Effectiveness of Trisodium Phosphate, Acidified Sodium Chlorite, Citric Acid, and Peroxyacids against Pathogenic Bacteria on Poultry during Refrigerated Storage

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ABSTRACT

The effects of dipping treatments (15 min) in potable water or in solutions (wt/vol) of 12% trisodium phosphate (TSP), 1,200 ppm acidified sodium chlorite (ASC), 2% citric acid (CA), and 220 ppm peroxyacids (PA) on inoculated pathogenic bacteria (Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Salmonella Enteritidis, Escherichia coli, and Yersinia enterocolitica) and skin pH were investigated throughout storage of chicken legs (days 0, 1, 3, and 5) at 3 ± 1°C. All chemical solutions reduced microbial populations (P < 0.001) as compared with the control (untreated) samples. Similar bacterial loads (P > 0.05) were observed on water-dipped and control legs. Type of treatment, microbial group, and sampling day influenced microbial counts (P < 0.001). Average reductions with regard to control samples were 0.28 to 2.41 log CFU/g with TSP, 0.33 to 3.15 log CFU/g with ASC, 0.82 to 1.97 log CFU/g with CA, and 0.07 to 0.96 log CFU/g with PA. Average reductions were lower (P < 0.001) for gram-positive (0.96 log CFU/g) than for gram-negative (1.33 log CFU/g) bacteria. CA and ASC were the most effective antimicrobial compounds against gram-positive and gram-negative bacteria, respectively. TSP was the second most effective compound for both bacterial groups. Average microbial reductions per gram of skin were 0.87 log CFU/g with TSP, 0.86 log CFU/g with ASC, 1.39 log CFU/g with CA, and 0.74 log CFU/g with PA for gram-positive bacteria, and 1.28 log CFU/g with TSP, 2.03 log CFU/g with ASC, 1.23 log CFU/g with CA, and 0.78 log CFU/g with PA for gram-negative bacteria. With only a few exceptions, microbial reductions in TSP- and ASC-treated samples decreased and those in samples treated with CA increased throughout storage. Samples treated with TSP and samples dipped in CA and ASC had the highest and lowest pH values, respectively, after treatment. The pH of the treated legs tended to return to normal (6.3 to 6.6) during storage. However, at the end of storage, the pH of legs treated with TSP remained higher and that of legs treated with CA remained lower than normal.

Poultry meat constitutes a substantial portion of present-day diets, and a high emphasis has been placed on marketing a good quality and safe product both for public health and trade reasons. The presence of pathogenic and spoilage microorganisms on poultry is undesirable although unavoidable as a result of the necessary procedures required to convert live animals into retail meat. Foodborne pathogens associated with poultry products include Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Salmonella, Campylobacter jejuni, Clostridium perfringens, Escherichia coli, and Yersinia enterocolitica (3, 45). Poultry products have been identified as the source of many foodborne outbreaks (26).

In addition to good manufacturing practices, the application of carcass decontamination technologies can reduce substantially the microbial load on fresh meat. Decontamination of carcasses is one of the most effective methods for producing pathogen-free meat products. Different chemical compounds have been developed to reduce microbial loads on poultry (16). Many of these compounds (e.g., acidified sodium chlorite and peroxyacids) are considered processing aids and have been approved by the U.S. Department of Agriculture Food Safety and Inspection Service as secondary direct food additives permitted in food for human consumption. Other compounds (e.g., trisodium phosphate and citric acid) have been designated as generally recognized as safe (34).

Although meat and poultry decontamination procedures have been authorized for years in United States, within the European Union (EU) antimicrobials are not permitted at present for treatment of red meat or poultry carcasses, neither parts nor viscera. The EU meat hygiene regulations do not allow any method or product decontamination other than washing with potable water or application of steam. Adoption of decontamination technologies has been perceived as compensating for poor hygienic practices in the slaughterhouse (7). However, Regulation (EC) No. 853/2004 of the European Parliament and Council laying down specific hygienic rules for the hygiene of foodstuffs (19), which is an application from January 2006, includes the possibility of using chemical substances for decontamination purposes provided they are approved by the Standing Committee on the Food Chain and Animal Health. In Annex II to the draft regulation, the Commission introduced a provision to authorize chlorite dioxide, acidified sodium chlorite, and trisodium phosphate as decontaminants for poultry carcasses. These compounds are currently under re-

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The objective of this study was to evaluate and compare the effectiveness of various decontaminant compounds (trisodium phosphate, acidified sodium chloride, citric acid, and peroxyacids) and potable water in reducing viable pathogenic bacteria on chicken to identify the best chemical decontamination treatments.

**MATERIALS AND METHODS**

**Samples.** A total of 180 chicken legs were collected from a local poultry processing plant immediately after carcass evisceration. Samples were transported to the laboratory in an ice chest and stored at 3 ± 1°C for no longer than 1 h before use.

**Bacterial cultures.** Bacterial cultures used to inoculate legs were *L. monocytogenes* NCTC 11994, *S. aureus* subsp. *aureus* ATCC 23235, *B. cereus* ATCC 21769, *Salmonella enterica* serovar Enteritidis CECT 556, *E. coli* ATCC 12806, and *Y. enterocolitica* subsp. *enterocolitica* NCTC 11174. Strains were maintained at 4°C in tryptic soy agar (TSA; Oxoid Ltd., Hampshire, UK) slants. Before inoculation, these cultures were transferred into tryptic soy broth (TSB; Oxoid) and incubated at 25°C for 18 h. Previous inoculations and dipping. Each sample was prepared by excising 5 g of skin with a sterile knife blade. The samples were placed in a sterile stomacher bag containing 45 ml of sterile 0.1% (wt/vol) peptone water (Oxoid) and homogenized (Masticator IUL, Barcelona, Spain) for 2 min. Serial dilutions in sterile 0.1% (wt/vol) buffered peptone water were prepared from this homogenate, and 0.1 ml was surface placed in duplicate onto plate count agar (PCA; Oxoid) and incubated for 48 h at 25°C (*B. cereus* and *Y. enterocolitica*) or 35°C. The pH of the homogenate was measured using a pH meter (Crison MicroPH 2001, Barcelona, Spain).

**Microbiological analysis and pH determinations.** All samples were evaluated for microbiological quality and pH after 0, 1, 3, and 5 days of storage. Day 0 legs were tested immediately after inoculation and dipping. Each sample was prepared by excising 5 g of skin with a sterile knife blade. The samples were placed in a sterile stomacher bag containing 45 ml of sterile 0.1% (wt/vol) peptone water (Oxoid) and homogenized (Masticator IUL, Barcelona, Spain) for 2 min. Serial dilutions in sterile 0.1% (wt/vol) buffered peptone water were prepared from this homogenate, and 0.1 ml was surface placed in duplicate onto plate count agar (PCA; Oxoid) and incubated for 48 h at 25°C (*B. cereus* and *Y. enterocolitica*) or 35°C. The pH of the homogenate was measured using a pH meter (Crison MicroPH 2001, Barcelona, Spain).

**Statistical analysis.** Five replicates were produced for each microbial group and treatment. Replications were performed on separate days. Microbial counts were converted to log CFU per gram. Means and standard deviations were calculated. The reduction in bacterial populations attributable to dipping treatments was calculated by subtracting the log values for dipped samples from the log values for control (untreated) samples. Data obtained (microbial reductions and pH values) were compared for significant differences (*P* < 0.05) using an analysis of variance (ANOVA) and the Duncan test. An ANOVA was performed for the three factors (microbial group [G], type of treatment [T], and temperature of the antimicrobial compound, and type of pathogen [P]) and their interactions. ANOVAs for all microbial groups and pH values also were conducted. Hypothesis tests were conducted to determine whether means were equal for type of treatment and day of storage. The interactions of *T* × *D* also were tested. All tests were conducted using the Statistica 6.0 software package (Statsoft, Inc., Tulsa, Okla.).

**RESULTS**

Initial microbial counts after inoculation were 6.89 ± 0.19 log CFU/g for *L. monocytogenes*, 6.74 ± 0.24 log CFU/g for *S. aureus*, 5.55 ± 0.40 log CFU/g for *B. cereus*, 6.93 ± 0.47 log CFU/g for *Salmonella Enteritidis*, 6.82 ± 0.42 log CFU/g for *E. coli*, and 6.96 ± 0.22 log CFU/g for *Y. enterocolitica*. All chemical solutions significantly reduced (*P* < 0.001) concentrations of inoculated bacteria on chicken legs compared with control samples. Similar microbial concentrations were obtained for control and water-dipped samples (*P* > 0.05).

ANOVA of the three factors (G, T, and D) revealed the significant influence (*P* < 0.001) of all factors and their interactions. Type of treatment was a significant factor (*P* < 0.001) for all microbial groups and pH values. Day of storage also was significant (*P* < 0.01) for all pathogens except *L. monocytogenes*, *S. aureus*, and *Salmonella Enteritidis* (*P* > 0.05). The T × D interactions were significant for all pathogens except *L. monocytogenes.*
TABLE 1. Population reductions on inoculated poultry legs treated with 12% trisodium phosphate as compared with untreated (control) legs during 5 days of storage (3 ± 1°C)

<table>
<thead>
<tr>
<th>Inoculated pathogen</th>
<th>Mean ± SD (log CFU/g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>1.09 ± 0.32 A a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.47 ± 0.29 A abc</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1.04 ± 0.28 A a</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>1.86 ± 1.22 A bc</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2.09 ± 0.81 A b</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>1.43 ± 0.51 A ac</td>
</tr>
</tbody>
</table>

*Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).

Mean microbial reductions (considering simultaneously all treatments) were lower (P < 0.001) for gram-positive (0.96 ± 0.76 log CFU/g) than for gram-negative (1.33 ± 0.99 log CFU/g) bacteria. CA and ASC were the most effective antimicrobial compounds against gram-positive and gram-negative bacteria, respectively. TSP was the second most effective compound against both bacterial groups. Mean microbial reductions were 0.87 ± 0.69 log CFU/g with TSP, 0.86 ± 0.61 log CFU/g with ASC, 1.39 ± 0.99 log CFU/g with CA, and 0.74 ± 0.51 log CFU/g with PA in gram-positive bacteria, and 1.28 ± 0.95 log CFU/g with TSP, 2.03 ± 1.01 log CFU/g with ASC, 1.23 ± 0.79 log CFU/g with CA, and 0.78 ± 0.75 log CFU/g with PA in gram-negative bacteria.

Tables 1 through 5 show the mean log reductions in pathogenic bacteria counts throughout storage following treatments with 12% TSP, 1,200 ppm ASC, 2% CA, 220 ppm PA, and water, respectively. Data for each microbial group and sampling day are compared in Figure 1.

L. monocytogenes. Among the treatments tested, CA was the most effective in reducing L. monocytogenes populations, especially from day 3 of storage (Fig. 1). No significant differences were observed in the reduction of L. monocytogenes following treatment with TSP or ASC (about 1 log CFU/g throughout storage). PA treatment was the least effective.

S. aureus. Results for S. aureus were similar to those for L. monocytogenes, with CA producing the largest reductions from day 3 of storage. However, immediately after treatment the highest microbial reductions were observed in samples dipped in TSP and ASC.

B. cereus. As for the other gram-positive microorganisms, CA was the most effective antimicrobial compound against B. cereus.

Salmonella. The use of ASC resulted in the largest reductions of Salmonella. TSP was the second most effective decontaminant studied for reducing Salmonella populations. CA and PA produced less marked reductions in Salmonella loads that did ASC and TSP.

E. coli. ASC and TSP were the most effective decontaminants against E. coli at the beginning (days 0 and 1) of storage. From day 3 of storage, the most effective antimicrobials for this pathogen were ASC and CA.

Y. enterocolitica. Findings for Y. enterocolitica were similar to those for other gram-negative bacteria, with the largest reductions in ASC-treated samples. CA had high antimicrobial activity from day 1 of storage.

Microbial reductions in samples treated with TSP and ASC tended to decrease throughout storage (with the exception of L. monocytogenes and Salmonella Enteritidis), and microbial counts increased, both on control and treated samples, throughout storage. However, the growth rate differed depending on the group of samples considered. In contrast, CA continued to have a lethal effect throughout the storage period, and reductions tended to increase toward

TABLE 2. Population reductions on inoculated poultry legs treated with 1,200 ppm acidified sodium chlorite as compared with untreated (control) legs during 5 days of storage (3 ± 1°C)

<table>
<thead>
<tr>
<th>Inoculated pathogen</th>
<th>Mean ± SD (log CFU/g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>1.03 ± 0.26 A a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.25 ± 0.16 A a</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1.11 ± 0.15 A a</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>2.05 ± 0.57 A b</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3.15 ± 0.62 A c</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>2.35 ± 0.67 AB b</td>
</tr>
</tbody>
</table>

*Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).
the end of storage (except for L. monocytogenes, B. cereus, and Y. enterocolitica). Effectiveness of PA throughout the storage period was highly pathogen dependent.

**pH values.** Table 6 gives the pH values of decontaminated and untreated chicken skin. No significant differences were observed in the pH of chicken leg skin as a function of pathogen. Untreated samples and those dipped in water and PA maintained a pH of 6.3 to 6.6 throughout storage. Samples treated with TSP and samples dipped in CA and ASC had the highest and the lowest pH values, respectively, after treatment. The pH of the treated legs tended to return toward normal (6.3 to 6.6) during the initial 24-h period and then remained relatively constant through the rest of the study period. However, at the end of storage the pH of legs treated with TSP remained higher and that of legs treated with CA remained lower than normal values.

**DISCUSSION**

The present work extends previous findings about the effects of TSP, ASC, CA, and PA solutions on pathogenic bacteria on poultry, indicating that all chemical decontaminants tested were effective in reducing pathogenic bacteria (compared with the control samples) both after treatment and during extended refrigerated storage. Similar results have been found by other authors on both red meat and poultry carcasses (12, 23, 27, 29, 31, 40).

Water-treated chicken legs were used as a physical parameter control. The lack of a significant difference in bacterial numbers (P > 0.05) between untreated and water-dipped samples suggests that the mechanical effect of water for removing bacterial was minimal. Similar findings have been obtained on both beef (12, 23, 31, 43) and poultry (27). In contrast, Ransom et al. (36) observed that dipping in water significantly reduced the population of E. coli O157:H7 (0.6 to 1.2 log CFU/cm²) on beef adipose tissue. No reductions were observed by these authors on boneless beef trimmings.

The influences of microbial group (G), type of treatment (T), and day of storage (D) on bacterial reductions in the present study agree with results from most authors for both poultry and red meat. The significant D × T interactions found suggest that mean differences among treatment reductions were generally of different magnitudes for the different days of storage.

The differences observed in initial bacterial loads on the chicken legs following inoculation, even though inocula of similar concentrations were used, might be due to differences in attachment of bacteria to meat surfaces, as previously suggested (10).

Microbial counts throughout the storage period should be considered total bacteria populations because they could include both the inoculated and natural flora present on fresh poultry meat. However, no significant interference by natural flora was expected because counts on PCA were not different (P > 0.05) from pathogen counts (on selective media), which is indicative of the high inoculated bacteria concentrations (about 100 times higher than those of natural flora on poultry throughout storage; data not shown). Similar results have been obtained on both beef (12, 23, 31, 43) and poultry (27). In contrast, Ransom et al. (36) observed that dipping in water significantly reduced the population of E. coli O157:H7 (0.6 to 1.2 log CFU/cm²) on beef adipose tissue. No reductions were observed by these authors on boneless beef trimmings.

**TABLE 3. Population reductions on inoculated poultry legs treated with 2% citric acid as compared with untreated (control) legs during 5 days of storage (3 ± 1°C)**

<table>
<thead>
<tr>
<th>Inoculated pathogen</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>1.27 ± 0.21 A b</td>
<td>1.24 ± 0.77 A ab</td>
<td>1.62 ± 0.70 A a</td>
<td>1.52 ± 0.36 A a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.86 ± 0.45 A b</td>
<td>0.82 ± 0.39 A bc</td>
<td>1.37 ± 0.55 B a</td>
<td>1.44 ± 0.51 B a</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>1.57 ± 0.21 A a</td>
<td>1.78 ± 0.32 A d</td>
<td>1.57 ± 0.20 A b</td>
<td>1.04 ± 0.80 A a</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>0.23 ± 0.64 A c</td>
<td>0.35 ± 0.33 A c</td>
<td>1.26 ± 0.85 B a</td>
<td>1.62 ± 0.91 B a</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.87 ± 0.53 A b</td>
<td>1.47 ± 0.46 B ad</td>
<td>1.83 ± 0.83 B ab</td>
<td>1.53 ± 0.52 B a</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>1.25 ± 0.24 A ab</td>
<td>1.97 ± 0.72 B d</td>
<td>1.31 ± 0.52 A a</td>
<td>1.08 ± 0.66 A a</td>
</tr>
</tbody>
</table>

* Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).

**TABLE 4. Population reductions on inoculated poultry legs treated with 220 ppm peroxyacids as compared with untreated (control) legs during 5 days of storage (3 ± 1°C)**

<table>
<thead>
<tr>
<th>Inoculated pathogen</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>0.81 ± 0.24 A ab</td>
<td>0.61 ± 0.32 AB ab</td>
<td>0.70 ± 0.19 A a</td>
<td>0.38 ± 0.29 B a</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.93 ± 0.37 A a</td>
<td>0.61 ± 0.68 A ab</td>
<td>0.68 ± 0.23 A a</td>
<td>0.94 ± 0.85 A ab</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0.54 ± 0.49 A a</td>
<td>1.23 ± 0.46 B c</td>
<td>0.91 ± 0.52 AB a</td>
<td>0.55 ± 0.61 A ab</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>0.36 ± 0.70 A b</td>
<td>0.07 ± 0.54 A a</td>
<td>0.66 ± 0.59 AB a</td>
<td>1.10 ± 0.59 B b</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.85 ± 0.58 A ab</td>
<td>1.09 ± 0.74 A bc</td>
<td>0.84 ± 0.39 A a</td>
<td>0.71 ± 0.64 A ab</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0.85 ± 0.34 AB a</td>
<td>1.19 ± 0.85 A bc</td>
<td>0.58 ± 0.41 B a</td>
<td>0.96 ± 0.73 AB ab</td>
</tr>
</tbody>
</table>

* Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).
TABLE 5. Population reductions on inoculated poultry legs treated with water as compared with untreated (control) legs during 5 days of storage (3 ± 1°C)

<table>
<thead>
<tr>
<th>Inoculated pathogen</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>0.36 ± 0.55</td>
<td>0.17 ± 0.49</td>
<td>0.41 ± 0.25</td>
<td>0.12 ± 0.18</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.17 ± 0.34</td>
<td>-0.36 ± 0.82</td>
<td>0.01 ± 0.32</td>
<td>0.31 ± 0.94</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0.15 ± 0.19</td>
<td>-0.10 ± 0.46</td>
<td>-0.41 ± 0.58</td>
<td>-0.21 ± 0.57</td>
</tr>
<tr>
<td>Salmonella Enteritidis</td>
<td>0.33 ± 0.35</td>
<td>-0.07 ± 0.27</td>
<td>-0.45 ± 0.41</td>
<td>-0.41 ± 0.12</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.41 ± 0.30</td>
<td>0.50 ± 0.60</td>
<td>-0.18 ± 0.47</td>
<td>-1.07 ± 0.65</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0.31 ± 0.19</td>
<td>0.09 ± 0.44</td>
<td>-0.24 ± 0.52</td>
<td>0.04 ± 0.63</td>
</tr>
</tbody>
</table>

* Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).

ilar findings have been found previously in both poultry (5) and red meat (43).

**L. monocytogenes.** Several researchers have evaluated the use of TSP on poultry products for controlling *L. monocytogenes* (8). The reductions found in the present study are lower than those previously reported for poultry: 2.1 (25), 2.5 (14), 1.52 to 3.63 (6), 1.12 to 3.34 (8), and 2.38 (21) log CFU/g. The differences among the results of these studies (observed for *Listeria* and other bacterial groups) probably are related to differences in type of sample, strain (type and physiological state), decontaminant exposure time, or application method, as previously indicated (9, 12, 20, 27, 32).

The antimicrobial effect of TSP may result from a combination of factors (7, 34). The high pH (about 12 to 13) of the TSP solutions appears to disrupt fatty molecules in the cell membrane, causing the bacterial cells to leak intracellular fluid. The ionic strength also can cause bacterial cell autolysis. The ability to remove a thin layer of lipids (“detergent” effect) from chicken skin, and thus expose any attached bacteria that may otherwise be protected in crevices and feather follicles, also is an important factor in the bactericidal activity of TSP. The removal of bacteria that are not yet firmly adherent to the surface also contributes to the decontaminant effect of TSP. *L. monocytogenes* is among the bacteria most resistant to TSP as a consequence of its high tolerance to conditions of alkaline pH (25).

*L. monocytogenes* reductions obtained after ASC treatment fill in the range of values obtained by other authors for red meat and poultry: 0.77 to 1.44 (31) and 1.5 (39). The antimicrobial activity of ASC is attributed to the oxidative effect of chlorous acid, which derives from the conversion of chlorite ions into the acid form under acidic conditions (12).

Organic acid rinses have been proposed as effective,
TABLE 6. Skin pH values for inoculated and decontaminated poultry legs during 5 days of storage (3 ± 1°C)

<table>
<thead>
<tr>
<th>Decontamination treatment</th>
<th>Mean ± SDa</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisodium phosphate</td>
<td>8.66 ± 0.53 A a</td>
<td>7.59 ± 0.31 B a</td>
<td>6.98 ± 0.22 C a</td>
<td>6.86 ± 0.24 C a</td>
<td></td>
</tr>
<tr>
<td>Acidified sodium chloride</td>
<td>5.78 ± 0.30 A b</td>
<td>6.16 ± 0.16 B b</td>
<td>6.25 ± 0.17 B b</td>
<td>6.47 ± 0.20 b b</td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>4.33 ± 0.23 A c</td>
<td>5.19 ± 0.16 B c</td>
<td>5.69 ± 0.26 C c</td>
<td>6.14 ± 0.17 b c</td>
<td></td>
</tr>
<tr>
<td>Peroxyacids</td>
<td>6.36 ± 0.13 A d</td>
<td>6.36 ± 0.10 A d</td>
<td>6.36 ± 0.16 A d</td>
<td>6.59 ± 0.21 b b</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>6.40 ± 0.12 A d</td>
<td>6.30 ± 0.15 A d</td>
<td>6.40 ± 0.20 b c</td>
<td>6.56 ± 0.15 c b</td>
<td></td>
</tr>
<tr>
<td>Untreated (control)</td>
<td>6.39 ± 0.12 A d</td>
<td>6.35 ± 0.16 A d</td>
<td>6.45 ± 0.14 A d</td>
<td>6.58 ± 0.21 b b</td>
<td></td>
</tr>
</tbody>
</table>

a Within the same row, means with no capital letters in common are significantly different (P < 0.05). Within the same column, means with no lowercase letters in common are significantly different (P < 0.05).

Inexpensive carcass decontamination interventions (11). The bactericidal effects of organic acids largely are due to the ability of undissociated acid to penetrate the bacterial cell membrane. Among organic acids, lactic and acetic acid are the most commonly used compounds in carcass decontamination, and to the best of our knowledge CA has not been studied for decontaminating poultry. The decrease in L. monocytogenes counts after treatment with 2% CA in the present study compares favorably with previously published results for other organic acids. Gonçalves et al. (21) studied the effect of 4% lactic acid on the growth of L. monocytogenes on poultry and found that immediately following the treatment, 2.38-log fewer bacteria could be recovered from chicken breast meat. Greer and Dilts (22) found a less marked reduction (1 log CFU/g) in L. monocytogenes when testing 3% lactic acid on pork.

In the present study, PA was not a strong decontaminant. The weak effect of PA against L. monocytogenes does not agree with findings obtained by an Ecolab laboratory (1): 2.11 log units after spray treatment and 0.60 to 1.25 log units after dipping. The weak effects observed in the present study suggest that further work is warranted to determine whether different PA application conditions would yield a greater antimicrobial response. The antimicrobial activity of PA is largely oxidative because of the presence of peryoxyacetic acid, hydrogen peroxide, and peryoxyctanic acid, which disrupt the permeability of cell membranes and alter protein synthesis. Indirect antimicrobial activity occurs through the acidification of the carcass surface and the penetration of the undissociated acids into the bacterial cell (41).

S. aureus. S. aureus reductions in the present study for samples treated with TSP compare favorably with results reported by Rodríguez de Ledesma et al. (37). These authors observed 1- to 2-log reductions on samples treated with 10% TSP for 15 s. Reductions of S. aureus obtained in the present study after treatment with ASC also agree with those reported by Hajmeer et al. (23), who found about 1-log lower concentrations on beef brisket immediately after treatment in comparison with untreated samples. These authors observed that, unlike other decontaminants, ASC caused similar S. aureus and E. coli reductions and suggested that ASC was able to suppress any physiological attachment differences of both pathogens to the meat, yielding similar responses to this treatment. Reductions of S. aureus observed by Lim and Mustapha (31) on beef after spraying with 1,200 ppm ASC solutions ranged from 1.26 to 3.76 log units. As far as we know, no studies have been carried out to determine the effect of CA and PA on S. aureus.

B. cereus. Although the effects of chemical decontaminants on various bacteria have been investigated, as far as we know no studies have evaluated their effect on B. cereus. As expected, the effect on this pathogen was similar to that on other gram-positive bacteria.

Salmonella. The reductions in Salmonella concentrations caused by TSP in the present study are consistent with the wide range of results previously found for poultry (7, 21, 32). Reductions very similar to those found in the present study were obtained by Kanellos and Burriel (27): 0.80 to 1.80 log units.

Reductions similar to those in the present study after ASC treatment (about 2 log CFU/g) were obtained by Schneider (39). Other authors have found very high reductions in Salmonella concentrations after ASC treatment both on poultry (5 log units) (33) and beef (4.6 log units) (35). In contrast, Pardue and Jones (35) observed minimal reductions in Salmonella Typhimurium counts (about 1 log unit) after treatment with a commercial compound containing sodium chlorite. As in the study being reported here, Mehyar et al. (32) found similar reductions in Salmonella (about 1 log CFU/g) after treatments with 10% TSP and with 1,200 ppm ASC for 30 min. A similar effect of TSP and ASC on Salmonella also was observed by Muller et al. (33) on chicken skin.

The weak effect of CA on Salmonella observed in our study was similar to that found by Kanellos and Burriel (27) with 1.5% lactic acid (0.5 to 0.75 log CFU). In contrast, King et al. (30) observed Salmonella Typhimurium reductions of 2.9 log CFU/cm² after spraying with 2% lactic acid. In Japan, Hiwaki et al. (24) reported an approximately 2-log reduction in inoculated Salmonella on chicken that was soaked in 0.6% fumaric acid. These authors observed similar reductions with 0.6% fumaric acid and 1.3% TSP, but significantly larger reductions were obtained by soaking in 1.37% ASC. In the present study, the stronger effect of TSP than of CA against Salmonella compares fa-
favorably with results of Kanellos and Burriel (27), who found that TSP was more effective than organic acids for reducing Salmonella loads.

Reductions in Salmonella observed in PA-treated samples (<1 log CFU/g) are coincident with results observed on both poultry (32) and beef (17). According to research commissioned by a private laboratory (Ecolab) (1), results vary with method of application. More marked Salmonella reductions (0.75 log unit) were obtained after spraying than after dipping (0.32 log unit). King et al. (30) observed that the application of 200 ppm peroxyacetic acid to chilled beef surfaces did not affect the concentration of Salmonella.

E. coli. E. coli reductions after TSP treatment in the present study were greater than the 0.3- to 1.8-log reductions reported by Colin and Salvat (13). E. coli reductions obtained after ASC treatment were higher than those reported by others on both poultry (1 to 1.6 log units) (32) and beef (1.1 to 1.9 (36), 0.45 to 0.89 (20), and 0.6 (17) log units). Schneider et al. (40) found that E. coli counts on beef decreased by 1.05 to 2.11 log units following dipping in 1,200 ppm ASC for 5 s. However, the microbial reductions obtained in that study reflected decreases in natural microflora rather than decreases in artificially inoculated laboratory cultures. E. coli reductions less marked than ours were also obtained by Gill and Badoni (20) (~0.30 to 1.16 log units) and Hajme et al. (23) (about 1 log unit) on beef. However, reductions similar to those found in the present study have been reported by Castillo et al. (12) (2.2 to 2.3 log units), Schneider (39) (2.28 log units), and Kemp et al. (28, 29) (2.18 to 2.21 log units) on poultry and by Rourke et al. (38) (2 to 2.1 log units) and Lim and Mustapha (31) (1.94 log units) on beef. Ransom et al. (36) found that ASC reduced the presence of E. coli O157:H7 by 1.3 to 2.1 log CFU/cm² in adipose tissue and by 1 to 1.1 log CFU/cm² in boneless beef trimmings, and Castillo et al. (12) reported E. coli reductions as high as 3.8 to 4.5 log CFU/cm². According to these authors, the force at which the ASC was sprayed in their study affected the reduction of E. coli on the surface of beef carcass tissue.

E. coli reductions after CA treatment in the present study were slightly less marked than those reported by Ransom et al. (36) (1.1 to 1.5 log CFU/cm² on adipose tissue and 0.6 to 1.1 log CFU/cm² in boneless trimmings) and by Stopforth et al. (43) (1.5 log units) in beef samples treated with lactic and acetic acids. Jiménez et al. (26) reported reductions in E. coli counts of approximately 0.80 log unit in chicken that was sprayed with 1 to 2% acetic acid.

Y. enterocolitica. Reduction in Y. enterocolitica populations were similar to those for other gram-negative bacteria and were within the range reported for Enterobacteriaceae by other authors (13, 14, 18). The strong effect of ASC on gram-negative bacteria is congruent with findings of other authors (12) and may be due to an additive effect of acidification of the sodium chlorite and the natural antimicrobial properties of CA. In contrast, the more marked reductions in gram-negative bacteria achieved with ASC than with TSP disagrees with the findings of Mehyar et al. (32), who reported that alkaline treatments were more effective than acidic treatments against these bacteria.

Gram-positive bacteria present on raw chicken might be less sensitive to decontamination procedures because of the characteristics of their outer membrane. The more marked reductions observed for gram-negative than for gram-positive bacteria are congruent with previous findings with most chemical compounds both in food (7, 13, 15, 31, 32) and in culture broth (42).

The results of the present study and those of Kanellos and Burriel (27) agree in that bacterial reductions (differences between treated and untreated samples) after TSP treatment tended to decrease during the refrigerated storage period. However, our results do not agree with those obtained by Lim and Mustapha (31), who found that S. aureus and E. coli reductions on beef increased throughout storage after treatment with 1,200 ppm ASC. Similar reductions of L. monocytogenes at the beginning and the end of the storage period were found by Su and Morrissey (44) in salmon treated with ASC.

pH values. The initial pH values on the skin of chicken legs in the present study agree with those reported by Mehyar et al. (32) and Kanellos and Burriel (27), who found that TSP caused the largest increase in skin pH after treatment, whereas lactic acid had the opposite effect. The pH values observed immediately after treatment with TSP and CA in the present study were within the range in which the multiplication of most bacteria is inhibited (27), thus adding to the bactericidal effect of these compounds. On the other hand, Mehyar et al. and Kanellos and Burriel found significant reductions in skin pH for chicken legs treated with PA. The weak antimicrobial effect of PA observed in the present study could be related to the less marked decrease in pH on poultry skin (17). In our study and in that of Lim and Mustapha (31), ASC treatments lowered the pH values of meat (as compared with control samples) as a consequence of the presence of CA used for the acidification of the sodium chlorite. The pH values of untreated or water-treated chicken skin (6.2 to 6.9) were in the range of values reported by others (27, 31, 32).

The change in pH for TSP-, CA-, and ASC-treated chicken skin as compared with normal skin pH agrees with previous results (4, 32) and could be due to the buffering capacity of the skin and meat tissue and to the progressive elimination of the decontaminant solution, as previously reported (6, 15). The maintenance of pH throughout storage observed for control and water-dipped samples in the present study is in agreement with the findings of others (4, 32). The potential for interference from the antimicrobial effect of residual compounds in samples used for microbiological testing, which may lead to underestimation of bacterial populations, was reduced by diluting the samples in buffered peptone water. This diluent neutralized the samples to ensure that the pH of any residual decontaminant solution remaining on the surface of the samples returned to normal, thereby eliminating the conditions suitable for ongoing antimicrobial activity (12).

All chemical decontaminants tested in this study pro-
duced significant reductions in populations of pathogenic bacteria when compared with the control and the water-treated samples. Such reductions would contribute to a marked decrease in the number of poultry carcasses contaminated by these microorganisms and would help increase the safety of poultry for human consumption. Among the decontaminants tested, CA and TSP were the most effective against gram-positive pathogenic bacteria, and ASC and TSP were the most effective against gram-negative bacteria.

ACKNOWLEDGMENT

This research was financially supported by the Spanish Ministerio de Sanidad y Consumo (FIS PI 040722).

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