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# Efficacy of Organic Acids Against *Escherichia coli* O157:H7 Attached to Beef Carcass Tissue Using a Pilot Scale Model Carcass Washer[1]

CATHERINE NETTLES CUTTER* and GREGORY R. SIRAGUSA

*Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 166, Clay Center, Nebraska 68933*

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ABSTRACT

The efficacy of organic acids for controlling *Escherichia coli* O157:H7 attached to beef carcass tissue was determined using a pilot scale model carcass washer. Lean or adipose surface tissues from beef carcasses were inoculated with three strains of *Escherichia coli* O157:H7 or *Pseudomonas fluorescens*. After spraying either water, 1, 3, or 5% acetic, lactic, or citric acids at 24°C, tissues were incubated for 24 h at 4°C and bacterial populations enumerated. Statistical analyses of the data indicated that acid type was not a significant treatment factor ($p \geq = 0.05$); however, concentration, tissue type, and bacterial strain were significant ($p \leq = 0.0001$ ) factors that influenced the reduction of bacterial populations on lean or adipose tissue. Of the concentrations tested on lean tissue, spray treatments with 5% were the most effective for reducing populations of *E. coli* O157:H7 or *P. fluorescens*. Differences in the resistances of the *E. coli* O157:H7 strains to acid washing also were observed. The magnitude of bacterial population reductions was consistently greater on adipose versus lean tissue for all bacterial strains. Surface pH data indicated that reductions of bacterial populations may have been due to the effects of acidic pH. This study demonstrates that, while organic acids did reduce populations of *E. coli* O157:H7 on red meat, treatments did not completely inactivate the pathogen.

*Escherichia coli* O157:H7 is a recognized etiologic agent of hemorrhagic colitis and hemolytic uremic syndrome (*19,23,27*). Livestock are known reservoirs of this organism (*15,27,28*). Therefore, foods of animal origin (milk and meat) can become contaminated with the bacteria during processing or slaughtering. Since 1982, contaminated raw milk and undercooked beef products have been implicated in outbreaks of *E. coli* O157:H7 infections and resulting sequelae (*5,6,20,22,23,28*). Presently, measures for controlling *E. coli* O157:H7 in foods are adequate heating and/or cooking procedures and preventing recontamination of cooked foods by contaminated equipment, water, or infected food handlers (*16*).

The application of organic acid sprays is one approach to decontaminate beef carcasses at commercial slaughterhouses. Several research reports have addressed the use of GRAS (generally recognized as safe) organic acids for reducing *E. coli* O157:H7 strains on red meat (*1-4,8-13,18,24*). Generally, treatments with acetic, lactic, or citric acids at varying concentrations resulted in population reductions ranging from 1- to 4-$\log_{10}$ CFU/cm$^2$ on meat surfaces (*13*). The effectiveness of organic acids for controlling meatborne pathogens varied between studies and may be attributable to differences of acid concentration, methods for acid delivery, temperature of acids, contact time, sampling techniques, tissue type, or organism (*18*). In November 1992, pre-evisceration organic acid rinses were approved by the Food Safety and Inspection Service of the U.S. Department of Agriculture (FSIS, USDA) for use in commercial slaughterhouses as a means of enhancing product safety and extending the shelf life of beef and pork carcasses (*17*). The following study was initiated to evaluate organic acid type, acid concentration, organism, and tissue type as factors for reducing *E. coli* O157:H7 on post rigor beef carcass surface tissue.

## MATERIALS AND METHODS

### *Bacterial cultures*

The following bacterial strains were obtained from American Type Culture Collection (ATCC; Rockville, MD): *Escherichia coli* O157:H7 ATCC 43895 (*E. coli* 43895); *Escherichia coli* O157:H7 ATCC 43889 (*E. coli 43889); Escherichia coli* O157:H7 ATCC 43890 (*E. coli* 43890), and *Pseudomonas fluorescens* ATCC 13525. Cultures were maintained in 75% glycerol at -20°C and propagated in tryptic soy broth (Troy Biologicals, Troy, MI) at 37°C, 18 h for *E. coli* O157:H7 strains and 26°C, 24 h for *P. fluorescens*. Because *Pseudomonas* spp. may be more susceptible to acid treatments than gram-negative pathogens (*18*), *P. fluorescens* ATCC 13525 was used as a gram-negative, control organism in our studies.

### *Inoculation of beef carcass tissue*

Beef carcass tissue (BCT) from the outer surfaces of post rigor beef carcasses was obtained from the abattoir of the Roman L. Hruska U.S. Meat Animal Research Center (RLHUSMARC).

[1] *Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.*

Lean BCT with intact fascia was cut from the *cutaneous trunci* muscle of beef carcasses. Adipose BCT with intact fascia was removed from the outer surface of the round, rib, and chuck areas of beef carcasses. Both tissues were considered representative of the beef carcass surface since the fascia covering was not removed. Tissues were trimmed to 7.5 cm × 7.5 cm × 0.5-cm pieces, sterilized by ultraviolet light (60 watt germicidal bulbs, General Electric; 51 cm distance from tissue, 20 min), and stored at -20°C. Cultures in early stationary phase of growth, were diluted 1:100 in sterile physiological saline (pH 7.0) to obtain a viable cell density of 7-$\log_{10}$ CFU/ml. After thawing to room temperature, individual pieces of lean and adipose BCT were inoculated by placing the fascia side of the BCT in 10 ml of the bacterial suspension and incubating for 15 min, 25°C. Bacterial counts of approximately 5-$\log_{10}$ CFU/cm$^2$ and 6-$\log_{10}$ CFU/cm$^2$ BCT were obtained for *E. coli* O157:H7 strains and *P. fluorescens*, respectively, using this inoculation procedure.

*Organic acids*

Solutions of DL-lactic (85%, wt/vol; Sigma Chemical Co., St. Louis, MO), acetic acid (glacial, vol/vol; Fisher Scientific, Pittsburgh, PA), and citric acid (monohydrate; wt/vol, Sigma), were prepared in distilled water to concentrations of 1, 3, and 5% and autoclaved. All solutions, including sterile-distilled water, were stored at 24°C until used. The calculated molarities and pH values (Corning Instruments, Corning, NY) of acid solutions at time of application are presented in Table 1.

TABLE 1. *Molarities and pH values of acids.*

| Solution | Molarity | pH[a] |
|---|---|---|
| 1% lactic | 111 mM | 2.39 |
| 3% lactic | 333 mM | 2.21 |
| 5% lactic | 555 mM | 2.14 |
| 1% acetic | 174 mM | 3.09 |
| 3% acetic | 522 mM | 2.68 |
| 5% acetic | 870 mM | 2.58 |
| 1% citric | 52.1 mM | 2.44 |
| 3% citric | 156 mM | 2.22 |
| 5% citric | 260 mM | 2.10 |

[a] Numbers represent an average pH reading obtained from five replications.

*Acid spray treatments and experimental design*

Efficacies of acids against *E. coli* O157:H7 attached to BCT were ascertained in two separate experiments. The first experiment investigated the interactive effects of 3 acids × 3 concentrations × 4 organisms on lean BCT as well as water against 4 organisms on lean BCT. The second experiment examined the interactive effects of 3 acids × 3 concentrations × 2 organisms attached to lean and adipose BCT and the effects of water against 2 organisms attached to the two tissues. A pilot scale model carcass washer (Carcass Acquired Pathogen Elimination/Reduction, CAPER unit) located at RLHUSMARC was used to apply the acids (*9*). Individual pieces of inoculated lean or adipose BCT were attached by alligator jaw clips (Radio Shack, Inc.) to a plastic board for mounting within the CAPER unit. Operation parameters for the CAPER unit were as follows: spray nozzle oscillation speed, 80 cycles/min; chain speed, 14 m/min; nozzle pressure, 80 psi; flow rate, 4.8 L/min; nozzle distance from sample, 17.8 cm; temperature of solutions, 24°C. Immediately after spray treatments, a 5 cm × 5 cm × 0.5-cm (25 cm$^2$ total surface area) piece was aseptically excised from the treated BCT, placed into a stomacher bag to prevent contamination and dehydration of tissue, and incubated at 4°C for 24 h. Remaining pieces of treated BCT were used to assess surface pH values (flat electrode, Corning Instruments, Corning, NY) after spray treatments (day 0) and again after storage at 4°C, 24 h (day 1).

*Bacterial enumeration*

Following incubation at 4°C, 24 h, each 25-cm$^2$ piece of BCT was pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH) in 50 ml of a neutralizing buffer consisting of buffered peptone water (BBL, Cockeysville, MD) with 0.1% Tween 20 (Fisher). Dilutions were made in 2% buffered peptone water and samples were plated on tryptic soy agar (Troy Biologicals) using a Model D Spiral Plater (Spiral Biosystems Instruments, Bethesda, MD). Plates were enumerated after incubation for 24 h at 37°C for *E. coli* and after 48 h at 26°C for *P. fluorescens*.

*Calculations and statistical analyses*

From plate count data, differences between untreated BCT and treated BCT were calculated as a log reduction factor (LRF; $\log_{10}$ CFU/cm$^2$). Least squared means (LSM) of LRFs were calculated from three experimental replications. Statistical data analysis (analysis of variance, ANOVA) was performed using the General Linear Models procedure of SAS (SAS Institute, 1982). Inoculum counts were used as a covariant to normalize data from treatment replications. LSM values calculated from surface pH data for both tissue types were used to determine differences (Δ) between untreated BCT and treated BCT. ANOVA was performed using General Linear Models procedure of SAS. The probability level was $p \leq = 0.05$ unless otherwise noted.

## RESULTS

*Effects of acids on experimentally inoculated lean BCT*

In the first aspect of this study, the interactive effects of 3 concentrations of 3 acids against 4 organisms experimentally attached to lean BCT were investigated. The effect of water spray treatments against the four organisms on lean BCT also was examined. When the 3 × 3 × 4 experiment was analyzed across all organisms and concentrations, acid type was not a significant ($p \geq = 0.05$) factor affecting LRFs. LRFs calculated for acetic acid (1.74) were not significantly different ($p \geq = 0.05$) than LRFs for citric (1.70) or lactic acid (1.90). Therefore, spray treatments with acetic acid were as effective as citric or lactic acid as applied in this study. Of the other individual variables evaluated, only organism and acid concentration were statistically significant ($p \leq = 0.0001$) factors. ANOVA showed a statistically significant 2-way interaction for concentration x organism ($p \leq = 0.001$). No statistically significant ($p \geq = 0.05$) 3-way interaction (acid × concentration × organism) was observed.

Figure 1 illustrates the interaction of organism × concentration for the four organisms tested on lean BCT. When the 3 concentrations were examined, 5% acid resulted in the greatest numerical reduction for each of the four organisms. While there were no statistical differences between the three *E. coli* strains for any of the concentrations examined, numerically, *E. coli* 43895 exhibited the greatest resistance to organic acid spray treatments. Comparatively, *P. fluorescens* was more sensitive to 1, 3, or 5% acid spray treatments than any of the *E. coli* O157:H7 strains tested.

When water was sprayed on lean BCT, *E. coli* 43889, *E. coli* 43890, *E. coli* 43895, and *P. fluorescens* were

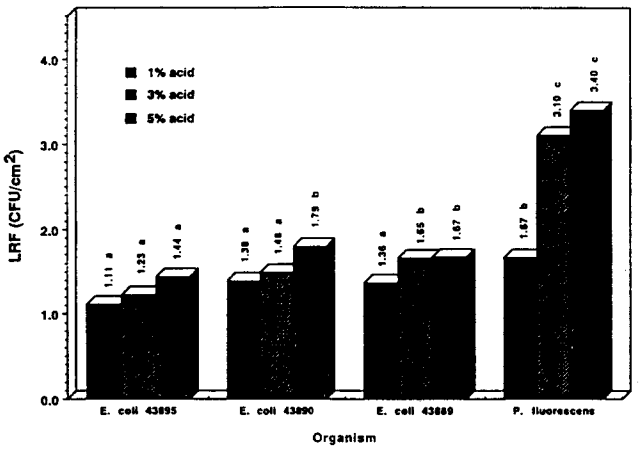


Figure 1. *The 2-way interaction of organism × concentration for experiments performed with lean BCT. Least square means (LSM) of log reduction factor (LRF; CFU/cm²) were calculated for* E. coli *O157:H7 ATCC 43895, 43890, 43889, and* P. fluorescens *ATCC 13525. Mean values with different letters (a, b, c) differ significantly (p ≤ = 0.001).*

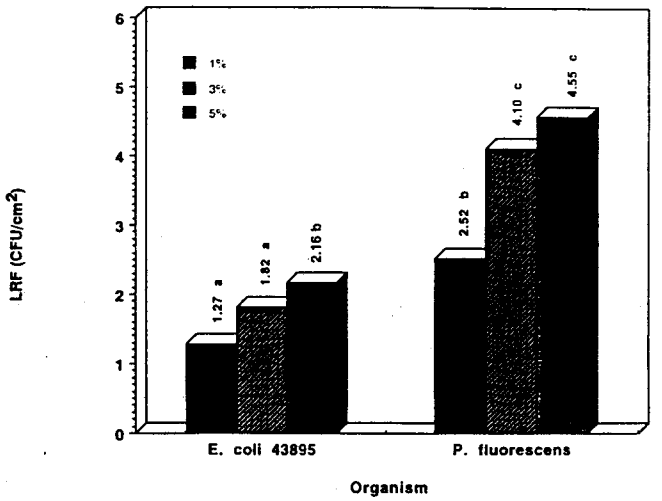


Figure 2. *The 2-way interaction of organism × concentration for lean and adipose BCT. LSM values of LRFs with different letters (a, b, c) differ significantly (p ≤ = 0.0008).*

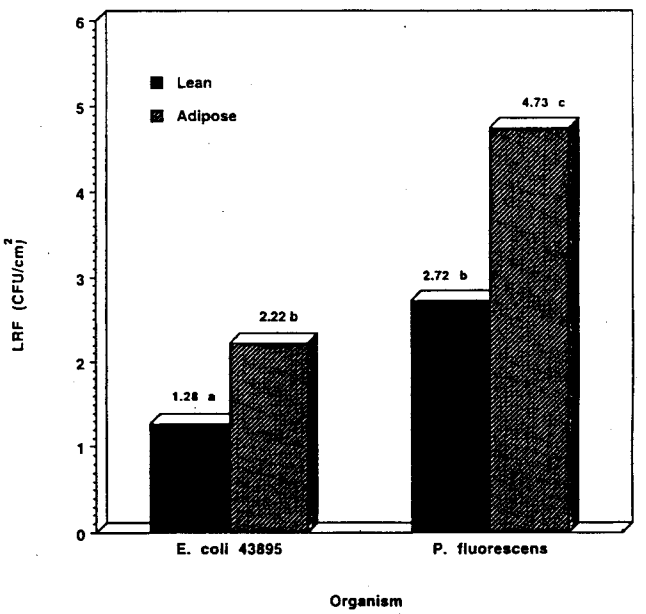


Figure 3. *The 2-way interaction of organism × tissue for lean and adipose BCT. LSM values of LRFs with different letters (a, b, c) differ significantly (p ≤ = 0.008).*

reduced by 1.41, 1.65, 1.06, and 1.90 LRFs, respectively. While there was no overall difference (p ≥ = 0.05) between the organisms, the LRFs of *P. fluorescens* and *E. coli* 43895 were statistically different (p ≤ = 0.05). Because of the greater resistance of *E. coli* 43895 and the sensitivity of *P. fluorescens* to spray treatments with either organic acids or water, these two organisms were used in subsequent experiments involving both lean and adipose BCT.

*Effects of acids on experimentally inoculated lean and adipose BCT*

The second aspect of this study involved the interactive effects of 3 acids × 3 concentrations × 2 organisms (*E. coli* 43895 and *P. fluorescens*) attached to adipose and lean BCT. Additional spray treatments with water against the 2 organisms attached to two tissues also were performed. Of the individual variables tested in the 3 × 3 × 2 experiment, organism (p ≤ = 0.0001), concentration (p ≤ = 0.0001), and tissue (p ≤ = 0.0001) were statistically significant factors. Two-way interactions of organism × concentration (p ≤ = 0.0001), organism × tissue (p ≤ = 0.0001), tissue × concentration (p ≤ = 0.05), and a 3-way interaction of acid × concentration × organism (p ≤ = 0.05) also were significant.

As indicated by the analysis of organism × concentration, increasing concentrations of acids to 5% afforded greater LRFs for either organism on lean or adipose BCT (Fig. 2). This analysis also demonstrated that despite tissue type, spray treatments of *E. coli* 43895 with 5% acid resulted in LRFs similar to those associated with 1% acid against *P. fluorescens*. Therefore, results indicated that *E. coli* 43895 was more resistant to the effects of the acids, while *P. fluorescens* was more sensitive. Following spray treatments with acids on both tissues, the analysis of organism × tissue (Fig. 3) demonstrated the resistance of *E. coli* 43895 and the sensitivity of *P. fluorescens* to organic acid spray treatments. Additionally, the magnitude of reductions was greatest when spray treatments were applied to bacteria attached to adipose BCT. Specifically, bacterial populations of *E. coli* 43895 were 1 $\log_{10}$ greater on adipose BCT versus lean BCT. LRFs for *P. fluorescens* were 2 $\log_{10}$ greater on adipose BCT as compared to lean BCT. This analysis also demonstrated that statistical similarities existed between LRFs of *E. coli* 43895 on adipose BCT and *P. fluorescens* on lean BCT. Analysis of tissue × concentration indicated that spray treatments with 3 and 5% acid on lean BCT afforded LRFs similar to spray treatments with 1% acid on adipose BCT (Fig. 4). Overall, the greatest LRFs were observed when 5% acid was sprayed onto bacteria attached to adipose BCT.

The 3-way interaction of acid × concentration × organism is depicted in Fig. 5. According to this analysis, spray treatments with 5% lactic resulted in the greatest LRFs for *E. coli* 43895, regardless of tissue type. Despite the maxi-

mum LRF associated with lactic acid, acid spray treatments were not as effective against *E. coli* as compared to *P. fluorescens*. Specifically, *P. fluorescens* was maximally reduced by 5% acetic acid, followed by 5% citric and 5% lactic acid on either BCT.

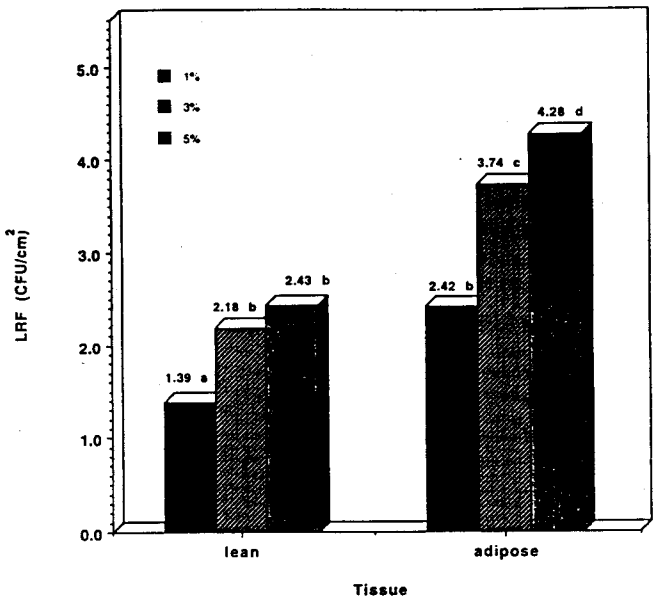


Figure 4. *The 2-way interaction of tissue × concentration for lean and adipose BCT. LSM values of LRFs with different letters (a, b, c, d) differ significantly (p ≤ = 0.04).*

Organism and tissue type were significant variables in the analysis of water treatments for both tissues. As was demonstrated earlier with acid spray treatments on lean and adipose BCT, *P. fluorescens* was more sensitive to spray treatments than *E. coli* 43895 (Fig. 6). Similarly, the magnitude of the LRFs obtained after spray treating with water was greater on adipose than lean BCT (Fig. 7). No significant ($p \geq = 0.05$) 2-way interaction of organism × tissue was observed when both tissues were spray treated with water.

*Analysis of surface pH data*

Surface pH values of both lean and adipose BCT were measured immediately after application of organic acids (day 0) and 24 h later (day 1) (Table 2A, 2B). Every treatment (acid × concentration) with organic acids or water significantly ($p \leq = 0.01$) altered the surface pH of lean or adipose BCT measured immediately after treatment. Higher acid concentrations affected lower surface pH values of both lean and adipose BCT at day 0. Surface pH values of treated lean BCT were higher at day 1 than day 0, with the exception of lean BCT treated with 5% citric and lactic acids. The Δ values for lean BCT treated with 5% citric and lactic acids remained consistently greater (i.e., lower surface pH) on day 0 and day 1 than other treatments.

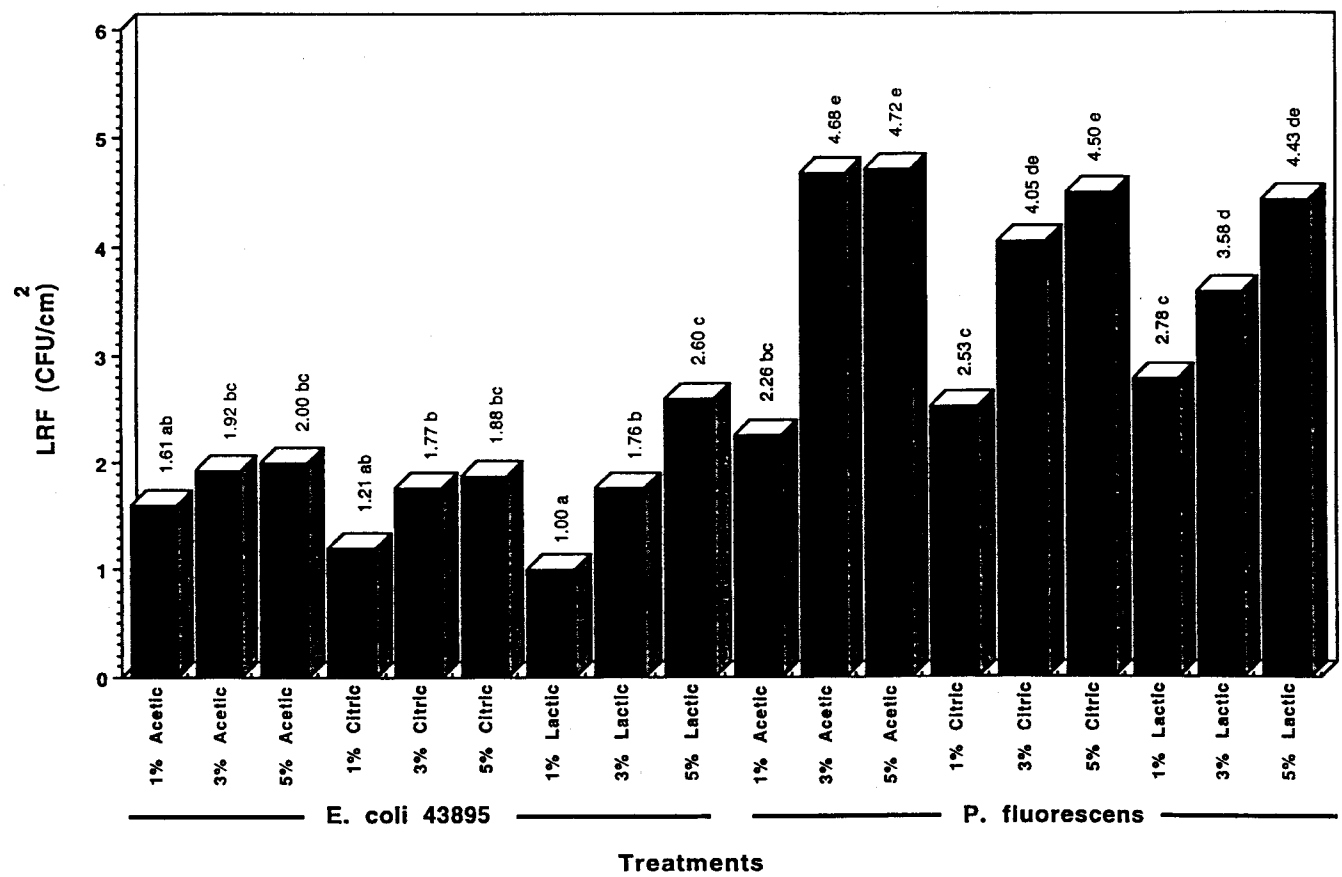


Figure 5. *The 3-way interaction of organism × acid × concentration for lean and adipose BCT. LSM values of LRFs with different letters (a, ab, b, bc, c, cd, d, de, e) differ significantly (p ≤ = 0.065).*

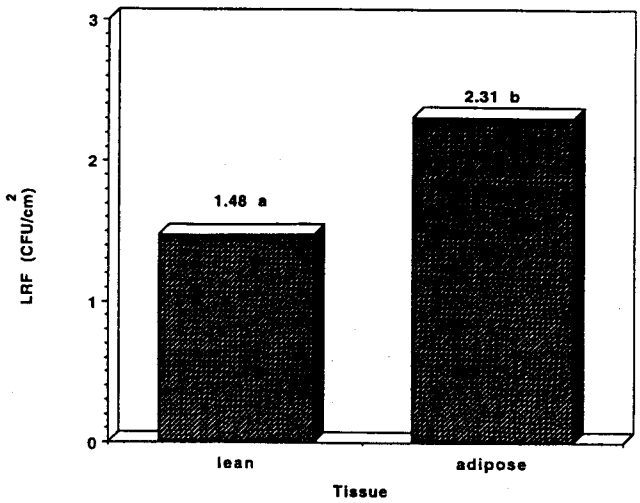


Figure 6. *The significant factor of tissue for lean and adipose BCT spray treated with water. LSM values of LRFs with different letters (a, b) differ significantly (p ≤ = 0.05).*

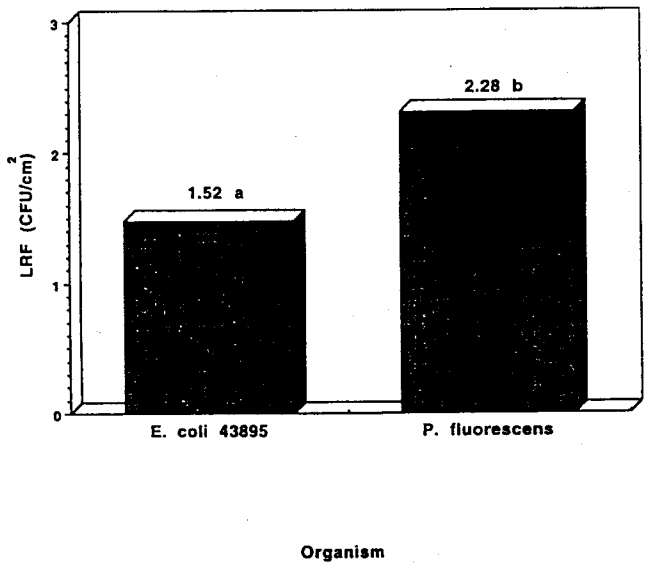


Figure 7. *The significant factor of organism for lean and adipose BCT spray treated with water. LSM values of LRFs with different letters (a, b) differ significantly (p ≤ = 0.07).*

Spray treatments with water were as effective for reducing surface pH of adipose tissue as 1% of any of the acids. With the exception of 1% citric acid, which had a greater Δ value than 1% acetic or lactic, all other acids behaved similarly within concentrations when applied to adipose tissue at day 0. The Δ values calculated from day 1 pH data of adipose tissue were greater (p ≤ = 0.01) than those calculated from lean tissue data.

Figure 8 illustrates the relationship between surface pH of lean and adipose BCT at day 1 and LRFs of *E. coli* 43895 after spray treated with 1, 3, and 5% lactic acid. The correlation coefficient of *E. coli* 43895 attached to both lean and adipose BCT was 0.86. Simple regression analysis suggests that linear relationships exist between acid concentration, surface pH values, and LRFs. Higher concentrations of acid (1 to 5%) resulted in lower day 1 surface pH, and ultimately, greater LRFs. A similar relationship was found for *P. fluorescens* 13525 (data not shown).

TABLE 2A. *Effect of acid spray treatment on the pH of lean beef carcass tissue.*

| Treatment | pH Day 0[a] | Δ[b] | Significance[c] | pH Day 1[d] | Δ[e] | Significance[f] |
|---|---|---|---|---|---|---|
| control | 5.59 | 0 | - | 5.68 | 0 | - |
| water | 5.01 | 0.58 | s | 5.59 | 0.10 | ns |
| 1% acetic | 4.96 | 0.62 | s | 5.39 | 0.31 | ns |
| 3% acetic | 4.67 | 0.92 | s | 5.57 | 0.11 | ns |
| 5% acetic | 4.38 | 1.21 | s | 5.33 | 0.35 | ns |
| 1% citric | 4.76 | 0.84 | s | 5.68 | 0 | ns |
| 3% citric | 4.36 | 1.23 | s | 5.54 | 0.14 | ns |
| 5% citric | 3.84 | 1.75 | s | 5.24 | 0.44 | s |
| 1% lactic | 5.15 | 0.44 | s | 5.66 | 0.02 | ns |
| 3% lactic | 4.30 | 1.29 | s | 5.51 | 0.17 | ns |
| 5% lactic | 3.79 | 1.80 | s | 5.29 | 0.40 | s |

TABLE 2B. *Effect of acid spray treatment on the pH of adipose beef carcass tissue.*

| Treatment | pH Day 0[a] | Δ[b] | Significance[c] | pH Day 1[d] | Δ[e] | Significance[f] |
|---|---|---|---|---|---|---|
| control | 6.78 | 0 | - | 6.57 | 0 | - |
| water | 4.99 | 1.75 | s | 5.50 | 0.81 | s |
| 1% acetic | 4.39 | 2.64 | s | 4.84 | 1.77 | s |
| 3% acetic | 3.80 | 3.20 | s | 4.44 | 2.14 | s |
| 5% acetic | 3.65 | 3.44 | s | 4.36 | 2.36 | s |
| 1% citric | 4.34 | 2.97 | s | 4.34 | 1.85 | s |
| 3% citric | 3.55 | 3.48 | s | 3.55 | 2.71 | s |
| 5% citric | 2.92 | 3.87 | s | 3.83 | 2.83 | s |
| 1% lactic | 4.95 | 2.24 | s | 5.55 | 1.46 | s |
| 3% lactic | 3.72 | 3.41 | s | 4.43 | 2.43 | s |
| 5% lactic | 3.24 | 3.98 | s | 3.74 | 2.96 | s |

[a] Surface pH value of BCT immediately after spray treatment. Number represents the least square means (LSM).
[b] Δ = LSM $control_{day\ 0}$-LSM $treatment_{day\ 0}$.
[c] s denotes a statistically significant difference (p ≤ = 0.01) between control and treated sample at day 0; **ns** denotes no significance (p ≥ = 0.01) between control and treated sample at day 0.
[d] Surface pH value of BCT 24 h after spray treatment (5°C). Number represents the least square means (LSM).
[e] Δ = LSM $control_{day\ 1}$-LSM $treatment_{day\ 1}$.
[f] s denotes a statistically significant difference (p ≤ = 0.01) between control and treated sample at day 1; **ns** denotes no significance (p ≥ = 0.01) between control and treated sample at day 1.

## DISCUSSION

Researchers have evaluated the efficacies of ascorbic, propionic, citric, lactic, and acetic acids, ranging from 0.1 to 24%, to reduce populations of bacteria on red meat (*13*). Bacterial reductions were greatest with higher concentrations of acids, combinations of acids, if the acid temperature was elevated, or if bacteria were attached to adipose tissue (*4,13*). The experimental design of this study was devised to mimic many elements (i.e., beef carcass surface, spray treatments, holding times, and temperatures) that may

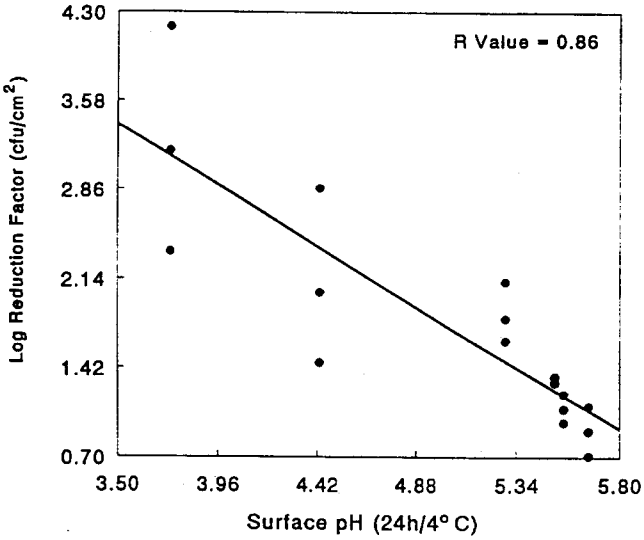


Figure 8. *Linear regression analysis of* E. coli *O157:H7 ATCC 43895 attached to lean and adipose BCT and treated with lactic acid; surface pH, day 1 vs. LRF.*

be encountered in a modern commercial slaughterhouse. Presently, some commercial meat processors use concentrations of 0.5 to 2% acetic acid, at 4 to 55°C for decontamination of beef carcasses. While the concentrations of the three acids were not equimolar, the range of acid concentrations used in this study reflects some of those used commercially.

In this study, sections of lean and adipose tissues with intact fascia covering, representative of the carcass surface, were used instead of the internal muscle or cores of beef tissue used in previous studies (*10-13*). The present investigation also found that acid type did not impact as significantly on bacterial reductions as did tissue type and acid concentration. Results showed that acetic, citric, and lactic acids were equally effective for reducing bacterial populations of the bacteria used in this study.

Of the bacteria tested in this study, strains of *E. coli* O157:H7 were more resistant to spray treatments with acids than *P. fluorescens* on either tissue type. Specifically, *E. coli* 43895 was the most resistant to organic acid sprays among the *E. coli* O157:H7 strains tested. *E. coli* 43895 (*21,25,27*), *E. coli* 43889 (*21*), and 43890 were isolated from raw hamburger and human feces, respectively. Strain differences may have contributed to the observed variations between the *E. coli* O157:H7 strains. Other researchers have documented that various strains of *E. coli* O157:H7 are more resistant to organic acids than the pathogens, Salmonella spp. and *Listeria monocytogenes* (*11,18*).

Overall, spray treatments with 5% acids effected the greatest bacterial reductions observed. Despite the resistance of *E. coli* 43895 to organic acid spray treatments, the magnitude of the reductions with 5% lactic acid was greater when the pathogen was attached to adipose BCT.

The antimicrobial effect of organic acids has been attributed to undissociated acid molecules that interfere with cellular metabolism or a decrease in biological activity as a result of pH changes of the cell's environment (*7,14*). In this study, the application of 5% acids reduced the surface pH of lean and adipose BCT immediately after treatment, thereby creating an unfavorable environment for bacterial growth. However, the surface pH of the treated adipose tissue was significantly less than the control after 24 h incubation at 4°C. The same was not found for treated lean BCT surface pH which was not significantly different ($p \geq = 0.01$) than the untreated control by 24 h. Simple regression analysis of the LRF values versus day 1 surface tissue pH of lean and adipose tissue suggests a linear relationship ($r = 0.86$) between sustained low pH and greater bacterial reduction. This observation is consistent with our results that greater LRFs were achieved against *E. coli* O157:H7 attached to adipose BCT which would maintain a higher level of undissociated lactic acid. The proportion of undissociated lactic acid is greater at lower pH values (*26*). For example, after 24 h the 5% lactic acid sprayed lean BCT had a surface pH of 5.29 versus 3.74 for adipose BCT. The calculated molar concentration of undissociated lactic acid applied to lean BCT is 19 mM versus 316 mM for adipose tissue (*26*). Previously, researchers have reported similar findings between lean and adipose tissues treated with organic acids (*10-12*). Dickson (*12*) attributed pH differences between the two tissues to dilution of the acid on lean tissue, which has a moisture content of 75%, compared to 20% for adipose. The pH differences observed between the two days may be explained by either evaporation of the acid over time or a buffering mechanism exerted by the tissue (*12*).

Dickson (*12*) reported that acetic acid resulted in the same reductions when beef tissue was artificially inoculated with *Salmonella typhimurium* at a population of 3 $\log_{10}$ or 7 $\log_{10}$, and therefore, bacterial numbers did not alter the overall effect of an acid. Recently, however, Greer and Dilts (*18*) demonstrated that initial bacterial numbers did influence the efficacy of organic acids against pathogenic or spoilage organisms. Specifically, initial inoculum levels of $\geq = \log_{10}4$ were the most susceptible to organic acid sprays (*18*). In the current study, experiments were performed with BCT inoculated with *E. coli* O157:H7 at a concentration of approximately 5-$\log_{10}$ CFU/cm$^2$. While this inoculum level did represent a worst-case scenario, it was not determined if organic acid treatments afforded similar reductions with lower initial bacterial numbers.

In the current study, it also was demonstrated that spray treatments with water resulted in reductions of approximately 1 $\log_{10}$, and that the observed reductions were greater on adipose BCT than lean BCT. Other researchers have reported that water reduced bacterial populations on meat (*2,18*). Spray treatments with water may effectively reduce bacterial populations by physically removing cells from the meat surface.

Our study confirms previous reports that, while spray treatments with organic acids did reduce populations of *E. coli* O157:H7 on red meat, neither lactic, citric, or acetic acid at concentrations up to 5%, reduced the pathogen to zero levels. Depending upon the strain and initial populations of the pathogen, reductions ranging from 1- to 2-$\log_{10}$ CFU/cm$^2$ may not be sufficient as the only means to improve the overall microbiological safety of beef car-

casses. However, organic acid spray treatments may be beneficial as part of an overall hazard analysis critical control point (HACCP) approach that can be implemented in order to enhance the microbiological safety and extend the shelf life of post rigor beef.


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