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Abstract

Thermal tolerance of acid-adapted *Escherichia coli* **O157:H7 or** *Salmonella* **in ground beef was evaluated during storage at 4°C or –20°C. Both pathogens were adapted to acidic conditions (pH ~4.6) by growing in tryptic soy broth supplemented with 1% glucose. A five-strain cocktail of** *E. coli* **O157:H7 or** *Salmonella* **was grown separately in TSB (pH ~6.6) and TSB + 1% glucose for 24 h at 37°C to provide cells with or without acid adaptation. Irradiated ground beef was inoculated with either acid-adapted or non-adapted** *E. coli* **O157:H7 or** *Salmonella***; the samples stored at 4°C were subjected to heat treatment at 62°C or 65°C on days 1, 7, 14, 21, and 28, and the samples stored at –20°C were subjected to heat treatment at 62°C or 65°C on days 1, 30, 60, 90, and 120. Decimal reduction time (D values) of the pathogens was determined as an indicator of thermal tolerance. Significantly higher D_{62} values were observed on days 21 and 28 for non-adapted** *E. coli* **O157:H7 stored at 4°C and on days 90 and 120 for non-adapted** *E. coli* **O157:H7 stored at –20°C ($P < 0.05$). Higher D_{62} values were observed on days 21 and 28 among non-adapted** *Salmonella* **strains stored at 4°C and on day 28 for acid-adapted strains of** *Salmonella* **stored at 4°C ($P < 0.05$). Higher D_{62} values for acid-adapted strains of** *Salmonella* **stored at –20°C were observed on days 30, 60, and 90 ($P < 0.05$), when while no differences were observed in the D_{65} values of acid-adapted and non-adapted strains of** *E. coli* **O157:H7 and** *Salmonella* **throughout storage at both temperatures ($P > 0.05$). This suggests that acid adaptation of foodborne pathogens provides a certain level of protection against heat treatment at lower cooking temperatures, while at higher temperatures there were no observed differences between the sensitivity of acid-adapted and non-adapted strains in an actual food system over an extended period of refrigerated and frozen storage.**

Disciplines

Agriculture | Food Chemistry | Food Processing | Meat Science | Parasitology

Comments

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Thermal Tolerance of Acid-Adapted and Non-adapted *Escherichia coli* O157:H7 and *Salmonella* in Ground Beef During Storage

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ABSTRACT

Thermal tolerance of acid-adapted *Escherichia coli* O157:H7 or *Salmonella* in ground beef was evaluated during storage at 4°C or –20°C. Both pathogens were adapted to acidic conditions (pH ~4.6) by growing in tryptic soy broth supplemented with 1% glucose. A five-strain cocktail of *E. coli* O157:H7 or *Salmonella* was grown separately in TSB (pH ~6.6) and TSB + 1% glucose for 24 h at 37°C to provide cells with or without acid adaptation. Irradiated ground beef was inoculated with either acid-adapted or non-adapted *E. coli* O157:H7 or *Salmonella*; the samples stored at 4°C were subjected to heat treatment at 62°C or 65°C on days 1, 7, 14, 21, and 28, and the samples stored at −20°C were subjected to heat treatment at 62°C or 65°C on days 1, 30, 60, 90, and 120. Decimal reduction time (D values) of the pathogens was determined as an indicator of thermal tolerance. Significantly higher D_{62} values were observed on days 21 and 28 for non-adapted *E. coli* O157:H7 stored at 4°C and on days 90 and 120 for non-adapted *E. coli* O157:H7 stored at –20°C ($P < 0.05$). Higher D_{62} values were observed on days 21 and 28 among non-adapted *Salmonella* strains stored at 4°C and on day 28 for acid-adapted strains of *Salmonella* stored at 4°C ($P < 0.05$). Higher D_{62} values for acid-adapted strains of *Salmonella* stored at –20°C were observed on days 30, 60, and 90 ($P < 0.05$), when while no differences were observed in the D_{65} values of acid-adapted and non-adapted strains of *E. coli* O157:H7 and *Salmonella* throughout storage at both temperatures ($P > 0.05$). This suggests that acid adaptation of foodborne pathogens provides a certain level of protection against heat treatment at lower cooking temperatures, while at higher temperatures there were no observed differences between the sensitivity of acid-adapted and non-adapted strains in an actual food system over an extended period of refrigerated and frozen storage.

INTRODUCTION

SINCE ITS FIRST RECOGNITION AS A FOODBORNE pathogen in the United States in 1982, *Escherichia coli* O157:H7 has emerged as one of the most important foodborne pathogens. The Centers for Disease Control and Prevention (CDC) reported that *E. coli* O157:H7 causes an estimated 73,000 infections, resulting in more than 2,000 hospitalizations and 61 deaths annually in the United States (Mead et al., 1999). Infection by *E. coli* O157:H7 can cause severe complications such as hemorrhagic colitis and hemorrhagic uremic syndrome (HUS) (Riley et al., 1983); the organism has been successfully isolated from foods associated with outbreaks of hemorrhagic colitis or HUS (Doyle and Schoeni, 1987). In 2003, he annual estimated cost of illness due to O157:H7 Shiga-like toxin producing *E. coli* (STEC) was $405 million, including $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity (Frenzen et al., 2005). According to Doyle and Schoeni (1987) *E. coli* O157:H7 has been isolated from 3.7% of beef, 1.5% of pork, 1.5% of poultry, and 2.0% of lamb samples, in-

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dicating that the bacterium is associated with foods of animal origin and not specifically beef.

In 1999, the Department of Agriculture Food Safety and Inspection Service (USDA/FSIS) established lethality regulations for fully and partially cooked meat and poultry products (USDA/FSIS, 1999). A 6.5-log unit reduction of *Salmonella* in cooked beef and roast beef and a 7.0-log unit reduction in certain fully and partially cooked poultry products were set as performance standards for lethality, stabilization, and product handling (Weche et al., 2005). Raw meat and poultry are usually considered ideal growth media for bacteria; however, all microbial contaminants are subjected to physical, chemical, and nutritional stresses during processing (Yousef and Courtney, 2003). Bacteria can face exposure to extremes of acidity in many situations in the environment, in foods, and in the bodies of animals or humans (Nojoumi et al., 1995). The USDA supports use of a mixture of pathogenic bacterial strains containing relatively heat-resistant serovars, particularly those that have been implicated in outbreaks, to verify compliance with performance standards (USDA/FSIS, 2001). Most of the cultures are prepared under optimal laboratory conditions, but a cocktail of bacteria containing stressed cells that more truly represents the physiological state of an organism that may contaminate the product during or after processing is a better choice for thermal inactivation or challenge studies (Juneja and Novak, 2003).

Acidic foods have long been considered generally safe for human consumption, and outbreaks related to these foods have rarely been recorded (Arvizu-Medrano, 2005). However, some acidic foods such as mayonnaise (Smittle, 2000), yogurt (Morgan et al., 1993), apple juice (CDC, 2000), and orange juice (Merrel and Camilli, 2000) have been associated with foodborne outbreaks: in 80% of these outbreaks, *Salmonella* and *E. coli* O157:H7 have been implicated, suggesting the acid tolerance of these pathogens. The ability of bacteria to survive in acid foods is of concern for pathogens with low infective doses, such as *S. typhi*. The acid adaptation response results in increased resistance of microorganisms to severe acid shocks and provides crossprotection against various other environmental stresses such as heat and surface active agents (Leyer and Johnson, 1993). Acid-adapted *E. coli* O157:H7 has shown increased resistance to irradiation (Buchanan et al., 1999) and heat (Buchanan and Edelson, 1999a) in laboratory media and in liquid food systems; however, varying results have been reported on the resistance of acid-adapted *Salmonella* to environmental stresses.

Information on the acid tolerance response of *S. typhi* is limited. Tiwari et al. (2004) found that *S. typhi* in the exponential phase is able to adapt and increase its acid tolerance in culture medium; however, it is important to investigate the acid responses in the stationary phase and in an actual food matrix, because of the possibility that the pathogen could contaminate food products when it is in its stationary phase. This study was undertaken to determine the thermal tolerance of acid-adapted pathogens in a food system that reflects current processing, storage, and distribution practices of food products. Our objective was to investigate the effects of storage temperature on the thermal tolerance of acid-adapted and non-adapted *Salmonella* spp. and *E. coli* O157:H7 in ground beef.

MATERIALS AND METHODS

Preparation of bacterial cultures

Five-strain cocktails of *E. coli* O157:H7 (ATCC 35150 and ATCC 43894 [human feces from outbreak of hemorrhagic colitis]; ATCC 43895 [isolate from raw hamburger implicated in hemorrhagic colitis]; WS 3062 and WS 3331 [clinical isolates]) and of *S. enterica* (*S*. Newport, *S*. Uganda, *S*. Heidelberg, *S*. Typhimurium, and *S. enteritidis*) were used. The five strains of *E. coli* O157:H7 were obtained from the Food Safety Research Laboratory (FSRL) and the five bovine strains of *Salmonella* spp. were obtained from the Veterinary Diagnostic Laboratory at Iowa State University, Ames. Iowa. All stock cultures were maintained on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD) slants at 4°C. Working cultures were maintained by daily transfers in tryptic soy broth (TSB; Difco) and TSB + 1% glucose for the non-adapted and acid-adapted strains, respectively.

Transfers of the non-adapted and acid-adapted cultures were done daily for 14 days prior to developing growth curves. The final pH of TSB ranged from 6.6 to 6.8 while the pH of TSB + 1% glucose was 4.6 after growing the cultures for 24 h. Growth curves for individual strains of *E. coli* O157:H7 and *Salmonella* spp. were constructed to determine the time at which the stationary phase was reached in order to make subsequent transfers (data not shown). The non-adapted and acid-adapted inocula were prepared by inoculating bacterial cultures into 10 mL TSB and TSB + 1% glucose, respectively, and then incubating at 37°C for 24 h. The addition of glucose to the growth medium allows the production of organic acids that lower the final pH of the culture medium significantly (Buchanan and Edelson, 1999b). Cultures (1 mL) were then transferred into 25-mL TSB centrifuge tubes and further incubated at 35°C for 18 h. The cultures were then centrifuged at 5,738 *g* for 10 min at 4°C (SORVALL SUPER T21, Newton, CT); supernatant was decanted and the resultant pellet resuspended with 10 mL of 0.1% sterile peptone water (PW; Difco). Each cocktail was prepared by mixing the five cultures in a sterile bottle to get a final volume of 50 mL of the inoculum.

Inoculation of ground beef

Ground beef was obtained from the Iowa State University Meat Laboratory and irradiated at the Iowa State University Linear Accelerator Facility to an average absorbed dose of 8.05 kGy. Ground beef was irradiated to remove any background gram-negative microflora. The irradiated ground beef was divided into four parts of 350 g each and inoculated with 50 mL of either non-adapted or acid-adapted *E. coli* O157:H7 and *Salmonella* spp., respectively. Inoculum was hand mixed into the ground beef to obtain a homogenous distribution into the product. Two-gram portions of inoculated ground beef were then placed into plastic pouches (2 × 2 inches), heat-sealed, and stored at 4°C for 4 weeks or −20°C for 120 days. Thermal resistance of samples stored at 4°C was analyzed at 7-day intervals for 28 days and those stored at −20°C were analyzed at 30-day intervals for 120 days.

Thermal resistance in ground beef

Thermal tolerance of the non-adapted and acid-adapted strains was determined by measuring decimal reduction values (D values, the time in minutes required to kill 1 log concentration of bacteria) at 62°C (143.6°F) and 65°C (149°F) for up to 10 minutes. The 2 g inoculated ground beef pouches were completely immersed in a water bath maintained at either 62°C or 65°C and removed from the water bath at 0, 1, 2, 3, 4, 5, 7.5, and 10 minutes. An additional 2 g ground beef pouch was placed in each water bath to monitor the temperature increase. A data logger (LI-1000; LI-COR, Lincoln, NE) was used to monitor temperature by inserting a thermocouple into a 2 g ground beef pouch and placing it in the water bath. Each experiment was not started until the 2 g portion of inoculated ground beef had reached the same temperature as the water bath. This was designated as time 0. The pouches were removed from the water bath and immediately placed in an ice water bath at each time interval. Each pouch was allowed to cool in the ice water bath for 10 min prior to sampling.

Microbial sampling and enumeration

The plastic pouch containing 2 g of ground beef that had been subjected to either 62°C or 65°C was aseptically opened into a filter stomacher bag and diluted with 10 mL 0.1% PW. The ground beef was homogenized with the diluent in a stomacher (Stomacher 400, Tekmar, Cincinnati, OH) for 1 min. Samples were serially diluted in 0.1% PW and spread-plated onto TSA: Clavero and Beuchat (1996) suggested that a higher recovery of *E. coli* was achieved on TSA compared to MacConkey agar and modified eosin methylene blue agar. The plates were incubated at 37°C for 48 h and colony forming units were manually counted and reported as $\log_{10}$ CFU/g.

Experimental design and statistical analysis

A randomized complete block design was used to prepare four 350 g batches of noninoculated ground beef that was then inoculated with 50 mL of either non-adapted or acid-adapted *E. coli* O157:H7 or *Salmonella* spp. The inoculated

Table 1. Decimal Reduction Time at 62°C of Acid-Adapted and Non-adapted *Escherichia Coli* O157:H7 and *Salmonella* in Ground Beef Stored at 4°C for Up to 28 Days

	Escherichia coli *O157:H7*		Salmonella	
Day	*Non-adapted*	*Acid-adapted*	*Non-adapted*	*Acid-adapted*
1	$1.97^{1,a}$	$2.20^{1,a}$	$1.98^{1,a}$	$1.93^{1,a}$
7	$2.46^{1,a}$	$2.53^{1,a}$	$2.00^{1,a}$	$2.24^{1,2,a}$
14	$2.91^{1,a}$	$2.88^{1,a}$	$2.43^{1,a}$	$2.29^{1,2,a}$
21	$4.50^{2,b}$	$2.71^{1,a}$	$3.55^{2,b}$	$2.35^{1,2,a}$
28	$4.60^{2,b}$	$2.66^{1,a}$	$4.29^{2,b}$	$2.98^{2,a}$

Numbers indicate significant differences ($p < 0.05$) within a group (acid adapted or non-adapted) for each pathogen.

Letters indicate significant differences ($p < 0.05$) between groups (acid adapted or non-adapted) for each pathogen.

ground beef was divided into 2 g pouches in a completely randomized design prior to D value testing on day 1 of storage at 4°C or −20°C. Survival curves were constructed for the organisms recovered on TSA, with the Y-axis representing $\log_{10}$ CFU/g of each inoculum tested and the X-axis representing time in minutes. D values were calculated as the reciprocal of the slope of the survivor curve for each inoculum type exposed to 62°C (D_{62}) or 65°C (D_{65}). Three replications of the experiment were performed and the mean D value of each inoculum was analyzed using analysis of variance (ANOVA) with the SAS PROC MIXED procedures (SAS Institute, Cary, NC).

RESULTS

There was no significant difference in D_{62} values of non-adapted and acid-adapted *E. coli* O157:H7 for up to 14 days of refrigerated storage at 4°C (Table 1). Significantly higher D_{62} values of non-adapted strains of *E. coli* O157:H7 were observed on days 21 and 28 of the storage period at 4°C. Among the non-adapted strains of *E. coli* O157:H7, significantly higher D_{62} values were observed after 21 days of refrigerated storage whereas no significant differences were observed for the acid-adapted strains over the 28-day storage period. There was no statistical differences in D_{65} values of non-adapted and acid-adapted strains of *Salmonella* throughout the 28 days of refrigerated storage (Table 2). D_{65} values of non-adapted and acid-adapted strains of *E. coli* O157:H7 did not change significantly over the 28-day storage period.

Significantly higher D_{62} values of non-adapted *Salmonella* stored at 4°C were observed in comparison to their acid-adapted counterparts on days 21 and 28. Higher D_{62} values

Table 2. Decimal Reduction Time at 65°C of Acid-Adapted and Non-adapted *Escherichia Coli* O157:H7 and *Salmonella* in Ground Beef Stored at 4°C for Up to 28 Days

	Escherichia coli *O157:H7*		Salmonella	
Day	*Non-adapted*	*Acid-adapted*	*Non-adapted*	*Acid-adapted*
1	$1.58^{1,a}$	$1.27^{1,a}$	$1.15^{1,a}$	$1.23^{1,a}$
7	$1.05^{1,a}$	$0.89^{1,a}$	$0.83^{1,a}$	$0.79^{1,a}$
14	$0.91^{1,a}$	$0.82^{1,a}$	$0.82^{1,a}$	$0.73^{1,a}$
21	$1.30^{1,a}$	$0.80^{1,a}$	$1.16^{1,a}$	$0.92^{1,a}$
28	$1.40^{1,a}$	$0.97^{1,a}$	$1.38^{1,a}$	$1.10^{1,a}$

Numbers indicate significant differences ($p < 0.05$) within a group (acid adapted or non-adapted) for each pathogen.

Letters indicate significant differences ($p < 0.05$) between groups (acid adapted or non-adapted) for each pathogen.

TABLE 3. DECIMAL REDUCTION TIME AT 62°C OF ACID-ADAPTED AND NON-ADAPTED *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN GROUND BEEF STORED AT −20°C FOR UP TO 120 DAYS

	Escherichia coli *O157:H7*		Salmonella	
Day	*Non-adapted*	*Acid-adapted*	*Non-adapted*	*Acid-adapted*
1	$1.85^{1,a}$	$1.86^{1,a}$	$1.98^{1,a}$	$1.93^{1,a}$
30	$2.25^{1,2,a}$	$2.40^{2,a}$	$1.65^{1,a}$	$2.56^{1,b}$
60	$2.29^{1,2,a}$	$2.34^{2,a}$	$1.65^{1,a}$	$2.54^{1,b}$
90	$2.36^{2,a}$	$2.73^{2,3,a}$	$1.85^{1,a}$	$2.58^{1,b}$
120	$2.35^{2,a}$	$2.85^{3,b}$	$2.03^{1,a}$	$2.62^{1,a}$

Numbers indicate significant differences ($p < 0.05$) within a group (acid adapted or non-adapted) for each pathogen.

Letters indicate significant differences ($p < 0.05$) between groups (acid adapted or non-adapted) for each pathogen.

were also observed among the non-adapted strains after 21 days of refrigerated storage. D_{62} values of acid-adapted *Salmonella* on day 1 were significantly lower than on day 28, while no statistical differences were seen between days 7, 14, and 21 during refrigerated storage. At 65°C there were no significant differences in the D values of non-adapted and acid-adapted *Salmonella* throughout the 28-day storage at 4°C. Also, there was no significant difference in D_{65} value as a result of storage time among the non-adapted *Salmonella* or their acid-adapted counterparts.

D_{62} values of non-adapted *E. coli* O157:H7 and *Salmonella* were comparable to their acid-adapted counterparts during the initial phase of storage at 4°C. D_{62} values of non-adapted pathogens tended to increase tremendously after 14 days and were significantly higher than the acid-adapted *E. coli* O157:H7 and *Salmonella* on days 21 and 28 of storage. Similar but not significant changes were also observed for D_{65} values of non-adapted *E. coli* O157:H7 on days 21 and 28 of storage, while *Salmonella* showed slight changes in D_{65} values for both acid-adapted and non-adapted strains throughout the 28-day storage at 4°C.

Tables 3 and 4 show the D values at 62°C and 65°C of acid-adapted and non-adapted *E. coli* O157:H7 and *Salmonella* in ground beef stored at −20°C over a 120-day period (Tables 3, 4). The acid-adapted strains of *E. coli* O157:H7 had significantly higher D_{62} values than their non-adapted counterparts on day 120 of frozen storage. Significant differences in the D_{62} values were also observed between days 1 and 90 and days 1 and 120 among the non-adapted strains of *E. coli* O157:H7 whereas in the acid-adapted strains significant differences were observed between day 1 and the rest of the storage period up to 120 days. Statistically significant differences in the D_{62} values were also observed

TABLE 4. DECIMAL REDUCTION TIME AT 65°C OF ACID-ADAPTED AND NON-ADAPTED *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA* IN GROUND BEEF STORED AT −20°C FOR UP TO 120 DAYS

	Escherichia coli *O157:H7*		Salmonella	
Day	*Non-adapted*	*Acid-adapted*	*Non-adapted*	*Acid-adapted*
1	$0.89^{1,a}$	$1.13^{2,a}$	$0.74^{1,a}$	$0.81^{1,a}$
30	$0.55^{1,a}$	$0.59^{1,a}$	$0.60^{1,a}$	$0.68^{1,a}$
60	$0.55^{1,a}$	$0.57^{1,a}$	$0.65^{1,a}$	$0.68^{1,a}$
90	$0.54^{1,a}$	$0.56^{1,a}$	$0.58^{1,a}$	$0.58^{1,a}$
120	$0.54^{1,a}$	$0.57^{1,a}$	$0.52^{1,a}$	$0.58^{1,a}$

Numbers indicate significant differences ($p < 0.05$) within a group (acid adapted or non-adapted) for each pathogen.

Letters indicate significant differences ($p < 0.05$) between groups (acid adapted or non-adapted) for each pathogen.

between days 30 and 120 and days 60 and 120 among the acid-adapted *E. coli* O157:H7 stored at −20°C. For D_{62} values of *Salmonella* significant differences were observed between the non-adapted and acid adapted strains on days 30, 60, and 90 of storage at −20°C. No significant differences in the D_{65} values were observed in the non-adapted or acid-adapted strains of *Salmonella* throughout the 120-day storage at −20°C.

No significant differences in D_{65} values were observed between the non-adapted and acid-adapted strains of *E. coli* O157:H7 as well as *Salmonella* throughout the 120-day storage at −20°C. In addition, no significant differences were observed as a result of storage in the non-adapted and acid-adapted strains. D_{65} values of non-adapted and acid-adapted *E. coli* O157:H7 and *Salmonella* were slightly higher on day 1 and tended to be lower but fairly constant for the remainder of the 120-day storage at −20°C.

DISCUSSION

Carcass decontamination and further processing of meat products exposes foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* to environmental stresses including starvation, low water activity, and low pH environments that may trigger protective responses to heat (Mazzotta, 2001). Numerous studies have reported that environmental factors such as pH can affect the thermal resistance of microorganisms (Bearson et al, 1997; Jay, 2000; Leyer and Johnson, 1993).

D values of non-adapted *E. coli* O157:H7 were higher at both 62°C and 65°C in our study compared to those reported by Juneja et al. (1997). Their study reported D values of 0.93 and 0.39 min for non-adapted *E. coli* O157:H7 at 62.5°C and 65°C, respectively. Differences in the D values of the non-adapted *E. coli* O157:H7 could be attributed to the variation in the type of strains used to conduct these thermal tolerance studies. Results from our study showed no differences between the D values of acid-adapted and non-adapted *E. coli* O157:H7 at 65°C irrespective of the storage temperature, while at 62°C the non-adapted cells had a higher D value than the acid-adapted cells after 21 days at 4°C. The D value of acid-adapted *E. coli* O157:H7 was higher than that of the non-adapted counterpart after 120 days at −20°C. These results were contrary to a study conducted by Buchanan and Edelson (1999a) that suggested a higher D value for three different strains of acid-adapted *E. coli* O157:H7 grown in laboratory media. The D values reported in their study were lower than those observed in our study; this may be attributed to the higher temperatures used in our study and/or to ground beef being used instead of laboratory media, which could potentially provide some level of protection to the pathogens against heat. D values at 62°C and 65°C in our study were also lower than those reported by Ahmed et al. (1995) and Line et al. (1991), who used a lower temperature in their studies.

A large amount of research has been done on the thermal tolerance of acid-adapted and non-adapted foodborne pathogens, especially *E. coli* O157:H7 and *Salmonella*, at low or sublethal temperatures: this leaves the need to investigate higher temperatures that are more indicative of current cooking practices. Cheng et al. (2002) demonstrated that acid-adapted *E. coli* O157:H7 were more thermally tolerant than their non-adapted counterparts in laboratory media and Ryu and Beuchat (1998) suggested that the heat tolerance of *E. coli* O157:H7 can be substantially enhanced by acid adaptation compared to acid shock at 52°C in apple cider and orange juice. Comparing our study to Cheng et al. (2002) and Ryu and Beuchat (1998), it can be concluded that acid adaptation might increase thermal tolerance in liquid foods, but the same effect is not observed in a solid food matrix such as ground beef.

Calicioglu et al. (2003) reported higher susceptibility of acid-adapted *Salmonella* than the non-adapted cells on beef jerky after drying, which is in agreement with the results from our study that indicated higher susceptibility of the acid-adapted *Salmonella* in ground beef stored at 4°C. Results on the D values in ground beef stored at −20°C showed that the acid-adapted strains of *Salmonella* were slightly more tolerant to heat than their non-adapted counterparts at 62°C and 65°C, concurrent with results from a study conducted by Leyer and Johnson (1993)

suggesting increased thermal tolerance of acid-adapted *Salmonella* at 50°C in cheese. Sharma et al. (2005) reported significant differences in D values of *Salmonella* and *E. coli* O157:H7 grown in TSB + 1% glucose in comparison to strains grown in TSB, regardless of the type of fruit juice they were heated in, whereas thermal resistance of *Listeria monocytogenes* was not affected by adaptation in acidified broth. As seen in our study, this is not necessarily true in the case of a food matrix such as ground beef over an extended period of refrigerated or frozen storage. Minimal or no differences in the thermal tolerance of acid-adapted and non-adapted *E. coli* O157:H7 and *Salmonella* in ground beef indicate the possibility that differences observed in laboratory media may not necessarily be extrapolated to a food system.

CONCLUSION

In this study there were no significant differences ($P > 0.05$) between the acid-adapted and non-adapted *E. coli* O157:H7 and *Salmonella* in ground beef throughout the 120-day storage period at –20°C. Since studies in the past have reported differences in the thermal tolerance of acid-adapted and non-adapted pathogens, it is important to consider the protective responses that are triggered as a result of environmental stresses that can be critical when choosing the most resistant target organism in order to calculate lethality of a heat treatment in a food matrix. A conservative approach that takes into consideration the most resistant target organisms can help in adding an extra safety factor to the minimum regulatory requirements for heating/cooking meat and poultry products. Most studies have reported thermal tolerance of acid-adapted pathogens in a laboratory medium or a liquid food system, but there is a lack of literature on the thermal behavior of these pathogens in an actual food matrix that has been subjected to refrigerated or frozen storage. Studies undertaken to determine thermal tolerance of acid-adapted *E. coli* O157:H7 have been conducted at lower temperatures ranging from 52°C to 58°C, in laboratory media, or in liquid foods such as juices. Our study was conducted in an actual food system (ground beef) that was subjected to handling conditions mimicking day-to-day practices followed in the industry, grocery stores, and consumer households. We have shown that acid adaptation of *E. coli* O157:H7 and *Salmonella* does not pose any additional threat for these pathogens surviving in foods if they have been cooked per the regulatory guidelines for non-adapted pathogens. Further studies need to be conducted to determine the pathogenicity of these acid-adapted pathogens to humans.

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