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Antimicrobial Effects of Trisodium Phosphate Against Bacteria Attached to Beef Tissue

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ABSTRACT

Sliced beef tissue was artificially contaminated with *Salmonella typhimurium, Listeria monocytogenes* and *Escherichia coli* O157:H7. The contaminated tissue was immersed in 8, 10 and 12% solutions of trisodium phosphate at 25, 40 and 55°C with contact times of up to 3 min. The concentration of the trisodium phosphate solution was not a significant factor in reducing the populations of the bacteria on either lean or adipose tissue. Reductions in bacterial populations of 1 to 1.5 $\log_{10}$ cycles were obtained on lean tissue contaminated with the gram-negative pathogens, although less reduction in population was seen with *L. monocytogenes*. Greater reductions in bacterial populations were observed on adipose tissue, with maximum reductions of 2 to 2.5 $\log_{10}$ cycles and 1 to 1.5 $\log_{10}$ cycles for the gram-negative and the gram-positive pathogens, respectively. Typically greater reductions in bacterial populations were seen as the temperature of the trisodium phosphate solution increased. Surface beef carcass tissue was inoculated with *E. coli* ATCC 25922 and sanitized with 8% trisodium phosphate using a model carcass washing system. Population reductions with the carcass washer and lean tissue were comparable to those observed in the laboratory with *E. coli* O157:H7. However, greater reductions were observed on adipose tissue from the model system, suggesting that the physical washing procedure may have contributed to the reduction in the bacterial population.

Key Words: Pathogens, beef, sanitizing, trisodium phosphate.

Microbial contamination of animal carcasses is a result of the necessary procedures required to process live animals into retail meat. The contamination can be minimized by good manufacturing processes, but the total elimination of foodborne pathogenic microorganisms is difficult if not impossible. A variety of methods have been developed to reduce the levels of contaminating bacteria on carcasses, although most of the current methods focus on washing and sanitizing procedures, as reviewed by Dickson and Anderson, 1992 (*7*).

A recent process has identified trisodium phosphate as an effective sanitizer in controlling salmonellae on poultry (*8*). The process consists of immersing processed poultry in 8 to 12% (wt/vol) solutions of trisodium phosphate for up to 15 min. The report cites a reduction in the number of carcasses testing positive for salmonellae from a control level of 35% to less than 1% after 15 s. The process has been patented for poultry (*2,3*), specifically covering solutions of trialkali metal ortho-phosphate solutions in concentrations of 4% (wt/vol) or greater with pH values in excess of 11.5.

Alkaline sanitizers have previously been shown to be effective in reducing bacterial contamination on beef tissue (*5*), although previous work with polyphosphates resulted in no significant change in the bacterial populations on beef (Dickson, unpublished data). The specific objectives of this research were to determine the effectiveness of trisodium phosphate on reducing bacterial contamination on beef tissue and evaluate the results of trisodium phosphate sanitizing sprays on contaminated beef.

MATERIALS AND METHODS

Bacterial cultures.

Salmonella typhimurium ATCC 14028, *E. coli* ATCC 25922 and *E. coli* O157:H7 (Food Research Institute, Madison, WI) were grown and maintained in tryptic soy broth (TSB; BBL). *Listeria monocytogenes* Scott A was grown and maintained in TSB + 0.5% (wt/vol) yeast extract (TSB-YE). Cultures were transferred to TSB and incubated at 37°C for 18 h prior to use. The cultures were harvested by centrifugation (1,000 × *g*, 25°C, 10 min) and the pellets re-suspended to the original volume in Butterfield's phosphate buffer (BPB) (*10*).

Tissue preparation.

Beef tissue was obtained as boneless trim from the abattoir at the Roman L. Hruska U.S. Meat Animal Research Center (RLHUSMARC) approximately 3 to 5 days after exsanguination. The tissue was separated into lean and fat tissue, sliced into 0.5 cm thick slices, frozen in sterile sealed bags, sterilized with gamma radiation at a minimum dose of 42 kGy, and stored at -20°C until use. Prior to use, the slices were cut into 1.0 × 1.0 cm squares (sample size 1.0 × 1.0 × 0.5 cm) and tempered to room temperature. Tissue produced in this manner had previously been determined to be representative of pre-rigor tissue, in terms of numbers of bacteria which would attach (*6*).

Experimental design.

Experiment 1: Each bacterium was evaluated individually.

[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.*

The harvested bacteria were diluted 1:100 in BPB to a final population of ca. $\log_{10}$ 7.0 colony forming units (CFU)/ml. Tissue samples were inoculated by immersion in the bacterial suspensions for 5 min, resulting in a bacterial population of approximately 5×10^4 CFU/cm^2 attached to the tissue surface. Samples were aseptically removed, allowed to drain briefly (ca. 5 s), and then immersed in sterile solutions of trisodium phosphate (TSP; Rhone-Poulenc, Cranbury, NJ). A $3 \times 3 \times 3 \times 2$ factorial design was used for *S. typhimurium*, which included the following variables: contact time (15 s, 60 s, 180 s); phosphate concentration (8%, 10%, 12% wt/vol); phosphate temperature (25°C, 40°C, 55°C) and tissue type (lean and adipose). The remaining experiments were conducted using phosphate concentrations of 8 and 12% (*L. monocytogenes*) and 8% (*E. coli* O157:H7). The pH values of the phosphate solutions were determined with a digital pH meter.

Experiment 2: Sections (approximately $10 \times 15 \times 0.5$ cm) of the *cutaneous trunci* and adipose tissue from the rib area were aseptically removed from *Bos taurus* cattle within 30 min of exsanguination. Unlike the tissue used in the previous experiments, this tissue was covered with intact fascia. The tissue was inoculated with a 1:100 dilution of the harvested culture (*E. coli* ATCC 25922) using a spray mister bottle, and allowed to air dry at 20°C for 15 min. The inoculated tissue was mounted on the polyethylene holder of the model Carcass Acquired Pathogen Elimination/Reduction (CAPER) unit, a model carcass washer described by De Zuniga et al. (*4*), and washed with 8% (wt/vol) trisodium phosphate at ca. 23°C. The CAPER unit was operated with a chain speed of 10 m/min and a line pressure of 690 kPa (4.9l/min) using a flat spray nozzle (UniJet type TP SS5008, Spraying Systems Co., Wheaton, IL) oscillated at 60 cycles/min. Tissue samples were obtained for analysis immediately before (control) and after (treated) washing by aseptically excising 25 cm^2 of tissue to an approximate depth of 3 mm.

Enumeration of bacteria.

All tissue samples were homogenized in 99 ml buffered peptone water (BPW, pH 7.2; BBL) for 2 min in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH). Preliminary tests of the homogenate indicated that BPW had sufficient buffering capacity to overcome any residual phosphate remaining on the sample, as the pH of the homogenate did not exceed pH 7.3. Samples were enumerated using tryptic soy agar (TSA) for *S. typhimurium* and *E. coli* and tryptic soy agar-yeast extract (TSA-YE) for *L. monocytogenes* using a Spiral Plater Model D (Spiral Systems Instruments, Inc., Bethesda, MD) (*9*). Plates were incubated at 37°C for 24 h and the total bacterial populations were estimated.

Statistical analysis.

Bacterial counts were converted to $\log_{10}$ CFU/cm^2 values. The reduction in bacterial populations attributable to trisodium phosphate treatment was calculated using the population estimates and the formula:

$$LR = ([\log_{10} \text{control CFU/cm}^2) - (\log_{10} \text{treated CFU/cm}^2]) \quad (1)$$

where LR = the $\log_{10}$ reduction in bacterial population. Statistical analysis was conducted using the General Linear Models procedure of the Statistical Analysis System (*11*), using models appropriate for a factorial arrangement of a completely randomized design. Reported means are the average of three independent replications of each experiment, with the population estimates of inoculated, untreated tissue samples used as covariates in the analysis to normalize the results between replications.

RESULTS AND DISCUSSION

Analysis of the data from the experiments with *S. typhimurium* revealed that the concentration of the trisodium phosphate solution had no significant effect on the results ($P>0.10$), so subsequent experiments limited the concentrations to 8 and 12% (*L. monocytogenes*) and 8% (both *E. coli* strains). There was a statistically significant difference between the 8 and 12% concentrations with *L. monocytogenes* ($P<0.10$), although the actual differences in populations and log reductions was generally less than 0.10 $\log_{10}$. Because such a small difference in bacterial populations is of questionable biological significance, irrespective of the statistical significance, the reported means for both *S. typhimurium* and *L. monocytogenes* are based on the pooled data from all of the concentrations used for each respective bacterium.

As previously stated, there was no difference in the LR values ($\log_{10}$ reductions in bacterial population) of *S. typhimurium* attributable to the concentration of trisodium phosphate. These results were not surprising since the pH values of the solutions were in a narrow range from 13.15 (8%) to 13.21 (12%). The pooled data set of all three concentrations of trisodium phosphate revealed a reduction in the population of *S. typhimurium* on lean beef tissue by approximately 1 $\log_{10}$ cycle (Fig. 1). Anderson and Marshall (*1*) had previously reported reductions in the populations of *S. typhimurium* of approximately 0.5 $\log_{10}$ CFU/cm^2, using a similar experimental design. There was no significant

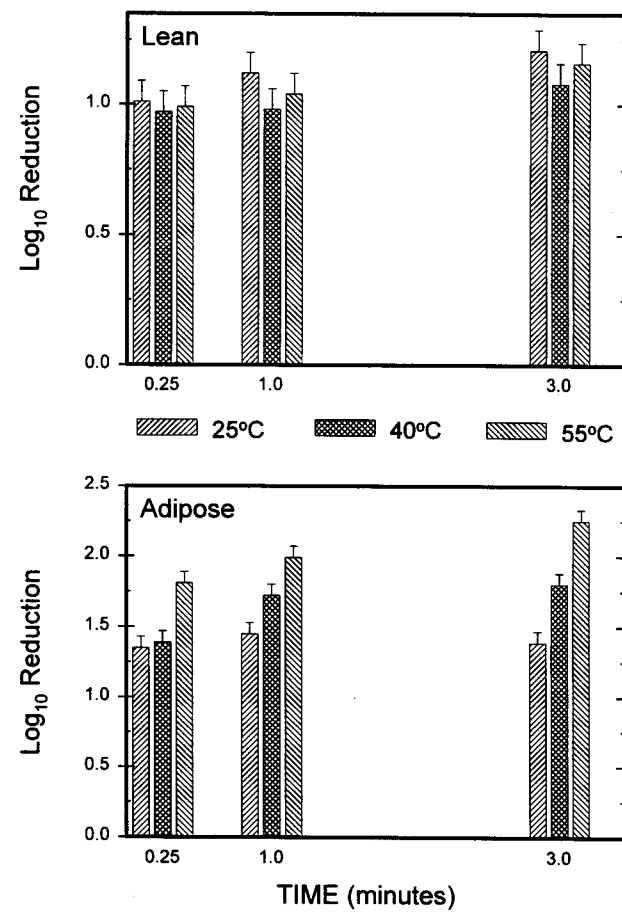

Figure 1. *Reduction of populations of* S. typhimurium *on beef tissue from initial populations by immersion in TSP solutions. (Pooled data from 8, 10 and 12% concentrations.).*

(P>0.1) increase in the LR values on lean tissue attributable to temperature. In contrast, there were significant (P<0.1) increases in the LR values on adipose tissue as the temperature of the phosphate solution increased, particularly as the contact time increased. The 25°C solutions consistently reduced the populations on adipose tissue by approximately 1.3 $\log_{10}$ cycles, while the 55°C solutions reduced the populations by approximately 1.3, 2.0 and 2.5 LRs after 0.25, 1.0 and 3.0 min, respectively, of contact time. The 40°C solutions reduced the populations by approximately 1.8 LR's after 3.0 minutes contact time.

Previous research has shown that solutions of sodium hydroxide (NaOH) and potassium hydroxide (KOH) reduced the populations of *S. typhimurium* by approximately 1.5 and 2.5 $\log_{10}$ cycles on lean and adipose tissue, respectively (*5*). These solutions were applied at ambient temperature with a contact time of 10 s. These reductions are similar to those described with the TSP solutions, although the reductions reported with 10% solutions of NaOH and KOH were greater than those for the TSP solutions. The pHs of the NaOH and KOH solutions were in the same range as those of the TSP solutions (ca. pH 13), which suggest that the reductions observed with all of these solutions were attributable to the extreme alkaline pH.

Listeria monocytogenes was less sensitive to the TSP solutions, with maximum reductions on lean tissue of <1 LR (Fig. 2). Application of TSP solutions at 25°C resulted in very slight reductions in populations, on the order of 0.3 LR. Application temperature and contact time were significant factors on lean tissue, with LRs of approximately 0.8 for the 55°C TSP solutions at 1 and 3 min, respectively. Trisodium phosphate solutions were significantly more effective on reducing bacterial populations on adipose tissue, with maximum LRs of 1.2 to 1.5 after 3 min. Application temperature was a significant factor, with greater reductions corresponding to higher application temperatures. These data are similar to the previous data with NaOH and KOH (*5*), with reported reductions of <1 $\log_{10}$ cycle on lean tissue and approximately 2 $\log_{10}$ cycles on adipose tissue. *Listeria monocytogenes* is generally more resistant to alkaline pH conditions than *S. typhimurium*, with D_{10} values in 1% NaOH of 8.13 (listeriae) compared to <1.34 (salmonellae) s (*5*).

The patterns of population reductions of *E. coli* O157:H7 (Fig. 3) were similar to those observed with *S. typhimurium* (Fig. 1). The LRs on lean muscle tissue were in the range of 1 to 1.5. The general trend at 0.25 and 1.0 min contact time was for greater LRs at higher application temperatures, although this effect was not always statistically significant. Increasing application times resulted in greater LRs on adipose tissue, although temperature generally had no effect at a specific application time (Fig. 3). The LRs on adipose tissue at each contact time were virtually identical to those observed with *S. typhimurium* (Fig. 1). Anderson and Marshall (*1*) had previously reported reductions in the populations of *E. coli* (non-O157:H7) of less than 0.5 $\log_{10}$ CFU/cm^2, using a similar experimental design. Although the previous study with alkaline sanitizer solutions did not examine the effects of NaOH and KOH on *E. coli* (*5*), studies with *Serratia*

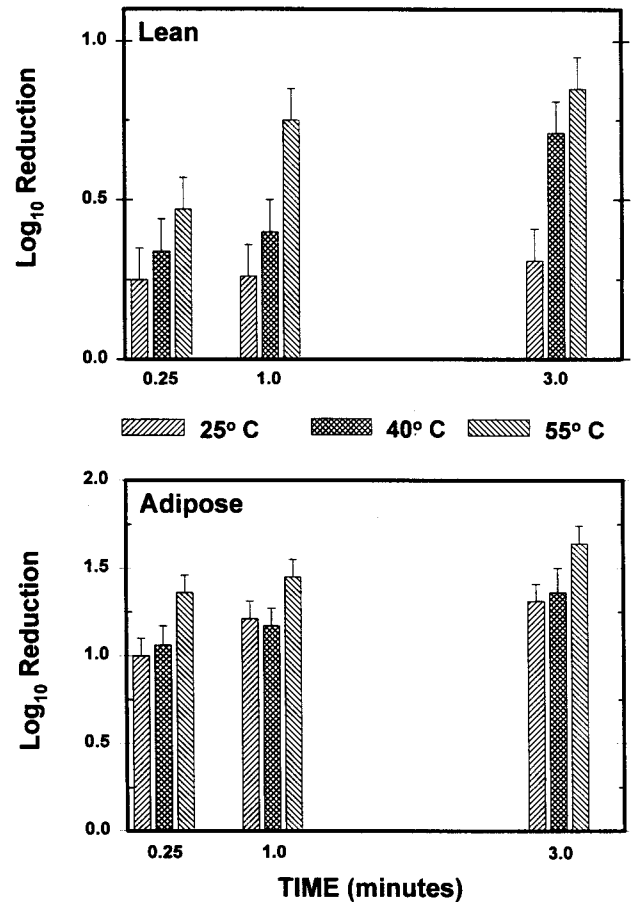

Figure 2. *Reduction of populations of* L. monocytogenes *Scott A on beef tissue from initial populations by immersion in TSP solutions. (Pooled data from 8 and 12% concentrations.)*

marcescens and *Staphylococcus aureus*, as well as the two previously discussed bacteria, indicated that gram-negative bacteria were much more sensitive to the effects of alkaline sanitizers than were the gram-positive bacteria.

When lean tissue with intact fascia was contaminated with *E. coli* ATCC 25922 and washed with 8% TSP solution in the model CAPER system, the LR values were similar to those reported with immersing lean muscle tissue in the same concentration of TSP for 0.25 min (Fig. 4). Washing reduced the bacterial populations by approximately 0.75 $\log_{10}$ cycles, compared to approximately 1 LR for the immersion experiment. However, washing adipose tissue in the model CAPER system resulted in substantially greater LR values than the 0.25 min immersion treatments, with an LR of approximately 2.5, compared to an LR of approximately 1.4 for the 25°C TSP immersion. The increased effectiveness of the TSP solution when applied by the model CAPER system may be a attributable to a direct, physical effect of the washing treatment, where the oscillation of the spray nozzle and the pressure of the wash may have either physically removed some of the bacteria or provided a more thorough coverage of the hydrophobic tissue.

Alternately, strain differences may account for the differences in observed reductions between the washing and immersion studies on adipose. Brief experiments indi-

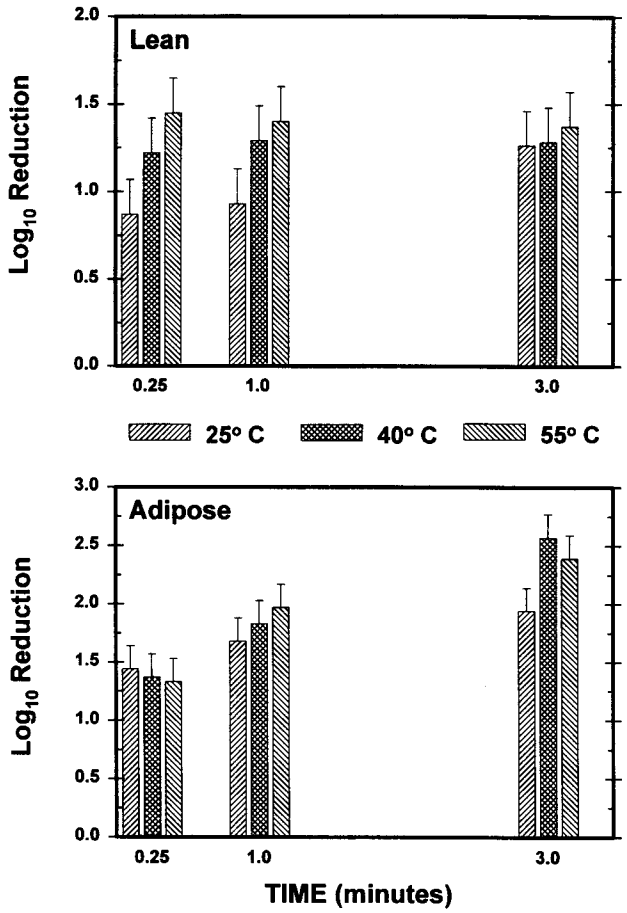

Figure 3. *Reduction of populations of* E. coli *O157:H7 on beef tissue from initial populations by immersion in 8% TSP solutions.*

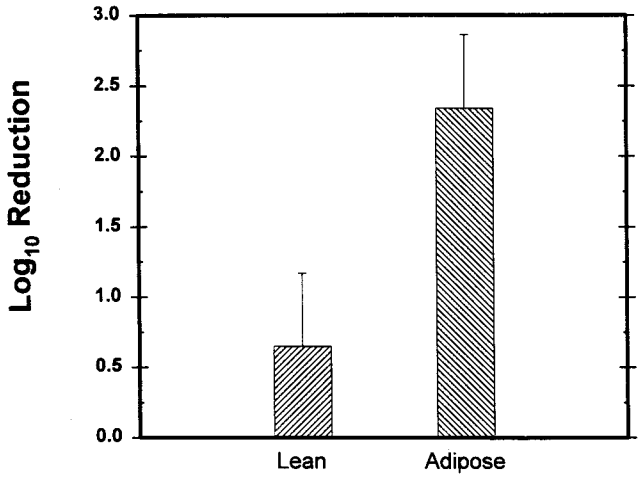

Figure 4. *Reduction of populations of* E. coli *ATCC 25922 on beef tissue by washing with TSP solutions at 23°C in the model CAPER system.*

cated that two strains of *E. coli* used in the immersion and washing treatments, O157:H7 and ATCC 25922, were equally sensitive to the sanitizing effects of 8% TSP solutions in immersion studies. However, it is possible that the ATCC strain did not attach as well to the fascia on the adipose tissue as to the sliced adipose tissue used in the immersion studies, and was therefore more easily removed. This would seem unlikely, since the two *E. coli* strains had approximately equal LRs in both the immersion and washing experiments. The most likely explanation, therefore, seems to be that washing adipose tissue with TSP is simply more effective than immersion, and that approximately equal LRs would have been observed with adipose tissue if both strains of *E. coli* would have been evaluated.

In summary, TSP solutions did reduce the populations of bacteria of public health significance on beef tissue from the initial levels, both in laboratory experiments and in simulated washing treatments. While no single treatment will eliminate all of the significant bacteria, sanitizing beef carcasses with TSP rinses does have a definite effect on these bacterial populations and is worthy of consideration as part of a Hazard Analysis Critical Control Points (HACCP) program to reduce bacteria on meat.

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