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PEER-REVIEWED ARTICLE

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Thermal Processing Parameters to Ensure a 5-log Reduction of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in Acidified Tomato-based Foods

ABSTRACT

Under 21 Code of Federal Regulations Part 114, manufacturers of acidified canned foods must apply a process that ensures destruction of pertinent vegetative bacterial pathogens and spoilage organisms. We used nonlinear (Weibull) modeling to calculate thermal processing parameters sufficient to inactivate *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella enterica* in tomato purée at 54°C. Inoculum (1 ml of a 5-strain single pathogen cocktail) was heated in 99 ml tomato purée at pH 4.50 (unacidified) or in purée acidified to pH 3.80 or 4.20 by use of acetic or citric acid. $D_{54°C}$ ranged from 20.85 min for *E. coli* O157:H7 in purée at pH 4.5; as well as 0.63 min for *S. enterica* in purée acidified to pH 3.8 by addition of acetic acid. Acetic acid was significantly more effective than citric acid in ensuring pathogen inactivation (P < 0.05). *E. coli* O157:H7 was significantly more heat- and acid-resistant than *S. enterica* and *L. monocytogenes* in tomato purée at pH 4.5, as well as in purée acidified to pH 4.2 by addition of citric or acetic acid (P < 0.05). *L. monocytogenes* was the most heat- and acid-resistant in purée acidified to pH 3.8 by addition of acetic acid, but in purée acidified to pH 3.8 with citric acid, there was no difference in the rate of pathogen inactivation. Extrapolating 5-log pathogen reduction times to relevant processing temperatures, *E. coli* O157:H7 was the most heat resistant at < 65°C (149°F), while *L. monocytogenes* was most heat resistant at temperatures above 65°C. Using a calculated z-value of 13.3°F (7.4°C) and F-value of 0.51 min at 160°F (71.1°C), time/temperature combinations to achieve a minimum 5-log pathogen reduction in tomato purée, pH ≤ 4.5, ranged from 13.83 min at 141°F (60.6°C) to 0.02 min at 180°F (82.2°C). Results can be used to inform development of scheduled processes and to support FDA process filings for tomato-based acidified foods.

INTRODUCTION

From January 2008 to October 2012, 1,693 new acidified tomato-based products were introduced into the United States market, including salsas, pasta sauces, and other table sauces. Consumer demand of these products is projected to increase and to prompt continued growth of this category *(13)*. Regulations require that acidified foods be thermally processed to an extent sufficient to destroy the vegetative cells of microorganisms of public health significance and those of non-health significance capable of reproducing in

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the food under the conditions in which the food is stored, distributed, retailed and held by the user *(22)*. For acidified foods, thermal processing is normally a mild heat treatment, such as steam pasteurization or an inverted hot-fill hold that, for low pH products, is generally sufficient to guarantee safety *(2)*.

In the manufacture of acidified canned foods, acidification is most often accomplished by addition of organic acids such as acetic, lactic or citric acid, singly or in combination, and employed to control microbial growth, improve sensory attributes, and reduce microbial spoilage of foods *(1, 9, 15)*. In a review of 179 tomato-based acidified food products found in local grocery stores, we noted that citric and acetic acids were the most commonly used acidulants, usually in the form of lime juice or distilled vinegar. Citric acid has been shown to inactivate *Salmonella* Typhimurium in tahini *(1)*, while acetic acid has been shown to inactivate *Escherichia coli* O157:H7 in simulated pickle products *(4)* and apple-carrot juice blends *(23)*. Acetic acid was also effective at inactivating *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* to various degrees in asparagus purée *(20)*. Acetic and citric acids have been shown to inactivate *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on fresh apples and lettuce *(18)* and in cucumber purée *(3)*.

Processors of acidified canned foods are required to register their manufacturing facility and file scheduled processes for their products with the Food and Drug Administration (FDA) *(22)*. FDA filings for acidified canned foods must be supported by research that validates destruction of important foodborne pathogens, and *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* are considered targets for thermal processing and acidification *(5–7)*. The work of Breidt and colleagues used a re-parameterized Weibull model to estimate thermal processing conditions necessary to achieve a 5-log reduction of target pathogens in an acidified cucumber juice medium *(5–7)*. Recent studies in our laboratory have confirmed the appropriateness of the non-linear Weibull approach for calculating accurate thermal processing D- and z-values *(10)*.

The process authority, in using laboratory data to establish a scheduled process for an acidified food, must rely on an understanding of heat penetration in a particular food matrix, the impact of formulation on microbial inactivation, and heat tolerance of spoilage organisms and target pathogens. It is in the best interest of manufacturers to have appropriate evidence to validate thermal processing conditions that produce a safe but not over-processed product, leading to a balance between food safety and food quality. In the absence of current recommendations developed with use of a tomato-based medium, this study was conducted to calculate thermal processing parameters that would inactivate vegetative cells of the target pathogens *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* in tomato purée. This study expanded on previous work *(10)* and examined the effect of acidification with citric and acetic acids on thermal inactivation of these vegetative pathogens in tomato purée at 54°C. Time/temperature processing conditions were estimated that would ensure a minimum 5-log pathogen reduction of vegetative pathogens and could be used to support safe manufacture of tomato-based acidified canned foods.

MATERIALS AND METHODS

Strain selection and maintenance

Strains of *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* were used in this study *(Table 1)*. With one exception, these strains were the ones used in work by Breidt et al. *(5–7)*; *E. coli* O157:H7 strain ATCC 43895 (American Type Culture Collection, Manassas, VA) replaced strain ATCC 43888 used in the prior work. Stock cultures of each strain 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. Bacterial strain identity and purity were assessed and confirmed by Gram reaction, cell and colony morphology, and responses to biochemical profiling kits (bioMerieux, Durham, NC) for *E. coli* O157:H7 and *S. enterica* (API 20E), and *L. monocytogenes* (API Listeria). Working cultures were prepared monthly by streaking for isolation from partially thawed stock cultures onto appropriate selective plating medium: *L. monocytogenes* on Listeria Selective agar (LSA; Oxoid LTD, Basingstoke, Hampshire, England) with added Listeria Selective Supplement (Oxoid), and *S. enterica* and *E. coli* O157:H7 on modified Levine's Eosin Methylene Blue agar (mLEMB; Difco). mLEMB was prepared from Levine's Eosin Methylene Blue agar, with addition of D-sorbitol (10 g/liter; Fisher) and NaCl (5 g/liter, Fisher). Working culture plates were incubated at 35°C for 24 h for *S. enterica* and *E. coli* O157:H7, or 48 h for *L. monocytogenes*, followed by storage at 4°C for ≤ 40 days. *E. coli* O157:H7 colonies appear colorless to pale pink on mLEMB agar, *S. enterica* 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.

Inoculum preparation

For each trial, a 5-strain single-pathogen cocktail was prepared. Inocula were prepared by the selection, for each strain, of a single colony from a working culture plate, the cells of which were suspended into 9 ml of TSB +1% added glucose (Fisher) and statically incubated at 35°C for 24 ± 2 h to obtain stationary-phase cells (10^8–10^9 CFU/ml) and expose growing cells to acid, as previously described *(5, 8)*. Contents of all five 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 re-suspended in 4.5 ml Butterfield's Phosphate Diluent (BPD; Nelson Jameson,

TABLE 1. Bacterial strains used in this study

Strain name	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
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.
[b]Previous publications included ATCC 43888, not 43895, as part of an *E. coli* O157:H7 cocktail in determination of 5-log pathogen reduction times for heat processed, acidified vegetable brine *(5)*.
[c]*Salmonella enterica* serotype.

Marshfield, WI) at 21°C and vortexed to obtain an inoculum cocktail (~10^{10} CFU/ml) (*n* = 116).

Preparation of tomato purée

Tomato purée was prepared from a single lot of locally grown late-harvest Roma tomatoes that had been blanched (98°C for 3 min), peeled, and placed into 3.8-liter freezer bags (Ziploc, S.C. Johnson, Racine, WI) in 453 g portions. Samples were held frozen at -20°C and used within 12 months. Prior to each experiment, tomatoes were thawed overnight (18–24 h) at 13°C, then brought to 21°C and homogenized for 5–7 s (Cuisinart Smart Stick Hand Blender, Stamford, CT). Tomato purée was adjusted to a target pH value prior to inoculation and heating. Unacidified tomato samples were adjusted to pH 4.50 ± 0.05 by addition of granular NaOH (Fisher) (0.15–0.35 g/99 ml tomato purée). Acidified samples were adjusted to the target pH (4.20 ± 0.05 or 3.80 ± 0.05) with addition of citric acid monohydrate (Fisher) (0.1–0.2 g, 0.4–0.5 g) or glacial acetic acid (17.5 N, Fisher) (0.3 ml, 2.7 ml). Target pH values of 4.5, 4.2, and 3.8 were based on a review of 117 scheduled processes for tomato-based acidified foods that had been developed by the corresponding author over a 1-year period.

Tomato samples at the target pH were aseptically transferred (99 ml) into 710 ml Whirl-Pak filter bags (Nasco, Fort Atkinson, WI), air was expelled, and each bag was preheated to the target temperature prior to inoculation. After thermal processing, each purée sample was cooled on ice to 21°C and the final pH was measured.

Thermal processing

For each trial, preheated tomato purée (99 ml) was inoculated with 1 ml of a 5-strain pathogen cocktail (~10^{10} CFU/ml) to produce a starting inoculum concentration of 10^8–10^9 CFU/ml. Inoculation and sampling occurred through a narrow (2.5–4 cm) access point in the top of the bag to restrict movement of air into the bag. Upon inoculation, bag contents were briefly stirred, using a pipette tip, and samples of inoculated purée (1 ml) were taken for enumeration at pre-determined intervals, depending on the pathogen, temperature, and pH/acidulant. Total heating times in unacidified tomato purée (pH 4.5) ranged

from 150 min for *E. coli* O157:H7 heated at 52°C, with 30 min sampling intervals, to 6 min for *S. enterica* heated at 58°C, with 1 min sampling intervals. Heating times in acidified tomato purée at 54°C ranged from 60 min for *E. coli* O157:H7 at pH 4.2 (citric acid), with 10 min sampling intervals, to 4 min for *S. enterica* at pH 3.8 (acetic acid), with 30 s sampling intervals. At least four independent trials were conducted for each of the temperature/pH/acidulant/pathogen combinations.

Whirl-Pak bags containing inoculated tomato purée were kept fully submerged in a circulating water bath (Thermo Scientific Phoenix II, Newington, NH) during heating. The water level in the circulating water bath was maintained at 1–2 cm above the level of the purée in the Whirl-Pak bag at all times. Internal purée temperature was measured using a digital data logger (HH506RA Multi-logger thermometer, OMEGA Engineering, Inc., Norwalk, CT) connected to sterile type "K-TC" thermocouple probes placed in the top 2–3 cm of the purée and in the geometric center of the purée. The water bath temperature was monitored using a calibrated mercury-in-glass thermometer. Preliminary experiments indicated no significant temperature differences (≤ 0.5°C) attributable to location or time in tomato purée; the system was isothermal and at steady state.

Enumeration of surviving cells

At designated intervals, 1 ml samples of inoculated and heated tomato purée were drawn and diluted into pre-chilled (2–3°C) 9 ml BPD. Time-zero sampling occurred within 20 s of inoculation and mixing. Comparison of the cocktail inoculum count to the time-zero count indicated an average recovery of 102.6% (range 92.6–108.2%) for inoculum in tomato purée (data not shown).

At each time point, samples were serially diluted in BPD at 21°C and 0.1 ml aliquots were spread plated onto tryptic soy agar (TSA; Difco) within 10 min of sampling. *E. coli* O157:H7 and *S. enterica* culture plates were incubated at 37°C for 24 h, and *L. monocytogenes* culture plates at 37°C for 48 h, and surviving pathogens enumerated. A sample of each 453 g bag of blanched, frozen, and thawed tomato purée was plated on TSA to estimate native microbiota. Neither native microbiota nor comtaminants were ever counted, and they were rarely encountered in inoculated samples.

Mathematical modeling and statistical analysis

Log-linear plots of the bacterial count (CFU/ml) versus time were made for all trials; 52 trials were conducted with pathogen cocktails in unacidified tomato purée (pH 4.5) at 52–58°C, and 64 trials were conducted with pathogen cocktails in tomato purée acidified to pH 4.2 or 3.8 with citric or acetic acid. A successful trial included at least six sampling points and resulted in a reduction of at least three-log units in surviving cells. Five-log reduction times were determined using the re-parameterized non-linear Weibull model of Breidt et al. *(5)* according to Eq. *(1)*:

$$\log(N) = \log(N_0) - 5(t/t^*)^{\beta}, \quad (1)$$

where N is the cell count (CFU/ml) at time t, N_0 is the cell count at time zero, t* is the time when the log reduction value (LRV = $\log(N_0/N)$) equals 5, and β is a curve shape parameter (β = 1 is linear, β < 1 is concave up, β > 1 is concave down). As described in *(10)*, D* = t*/5, where D* is the decimal reduction time.

For experiments with unacidified tomato purée (pH 4.5), analysis of the temperature dependence of D* was according to the z-value of Eq. *(2)*:

$$\log(D^*_{T1}/D^*_{T2}) = (T2\text{-}T1)/z, \quad (2)$$

where D^*_T is the D*-value at temperature T, and z is the temperature increase required for a decimal reduction in the D*-value.

The mean and standard error for each of the three parameters of the re-parameterized nonlinear Weibull model ($\log(N_0)$, t*, β) were determined by use of the regression protocol of the R software package (R Foundation for Statistical Computing, Vienna, Austria) from all time points of the thermal death curve. Significant differences of t* values for all trials in unacidified tomato purée at pH 4.5 were determined with use of Tukey's HSD test in the R statistical software. Statistical differences between curve shape parameter β and 1 for assessment of linearity for each of the 116 trials were calculated by use of a one-sample Wald z-test. $D_{54°C}$ values from acidification trials were compared in PROC MIXED *(20)* (SAS version 9.4, SAS Institute, Cary, NC) using a 3*2*2 factorial design with all possible interactions for three pathogen types (*E. coli* O157:H7, *Salmonella*, *Listeria*) * two acidulants (acetic and citric acids) * two pH levels (4.2 and 3.8). Additionally, $D_{54°C}$ –values for tomato purée at pH 4.5 (acid control) and at pH 4.2 and 3.8, acidified with citrate or acetate, were compared using a 3*5 full factorial design for all five treatments for each pathogen, with no treatment factorial structure. Differences in $D_{54°C}$ –values for acidification trials were determined by Fisher's LSD test in the SAS statistical software. A significance cut-off value of 0.05 was used for all analyses.

RESULTS

Pathogen D-values in tomato purée at 54°C ranged from 20.98 min for *E. coli* O157:H7 (pH 4.5, unacidified) to 0.63 min for *S. enterica* (pH 3.8, acidified with acetic acid) *(Table 2)*. Pathogen thermal inactivation curves were often not linear (Weibull shape parameter (β) significantly different from 1, *P* < 0.05) As Dufort et al. reported *(10)*, 16 of 52 (31%) pathogen inactivation trials in unacidified tomato purée resulted in nonlinear inactivation curves; likewise,

TABLE 2. Decimal reduction times (D-value) for pathogen cocktails in tomato purée at 54°C

Pathogen		*E. coli* O157:H7		*L. monocytogenes*		*S. enterica*	
pH	Acidulant[a]	*n*[b]	D* (SD) min[c]	*n*	D* (SD) min	*n*	D* (SD) min
4.5[d]	None	4	20.85 (1.67)^{A,a}	6	10.63 (0.71)^{A,b}	4	6.97 (0.81)^{B,c}
4.2	Citric	4	16.39 (2.23)^{B,a}	7	10.63 (1.20)^{A,b}	4	6.12 (0.26)^{B,c}
4.2	Acetic	4	9.40 (0.76)^{C,a}	4	6.73 (1.10)^{B,b}	14	3.79 (1.49)^{C,c}
3.8	Citric	4	10.05 (0.87)^{C,a}	4	8.68 (0.90)^{B,a}	4	9.16 (2.24)^{A,a}
3.8	Acetic	4	2.71 (0.32)^{D,b}	4	3.49 (0.19)^{C,a}	7	0.63 (0.21)^{D,c}

[a]Acidulant: None = unacidified, Citric = citric acid monohydrate, Acetic = glacial acetic acid.
[b]*n* = Number of independent experiments.
[c]Mean (standard deviation) of the D-value (min). Mean values within a column with different uppercase superscripts (A–D) are significantly different ($P < 0.05$). Mean values within a row with different lowercase superscripts (a–c) are significantly different ($P < 0.05$).
[d]Data at pH 4.5 (unacidified) from Dufort et al. *(10)*.

in 38 of 64 (59%) thermal inactivation trials in acidified tomato purée (this study), the shape of the pathogen inactivation curve differed significantly from linearity ($P < 0.05$; data not shown).

β values averaged 0.93 (± 0.20), 0.55 (± 0.15) and 0.91 (± 0.14) across all pH/acid combinations for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, respectively. Inactivation kinetics for *E. coli* O157:H7 in tomato purée acidified to pH 3.8 with citric acid are depicted in *Fig. 1*. β values across the four trials ranged from 1.20 to 1.38; β = 1.30 for the average curve fit with the Weibull model *(Fig. 1)*. Post-processing pH measurements indicated that pH of heated tomato purée deviated only slightly from the initial pH value, fluctuating -0.06 to 0.05 pH units across all heated purée samples and all target pH values (n = 116; data not shown).

Results from a 3*2*2 factorial statistical analysis indicated a high level of interaction between the acidulant, pH level, and pathogen (data not shown; $P < 0.01$). Pathogen inactivation trends were not uniform across acidulant and pH level combinations. Subsequently, using a 3*5 factorial design, all D-values at 54°C were statistically compared as a one-way ANOVA with 15 treatments: three pathogens processed in five different tomato purée conditions (unacidified at pH 4.5, citrate-acidified at pH 4.2 and 3.8, and acetate-acidified at pH 4.2 and 3.8). $D_{54°C}$–values were not significantly different at several combinations of pathogens and treatments ($P > 0.05$) (data not shown). Meaningful and relevant statistical comparisons of pathogen inactivation across treatments are shown in *Table 2*. Under experimental conditions, *E. coli* O157:H7 was significantly more heat- and acid-resistant than *S. enterica* and *L. monocytogenes* in tomato purée at pH 4.5 (unacidified) and in purée acidified to pH 4.2 using citric or acetic acid ($P < 0.05$). *L. monocytogenes* was the most heat- and acid-resistant in purée acidified to pH 3.8 using acetic acid ($P < 0.05$), but in purée acidified to pH 3.8 using citric acid, there was no difference in pathogen inactivation ($P > 0.05$). Thermal tolerance in heated tomato purée at pH 4.5 or 4.2 followed the trend *E. coli* O157:H7 > *L. monocytogenes* > *S. enterica* ($P < 0.05$); at pH 3.8 with acetic acid as the acidulant, thermal tolerance followed the trend *L. monocytogenes* > *E. coli* O157:H7 > *S. enterica* ($P < 0.05$).

Data from previous work *(10)* were used to calculate 5-log reduction times for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* in tomato purée, pH 4.5 *(Table 3)*. From data on each pathogen, z-values were estimated based on the D-values from nonlinear modeling of microbial inactivation as described previously (*10, Table 4*). The z-value for *L. monocytogenes*, 7.95°C (14.3°F), was significantly higher than the z-value for *E. coli* O157:H7 and *S. enterica* ($P < 0.05$), which were not significantly different ($P > 0.05$). Using a reference temperature of 71.1°C (160°F), the F-160 value was estimated for each pathogen using the respective z-values in °C *(Table 4)*. Using pathogen z-values and D^*_{ref}, the data were extrapolated to the range of common processing temperatures (141–181°F/60.6–82.2°C); data for *E. coli* O157:H7 and *L. monocytogenes* are shown in *Fig. 2*. *E. coli* O157:H7 was the most heat resistant of the three pathogens at temperatures below 65°C (149°F), but *L. monocytogenes* was the most heat resistant at temperatures above 65°C *(Fig. 2)*. To establish a single set of parameters that ensure lethality of vegetative pathogens in the range of temperatures tested as well as those relevant to commercial processors, the two endpoints for the plotted 5 log-reduction times for the most heat tolerant organism at 52°C and 82.2°C, *E. coli*

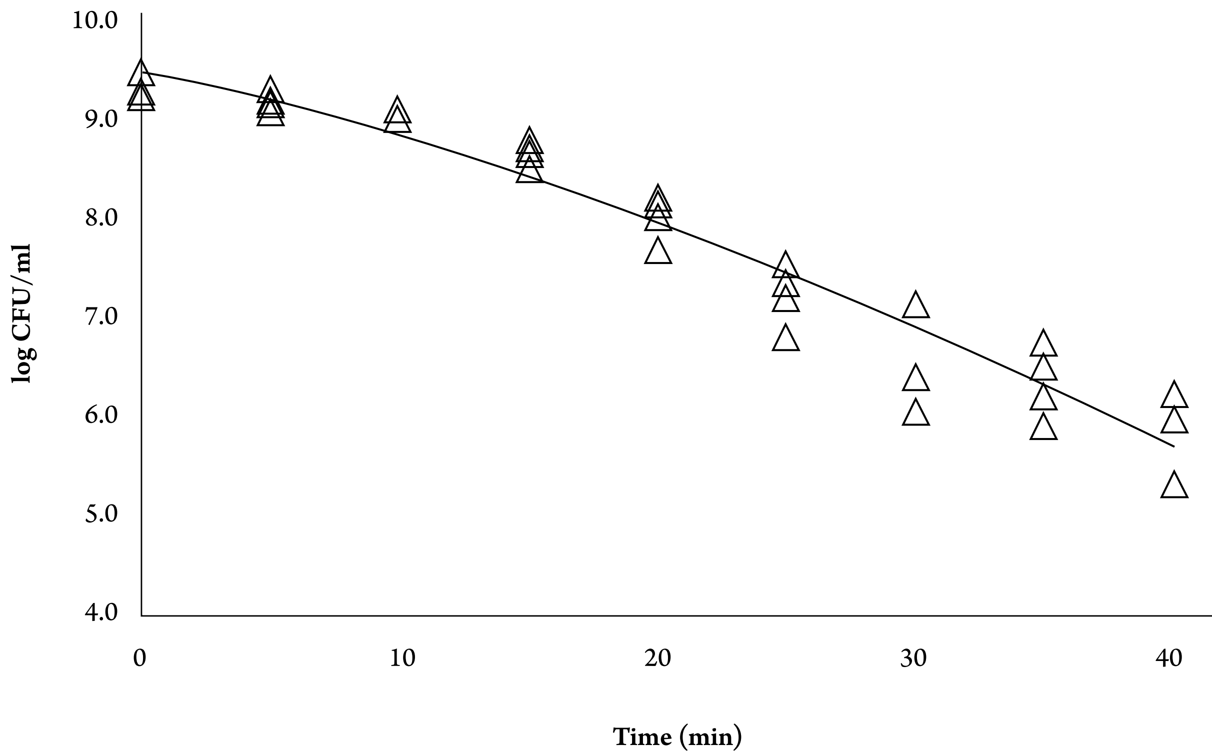

FIGURE 1. Thermal inactivation curve for E. coli *O157:H7 pathogen cocktail at 54°C (129.2°F) in tomato purée at pH 3.8, acidified with citric acid monohydrate. Four independent replications of the data are shown (Δ) and then fit using the re-parameterized Weibull model (–).*

TABLE 3. Estimated 5-log reduction times in unacidified tomato purée pH 4.5[a]

Pathogen	Temp °C	Temp °F	5LR (SD)[b]
Escherichia coli O157:H7	52	125.6	206.38 (22.44)
	54	129.2	104.27 (8.36)
	56	132.8	42.59 (2.21)
	58	136.4	27.63 (2.30)
Listeria monocytogenes	52	125.6	100.75 (5.09)
	54	129.2	53.14 (3.55)
	56	132.8	33.14 (2.74)
	58	136.4	17.09 (1.79)
Salmonella enterica	52	125.6	74.66 (6.40)
	54	129.2	38.84 (4.06)
	56	132.8	19.24 (2.70)
	58	136.4	9.00 (2.31)

[a]Based on data from Dufort et al. *(10)*.
[b]Mean (standard deviation) calculated 5-log reduction (5LR) time (min).

TABLE 4. z and F values in unacidified tomato purée at pH 4.5[a]

Pathogen	z-val (°C)[b]	z-val (°F)[b]	F-160[c] (min)
Listeria monocytogenes	7.95[A]	14.3[A]	0.39
Escherichia coli O157:H7	6.64[B]	12.0[B]	0.27
Salmonella enterica	6.57[B]	11.8[B]	0.09
Calculated	7.37	13.3	0.51

[a]Summarized from Dufort et al. *(10)*.
[b]Estimated z-values within a column with different superscripts (A–B) are significantly different ($P < 0.05$).
[c]Estimated 5-log reduction time at 160°F (71.1°C) (min).
[d]Calculated from fitted line (*Fig. 2*).

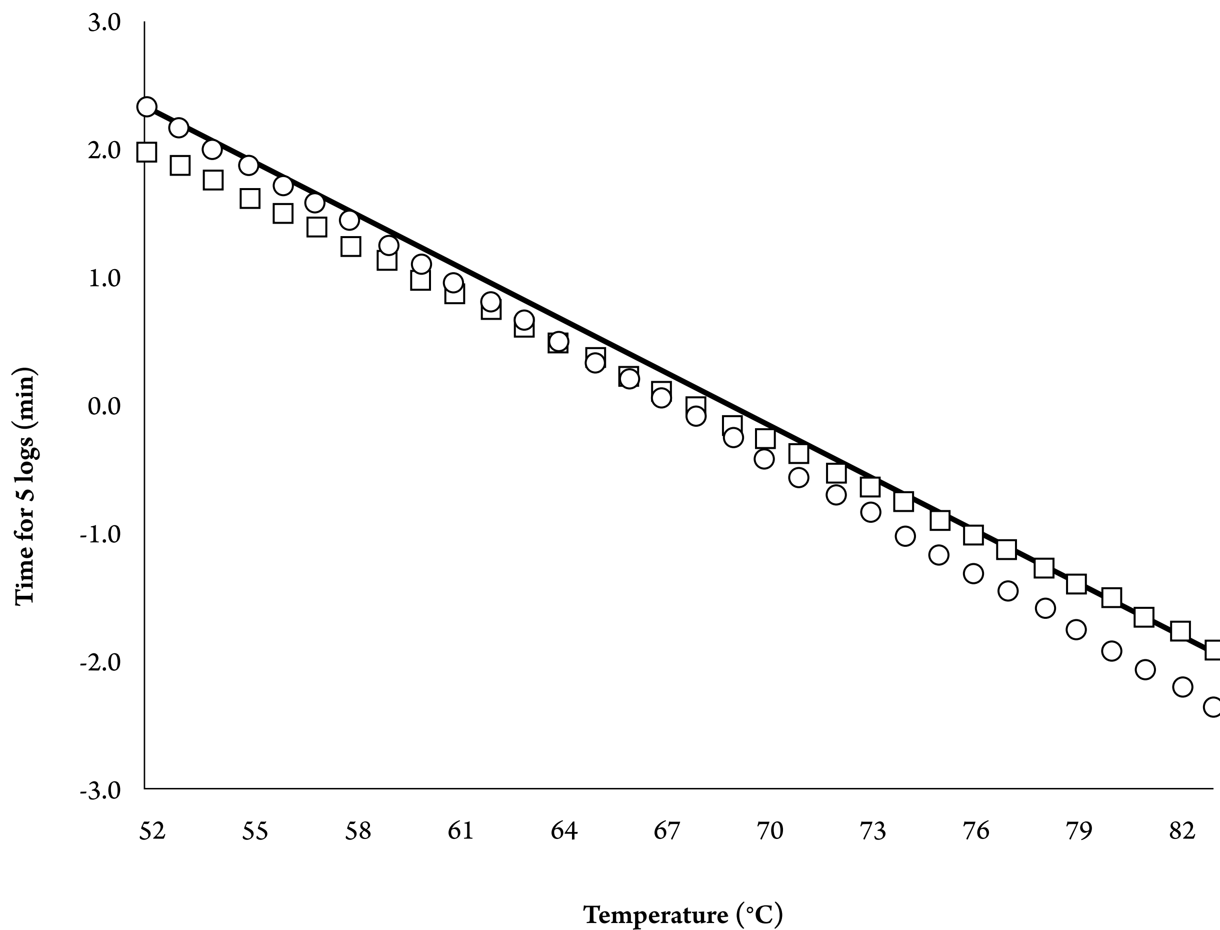

FIGURE 2. The 5-log reduction times for cocktails of E. coli *O157:H7 (circles) and* L. monocytogenes *(squares) in the range of temperatures tested and at typical processing temperatures. The solid line is the time to attain at least a 5-log pathogen reduction for* E. coli *O157:H7,* L. monocytogenes*, and* S. enterica *across all temperatures.*

TABLE 5. Calculated processing times to achieve at least a 5-log pathogen reduction versus temperature in tomato-based acidified foods (pH 4.5 and below)

T (°F)	T (°C)	Time (min[a])	T (°F)	T (°C)	Time (min)
141	60.6	13.83	161	71.7	0.43
142	61.1	11.63	162	72.2	0.36
143	61.7	9.78	163	72.8	0.30
144	62.2	8.22	164	73.3	0.26
145	62.8	6.91	165	73.9	0.21
146	63.3	5.81	166	74.4	0.18
147	63.9	4.88	167	75.0	0.15
148	64.4	4.10	168	75.6	0.13
149	65.0	3.45	169	76.1	0.11
150	65.6	2.90	170	76.7	0.09
151	66.1	2.44	171	77.2	0.08
152	66.7	2.05	172	77.8	0.06
153	67.2	1.72	173	78.3	0.05
154	67.8	1.45	174	78.9	0.04
155	68.3	1.22	175	79.4	0.04
156	68.9	1.02	176	80.0	0.03
157	69.4	0.86	177	80.6	0.03
158	70.0	0.72	178	81.1	0.02
159	70.6	0.61	179	81.7	0.02
160	71.1	0.51	180	82.2	0.02

O157:H7 and *L. monocytogenes*, respectively, were used to fit a straight line across the range of temperatures (solid line, Fig. 2). The fitted line was used to generate a table of processing conditions that ensure a reduction of at least 5 log units for all pathogens tested across all temperatures *(Table 5)* and, along with calculated values for z = 13.3°F (7.4°C) and F_{160} = 0.51 min *(Table 4)*, can be used to support scheduled process development and FDA process filing for tomato-based acidified foods.

DISCUSSION

Previous studies that established thermal processing conditions for acidified foods (pH 4.1–4.6) were conducted using a cucumber juice medium with acetic acid as the acidulant (pH 4.6) *(6)*. The 5-log reduction times in cucumber juice medium at 56°C were 126.10 min, 150.73 min, and 156.70 min, for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, respectively. The calculated 5-log reduction times for similar pathogen cocktails in tomato purée at 56°C were significantly shorter, ranging from 42.59 min for *E. coli* 157:H7 to 19.24 min for *S. enterica* *(Table 3)*. Additionally, the calculated z-values for pathogen reduction in cucumber juice medium (17.4, 15.6, and 16.7°F for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively), were higher than the z-values for the same pathogens in tomato purée, 11.9, 11.8 and 14.3°F *(Table 4)*. The differences in D- and z-values may be attributed to the presence of natural inhibitory organic acids present in the tomato purée that were not present in the cucumber juice medium as well as to differences in experimental design and methodology that could have impacted pathogen thermal tolerance, such as the level of dissolved oxygen present in the system *(14)*.

The D-values of all three pathogens decreased significantly ($P < 0.05$) as the pH decreased *(Table 2)*. Usaga et al. *(24)*, Gabriel *(11)*, and Steenstrup et al. *(21)* each studied the thermal tolerance of a single strain of *E. coli* O157:H7 and observed a similar pH-dependent trend when the pathogen was heated in an apple-carrot juice blend (pH 3.3 to 4.5), in a model fruit juice (pH 3.0 to 6.0), and in apple cider (pH 3.1 to 4.2), respectively. Across the pH and acid treatment combinations in this study at 54°C, *E. coli* O157:H7 was significantly more heat and acid resistant than *S. enterica*

and *L. monocytogenes* ($P < 0.05$) *(Table 2)*. Breidt et al. *(4)* found that thermal inactivation rates of *E. coli* O157:H7 and *L. monocytogenes* in pickle brine (pH 4.1) were identical, while *S. enterica* was significantly less heat tolerant. Similarly, Mazzotta *(16)* found that, in heated fruit juices (pH 3.9), *S. enterica* was heat sensitive while *E. coli* O157:H7 was heat resistant. Acidification of a product reduces the heat resistance of vegetative cells and reduces the possibility of recovery from sub-lethal injury *(24)*. In acidified tomato purée samples, all three pathogens were significantly more heat tolerant when purée was acidified with citric acid rather than with acetic acid ($P < 0.05$; *Table 2*). When tomato purée was acidified to pH 3.8 with acetic acid rather than with citric acid, the average $D_{54°C}$ –values were 7.34 min, 8.53 min, and 5.19 min lower for *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, respectively. In heated cucumber purée, Bae et al. also found that *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* were more resistant to citric acid than to acetic acid *(3)*. The antimicrobial effects of citric and acetic acid are dependent on their pKa values *(23)*. Acetic acid (pK_a 4.76) is a stronger acid than citric acid, which is a triprotic acid (pK_{a1} 3.13, pK_{a2} 4.77, pK_{a3} 6.39). In acidified tomato-based foods with pH 4.5 and below, acetic acid has a greater proportion of undissociated acid than citric acid has, and acetic acid is more likely than citric acid to diffuse into the cell, lower cytoplasmic pH, and inhibit metabolic reactions *(1, 17)*. While this phenomenon may at least partially explain our findings, our experimental design was aimed only at achieving a target pH-value and did not take into account either buffering capacity of the system or actual quantity of acid added. For industrial food safety applications, it is advantageous to use organic acids with a pK_a near the pH of the food environment. However, the amount and concentration of acid required to achieve the target pH value, the associated flavor characteristics, and the labeling provision of an acidulant are also important factors to consider when selecting acidulants for product formulation *(12)*.

In previous work from our laboratory, pathogen inactivation in unacidified tomato purée (pH 4.5) was modeled over a range of temperatures (52–58°C) using the re-parameterized Weibull method and the shape of the inactivation curve (β) was evaluated, with nonlinearity frequently observed *(10)*. In the current study, when pathogen inactivation was modeled in acidified tomato purée (pH 4.2 and 3.8) as compared to unacidified purée, nonlinearity in heat inactivation curves increased from 31% to 59% of trials (data not shown), suggesting that a nonlinear curve-fit model, such as the Weibull method, may be of even greater utility when establishing accurate thermal processing parameters in low pH food systems.

Using data derived from a cucumber juice medium, Breidt et al. *(6)* calculated thermal processing parameters for pathogen destruction in acidified cucumber juice across a wide range of potential processing temperatures (60.6–82.2°C/141–180°F) and showed that *L. monocytogenes* was more heat resistant than *E. coli* O157:H7 and *S. enterica* at temperatures below 74°C (166°F), but *E. coli* O157:H7 was the most heat resistant at temperatures above 74°C. The present work found that when 5-log reduction values were plotted against a wide range of typical processing temperatures, data lines for *E. coli* O157:H7 and *L. monocytogenes* intersected at 65°C; *E. coli* O157:H7 was the most heat-resistant pathogen at temperatures below 65°C (149°F), but *L. monocytogenes* was the most heat resistant above 65°C *(Fig. 2)*. While the physiological explanation for this difference in z-values between the two pathogens is not completely understood, the difference in z-values is important to consider when calculating processing time/temperature combinations. Therefore, to establish a single set of parameters that ensure lethality of vegetative pathogens across a wide range of relevant temperatures, a straight line was created that ensured at least a 5-log pathogen reduction across all temperatures. The linear equation that defined the straight line was used to generate a table of calculated time-temperature pairs *(Table 5)* that would ensure at least a 5-log reduction for all pathogens tested, similar to the method of Breidt et al. *(6)*. Processors of tomato-based acidified foods should consult a competent process authority to determine additional heating requirements beyond the minimal recommended here, to account for formulation effects on thermal processing, to address spoilage, and/or to address destruction of acid-resistant microbiota and other relevant factors in the establishment of the scheduled process.

RECOMMENDATIONS

Using the re-parameterized Weibull model, we have calculated time/temperature conditions that ensure at least a 5-log reduction of vegetative pathogens of concern in tomato-based acidified canned foods (pH ≤ 4.5).

- **Processing time and temperature combinations were calculated that can be applied to acidified products with tomato as the primary ingredient/formulation base.** These recommendations support safety while avoiding over-processing. Processors will wish to take into account product formulation and the potential presence of spoilage organisms in using these minimal to establish operational processing parameters.
- **Acidification of tomato-based canned foods using citric or acetic acid will increase pathogen lethality across all heating temperatures,** with acetic acid having greater antimicrobial activity than citric acid.
- **Processing times and temperatures achieve at least a 5-log pathogen reduction across a wide range of anticipated processing conditions.** Modeling pathogen survival across typical commercial processing temperatures indicated that the target organism depends on temperature; *E. coli* O157:H7 had the greatest

heat tolerance at temperatures below 65°C, while *L. monocytogenes* had the greatest heat tolerance at temperatures above 65°C.

- **Calculated minimum processing time/temperature combinations** *(Table 5)* **and use of a z-value of 13.3°F (7.4°C),** along with consideration given to intrinsic product characteristics and acid tolerant spoilage microbiota, can be used by Process Authorities to develop scheduled processes or support FDA process filing for tomato-based acidified food products.

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