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Thermal Inactivation of Stationary-Phase and Acid-Adapted *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* in Fruit Juices

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ABSTRACT

The heat resistance of stationary-phase and acid-adapted *Escherichia coli* O157:H7, *Salmonella enterica* (serotypes Typhimurium, Enteritidis, Gaminara, Rubislaw, and Hartford), and *Listeria monocytogenes* was evaluated in single-strength apple, orange, and white grape juices adjusted to pH 3.9. The heat resistance increased significantly ($P < 0.05$) after acid adaptation. *Salmonella* had an overall lower heat resistance than the other pathogens. Acid-adapted *E. coli* O157:H7 presented the highest heat resistance in all juices at the temperatures tested, with lower *z*-values than *Salmonella* and *L. monocytogenes*. The heat resistance ($D_{60°C}$-values) of all three pathogens, assessed in tryptic soy broth adjusted to different pH values, increased above pH 4.0. From the results obtained in this study, one example of a treatment that will inactivate 5 logs of vegetative pathogens was calculated as 3 s at 71.1°C (*z*-value of 5.3°C). Normal processing conditions calculated for hot-filled, shelf-stable juices achieve a lethality in excess of 50,000 *D* for all three pathogens.

Acidic foods such as fruit juices were not recognized as vehicles of foodborne illness until recently when major outbreaks involving *Escherichia coli* O157:H7 and *Salmonella enterica* occurred in apple and orange juices and in apple cider. *E. coli* O157:H7 was first confirmed as the epidemiological agent in juices after an apple cider outbreak in 1991 *(3).* However, it was also suspected as the microbial agent responsible for an earlier outbreak in apple cider *(20).* Two other major multistate outbreaks involving this pathogen in fresh apple juice occurred in 1996 *(6, 7).* Different serotypes of *S. enterica* were also implicated in foodborne disease transmitted by apple and orange juices, including *Salmonella* Typhimurium in commercial apple cider *(4)* and *Salmonella* Hartford, *Salmonella* Gaminara, and *Salmonella* Rubislaw in fresh orange juice *(5).* All incidents of foodborne illness reported in fruit juices were from unpasteurized juice *(11).*

These and other incidents of foodborne disease involving fruit juices prompted the U.S. Food and Drug Administration (FDA) to propose a hazard analysis and critical control point (HACCP) regulation that includes a performance criterion to assure juice safety *(11).* If this rule becomes final, juice producers will be required to implement a system that will achieve a 5-$\log_{10}$ reduction of the most resistant organism of public health significance. In selecting the target organism, juice processors would have to conduct a hazard analysis to determine what pathogenic organism is most likely to occur in their particular juice plant operation. No outbreaks involving *Listeria monocytogenes* in fruit juices have been reported; however, the National Advisory Committee on Microbiological Criteria for Foods recommended that, in the absence of known specific pathogen-product associations, *E. coli* O157:H7 or *L. monocytogenes* should be used as target organisms, as appropriate *(11).*

After the proposed regulation was published, two major outbreaks occurred in orange juice; a multistate outbreak of *S. enterica* serotype Muenchen from orange juice produced in a plant in Arizona *(8),* and a major outbreak of *Salmonella* Typhimurium that affected more than 400 people in South Australia *(17).* Once again, the production of these juices did not include a kill step, and these outbreaks further emphasize the need to inactivate pathogens in juice.

Taking into account the safety record of pasteurized fruit juices, pasteurization seems an effective way to assure juice safety. However, the minimum time-temperature combinations necessary to inactivate 5 logs of vegetative pathogens in juices deserved more study. The heat resistance of *E. coli* O157:H7 has been evaluated only in apple cider and juice *(14, 22)* for stationary-phase cells, while we could find no reference to indicate that the thermal resistance of *Salmonella* or *L. monocytogenes* in fruit juices had been investigated.

Microorganisms may contaminate the fruit and be transferred to the juice during or after extraction. Pathogenic microorganisms do not grow in fruit juices due to their low pH but can survive and become adapted to the acidic environment *(15, 18, 23).* The extended survival of these pathogens in acidic foods and the increased tolerance of acid-adapted cells to unfavorable growth conditions is well established *(2, 9, 15, 18, 21).* Acid adaptation may

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also increase the heat resistance of these organisms, and processing conditions should account for this fact.

This study evaluated the heat resistance of stationary-phase and acid-adapted *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* in single-strength apple, orange, and white grape juices. The results presented here may help juice producers to determine the appropriate target pathogen to establish a heat process, as well as the parameters required to inactivate the pathogen in a fruit juice using heat.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *E. coli* O157:H7 strains used were C7927, isolated from a patient in an apple cider-associated outbreak in Massachusetts (obtained from M. P. Doyle, University of Georgia); and National Food Processors Association strains N-4070, isolated from juice from the 1996 Odwalla-associated outbreak; N-4073, isolated from juice from the 1996 Connecticut apple cider-associated outbreak; and N-4063, an acid-tolerant strain isolated from an outbreak involving salami. The heat resistance of these strains was tested individually in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.); a composite of strains C7927 and N-4063 was used to test the thermal resistance in juices. Two composites of different *Salmonella* serotypes were used. Composite 1 included serotypes isolated from juices: *Salmonella* Gaminara, *Salmonella* Rubislaw, and *Salmonella* Hartford (obtained from M. E. Parish, University of Florida). Composite 2 was prepared using clinical isolates of *Salmonella* Typhimurium American Type Culture Collection (ATCC) 13311, *Salmonella* Enteritidis ATCC 13076, and *Salmonella* Enteritidis strain N-4016. The *L. monocytogenes* composite included four clinical isolates: strain N-7285 (serotype 1/2a), strain N-7298 (serotype 1/2b), strain N-7004 (Scott A, serotype 4b), and strain N-7017 (Murray B, serotype 4b).

Working cultures of all strains were made from freeze-dried stocks and maintained on slants of tryptic soy agar (Difco) at 4°C and transferred monthly. Prior to the inoculum preparation, *E. coli* O157:H7 and *Salmonella* strains were grown in TSB aerobically at 35°C; *L. monocytogenes* strains were grown in TSB supplemented with 0.2% dextrose and 0.1% yeast extract (TSBDY) and incubated at 30°C. For a stationary-phase inoculum, the strains were individually grown overnight. Acid-adapted cultures were grown as above in media adjusted to pH 5 with 1 N HCl. For compositing, equal volumes of each strain were mixed. Each strain was enumerated on tryptic soy agar prior to compositing to ensure approximately equal numbers of each strain in the composite. Dilutions were made in 0.1% peptone water.

Juice preparation. Different brands of shelf-stable, single-strength 100% apple, orange, or white grape juices without preservatives were purchased at a local supermarket. The pH of juices was aseptically adjusted to 3.9 with 1 N NaOH before use.

Experimental procedure. Thermal death time experiments were conducted using an end-point procedure in three-neck flasks (1,000 ml). The flasks were fitted with a thermocouple (type-T) through a cotton plug in one of the openings, cotton plugs in the other two openings, and a stir-bar, and were sterilized in an autoclave before use. The test juice (150 ml) was dispensed aseptically into the flask. The flask was immersed in a thermostatically controlled water bath (model DL 30; Haake, Hamburg, Germany) to a depth above the level of the juice, and allowed to equilibrate. Test temperatures selected were 58, 60, and 62°C for *E. coli* O157:H7 and 56, 60, and 62°C for *Salmonella* and *L. monocytogenes.* The juice was agitated constantly with a submersible stirrer (model 230; VWR Scientific, Bridgeport, N.J.). The juice temperature was recorded using a Validator (Kaye Instruments, Bedford, Mass.). When thermal equilibrium was achieved, 1 ml of the inoculum was added to the juice at a final concentration of approximately 10^5 cells per ml, to evaluate the thermal resistance of either stationary-phase or acid-adapted cells. At five predetermined time intervals, 6-ml samples were removed with a single-use sterile pipette and dispensed into an empty test tube in an ice-water bath. The tube was shaken in the ice-water bath to cool the juice rapidly, and 1-ml aliquots were dispensed into five tubes containing 9 ml of the recovery medium. *E. coli* O157:H7 and *Salmonella* were recovered in TSB and incubated at 35°C and *L. monocytogenes* was recovered in TSBDY and incubated at 30°C.

Negative controls were prepared by dispensing 1 ml of the unheated, uninoculated juice into each of 5 tubes containing 9 ml of the appropriate recovery medium. Positive controls were prepared similarly, using unheated, inoculated juice. Positive and negative controls were incubated along with the test samples.

To determine the initial concentration of cells, 0.1 ml of the inoculum was dispensed in 15 ml of the juice (to maintain the 1:150 ratio) and, after appropriate decimal dilutions, pour-plated with tempered tryptic soy agar, and incubated overnight at the incubation temperature appropriate for each organism.

Influence of pH on thermal resistance. The heat resistance of stationary-phase and acid-adapted *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* composites was assessed at 60°C, following the same procedure described above. The *Salmonella* composite used included all strains (composite 1 plus composite 2). Due to the buffering capacity of juices, it was not possible to adjust their pH to values above 4.5. Hence, the experiments were done in TSB adjusted to pH 3, 3.5, 4, 4.5, 5, 6, and 7 with 1 N or 0.1 N HCl, using a Future Plus electrode connected to a model Φ 44 pH meter (Beckman Instruments, Inc., Fullerton, Calif.). After the heat treatment, cells were recovered as above. For each condition two independent experiments were performed.

Identity of organisms in positive tubes. Recovery tubes developing turbidity (and gas in the case of *E. coli* O157:H7 and *Salmonella*) were considered presumptive positive for growth of the test organism. For *E. coli* O157:H7, a loopful from positive tubes at the longest time interval from each temperature was streaked onto MacConkey-Sorbitol agar (Difco); colonies were confirmed as *E. coli* O157:H7 by testing for antigens O157 and H7 using the Rim *E. coli* O157:H7 latex test (Remel, Lenexa, Kans.). For *Salmonella,* a loopful was streaked on Hektoen enteric agar (Difco) and incubated at 35°C. Colonies were identified as *Salmonella* following the procedures in the FDA Bacteriological Analytical Manual *(1),* and compared to pure cultures. For *L. monocytogenes,* a loopful was streaked onto Oxford medium base with Oxford antimicrobial supplement (Difco) and incubated at 30°C. *L. monocytogenes* colonies were identified following the procedures in the FDA Bacteriological Analytical Manual *(13)* and compared to pure cultures. Additional tests were also performed using Vitek (model 32; bioMérieux, Vitek, Inc., Hazelwood, Mo.) with GNI+ and GPI cards.

Calculation of *D*- and *z*-values. At each temperature, decimal reduction times (*D*-values) were determined by the formula: $D = t/(\log A - \log B)$, where t is the heating time in minutes; A is the initial number of organisms in the flask (CFU/ml); and B is the final number of cells that survived at the last time point. B was estimated by the most probable number procedure of Halvorson and Ziegler *(12),* using the formula: $B = \ln(\text{total number}$

TABLE 1. *Heat resistance of* E. coli *O157:H7 in single-strength fruit juices adjusted to pH 3.9*

Juice	Inoculum growth condition	*D*-value ± SD (min) at temperature (°C) of: 56	58	60	*z*-value (°C)
Apple	Stationary phase[a]	4.1 ± 0.70	1.9 ± 0.16	0.8 ± 0.09	5.6
	Acid adapted[b]	7.0 ± 0.21	3.5 ± 0.53	1.5 ± 0.39	5.9
Orange	Stationary phase	7.5 ± 0.39[c]	3.2 ± 0.30[c]	1.1 ± 0.35[c]	4.8
	Acid adapted	11.0 ± 1.29[d]	5.0 ± 1.10[d]	1.7 ± 0.34[d]	4.9
White grape	Stationary phase	4.0 ± 0.78	1.6 ± 0.35	0.7 ± 0.34	5.3
	Acid adapted	6.1 ± 1.30	2.7 ± 0.73	1.2 ± 0.20	5.7

[a] Grown overnight in pH 7.2 TSB.
[b] Grown overnight in pH 5.0 TSB.
[c] Significantly different from values obtained with stationary cells in apple and white grape ($P < 0.5$).
[d] Significantly different from values obtained with acid-adapted cells in apple and white grape ($P < 0.5$).

of tubes for an interval/number of sterile tubes). In experiments where all samples were positive at one time point and all negative at the next time point, the *D*-value was estimated by assuming one negative at the last all-positive interval, and one positive at the first all negative time point, calculating separate *D*-values for each assumption, and averaging the values. The *z*-values (°C) were calculated as the negative inverse slope of the linear regression line for the log *D*-values over the range of heating temperatures tested.

Statistics. Values given are the means for three independent experiments. Variances were analyzed and compared by a general linear model procedure at the 95% confidence interval with SAS statistical software (SAS Institute Inc., Cary, N.C.).

RESULTS

During preliminary thermal death time experiments, recovery tubes that did not become positive after 10 days of incubation did not turn positive during a continued incubation period of 30 days (longest time to positive was 4 days). In subsequent experiments, recovery tubes were incubated for 10 days. All three strains of *E. coli* O157:H7 isolated from outbreaks associated with juice had similar heat resistance after growth to stationary phase or acid adaptation in TSB (data not shown). Therefore, only strain C7929 was composited with N-4063 to evaluate the heat resistance of *E. coli* O157:H7 in juices.

D- and *z*-values in the three juices for *E. coli* O157:H7 are presented in Table 1, for *L. monocytogenes* in Table 2, for *Salmonella* composite 1 in Table 3 and for *Salmonella* composite 2 in Table 4. An analysis of variance ($\alpha = 0.05$) showed that acid adaptation increased the heat resistance of all three pathogens at all temperatures tested. The increase in the heat resistance was higher for *E. coli* O157:H7 and *L. monocytogenes* than for the two *Salmonella* composites tested. The average *z*-value for *L. monocytogenes* was 6.1 ± 0.3°C, for *Salmonella* 5.8 ± 0.3°C, and for *E. coli* O157:H7 5.3 ± 0.4°C. An analysis of variance found no significant differences ($P < 0.05$) between the *z*-values of *L. monocytogenes* and *Salmonella,* but these were to some extent higher than that of *E. coli* O157:H7.

Thermal death time experiments in TSB at different pH values confirmed that acid adaptation increased the $D_{60°C}$-value of all three pathogens. At pH values lower than 4.0, the difference between stationary-phase and acid-adapted cultures was greater for *E. coli* O157:H7 and *L. monocytogenes* than for *Salmonella* (Fig. 1). The *Salmonella* composite used in this experiment included all *Salmonella* serotypes listed in the methodology section. The $D_{60°C}$-values of the three pathogens increased at pH values above 4.0 for stationary-phase and acid-adapted cultures (Fig. 1), although this increase was less dramatic for *Salmonella* stationary-phase cells.

The heat resistance of *E. coli* O157:H7 was slightly but significantly ($P < 0.05$) higher in orange juice than in apple or white grape (Table 1). There were no differences in the heat resistance of the other organisms in orange, apple, or white grape at any of the temperatures tested.

To compare the heat resistance of the three pathogens, the *D*-values obtained from the acid-adapted inocula were

TABLE 2. *Heat resistance of* L. monocytogenes *in single-strength juices adjusted to pH 3.9*

Juice	Inoculum growth condition	*D*-value ± SD (min) at temperature (°C) of: 56	60	62	*z*-value (°C)
Apple	Stationary phase[a]	1.59 ± 0.15	0.45 ± 0.07	0.17 ± 0.04	6.3
	Acid adapted[b]	5.00 ± 0.45	0.90 ± 0.30	0.43 ± 0.03	5.6
Orange	Stationary phase	2.05 ± 0.49	0.43 ± 0.06	0.21 ± 0.02	6.0
	Acid adapted	3.83 ± 0.41	0.67 ± 0.12	0.38 ± 0.09	5.9
White grape	Stationary phase	2.29 ± 0.37	0.59 ± 0.07	0.29 ± 0.01	6.6
	Acid adapted	4.59 ± 0.70	1.38 ± 0.17	0.48 ± 0.11	6.3

[a] Grown overnight in pH 7.2 TSBDY.
[b] Grown overnight in pH 5.0 TSBDY.

TABLE 3. *Heat resistance of* Salmonella *(composite of serotypes Gaminara, Rubislaw, and Hartford) in single-strength fruit juices adjusted to pH 3.9*

Juice	Inoculum growth condition	*D*-value ± SD (min) at temperature (°C) of: 56	60	62	*z*-value (°C)
Apple	Stationary phase[a]	0.96 ± 0.27	0.28 ± 0.17	0.09 ± 0.05	6.0
	Acid adapted[b]	1.07 ± 0.18	0.32 ± 0.06	0.09 ± 0.02	5.8
Orange	Stationary phase	1.61 ± 0.38	0.21 ± 0.10	0.08 ± 0.02	4.6
	Acid adapted	1.40 ± 0.46	0.35 ± 0.27	0.10 ± 0.01	5.4
White grape	Stationary phase	2.43 ± 0.29	0.44 ± 0.12	0.28 ± 0.04	6.2
	Acid adapted	3.62 ± 0.87	0.95 ± 0.42	0.36 ± 0.18	6.1

[a] Grown overnight in pH 7.2 TSB.
[b] Grown overnight in pH 5.0 TSB.

plotted versus temperature (Fig. 2). Independently of the juice, the highest *D*-values of each pathogen were fitted to a linear regression, and the fitted line was extrapolated to higher processing temperatures. Acid-adapted *E. coli* O157:H7 had greater heat resistance at the temperatures tested than *Salmonella* and *L. monocytogenes.* This curve fit yielded a *z*-value of 5°C for *E. coli* O157:H7 and a *z*-value of 6°C for the other two pathogens. When extrapolating the results to higher temperatures the slopes of the fit of the highest *D*-values for *E. coli* O157:H7 cross that of *L. monocytogenes* at 65°C. This may indicate that *L. monocytogenes* is more heat resistant than *E. coli* O157:H7 at typical juice-processing temperatures. The *Salmonella* serotypes tested (Gaminara, Rubislaw, Hartford, Enteritidis, and Typhimurium) had the lowest heat resistance at all temperatures (tested and extrapolated) (Fig. 2). To determine a process that would inactivate all three pathogens at all processing temperatures, a line above the experimental *D*-values of *E. coli* O157:H7 and the extrapolated heat resistance of *L. monocytogenes* was drawn and yielded the equation $\log D = 11.5 - 0.19T$ (°C). From this equation a general process for juices can be calculated as $5D = 3$ s at 71.1°C (160°F, milk pasteurization temperature) with a *z*-value of 5.3°C (9.5°F).

DISCUSSION

Pasteurization is a treatment that can increase the safety of fruit juices. In choosing the target organism to calculate the lethality of a pasteurization treatment, juice processors may consider either *E. coli* O157:H7 or *Salmonella,* due to the numerous outbreaks that these organisms have caused in unpasteurized juices or *L. monocytogenes* due to its ubiquitous nature. In fact, the target organism should be the most resistant pathogen likely to occur in the juice, so a process effective against the target organism will also control other pathogens *(19).*

Acid adaptation in *E. coli* O157:H7 *(9)* and in *L. monocytogenes (16)* cross-protects the cells to heat, increasing the *D*-values. During the production of juices, these pathogens, if present, will be exposed to a low pH environment that may trigger a protective response to a heat process. Acid adaptation significantly increased the heat resistance of *E. coli* O157:H7 and *L. monocytogenes* in the three juices tested. It will be unlikely, under good manufacturing practices and sanitation standard operating procedures, that a juice will be contaminated with 5 logs of any of these pathogens. Moreover, the calculation of a process that considers the heat resistance of acid-adapted cells instead of stationary-phase cells will include an extra safety factor to the minimum requirements for pasteurization.

From all data obtained for the three pathogens in the three juices, it is clear that *Salmonella* was the most heat-sensitive organism under the conditions tested. Based on a review of the data in Tables 1 to 4, it would appear that acid-adapted *E. coli* O157:H7 should be considered as the target organism in juices, because the heat resistance at the temperatures tested was higher than that of both *Salmonella* and *L. monocytogenes.* However, acid-adapted *L. monocy-*

TABLE 4. *Heat resistance of* Salmonella *(composite of serotypes Typhimurium and Enteritidis) in single-strength fruit juices adjusted to pH 3.9*

Juice	Inoculum growth condition	*D*-value ± SD (min) at temperature (°C) of: 56	60	62	*z*-value (°C)
Apple	Stationary phase[a]	1.21 ± 0.42	0.23 ± 0.10	0.11 ± 0.05	5.7
	Acid adapted[b]	2.28 ± 0.36	0.81 ± 0.23	0.19 ± 0.10	5.9
Orange	Stationary phase	2.52 ± 1.32	0.45 ± 0.23	0.22 ± 0.04	5.4
	Acid adapted	4.54 ± 1.07	0.98 ± 0.45	0.49 ± 0.15	6.0
White grape	Stationary phase	1.38 ± 0.18	0.28 ± 0.16	0.10 ± 0.04	5.3
	Acid adapted	1.60 ± 0.58	0.45 ± 0.13	0.12 ± 0.03	5.5

[a] Grown overnight in pH 7.2 TSB.
[b] Grown overnight in pH 5.0 TSB.

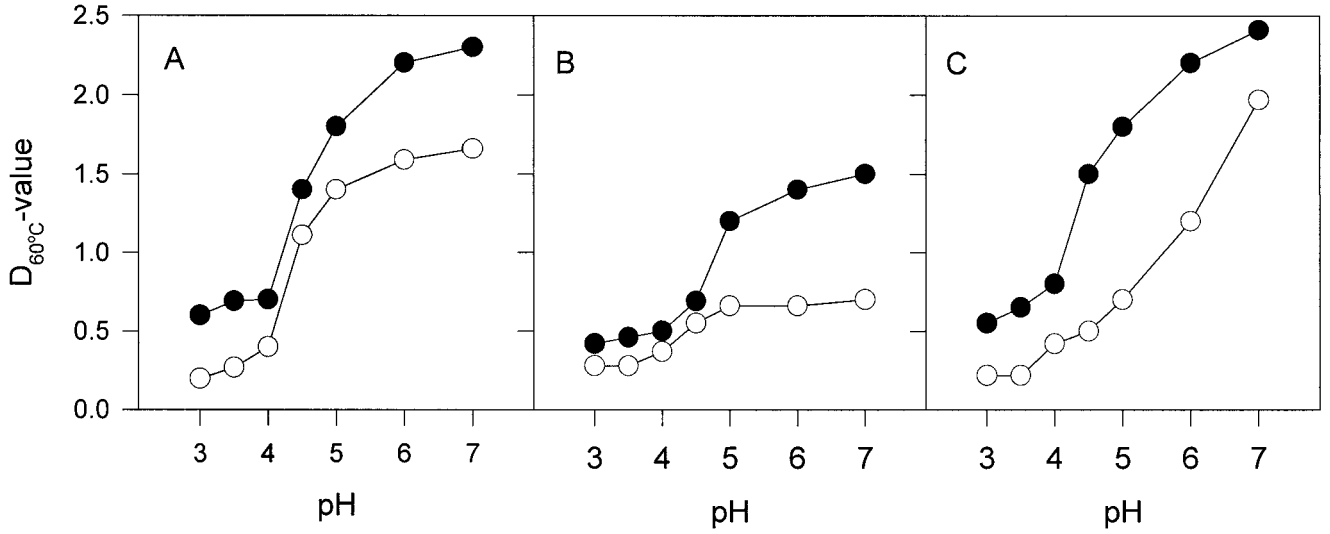

FIGURE 1. *Influence of pH on the* $D_{60°C}$*-value of stationary-phase (○) and acid-adapted (●)* E. coli *O157:H7 (panel A),* Salmonella *(panel B), and* L. monocytogenes *(panel C).*

togenes had a higher *z*-value, and, when extrapolating the heat resistance to temperatures above 65°C, it had a greater heat resistance than acid-adapted *E. coli* O157:H7. The overall process of 3 s at 71.1°C (z = 5.3°C) calculated by combining the results obtained at the test temperatures with the data extrapolated to higher temperatures of all three pathogens provides guidelines to establish effective heat treatments. However, juice processors may want to obtain data specific to a juice to optimize the processes.

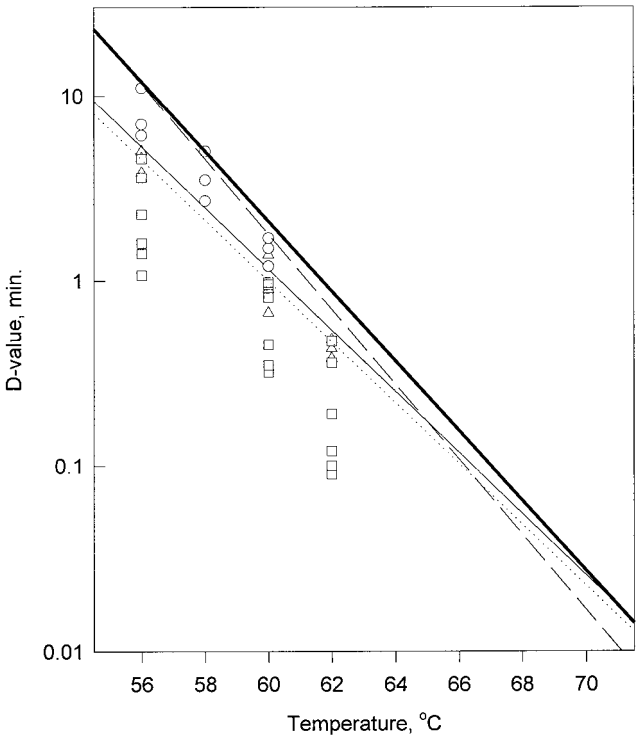

FIGURE 2. *Semilogarithmic plot of* D*-values versus temperature for (○)* E. coli *O157:H7, (□)* Salmonella *composite 1 and 2, and (△)* L. monocytogenes. *The highest* D*-values obtained for each pathogen, independently of the juice, were fitted to a linear curve to calculate a minimum process at 71.1°C. The dashed line represents* E. coli *O157:H7, the dotted line* Salmonella, *and the solid fine line* L. monocytogenes. *A thick solid line was drawn above all the pathogen regression lines to calculate an overall process for juices.*

Considering that it is not likely to find 10^5 pathogens per ml of juice produced under good manufacturing practices and good sanitary plant conditions, this process is very conservative. During an outbreak of salmonellosis from orange juice contaminated with *Salmonella* Hartford, counts recovered from the juice were below 10 CFU/100 ml of juice *(10).* Moreover, the process calculation was based on data for acid-adapted cells; cells that have not adapted to a stress shock would be more sensitive.

The juice type and juice composition may influence the heat resistance parameters of these organisms. Degrees Brix in the range of 11.8 to 16.5 were reported to have no significant effect on the heat resistance of *E. coli* O157:H7 in apple juice *(22).* The pH in the range of 3.6 to 4.0 was also reported as a nonsignificant variable in the heat resistance of *E. coli* O157:H7 in apple juice. However, the heat resistance increased at pH 4.4 *(22).* The heat resistance of the three pathogens tested, as influenced by the pH, increased considerably above pH 4, so processors of juices or juice mixes with pH above 4.0 should expect these pathogens to demonstrate higher *D*-values.

Shelf-stable, hot-filled juices are processed to inactivate microorganisms such as molds, yeasts, and other bacteria that may spoil the product. A typical pasteurization process might be 90°C for 2 s, followed by filling at 85°C and holding at that temperature for 1 min before cooling. Without taking into account the cumulative lethality during the cooling period, shelf-stable, hot-filled juices receive a lethality sufficient to inactivate 50,000 logs of acid-adapted vegetative pathogens such as *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes.* There is, thus, no question about the microbial safety of these juices.

If the pasteurization process is below that necessary to kill pathogens present in juices, refrigeration temperatures

prolong the survival of healthy, stressed, and heat-treated cells in the juice *(16).* None of the pathogens tested grew at pH 3.9 but remained viable for more than 30 days at refrigeration temperatures (data not shown). None of the strains tested survived in juice beyond 2 days at 35°C (data not shown), but the results confirm previous observations that refrigerated storage can considerably extend the survival of these pathogens in juices *(18, 23).* Processing temperatures and postprocess storage conditions need to be selected carefully to prevent the survival of cells that may potentially be present in juice.

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