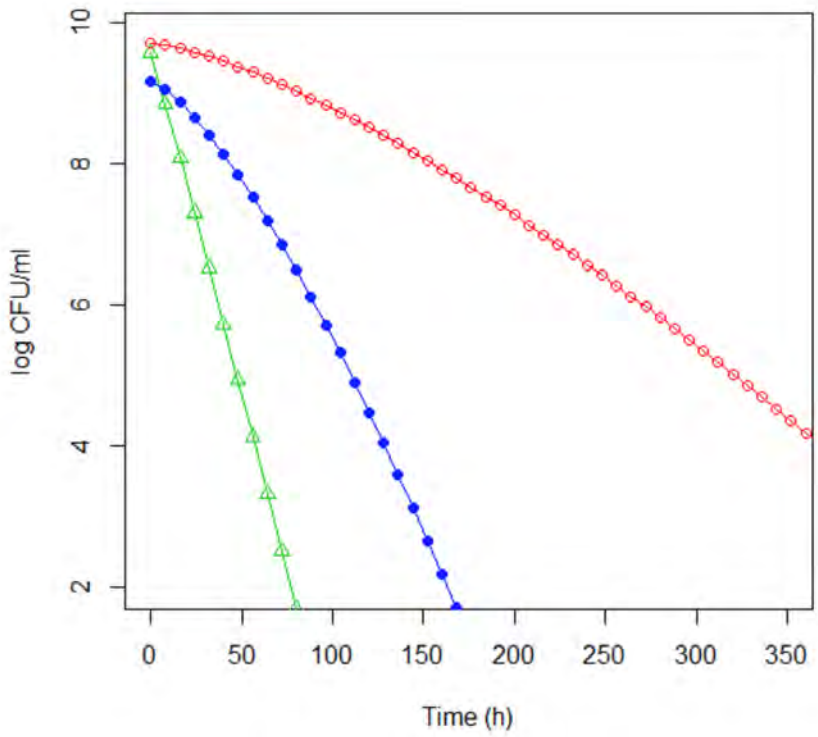

Figure 2. Average survival of *E. coli* O157:H7 (open circles), *L. monocytogenes* (triangles), and *Salmonella* spp. (filled circles) in a citric acid solution, with added fumaric and benzoic acids, pH 3.8, at 5°C (n = 3)

each acid solution, survival of *E. coli* O157:H7 was greater than that of *L. monocytogenes*, which was in turn greater than that of *Salmonella*. Across all pathogen/acid combinations, *E. coli* O157:H7 showed the highest decimal reduction value in citric acid, significantly more so than other pathogen/acid combinations ($P < 0.05$). The estimated 5-log reduction time for *E. coli* O157:H7 in a solution of citric acid, pH 3.8 at 5°C, was 282 h. *Salmonella* had the lowest survival of any pathogen in acid solution; an estimated 5-log reduction time for *Salmonella* at pH 3.8 (5°C) was 53–59 h, depending on the identity of the acid solution.

An additional trial was run to investigate the pattern of survival of a cocktail of various native microbiota *(Table 2)* in the acid solutions. Strains of native microbiota were isolated as a result of extensive sampling of fresh produce *(20)*. A 5-log reduction of the native cocktail was achieved in less than 48 h in all acid solutions, and additional experiments were not conducted (data not shown). Although not fully investigated, this preliminary finding suggests that native microbiota would not affect pathogen survival during extended storage of raw fruits and vegetables in acid solutions at 5°C.

DISCUSSION

Several outbreaks of illness have been linked to non-heat treated, pickled vegetables *(11, 34)*. The extended survival of *E. coli* O157:H7 in acidic environments and at refrigerated temperatures is well documented in the literature *(13, 17, 27, 28)*. In our preliminary study, we showed that *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* could all survive in acid solutions at pH 4.0 and 5°C with no loss of viability.

The use of fumaric acid and sodium benzoate as antimicrobial compounds to increase destruction of pathogens in apple cider was attempted by Chikthimmah and colleagues. At 5°C, destruction of *E. coli* O157:H7 in the presence of preservatives increased with time, whereas without preservatives there was little decline in *E. coli* O157:H7 populations *(12)*. Lu et al. developed a novel pickling solution based on commercially available refrigerated pickle brines that contained 25 mM fumaric acid, 5 mM benzoic acid, 70 mM acetic acid and 2% NaCl, pH 3.8 *(25)*. In this brine, at 10°C, a 5-log reduction of a 5-strain cocktail of *E. coli* O157:H7 was achieved in 8.83 days. In our study, the 5-log reduction time for *E. coli* O157:H7 in acetic acid solution, pH 3.8, containing 25 mM fumaric acid, 5 mM benzoic acid, and 2% NaCl, was 236 h, or 9.87 days. In an attempt to simulate the atmospheric conditions in vacuum-sealed refrigerated pickle jars, Lu et al. employed an anaerobic environment for their survival studies, which would have enhanced pathogen survival *(21)*. We chose a different approach, as our interaction with retail food establishments indicated that manufacturers were placing cut and sliced vegetables and/or fruits in large plastic food-grade containers, adding brine, and placing the covered container in the refrigerator to allow for flavor development; the container was covered but not vacuum-sealed.

Decimal reduction times indicate that *E. coli* O157:H7 was the most acid-resistant pathogen, followed by *L. monocytogenes* and then *Salmonella*. This order of acid resistance confirms previous work by Breidt and colleagues *(6, 7)*. Lin et al. tested 11 O157:H7 strains and four commensal strains of *E. coli* for their ability to survive extreme acid exposures (pH 3) *(24)*. Three previously characterized acid resistance systems were investigated: an acid-induced oxidative system, an acid-induced arginine-dependent system, and a glutamate-dependent system. Once induced, the acid resistance systems remained active for prolonged periods at 4°C. These acid resistance systems may help explain why *E. coli* O157:H7 was found to be more acid resistant than *L. monocytogenes* and *Salmonella*. *L. monocytogenes* was most susceptible to storage in acetic acid, with significantly shorter log reduction times noted on storage in this acid vs. other acids tested *(Table 3)*. Farber et al. also noted that acetic acid was a more effective growth inhibitor against *L. monocytogenes* than lactic, citric, and hydrochloric acids at either 4 or 30°C *(18)*. Regardless of the acid challenge, there was no significant difference in *Salmonella* survival. While significantly greater overall survival was seen with *E. coli* O157:H7, there were significant differences in survival of this pathogen depending on the acid challenge. In this study, survival was significantly less in acetic acid than in citric acid, and survival in lactic and malic acids was not different from that in acetic acid. Lu et al. *(26)* investigated the antimicrobial effects of acids (pH 3.2) on the survival of *E. coli* O157:H7 under anaerobic conditions. Lu et al. ranked the effectiveness of weak acids as: L- and D-lactic > acetic > malic acid at 30°C. Ryu et al. *(31)*, however, found that acetic was the most lethal acid compared with lactic, citric, and malic acids against unadapted and acid-adapted cells of *E. coli* O157:H7. Adams and Hall challenged *E. coli* and *S.* Enteritidis with solutions of lactic and acetic acids (pH 5 and 6). Acetic acid decreased the growth rate of the pathogens significantly more than lactic acid *(1)*. Experiments conducted under different environmental conditions, with different pathogens and with different strains, at different pH values, and with or without the presence of added antimicrobials, all contributed to the varying acid effects.

The inhibitory action of organic acids is thought to be due to the ability of undissociated acid to freely cross the cell membrane and release anions and protons, which subsequently accumulate in the cell *(8)*. Ridding the cytoplasm of accumulated protons drains the cells of ATP resulting in death *(3, 5, 30)*. Ahamad and Marth challenged *L. monocytogenes* with acid solutions of up to 0.5% acetic, citric or lactic acid *(2)*. The effectiveness of the acids coincided with their degree of undissociation. Citric and lactic acids, with larger dissociation constants, were less

detrimental to the pathogen than was acetic acid. Studies by Eklund found undissociated acid to be 10 to 600 times more inhibitory than dissociated acid against *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* *(16)*. Conner and Kotrola noted that pH alone could not account for acid inhibition of *E. coli* O157:H7 *(13)*. Breidt et al. demonstrated that acetic acid can decrease the survival time for *E. coli* at a given pH, compared with the effect of pH alone *(5)*.

Acetic acid with a pKa of 4.76 *(33)*, would have easily diffused across the bacterial cell membrane at the experimental pH, and its lethality against all pathogens was significant. The other acids (lactic (pKa 3.86), citric (pKa_1 3.06, pKa_2 4.74, pKa_3 5.40), malic (pKa_1 3.06, pKa_2 5.05) and hydrochloric (pKa < 1.0 *(33)*) were at least partially dissociated at pH 3.8. While the ability of the bacterial strains used in these experiments to modulate intracellular pH is not known, *E. coli* O157:H7 has been shown to be more resistant than generic *E. coli* to the toxicity of acetic acid *(14)*. This could account for the significantly greater overall survival of *E. coli* O157:H7 compared with *Salmonella* and *L. monocytogenes*. Adams and Hall suggested that because lactic acid (pK_a 3.86) is a stronger acid than acetic (pK_a 4.76), in foods with moderately low pH (4–6), acetic acid would be expected to be the more potent antimicrobial, since a greater proportion would be undissociated *(1)*. However, the antimicrobial activity of a variety of organic acids against a particular pathogen is affected by more than just the pK_a value of the acid; temperature, specific type of acid, acid concentration, pH, and ionic strength are also important *(21)*.

In contrast to our results, results of some studies suggest a protective effect of organic acids. Work with *L. monocytogenes* has suggested that polycarboxylic acids may have two effects: antimicrobial activity associated with the undissociated form of the molecule, and a protective effect due to chelation of metal ions by the dissociated form *(9, 10)*. Bjornsdottir et al. challenged five *E. coli* O157:H7 strains with organic acids (pH 3.2) and found reduced lethality for *E. coli* when low concentrations (5 mM) of fully protonated acetic, malic, or L-lactic acid were present *(4)*. In another study, the presence of organic acids in tryptic soy broth with 0.6% yeast extract stored at 4°C enhanced survival of *E. coli* O157:H7, compared with the control *(13)*.

Overall, our results support the ability of retailers to store cut fruit and/or vegetables in organic acid solutions with added antimicrobials, at pH 3.8 or below and at 5°C or below, as a way to help ensure the safety of products pickled on-site. In a review of 41 retail pickle products in hermetically sealed shelf-stable containers at three local grocery stores, 85% of products contained acetic acid, 12.5% contained lactic acid, and 17.5% contained citric acid (data not shown). Although the flavor profile of each acid has been determined *(19)*, we did not consider the sensory implications in this work. Our work does support the safety of refrigerated pickle products as long as certain critical parameters are adhered to.

CONCLUSIONS

Our goal was to study a process that might be used for pickling in a retail food establishment and to ascertain whether various combinations of organic acids with added antimicrobial compounds could be used to attain a 5-log reduction of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* during storage at 5°C. We observed the following:

- The target pathogen for evaluating the safety of refrigerated pickles is clearly *E. coli* O157:H7. This pathogen survived significantly longer than either *L. monocytogenes* or *Salmonella* under the conditions of the study.
- The effect of acids on pathogen survival did not solely depend on pH. In this study, across acetic, lactic, citric, and malic acid solutions, all at pH 3.8, we noticed some significant differences ($P < 0.05$) in pathogen survival based on the acid challenge. Most notably, acetic acid was significantly more lethal to *E. coli* O157:H7 than was citric acid. Survival of *E. coli* O157:H7 in lactic acid solution was not different from that in acetic acid.
- The addition of antimicrobial compounds (fumaric and benzoic acids) are necessary to ensure a 5-log reduction of pathogens. In preliminary experiments, no reduction of *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* was observed in acid solution at 5°C without these components.
- A retailer who formulates a pickling brine with 25 mM fumaric acid, 5 mM benzoic acid and 2% NaCl, and with a pH of 3.8 or below, using citric acid as the acidulant, would require a holding period of at least 282 h (11.75 days) at 5°C to effectively reduce pathogens in acidified products prepared using fresh produce. Similarly, a holding time of at least 237 h or 9.87 days would ensure a 5-log pathogen reduction in acetic acid-acidified brine.

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