

Survival and Growth of *Escherichia coli* O157:H7 in Ground, Roasted Beef as Affected by pH, Acidulants, and Temperature

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A study was undertaken to determine the fate of *Escherichia coli* O157:H7 in ground, roasted beef as influenced by the combined effects of pH, acidulants, temperature, and time. There was essentially no change in the viable population of *E. coli* O157:H7 when beef salads (pH 5.40 to 6.07) containing up to 40% mayonnaise were incubated at 5°C for up to 72 h. At 21 and 30°C, significant ($P \leq 0.05$) increases in populations of the organism occurred in salads containing 16 to 32% mayonnaise (pH 5.94 to 5.55) between 10 and 24 h of incubation. Death was more rapid as the pH of acidified beef slurries incubated at 5°C was decreased from 5.98 to 4.70. *E. coli* O157:H7 grew in control slurries (pH 5.98) and in slurries containing citric and lactic acids (pHs 5.00 and 5.40) incubated at 21°C for 24 h; decreases occurred in slurries acidified to pHs 4.70, 5.00, and 5.40 with acetic acid or pH 4.70 with citric or lactic acid. At 30°C, populations decreased in slurries acidified to pHs 4.70 and 5.00 with acetic acid. Citric and lactic acids failed to prevent significant increases in populations in slurries at pH 4.70 to 5.40 between 10 and 24 h of incubation. The order of effectiveness of acidulants in inhibiting growth was acetic acid > lactic acid \geq citric acid. The same order was observed for inactivation of *E. coli* O157:H7 in acidified (pH 5.00) beef slurry heated at 54°C. Sorbitol MacConkey agar supplemented with 4-methylumbelliferyl- β -D-glucuronide was unable to resuscitate some heat-stressed *E. coli* O157:H7 cells, indicating its limited value as a recovery medium for these organisms. Results indicate that *E. coli* O157:H7 can grow at pH values characteristic of beef salads.

In 1982, two outbreaks of hemorrhagic colitis in the United States were associated with the consumption of ground beef sandwiches (2). *Escherichia coli* O157:H7 was isolated from a frozen raw beef patty of the kind implicated in these outbreaks (32). It was suggested that the organism survived the grilling process used to cook the hamburgers. Several additional foodborne outbreaks caused by *E. coli* O157:H7 have since been reported (7). Foods suspected as vehicles for transmitting the pathogen have included raw milk, turkey sandwiches, and cooked potatoes. Data published by Bryant et al. (5) suggested an association between ingestion of undercooked ground beef and human infections. Manifestations of illness caused by *E. coli* O157:H7 include nonbloody diarrhea, hemorrhagic colitis, cystitis and balanitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (7, 27).

Foods of animal origin appear to be primary sources of *E. coli* O157:H7 infections. The organism has been isolated from dairy cattle (4), calves (24), chickens (3), swine, and sheep (9). Retail meats from these animals have been documented to harbor *E. coli* O157:H7 (9, 28).

The presence of coliforms, occasionally including enteropathogenic *E. coli*, on salad vegetables has been documented by numerous investigators (10, 11, 14, 18, 20, 22). Confirmation of a recent outbreak of *E. coli* O157:H7 infection due to waterborne contamination (15, 26) further supports the possibility of cross-contamination of salad vegetables and other foods during processing and marketing and in food service operations. The presence of various pathogenic

bacteria on a wide range of raw and cooked delicatessen foods (13, 19), presumably because of cross-contamination during preparation and handling in food service and retail outlets, reaffirms the need to investigate the survival and growth characteristics of *E. coli* O157:H7 on such foods. The study reported here was undertaken to determine the fate of *E. coli* O157:H7 in ground, roasted beef as influenced by the combined effects of pH, acidulants, temperature, and time.

MATERIALS AND METHODS

Inoculum preparation. Five strains of *E. coli* O157:H7 were studied. Strain 301C (chicken isolate) was provided by Donald E. Conner, Auburn University. Strains 204P (pork isolate), 505B (beef isolate), A9218-CL, and 45753-35 (human isolates) were also investigated. Cultures were maintained on tryptic soy agar (TSA; pH 7.0) (Difco, Detroit, Mich.) slants at 5°C and activated by transferring loop inocula grown in 10 ml of tryptic soy broth (pH 7.0) at 30°C at three successive 24-h intervals immediately prior to experiments. Equal volumes of cultures of each strain were combined to serve as an inoculum for all experiments.

Preparation of beef. Boneless beef chuck meat (11 to 12 kg), purchased at a local supermarket, was placed on a rack in an aluminum pan, covered with aluminum foil, and roasted in an oven at 163°C until the internal temperature reached 77°C. Roasts were then placed in a refrigerator (1°C) for 16 to 18 h to cool before grinding with a 2250 electric food grinder equipped with a plate with 0.5-cm-diameter holes (Rival Manufacturing Co., Kansas City, Mo.). Ground, roasted beef was placed in polyethylene bags, sealed, and stored at -18°C until used, i.e., never for more than 3 days.

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Care was taken to handle the beef aseptically between the time it was roasted and the time it was used in various experiments.

Beef salad studies. Ground, roasted beef at 1 to 5°C and commercial mayonnaise (Kraft General Foods, Inc., Glenview, Ill.) that contained soybean oil, eggs, vinegar, water, egg yolks, salt, sugar, pure lemon juice concentrate, dried garlic, dried onion, calcium disodium EDTA, paprika, and natural flavor were combined to prepare salads containing 0 to 40% (wt/wt) mayonnaise in 4% increments. The salad pH was measured within 10 min of preparation (0 h) and after 24 h of storage at 1°C.

Salad formulations containing 0, 16, 24, 32, and 40% mayonnaise were selected for studies designed to determine survival and growth patterns of *E. coli* O157:H7. Salads (700 g) were inoculated with 7.0 ml of a five-strain mixture of *E. coli* O157:H7 which had been diluted 100-fold in sterile 0.1% peptone water (pH 7.0). After thorough mixing, 25-g samples were placed into sterile polyethylene stomacher bags and placed in incubators at 5, 21, and 30°C. The tops of the bags were folded such that atmosphere exchange between the inside and outside of the bags was minimized during incubation.

Salads were analyzed for pH and viable populations of *E. coli* O157:H7 within 10 min of preparation and after incubation for 4, 10, 24, 48, and 72 h at 5°C and 4, 10, and 24 h at 21 and 30°C. Duplicate 25-g samples were individually combined with 100 ml of sterile 0.1% peptone and pummeled with a stomacher for 1 min. Sorbitol MacConkey agar (SMA; pH 7.1) (Unipath Oxoid US, Columbia, Md.) supplemented (0.2 g/liter) with 4-methylumbelliferyl- β -D-glucuronide was used to enumerate *E. coli* O157:H7. 4-Methylumbelliferyl- β -D-glucuronide reagent was added to heat-sterilized (121°C, 15 min), molten (47 to 50°C) SMA to yield modified SMA (MSMA). Serially (1:10) diluted samples (0.1 ml) were surface plated in duplicate on MSMA. Plates were incubated at 35°C for 20 to 24 h before presumptive colonies of *E. coli* O157:H7 were counted.

Presumptive colonies (one per duplicate plate containing 30 to 300 colonies) were randomly selected from MSMA plates and subjected to biochemical and serological tests for confirmation. These tests consisted of the API 20E miniaturized diagnostic kit (Analytab, Div. of Sherwood Medical, Plainview, N.Y.), the *E. coli* O157:H7 latex agglutination assay (Unipath Oxoid US), and the Bacto *E. coli* H antiserum H7 assay (Difco).

Beef slurry studies. Ground, roasted beef (25 g) was combined with 100 ml of sterile water adjusted to 5, 21, or 30°C, the temperatures at which inoculated slurries would eventually be incubated. Sufficient amounts of 10% acetic, citric, or lactic acid were added to slurries to result in pH values of 4.70, 5.00, and 5.40 (± 0.03). Slurries (pH 5.98 ± 0.02) to which no acid was added served as controls. Acidified and control slurries were pummeled with a stomacher for 30 s, inoculated with 1.0 ml of a diluted suspension (ca. \log_{10} 6.80 CFU/ml) of the five-strain inoculum, and pummeled again for 15 s. Slurries were then placed in incubators at 5, 21, and 30°C. Duplicate samples were analyzed for populations of *E. coli* O157:H7 and pH within 5 min of preparation and after 4, 10, and 24 h. Samples were stomached for 30 s, serially diluted, and subjected to the analytical procedures described above for beef salads.

Heat inactivation studies. Ground, roasted beef (25 g) was combined with 100 ml of sterile water in 250-ml Erlenmeyer flasks. Slurries were adjusted to pH $5.00 (\pm 0.02)$ by adding 10% solutions of acetic, citric, or lactic acid. The unacidified

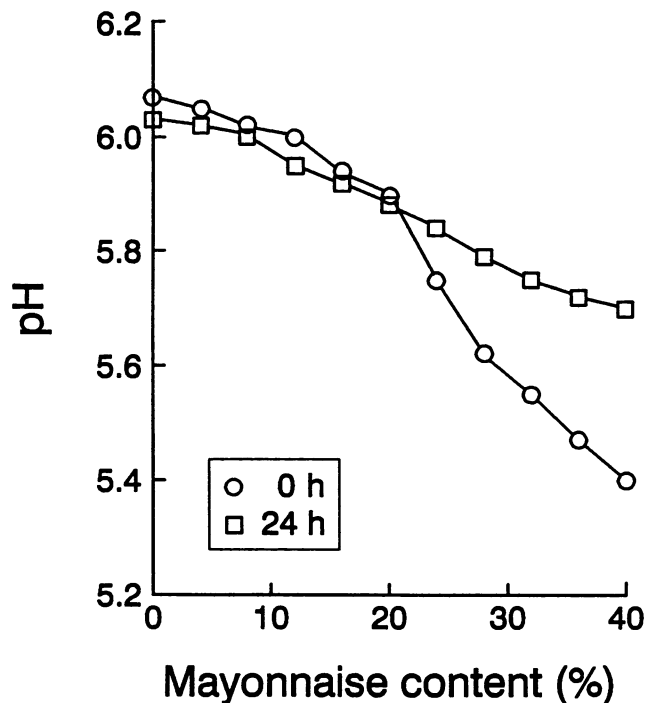


FIG. 1. Changes in the pH of ground, roasted beef containing up to 40% mayonnaise as affected by incubation for 24 h at 1°C.

slurry (pH 6.03) served as a control. Flasks were placed in a water bath shaker such that the level of the water in the bath was at least 1-cm above the level of the slurry. After slurries were adjusted to the desired temperature (52, 54, or 56°C), 1.0 ml of the five-strain mixture of *E. coli* O157:H7 was added. Samples were withdrawn from inoculated slurries after 14, 28, 42, 56, or 70 min of constant mixing (150 rpm), serially diluted in 0.1% peptone, and surface plated (0.1 ml) in duplicate on MSMA and TSA. Procedures for incubating, enumerating, and confirming *E. coli* O157:H7 colonies were as described for beef salads.

Statistical analyses. Data were subjected to the Statistical Analysis System (SAS Institute, Cary, N.C.) for analysis of variance and Duncan's multiple range tests. Each value presented represents a mean of eight values (duplicate values from duplicate samples analyzed from two replicate trials.)

RESULTS

Preliminary experiments were done to determine the pHs of mixtures of various ratios of ground, roasted beef (pH 6.07) and mayonnaise (pH 4.04) immediately after thoroughly mixing the two ingredients and after incubating the mixtures at 1°C for 24 h. Results are illustrated in Fig. 1. The initial (0 h) pH of beef salads containing up to 16% mayonnaise decreased slightly during the 24-h storage period, while the pH of salads containing 20 to 40% mayonnaise increased substantially during the same period. Salads containing 0, 16, 24, 32, and 40% mayonnaise were selected for studies designed to determine the fate of *E. coli* O157:H7. Inoculation of these salads was done within 1 h of combining beef with mayonnaise.

Survival and growth in beef salads. Preliminary studies indicated that all five test strains of *E. coli* O157:H7 had

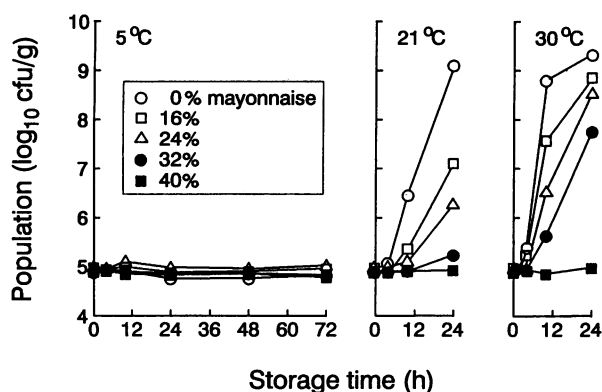


FIG. 2. Populations of *E. coli* O157:H7 in ground, roasted beef salads containing up to 40% mayonnaise and incubated at 5, 21, and 30°C for up to 72 h.

similar growth rates in tryptic soy broth. Shown in Fig. 2 are populations of the five-strain mixture of *E. coli* O157:H7 in beef salads containing up to 40% mayonnaise which were incubated at 5, 21, and 30°C for up to 72 h. The initial mean \log_{10} population was 5.04 CFU/g. Populations immediately recoverable from salads were reduced, particularly in samples with reduced pH, indicating that acetic and/or citric acid present in the mayonnaise may have had a lethal effect on *E. coli* O157:H7. At 5°C, there was essentially no change in population during 72 h of incubation, regardless of the mayonnaise content of a salad. The initial pH range of 6.07 to 5.40 in salads containing 0 to 40% mayonnaise, respectively, had neither a lethal nor a stimulatory effect on *E. coli* O157:H7.

At 21°C, significant ($P \leq 0.05$) increases in populations of *E. coli* O157:H7 were detected in salads containing 0 to 32% mayonnaise (pH 6.07 to 5.55, respectively). The largest populations were achieved in beef to which no mayonnaise was added. The population of *E. coli* O157:H7 in a salad containing 40% mayonnaise did not change during 24 h of incubation.

At 30°C, there was a tremendous increase in the *E. coli* O157:H7 populations of salads containing 0 to 32% mayonnaise during 24 h of incubation. The largest populations were detected in beef containing no mayonnaise. No significant change in population occurred when beef salad containing 40% mayonnaise was incubated at 30°C.

Survival and growth in beef slurries. Vinegar and/or lemon juice are normally used in mayonnaise formulations to provide flavor and reduce the pH. In the present study, 10% solutions of acetic and citric acids were used instead of vinegar and lemon juice, respectively, to acidify 20% beef slurries. Lactic acid was also evaluated. The amounts of each acidulant required to reduce the pH of 125 ml of beef slurry from 5.98 (± 0.02) to 5.40, 5.00, and 4.70 (± 0.03) are listed in Table 1.

Data from experiments designed to determine the survival and growth characteristics of *E. coli* O157:H7 in acidified beef slurries incubated at 5, 21, and 30°C for up to 24 days are presented in Fig. 3. On the basis of populations in five-strain mixtures used to inoculate beef slurries, the calculated mean populations (\log_{10} CFU per gram) in slurries prepared for incubation at 5, 21, and 30°C were 4.79, 4.81, and 4.78, respectively. Populations of *E. coli* O157:H7 recovered from slurries within 5 min of incubation (0 h of storage) were reduced, indicating that some of the cells died

TABLE 1. Amounts of acidulants required to reduce the pH of 125 ml of a 20% slurry of ground, roasted beef

Acidulant (concn [%])	Vol (ml) required for beef slurry pH (± 0.03) (resulting molarity) of ^a :		
	5.40	5.00	4.70
Vinegar ^b	0.57	1.58	3.53
Acetic acid (10)	0.42 (0.0056)	0.95 (0.0127)	2.33 (0.0311)
Lemon juice ^c	0.80	2.12	4.35
Citric acid (10)	0.46 (0.0019)	1.38 (0.0057)	2.66 (0.0111)
Lactic acid (10)	0.50 (0.0044)	1.10 (0.0089)	1.90 (0.0169)

^a Initial pH, 5.98 \pm 0.02.

^b Distilled white vinegar, 5% acidity (50 grains [1 grain is 0.0648 g]) (H. J. Heinz Co., Pittsburgh, Pa.).

^c ReaLemon natural-strength lemon juice (contains water, lemon juice concentrate, lemon oil, 250 ppm of sodium benzoate, and 250 ppm of sodium bisulfite) (Borden, Inc., Columbus, Ohio).

between the time of inoculation and the time of analysis or that MSMA did not adequately support resuscitation and eventual colony formation by cells which may have been injured upon exposure to reduced pH.

At 5°C, significant ($P \leq 0.05$) decreases in populations occurred within 4 h, regardless of the pH or type of acidulant present in slurries. Additional significant decreases were noted at 10 and 24 h of storage. Death was more rapid as the pH was decreased from 5.98 to 4.70, and the order of effectiveness of acids in reducing viable populations was acetic acid > lactic acid > citric acid. In no instance, however, was the reduction in population greater than 2 \log_{10} CFU/ml during the 24-h storage period.

E. coli O157:H7 grew in control samples of beef slurry (pH 5.98) and in slurries containing citric and lactic acids (pHs 5.00 and 5.40) stored at 21°C for 24 h. Significant population

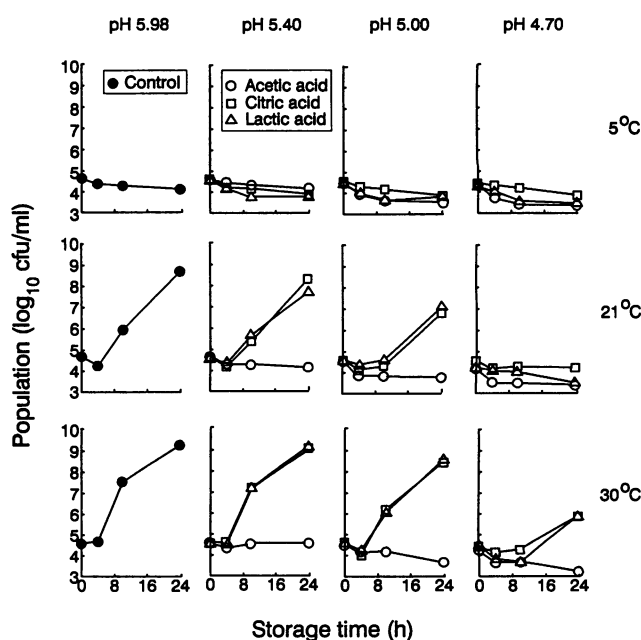


FIG. 3. Populations of *E. coli* O157:H7 in 20% ground, roasted beef slurries acidified with acetic, citric, and lactic acids during incubation at 5, 21, or 30°C for up to 24 h.

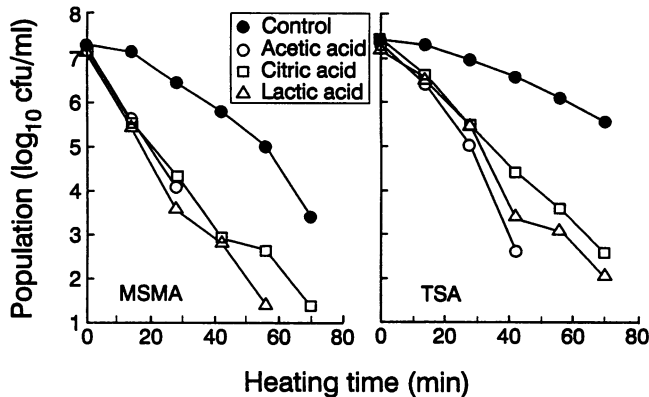


FIG. 4. Inactivation curves for *E. coli* O157:H7 in 20% control (pH 6.03) and acidified (pH 5.00 \pm 0.02) ground, roasted beef slurries heated at 54°C. Enumeration of survivors was done with MSMA and TSA. No viable *E. coli* O157:H7 cells were detected in slurry acidified with acetic acid after heating for more than 28 or 42 min and plating on MSMA or TSA, respectively.

decreases were observed in slurries acidified to pHs 4.70, 5.00, and 5.40 with acetic acid or to pH 4.70 with citric or lactic acid. Acetic acid was more inhibitory to *E. coli* O157:H7 than were citric and lactic acids, although all acidulants were inhibitory or lethal. Effectiveness (acetic acid > lactic acid \geq citric acid) was magnified as the pH decreased and the storage time increased.

Viable populations of *E. coli* O157:H7 decreased in beef slurries acidified with acetic acid (pHs 4.70 and 5.00) and stored at 30°C. Populations in slurry acidified to pH 5.40 with acetic acid essentially remained constant throughout the study. Citric and lactic acids failed to prevent significant increases in population between 10 and 24 h of incubation. The order of effectiveness in killing or retarding the rate of growth of *E. coli* O157:H7 in beef slurries stored at 30°C was acetic acid > lactic acid \geq citric acid.

Heat inactivation studies. Treatment of control (pH 6.03) and acidified (pH 5.00 \pm 0.02) beef slurries at 52°C for 70 min resulted in a less than 10% reduction of populations of individual strains of *E. coli* O157:H7, whereas treatment at 56°C resulted in reductions of up to 10⁶ CFU/ml within 14 min. Changes in viable populations of a five-strain mixture of *E. coli* O157:H7 in slurries heated at 54°C for up to 70 min are shown in Fig. 4. Inactivation was more rapid in acidified slurries than in the control slurry. Tolerance to heat stress was dependent upon the acid used to achieve pH 5.00. The order of tolerance was acetic acid < lactic acid < citric acid.

Perhaps the most revealing finding from these experiments was that MSMA is an extremely poor medium for recovering heat-stressed and/or acid-stressed cells of *E. coli* O157:H7. Within 14 min of heat treatment in acidified slurries, significant ($P \leq 0.05$) smaller populations of the organism were recovered on MSMA than on TSA. When *E. coli* O157:H7 was heated in the control slurry (pH 6.03) for 28 min, significantly smaller populations were recovered on MSMA than on TSA. These differences persisted throughout the duration of the heat treatment.

DISCUSSION

The fate of other pathogenic bacteria in or on meats containing mayonnaise has been studied in other laboratories. Swaminathan et al. (31) reported that mayonnaise had a

significant inhibitory effect on growth of *Salmonella typhimurium* in sandwiches prepared with turkey breast meat. However, growth was not prevented when sandwiches were stored at 21 or 30°C. The pH of the mayonnaise used in these studies ranged from 3.79 to 3.91, whereas the pH of turkey meat was 6.24 to 6.29. The fate of *S. typhimurium* and *Staphylococcus aureus* in chicken and ham salads was investigated by Doyle et al. (8). Storage of salads containing no mayonnaise at 22 or 32°C for 5 h resulted in increases in populations, even when the pH of chicken and ham was reduced from 6.4 to 6.1 and 5.6 to 5.2, respectively, by adding mayonnaise. Increases were greater in meats stored at 32°C than in those stored at 22°C. *Salmonella* species and *Listeria monocytogenes* have been shown to die rapidly (>10⁷/g died within 72 h at 23.9°C) in acidified (pH < 4.1) reduced-calorie mayonnaise containing 0.7% acetic acid in the aqueous phase (16). Our findings on *E. coli* O157:H7 indicate that the organism behaves similarly to *S. typhimurium* and *S. aureus* in that it does not die at 5°C in beef salads containing mayonnaise at levels normally used in household and commercial recipes. Salads containing 32% or less mayonnaise (pH 5.55 to 6.07) supported the growth of *E. coli* O157:H7 when incubated at 21 or 30°C.

Acetic acid, present in most mayonnaise formulations at concentrations ranging from 0.31 to 0.32% (29), retards the growth of microorganisms. Findings from our studies on beef salad formulations containing up to 40% mayonnaise, however, indicate that the acidity and pH achieved in such formulations cannot be relied upon to control the growth of *E. coli* O157:H7. Admittedly, beef salads normally would not be consumed in the form of a slurry and would contain other ingredients in addition to beef and mayonnaise. These ingredients may change the acidity and pH of formulations and should be investigated in terms of their effect on survival and growth of *E. coli* O157:H7.

Studies involving acidified beef slurries were designed to determine whether survival and growth of *E. coli* O157:H7 is affected by acidity and pH. Our findings on the behavior of *E. coli* O157:H7 under the conditions administered in this study should serve as a preliminary model for predicting behavior in beef salads. The order of effectiveness of acids, i.e., acetic acid > lactic acid > citric acid, in controlling growth was the same at all of the pH values and incubation temperatures evaluated. The same order of effectiveness has been reported for *L. monocytogenes* (1, 21, 30) and *S. aureus* (23). Farber et al. (12) demonstrated that acetic acid was more effective than lactic, citric, and hydrochloric acids in inhibiting growth of *L. monocytogenes*. At equivalent pH values, wine vinegar has been shown to be more inhibitory than lemon juice in controlling the growth of *Salmonella enteritidis* in mayonnaise (25). Effectiveness of these acids as inhibitors of bacterial growth is enhanced as the pH is decreased and/or the incubation temperature is increased.

On the basis of the molarity of acetic, citric, and lactic acids rather than percentage (Table 1), the relative differences in effectiveness of acidulants in inhibiting *E. coli* O157:H7 are not as great. Still, acetic acid appears to be the most effective of the three acids, an attribute undoubtedly associated with its ease of entry into cells, which is facilitated by its low molecular weight compared with those of citric and lactic acids.

Glass et al. (17) reported that *E. coli* O157:H7 grew at 37°C in Trypticase soy broth adjusted to pH 4.6 with lactic acid. The organism survived but did not grow during fermentation of sausage to pH 4.8 and subsequent storage at 4°C for 8 weeks. Results from our studies are not inconsistent with

those of Glass et al. (17) in that survival of *E. coli* O157:H7 was essentially unaffected in beef slurries (pH 4.70) stored at 5°C for 24 h. Use of acetic acid as a sanitizer in spray chilling treatments of raw beef has been shown to reduce populations of bacterial pathogens, including *E. coli* O157:H7 (6). Bactericidal activity was more pronounced on lean tissue than on fat tissue.

Ahamad and Marth (2) studied the susceptibility of *L. monocytogenes* to injury upon exposure to 0.3 and 0.5% solutions of acetic, citric, and lactic acids. Acetic acid caused the greatest inactivation, but citric acid generally caused the greatest degree of injury, followed by lactic and acetic acids. Results from our studies with acidified beef slurry as a medium to suspend *E. coli* O157:H7 during heat treatment indicate that the order of lethal effectiveness was acetic acid > lactic acid > citric acid. Acids were similar in terms of causing injury, at least as evidenced by the MSMA-TSA detection system used in our experiments.

The poor performance of MSMA in supporting colony development by heat-injured *E. coli* O157:H7 was not entirely unexpected. The medium is formulated to select for the organism, but inhibition of stressed cells could occur. However, the extent of inhibition of unstressed and heat-stressed cells of *E. coli* O157:H7 was somewhat surprising. Recovery media other than or in addition to MSMA should be considered for enumeration of *E. coli* O157:H7 in foods, particularly if the organism is stressed or injured.

Findings made in this study indicate that *E. coli* O157:H7 is capable of growing in ground, roasted beef with a mayonnaise content commonly used in salad recipes. Caution should be taken to handle ground beef products in a manner that prevents cross-contamination and growth of the pathogen during marketing and handling in the home.

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