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Thermal Processing of Acidified Foods with pH 4.1 to pH 4.6

ABSTRACT

Shelf-stable acidified foods with a pH at or below 4.6 must be processed to achieve a 5-log reduction for vegetative bacterial pathogens. Published research does not exist to adequately support the Food and Drug Administration process filings for products with pH 4.1–4.6 or to define critical limits for acid and acidified foods with pH values in this range. Using a non-inhibitory vegetable-based medium, we developed models and data for the thermal destruction of acid-resistant vegetative microbial pathogens, including 5-strain cocktails of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in acidified foods with pH values of 4.1 to 4.6. Under the experimental conditions, *Listeria monocytogenes* was the most heat- and acid-resistant pathogen. A z-value of 16.7°F, an F-value (at 160°F) of 5.6 min, and a table of recommended processing conditions were estimated from the thermal processing data. This work addresses a lack of documentation that is challenging to all areas of the industry, especially small processors.

INTRODUCTION

All processors of acidified and low-acid canned foods must register with the Food and Drug Administration (FDA) and file scheduled processes for each product that they manufacture. These process filings must be supported by research that substantiates the lethality of the scheduled process for microbial pathogens. For acidified foods with a pH of 4.1 or lower, research has defined heat processing conditions that ensure a 5-log reduction of the pathogens of concern, *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* (3, 5). These data identify the holding time at the recommended temperature for the “cold spot” in the product container, as defined by a process authority. However, no published peer-reviewed documented research exists to support FDA process filings for processing acidified food products with pH 4.1–4.6. This lack of documentation is challenging to all areas of the industry but especially so for small food processors that lack the resources to support challenge studies conducted by private laboratories.

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To conduct studies on the safety of acidified food, laboratory conditions should be established that allow equal or greater survival of the target vegetative microbial pathogens, compared with survival under the conditions of the relevant food products. For this reason, previous studies of thermal processing or acid killing of vegetative pathogens in acidified vegetables were done with cucumber juice medium (CJ, to be described later) or cucumbers in a low-salt (2% or less) brine as a medium representative of various acidified vegetable products *(1–4)*. Cucumbers do not contain compounds that are known to be inhibitors of microbial survival or growth, but cucumbers do contain amino acids, sugars, and other compounds that may aid in the survival of bacterial pathogens *(1, 7)*. Many other vegetables or ingredients typically present in acidified vegetable products, including cabbage, peppers, garlic, horseradish and others, contain antimicrobial compounds *(8, 9)*. However, use of media containing these natural antimicrobials for pathogen reduction studies with acidified foods is problematic, because the active concentration of these natural antimicrobials is hard to quantify and may vary with cultivar, growing season, weather, and other factors. Therefore, CJ was chosen to represent a "worst-case" condition for the survival of bacterial pathogens in thermal processing studies. Results of studies that use CJ medium can be viewed as applicable to a variety of acidified foods. Research with acetic acid and gluconic acid (as a non-inhibitory buffer) in CJ medium *(2–4)* has been used to support process filings for a variety of acidified food products that may or may not contain cucumbers, including flavorings, syrups, dressings, toppings, sauces, salsas, and others
(F. Arritt and B. Ingham, personal communication).
The objective of this research was to define thermal processing conditions that will ensure a 5-log reduction of vegetative bacterial pathogens in acidified food products that have pH values above 4.1 and below 4.6. Acetic acid is perhaps the most common organic acid used in acidified foods, particularly acidified vegetables. Acetic acid was therefore included in CJ for thermal processing studies. Gluconic acid, which has been shown to function as a non-

TABLE 1. Bacterial strains

Strain ID	Strain Name	Previous ID[a]	Food Origin
B0195	*Listeria monocytogenes*	SRCC 529	Pepperoni
B0196	*Listeria monocytogenes*	SRCC1791	Yogurt
B0197	*Listeria monocytogenes*	SRCC 1506	Ice cream
B0198	*Listeria monocytogenes*	SRCC 1838	Cabbage
B0199	*Listeria monocytogenes*	SRCC 2075	Diced coleslaw
B0200	*Escherichia coli* O157:H7	ATCC 43888	Laboratory strain
B0201	*Escherichia coli* O157:H7	SRCC 1675	Apple cider outbreak
B0202	*Escherichia coli* O157:H7	SRCC 1486	Salami outbreak
B0203	*Escherichia coli* O157:H7	SRCC 2061	Ground beef
B0204	*Escherichia coli* O157:H7	SRCC 1941	Pork
B0206	*Salmonella*[b] Braenderup	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, IL; ATCC, American Type Culture Collection, Manassas, VA; ID, identification

[b]*Salmonella enterica* strains with the serotype (non-italicized)

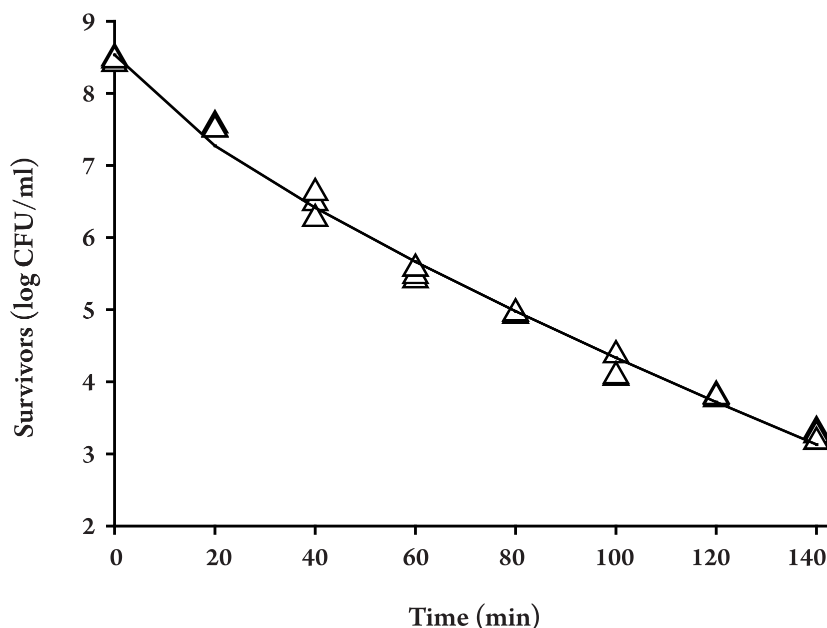

FIGURE 1. Thermal death time data for E. coli *O157:H7 in 100 mM (0.6%) acetic acid, pH 4.6, at 133°F (56°C). Three independent replications of the data are shown (triangles) for the log CFU/ml survivors vs. time (min). The fitted Weibull model for these data is also shown (solid line).*

inhibitory buffer in CJ in studies of *E. coli* O157:H7 and related strains of this species under acid conditions *(1)*, was also tested in place of acetic acid with *E. coli* O157:H7 to determine the effect of pH on the effectiveness of heat processing in the absence of an inhibitory organic acid effect on bacterial survival.

MATERIALS AND METHODS

Media

The CJ medium used to determine 5-log reduction values for target pathogens was prepared from brined cucumbers as previously described *(2)*. Size 2A pickling cucumbers of mixed varieties (about 2 cm in diameter) were obtained from a commercial processor. Approximately 640 g of cucumbers (*Cucumis sativus*) were placed in a 1.4-liter jar with 640 ml of brine. The brine contained 2% NaCl and 100 mM (0.6%) acetic acid, with the balance sterile H_2O, at pH 4.6 after equilibration. For some *E. coli* O157:H7 experiments, the acetic acid was replaced with 20 mM (0.4%) gluconic acid. The pH was adjusted by adding predetermined amounts of HCl, based on a titration of the blended cucumbers. The brined cucumbers were pasteurized at 165°F (74°C); internal temperature at the cold spot in the jar) for 15 min in a steam-heated waterbath and left at room temperature (23° + 2°C) for 1 week or longer to allow equilibration of sugars, amino acids, and other compounds between the brine and cucumbers. Following equilibration, the brine was removed from the jars as needed and aseptically transferred to double walled sterilized fermentation jars for thermal processing studies. Tryptic soy agar, used for plating *L. monocytogens* and *S. enterica* strain cocktails, and Luria agar for plating *E. coli* O157:H7 cocktails, were obtained from BD Biosciences (BD Biosciences, San Jose, CA). All chemicals used in the study were obtained from Fisher Scientific (Itasca, IL) unless otherwise specified.

Thermal processing

Bacteria used in this experiment consisted of cocktails of five strains each of three foodborne pathogens, as shown in *Table 1*. Bacteria were grown statically at 37°C for 16 h in 5 ml tryptic soy broth (TSB, Difco, Becton Dickinson, Sparks, MD, for *Listeria*) or Luria broth (LB, Becton Dickinson, for *Salmonella* and *E. coli*) modified to contain 1% glucose to induce acid tolerance, as previously described *(1, 3, 6)*. The five cultures of each species were each grown separately, harvested by centrifugation (3,000 × g, 10 min, 10°C, Sorvall Superspeed Centrifuge, SS-34 rotor, DuPont Instruments, Newton CT), re-suspended in 0.5 ml sterile saline (0.85% NaCl) and combined into a single-pathogen inoculum cocktail. A 1.5 ml aliquot was added to 150 ml of CJ medium in a 300 ml water-jacketed fermentation flask, to give a final cell concentration of approximately 10^8 CFU/ml. Prior to inoculation, flasks were equilibrated at a temperature of 133° to 151°F (56° to 66°C, to be indicated later), with magnetic stirring, using a heating-cooling waterbath (Neslab RTE-111, Newington, NH). CJ temperature was confirmed using sterile type 'T' thermocouples inserted into the medium, and recorded with a data-logging apparatus (Omega 3000, Omega Engineering, Inc., Stamford, CT). The CJ in the flasks was inoculated through a rubber septum, using a

TABLE 2. Estimated 5-log reduction times

Pathogen Name	Temp°C	Temp°F	5LR[a]	Std. Err.[b]
Escherichia coli O157:H7	56	132.8	126.10	5.30
	58	136.4	88.79	4.23
	60	140	95.74	4.32
	62	143.6	56.00	1.82
	64	147.2	24.06	1.07
	66	150.8	11.71	0.64
Salmonella enterica	56	132.8	150.73	7.74
	60	140	87.46	4.77
	64	147.2	24.55	1.14
	66	150.8	10.55	0.50
Listeria monocytogenes	56	132.8	156.70	8.66
	60	140	125.31	8.64
	64	147.2	28.75	1.46
	66	150.8	14.32	0.86

[a]Estimated 5-log reduction time in minutes
[b]Standard error for the 5-log reduction time

3 ml syringe. A nitrogen gas blanket was maintained over the liquid using filtered (0.2 um) industrial grade N_2 gas (≤20 ppm O_2; Air Products, Raleigh, NC). At indicated time intervals, depending on temperature, 1 ml of the cell suspension was withdrawn through the septum and 0.1 ml was immediately cooled by dilution into 0.9 ml of pH 7.0 MOPS (3-[N-morpholino] propanesulfonic acid, Sigma-Aldrich Chemical Co., St. Louis, MO) at room temperature (23 + 2°C), followed by serial dilution in sterile saline (0.85% NaCl) and plating on tryptic soy agar or Luria agar, using an automated spiral plater (Autoplate 4000, Advanced Instruments, Norwood, MA). Plates were incubated at 37°C for 24 h prior to determination of log CFU/ml with an automated plate reader (QCount, Advanced Instruments). Three or more independent replications of each experiment were carried out.

Modeling

To determine D- and z-values, a combination of Weibull and linear models was used, as previously described *(3)*. A Weibull nonlinear regression model for time-temperature survival data (5-log reduction values, 5D) was fit, assuming normally distributed errors with constant variance and mean function:

$$log_{10}(S(\tau)) = No - 5(\tau / t^{*})\beta$$

with N_o = the initial cell count, τ representing the observation times, t^{*} the 5-log reduction time, and β a shape parameter for the Weibull curve. Linear regression was used for estimating the z-value, using the log_{10} 5-D values estimated for each temperature, as previously described *(3, 5)*. A reference temperature of 160°F (71.1°C) was used for F-values. Curve fitting was done with custom Matlab algorithms or SAS software (SAS Institute, Cary, NC), using proc NLIN for the Weibull model.

RESULTS

Thermal death-time curves were generated for the 5-strain single-pathogen cocktails of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*. A typical survival curve (for *E. coli* O157:H7 in CJ with acetic acid

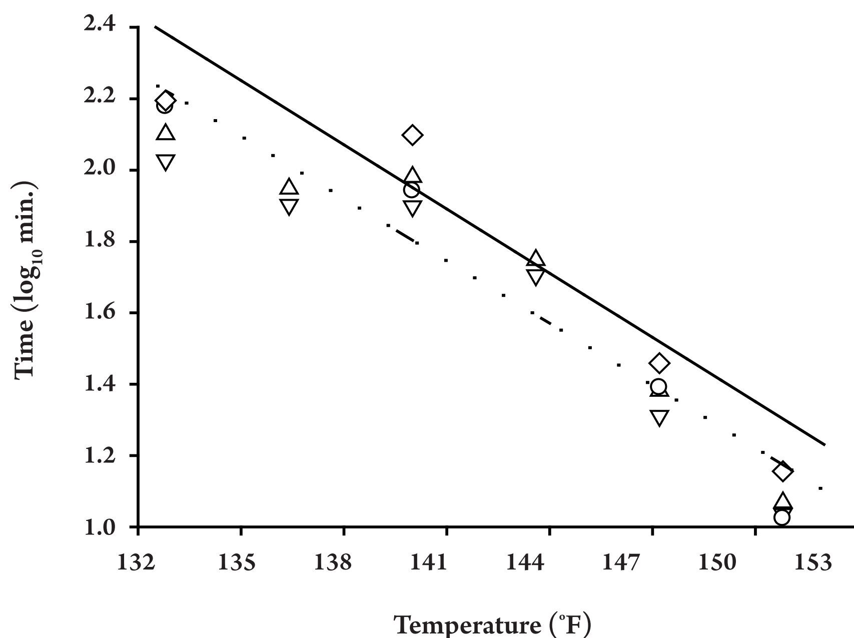

FIGURE 2. The log_{10} 5-log reduction times for cocktails of E. coli O157:H7 at pH 4.6 for 131°F to 151°F (56–66°C) in 0.6% acetic acid (Triangle up), E. coli O157:H7 in gluconic acid buffer (Triangle down), S. enterica in 0.6% acetic acid (circles), and L. monocytogenes in 0.6% acetic acid (diamonds) are shown. The dashed line was determined by linear regression for the entire data set, with an R^2 = 0.90; the solid line was based on the L. monocytogenes regression (R^2 = 0.91) with F_{160} increased to 5.6.

TABLE 3. Table of z and F values at pH 4.6

Pathogen Name	z-val (°F)[a]	Rsq[b]	F160[c]
Escherichia coli O157:H7[d]	17.4	0.91	4.44
Salmonella enterica	15.6	0.96	3.34
Listeria monocytogenes	16.7	0.91	4.89
Combined[e]	17.1	0.90	4.30

[a]Estimated z-value in °F

[b]R-squared value for the regression line used to estimate the z-value

[c]The processing time at 160°F in min

[d]Combined data for acetic acid and gluconic acid buffer experiments

[e]Combined data for all experiments

at 56°C, 133°F) is shown in Fig. 1. The 5-log reduction times for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* in CJ with acetic acid at pH 4.6 are presented in Table 2. The results for thermal death experiments for *E. coli* in CJ with gluconic acid showed that the 5-log reduction times were equal to or less than the 5-log reduction times for each temperature tested with acetic acid (data not shown). For all temperatures and treatments, *L. monocytogenes* was the most heat and acid-resistant organism tested, followed by *S. enterica* or *E. coli* O157:H7, depending on temperature (Table 2).

For data on each pathogen, as well as for the combined data on all species tested, z-values based on the 5-log reduction times were estimated (Table 3). *E. coli* O157:H7 had the highest z-value (17.4°F) with a standard error (SE) of 2.0°F. The z-values for *S. enterica* and *L. monocytogenes* were 15.6°F (1.4) and 16.7°F (2.1), respectively (SE in parentheses). Figure 2 shows the z-value plot for the

TABLE 4. Recommended processing conditions for acidified foods (pH 4.1 to 4.6) to achieve a 5-log pathogen reduction

Temp°C	Temp°F	5LR (min)[a]	Temp°C	Temp°F	5LR (min)
60.6	141	77.8	71.7	161	4.9
61.1	142	67.7	72.2	162	4.3
61.7	143	59.0	72.8	163	3.7
62.2	144	51.4	73.3	164	3.2
62.8	145	44.8	73.9	165	2.8
63.3	146	39.0	74.4	166	2.5
63.9	147	34.0	75.0	167	2.1
64.4	148	29.6	75.6	168	1.9
65.0	149	25.8	76.1	169	1.6
65.6	150	22.4	76.7	170	1.4
66.1	151	19.5	77.2	171	1.2
66.7	152	17.0	77.8	172	1.1
67.2	153	14.8	78.3	173	0.9
67.8	154	12.9	78.9	174	0.8
68.3	155	11.2	79.4	175	0.7
68.9	156	9.8	80.0	176	0.6
69.4	157	8.5	80.6	177	0.5
70.0	158	7.4	81.1	178	0.5
70.6	159	6.5	81.7	179	0.4
71.1	160	5.6	82.2	180	0.4

[a]Recommended processing time (5-log reduction time) in min

combined data set (z = 17.1°F) as well as the z-value line for recommended processing conditions, which had a z-value of 16.7°F and an F-value at 160°F (F_{160}) of 5.6 min. The processing time at a reference temperature of 71°C (F_{160}) was estimated from observed data based on the average 5-log reduction time being 4.4 min for *E. coli* O157:H7, 3.3 min for *S. enterica*, and 4.9 min for *L. monocytogenes* (*Table 3*).

DISCUSSION

Results from previous studies of non-thermal processing conditions for acidified foods showed that *E. coli*

O157:H7 was significantly more acid resistant than *S. enterica* or *L. monocytogenes* at 10°C (50°F) in cold-fill-hold acidified food products at pH 3.3 to 3.8 *(3, 5)*. Data on previous thermal processing at pH 4.1 showed that *E. coli* O157:H7 and *L. monocytogenes* had similar heat and acid resistance *(3)*. By analysis of the estimated z and F values from the current study, it was found that *E. coli* O157:H7 was more heat resistant than *S. enterica* and *L. monocytogenes* at temperatures above 74°C (166°F), but less resistant than *L. monocytogenes* below that temperature. Below 74°C (166°F), *L. monocytogenes* was more heat resistant than cells of the other two species. The physiological basis for this difference is unclear. To derive a single set of parameters that will ensure safety under all processing conditions, the F160 for *L. monocytogenes* (which had a z-value of 16.7°F) was increased from 4.8 min (as shown in *Table 3*) to 5.6 min. The resulting thermal processing recommendation, a z-value of 16.7°F and a F_{160} of 5.6 min, allowed achievement of a 5-log reduction of all strains tested for any temperature in the range tested and used for the recommended processing times and temperatures. A linear model, corresponding to a z-value of 16.7°F and an F_{160} of 5.6 min is shown as the solid line in *Fig. 2*. Based on these z- and F-values, a table of recommended processing conditions was generated in the range of temperatures typically used for commercial processing of acidified vegetables *(Table 4)*.

Previously, five times the standard error estimate was added to the 5-log reduction times as an arbitrary safety factor for recommended thermal processes *(3)*. For recommended thermal processing times and temperatures for acidified foods using the data in *Table 4*, the authors suggest that a competent process authority should be consulted to determine if any additional safety factor is needed.

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