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Effects of Cetylpyridinium Chloride, Acidified Sodium Chlorite, and Potassium Sorbate on Populations of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Staphylococcus aureus* on Fresh Beef †

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ABSTRACT

The effects of selected food-grade antimicrobial agents at decreasing the number of pathogenic bacteria on fresh beef were determined. Beef cubes inoculated with *Escherichia coli* O157:H7, *Listeria monocytogenes,* or *Staphylococcus aureus* were sprayed with 0.5% cetylpyridinium chloride (CPC), 0.12% acidified sodium chlorite (ASC), 0.1% potassium sorbate (PS), or an equal mix of any two solutions. The beef samples were placed on absorbent tray pads sprayed with each single or mixed solution, wrapped with polyvinyl chloride film, heat sealed, and stored at 4°C for 2 weeks. Surface sanitization using CPC, ASC, or an equal mix of these two agents effectively reduced microbial numbers on the beef during storage. At day 0, ASC and the CPC-ASC mix reduced the number of *E. coli* O157:H7 by 2.50 and 1.58 log CFU/cm^2, respectively. CPC demonstrated a 3.25-log reduction of *L. monocytogenes* and a 4.70-log reduction of *S. aureus* at 14 days. The CPC-PS mix reduced *E. coli* O157:H7 numbers by 1.46, *L. monocytogenes* by 2.95, and *S. aureus* by 4.41 log CFU/cm^2 at 14 days. PS alone and the mixed solutions, CPC-ASC, CPC-PS, or ASC-PS, were not as effective as ASC or CPC alone. To effectively reduce *E. coli* O157:H7, *L. monocytogenes,* or *S. aureus* numbers, higher (>0.1%) concentrations of PS were necessary. Loss of redness and light color of beef surfaces consistently coincided with decreases in pH for ASC-treated beef samples.

An estimated 10,000 to 20,000 cases of *Escherichia coli* infections occur in the United States each year. *E. coli* O157:H7 remains a significant human pathogen even though the first well-publicized outbreaks due to contaminated hamburgers occurred more than a decade ago. *L. monocytogenes* recently emerged as a problem in deli meats and other processed foods. Cold-tolerant *L. monocytogenes* has been isolated from beef-processing equipment and raw beef and identified as a contaminant of retail cuts *(19, 22).* The most serious risk related to *L. monocytogenes* is due to its ability to multiply to large numbers with minor contamination of food products under low temperature conditions (4 to 10°C). *S. aureus* outbreaks are typically a result of contamination from food handlers and the production of a heat-stable toxin in the contaminated food *(11). S. aureus* is of particular importance for raw and ready-to-eat beef safety because of its common occurrence and unique ability to survive and grow in an environment with a relatively low water activity *(23).*

For maintaining and ensuring food safety, the multiple-hurdles concept has been shown to be practical and effective. Cetylpyridinium chloride (CPC) is a versatile ingredient that can be used in ready-to-cook, ready-to-eat, and processed products manufactured from poultry, meat, and fish as well as fruits and vegetables *(1).* CPC is effective against many pathogens, including *Salmonella, L. monocytogenes, Campylobacter,* and *E. coli* O157:H7 and does not adversely affect the flavor, texture, appearance, or odor of foods *(3, 17).* As a germicidal surfactant agent, the antibacterial activity of CPC is caused by its cations *(9, 16, 27).* CPC has both hydrophilic and lipophilic properties derived from the chloride and cetylpyridinium cations, respectively. Along with its amphiphilic properties, the low surface tension of CPC allows it to effectively penetrate cells *(17).* Acidified sodium chlorite (ASC), which is a combination of any generally recognized as safe (GRAS) acid and sodium chlorite in an aqueous solution, is approved as a direct food additive by the U.S. Department of Agriculture and the Food and Drug Administration to be used for decontamination of poultry and red-meat carcasses *(6).* It is used at levels that result in sodium chlorite concentrations of 500 to 1,200 ppm in combination with any GRAS acid at levels sufficient to achieve a pH of 2.3 to 3.2 on red meat, poultry, seafood, and fruit and vegetables *(10).* It has also been proven to be an effective microbial control agent in meat products as well as a surface disinfectant and sanitizer resulting from a combination of antimicrobial effects due to the acid content and antimicrobial properties of chlorine. The antimicrobial mechanism of ASC is due to the conversion of chlorite ions to chlorine dioxide that inhibits protein synthesis in the cell *(24, 25).* Potassium sorbate (PS) is the potassium salt of sorbic acid, a naturally occurring organic acid that has been used as a

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fungistatic agent for foods at levels of 0.025 to 0.3% *(11, 28).* PS is 50% more soluble in water than is sorbic acid. PS has about 74% of the antimicrobial activity of sorbic acid *(11, 28).* Although sorbic acid is more effective over a wide range of pH conditions than its salts, sorbates are used in many cases due to their higher solubility in foods.

In addition to the use of antimicrobial agents, developments in food packaging technology are also contributing to augment the stability and quality of shelf-life-enhanced foods. A suitable food package should be able to extend the microbial lag phase and reduce the growth rate of microorganisms to prevent postcontamination and maintain the sensory properties of the food *(8).* To control undesirable spoilage and pathogenic microorganisms, the incorporation or coating of antimicrobial agents on packaging materials can be considered as effective methods. Additionally, absorbent tray pads are used extensively in the meat industry because of their wide range of controllable absorbencies, full lock-way characteristics, and appealing appearance *(2).* These packaging systems could be made more effective if combined with an antimicrobial agent, which could consequently extend the shelf life and safety of meats. In our previous study, a 7.3- and 1.2-log reduction of *E. coli* O157:H7 and *Lactobacillus plantarum* numbers, respectively, were demonstrated over a storage period of 3 weeks on raw beef vacuum packaged in film that had been sprayed with 2% polylactic acid *(15).* The objective of this current study was to further explore the use of antimicrobial-sprayed packaging materials on reducing the numbers of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* on raw beef.

MATERIALS AND METHODS

Bacterial strains. *E. coli* O157:H7 505B, *L. monocytogenes* Scott A, and *S. aureus* FR1 were obtained from the Microbiology Laboratory of the Food Science Department, University of Missouri–Columbia. Bacterial cultures were maintained at 4°C in tryptic soy broth (Difco, BD Diagnostic Systems, Sparks, Md.) supplemented with 0.1% yeast extract (Difco, BD Diagnostic Systems) (TSBYE) and transferred to fresh TSBYE broth prior to use. Bacteria in the early stationary phase of growth were used as inocula to conduct experiments. All three strains were aerobically grown at 37°C.

Preparation of antimicrobial solutions. Three antimicrobial agents to be evaluated included CPC, ASC, and PS. All solutions were made fresh prior to conducting experiments. CPC (ICN Biomedicals Inc., Aurora, Ohio) was prepared in sterilized distilled water at a concentration of 0.5% (wt/vol). ASC solution was made by mixing sodium chlorite (Alfa Aesar, Ward Hill, Mass.) and citric acid (Acros Organics, Geel, Belgium) to a final concentration of 0.12% sodium chlorite and 0.9% citric acid. The final pH of ASC solution and all ASC-containing mixes were in the range of 2.5 to 2.9. PS (Sigma Chemical Co., St. Louis, Mo.) (0.1% wt/vol) solution was prepared in sterilized distilled water. Each antimicrobial formulation was used separately or as an equal mix of any two solutions. Treatment formulations were prepared fresh for each experiment and were composed of (i) untreated control, (ii) sterilized distilled water, (iii) 0.5% CPC, (iv) 0.12% ASC, (v) 0.1% PS, (vi) an equal mix of 0.5% CPC and 0.12% ASC (CPC-ASC), (vii) an equal mix of 0.5% CPC and 0.1% PS (CPC-PS), and (viii) an equal mix of 0.12% ASC and 0.1% PS (ASC-PS). Solutions were stored at 4°C and used within 4 h. All solutions were tempered at room temperature before using.

Preparation of beef samples and microbial inocula. Whole beef round was purchased from a local retail establishment (Tiger Packing, Inc., Columbia, Mo.) and the surface of the beef was aseptically trimmed to minimize the initial numbers of microflora. The trimmed beef was cut into cubes of 2.5 by 2.5 by 2.5 cm, and to further eliminate natural surface microflora, exposed to short-wave ultraviolet light (15 W, Sylvania germicidal UV lamp, GTE Products Co., Danvers, Mass.) at a distance of 20 cm for 3 min on each side. The UV-treated beef samples were stored at 4°C, sealed in vacuum package films (low density polyethylene, Koch Supplies Inc., Kansas City, Mo.) having an oxygen transmission rate of 0.6 $cm^3/100\ in^2/24$ h at 0°C and a water vapor transmission rate of 0.6 $g/100\ in^2/24$ h at 100% relative humidity at 37.8°C under vacuum (Vac Master SVP20, Specialty Food Equipment Co., Kansas City, Kans.), and tempered at room temperature before using. Fresh cells of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* were grown in TSBYE broth for 9 h at 37°C to obtain an initial viable cell density of approximately 10^7 to 10^8 CFU/g of each bacterial suspension. Cultures were pelleted by centrifugation (Model J2–21, Beckman Instr. Inc., Schaumburg, Ill.) at 10,000 × *g,* at 4°C for 15 min and washed twice with sterile peptone water (0.1%, Difco, BD Diagnostic Systems) by centrifugation under the same conditions. Cells were resuspended in sterile peptone water, in a volume equal to the original, to a final concentration of 10^7 to 10^8 CFU/g. The washed cells were used to inoculate the beef samples by dipping for 1 min and drip drying for 10 min to obtain a final concentration of 10^5 to 10^6 CFU/g on each raw beef cube surface. Following inoculation, the samples were aseptically sprayed with 10 ml of each antimicrobial formulation and wrapped in antimicrobial-treated packaging materials as described below.

Preparation of antimicrobial sprayed packaging materials. Dri-Loc AC-50 absorbent pads (50 to 55 g absorbancy, Cryovac, Saddle Brook, N.J.) were separately sprayed with 20 ml of each of the six liquid antimicrobial formulations using a handheld, sterile plastic sprayer that delivered a 140-ml spray. The beef samples were sprayed with 10 ml of each liquid antimicrobial agent. Two pieces of inoculated sprayed beef samples were aseptically packed in a commercial foam tray pack (Model W2 [8.0 by 5.75 by 1.11 in.], Cryovac, Saddle Brook, N.J.), which contained a commercial absorbent pad sprayed with each antimicrobial agent. The trays were overwrapped on a heat-sealing station (Model HS 625A, Cleveland, Ohio) with polyvinyl chloride film (ClearView Stretch Wrap Film, Hobart Co., Troy, Ohio) that had been aseptically cut into 15-in. pieces. The polyvinyl chloride film had an oxygen permeability of 600 $cm^3/100\ in^2/24$ h at atm, 73°F, 0% relative humidity, and water permeability of 4 $g/100\ in^2/24$ h at 90% relative humidity. Another set of inoculated and water-sprayed beef cubes was similarly wrapped after placing the samples on water-treated tray pads in the foam tray packs. Untreated control samples were similarly packed using untreated tray pads. Samples were stored in a commercial display refrigerator (Model True, True Manufacturing Co., St. Charles Industrial Center, O'Fallon, Mo.) that was lit by a fluorescent lamp (40 W, 48 in., Natural Color Fluorescent for Kitchen & Bath Ultra, General Electric Co., Douglas, Ariz.) to simulate grocery store conditions (4°C).

pH determination and microbial enumeration. After spray treatments, each sample was analyzed for surface pH and bacterial

TABLE 1. *Effect of antimicrobial treatments on the numbers of* Escherichia coli *O157:H7 on fresh beef stored at 4°C for up to 14 days*

Day	Mean number of *E. coli* O157:H7 for each treatment							
	Control	Water	CPC	ASC	PS	CPC-ASC	CPC-PS	ASC-PS
0	6.89 A[a]	6.85 A	5.64 CD	4.95 D	6.58 AB	5.11 CD	5.75 CD	5.86 BC
2	6.85 A	6.4 AB	4.87 CD	3.42 E	6.57 A	5.56 C	5.60 BC	4.48 D
4	6.67 A	6.28 AB	5.07 C	3.1 E	6.41 A	5.23 C	5.52 BC	4.13 D
6	6.63 A	6.2 A	4.32 B	2.42 D	6.1 A	4.35 B	5.04 B	3.41 C
8	6.56 A	6.18 A	3.92 C	2.76 D	6.18 A	1.73 E	4.76 B	3.73 C
10	6.53 A	6.1 A	3.89 C	1.98 D	6.19 A	1.55 D	5.1 B	3.12 C
14	6.44 A	5.9 A	4.11 B	2.28 D	6.25 A	2.89 CD	4.8 B	3.22 C

[a] Means within a row with common letters do not differ ($P > 0.05$).

counts every other day from day 0 to day 10, and on day 14 to see the poststorage trend. pH was measured on six areas of the beef surfaces using a surface pH probe (Model 955, Fisher Scientific, St. Louis, Mo.) and the mean values were used for determining representative surface pH values. Each beef cube was individually placed in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, Wis.) containing 20 ml of 0.1% sterile peptone water, pH 7.0, and stomached for 2 min (Stomacher 400, Tekmar Inc., Cincinnati, Ohio). The solutions were serially diluted in 0.1% sterile peptone water to appropriate dilutions for plating and each dilution was spread plated in duplicate. Cells of *E. coli* O157:H7 were plated on MacConkey sorbitol agar (Difco, BD Diagnostic Systems), *L. monocytogenes* was plated on modified Oxford agar (Difco, BD Diagnostic Systems), and *S. aureus* was plated on Baird-Parker agar (Difco, BD Diagnostic Systems) for enumeration. Plates were incubated at 37°C for 24 to 48 h and colonies were counted. Counted microbial values were transformed to common logarithms of bacterial count values before analysis with SAS. Three experimental replications were performed on different days.

Color evaluation. The color of fresh beef was evaluated every other day for up to 14 days except for day 12. Light reflectance (CIE L*, a*, and b*) was measured using a HunterLab Miniscan XE Plus Colormeter set at D65/10° (Hunter Associates Lab., Reston, Va.) and standardized to a black and white tile. Lightness (L*), redness (a*), and yellowness (b*) readings were taken from all six surfaces of the raw beef to minimize variation. Three experimental replications were performed on different days.

Statistical analysis. All experiments were replicated three times and plating was performed in duplicate at each sampling time. Averaged surface pH, mean log CFU/cm^2, and color were analyzed by least significant difference using the General Linear Model Procedure of a statistical-analysis software package. Mean separation was conducted among treatments and storage times when the analysis of treatment effects and storage times indicated a significant difference at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of treatments on pH of beef surfaces. No significant difference in pH was found among all beef samples inoculated with *E. coli* O157:H7 immediately after treatments (data not shown). However, on day 2, ASC-treated beef showed significantly lower pH than all the other samples except for those treated with the CPC-ASC or ASC-PS mix. ASC-treated beef surfaces exhibited a pH of 4.99, which was significantly lower than those of all the other treatments on day 6. At the end of storage, CPC-treated beef had a similar pH as all the other treatments except for ASC and the untreated control, but they were significantly higher than those of the ASC-treated samples and significantly lower than that of the untreated control. Similarly, there was no significant difference in pH among all treatments for beef inoculated with *L. monocytogenes* at 0 day (data not shown). The CPC-ASC mix resulted in the lowest surface pH from day 2 to day 4. On the other hand, after day 8, ASC treatments, including the CPC-ASC and ASC-PS mixes, had the lowest pH but they were not significantly different from one another. At 8 days, *S. aureus*-inoculated beef treated with ASC showed the lowest pH value, which was significantly different from those of all the other treatments (data not shown). As expected, ASC treatments lowered meat pH values more than did the other treatments. This may have been due to the presence of citric acid used for the acidification of sodium chlorite. Because the CPC solution has almost neutral pH values, which means less pH dependence for its activity than other common antimicrobial agents such as ASC, it did not cause a great pH decline on the beef surfaces. The pH of CPC-treated beef surfaces were not significantly different from those of the untreated control beef for all three strains except *L. monocytogenes* on days 10 to 14. Thus, the use of CPC as an effective antimicrobial agent is promising for this type of product. In addition, the surface pH of PS-treated beef showed similar trends as that of the CPC treatment.

Effect of treatments on microbial counts. Bacterial counts of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* on fresh beef were compared for effects of CPC, ASC, PS, and an equal mix of any two antimicrobial solutions. The results showed that the numbers of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* on beef surfaces, following antimicrobial spraying on absorbent tray pads and beef surfaces, were significantly lower than those of the untreated controls. Water-treated beef samples were used as a physical parameter control and no difference in bacterial numbers ($P > 0.05$) was found between the untreated control and the water control. Viable populations of *E. coli* O157:H7 were significantly lower on beef surfaces treated with CPC, ASC, or the CPC-ASC mix compared with populations on beef surfaces treated with water or PS through-

TABLE 2. *Effect of antimicrobial treatments on the numbers of* Listeria monocytogenes *on fresh beef stored at 4°C for up to 14 days*

	Mean number of *L. monocytogenes* for each treatment							
Day	Control	Water	CPC	ASC	PS	CPC-ASC	CPC-PS	ASC-PS
0	6.07 A[a]	5.83 A	2.8 D	5.07 AB	5.47 A	4.10 BC	3.7 CD	5.23 A
2	5.86 A	5.81 A	3.96 C	4.94 ABC	5.45 A	4.34 BC	4.09 BC	5.0 AB
4	5.95 A	5.7 A	2.69 D	5.18 AB	5.72 A	4.37 BC	3.65 CD	5.11 AB
6	5.85 A	5.18 AB	3.25 D	4.83 BC	4.63 BC	4.17 CD	3.32 D	4.55 BC
8	5.88 A	5.85 A	3.18 D	4.40 BC	5.04 AB	3.59 CD	4.48 BC	4.78 B
10	5.57 A	5.41 AB	3.30 E	4.39 CD	5.32 ABC	3.97 DE	4.46 BCD	4.99 ABC
14	5.49 A	5.04 AB	3.17 C	4.26 B	4.93 AB	4.17 BC	4.54 AB	4.98 AB

[a] Means within a row with common letters do not differ ($P > 0.05$).

out storage (Table 1). CPC reduced numbers of *E. coli* O157:H7 by 2.78 log, ASC resulted in a 4.62-log reduction of *E. coli* O157:H7, and PS resulted in a 0.64-log reduction of *E. coli* O157:H7 during storage. CPC mixed with ASC resulted in a 4.0-log reduction of *E. coli* O157:H7, CPC mixed with PS caused a 2.09-log reduction of *E. coli* O157:H7, and the ASC-PS mix resulted in a 3.67-log reduction of *E. coli* O157:H7 at the end of storage (Table 1).

At day 0, a significant decrease in *L. monocytogenes* counts was evident on CPC-treated samples but it was not significantly different from that of the CPC-PS mix treatment (Table 2). However, throughout storage, CPC was found to be significantly more effective against *L. monocytogenes* than ASC or PS except on day 2. CPC, CPC-ASC, and CPC-PS spray treatments resulted in lower counts of *S. aureus* than ASC alone at the beginning of storage but the differences were not always significant (Table 3). During storage, the greatest reductions of *S. aureus* by CPC, ASC, PS, CPC-ASC, CPC-PS, and ASC-PS spraying were 4.01, 5.09, 2.36, 4.03, 3.99, and 4.27 log, respectively. The log reductions in *S. aureus* numbers by the equal mix of any two solutions were not significantly different from one another at the end of storage.

Cutter et al. *(7)* demonstrated a 5- to 6-log reduction of *E. coli* O157:H7 and undetectable levels of *Salmonella* Typhimurium on lean beef surfaces immediately after CPC washes and during 35-day storage. Compared with this observation by Cutter et al. *(7),* even though *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* populations were less suppressed by CPC treatment in this study (a 2.78, 2.9, and 4.01 log CFU/cm^2, respectively, at the end of a 14-day storage), the log reductions by CPC treatment were still significantly different from those of the controls and water-treated samples.

The effectiveness of CPC has also been widely investigated in poultry processing *(4, 13, 17, 26, 27).* Breen et al. *(4)* reported that CPC showed a 4.9-log reduction of *Salmonella* Typhimurium from poultry tissues at a concentration of 8 mg/ml for 10 min. Kim and Slavik *(13)* investigated the mechanism of how CPC lowered the number of salmonellae on chicken skins via scanning electronic microscopy. These authors mentioned that, although CPC did not detach the cells from chicken skin, the treatment might cause metabolic malfunctions resulting from morphological structural damage of salmonellae cells because indentations on cells treated with 0.1% CPC solutions for 5 min were observed via scanning electron microscopy *(13).* Although no scanning electron microscopic studies were conducted in our current work, the observations by Kim and Slavic *(13)* may help to explain how CPC might have effectively reduced the number of the pathogens immediately after treatment and during refrigerated storage when sprayed on absorbent tray pads and beef surfaces.

From days 2 to 6, 0.12% ASC significantly reduced the number of *E. coli* O157:H7, but when used in combination with CPC, the mix significantly reduced the population of *E. coli* O157:H7 at day 8 (Table 1). Beef cubes packaged in ASC-, CPC-ASC-, and ASC-PS-sprayed absorbent tray pads showed a 4.62-, 4.0-, and 3.67-log reduction on *E. coli* O157:H7, respectively. The activity of ASC on *L. monocytogenes* was not as great as it was on *E. coli* O157:H7. A 1.81-log reduction of *L. monocytogenes* was

TABLE 3. *Effect of antimicrobial treatments on the numbers of* Staphylococcus aureus *on fresh beef stored at 4°C for up to 14 days*

	Mean number of *S. aureus* for each treatment							
Day	Control	Water	CPC	ASC	PS	CPC-ASC	CPC-PS	ASC-PS
0	5.8 A[a]	5.47 AB	4.13 B	4.54 AB	5.33 AB	4.2 B	4.23 B	5.25 AB
2	6.12 A	5.43 AB	3.56 C	4.86 ABC	5.47 AB	4.43 BC	4.28 BC	5.28 AB
4	5.88 A	5.77 AB	3.32 D	4.51 BCD	5.58 AB	4.02 CD	3.81 CD	5.11 ABC
6	5.93 A	5.52 A	3.78 C	4.13 BC	5.47 AB	3.62 C	4.09 C	4.57 ABC
8	5.69 A	5.37 A	2.89 DE	2.54 E	5.29 AB	3.4 CDE	4.63 ABC	3.99 BCD
10	5.05 A	4.93 A	3.31 BC	2.0 BC	4.94 A	1.83 C	3.29 B	2.74 ABC
14	4.37 A	4.76 A	1.79 B	0.71 B	3.43 A	1.77 B	1.8 B	1.53 B

[a] Means within a row with common letters do not differ ($P > 0.05$).

caused by ASC treatment at day 14, but this was not significantly different from those caused by water, PS, CPC-PS, or ASC-PS treatments (Table 2). Although ASC reduced populations of *S. aureus* at day 14 by 5.09 log, this reduction was not significantly different from those caused by any other treatments except the untreated control, water, and PS treatments (Table 3). These results may be explained by the amount of acidity, the rate of greater decomposition of chlorous acid, and the lower pH of the solution have an effect on the intermediate chlorous acid formation from ASC *(12).*

The results of our study indicated that PS did not have an inhibitory effect on *E. coli* O157:H7, *L. monocytogenes,* or *S. aureus* during storage. There were no significant differences in the reduction of these three pathogens between the control and water spray. Therefore, a higher concentration of PS or a combination of PS with other antimicrobial agents may be more effective at inhibiting the cultures in this study. On the basis of this idea, Morad et al. *(21)* demonstrated that the antimicrobial activity of a combination of 0.1% sorbate with 0.01% butylated hydroxyanisole was greater than that of sorbate alone against the natural microflora on fresh turkey meat, and at reducing the survival of *Salmonella* Typhimurium in cooked turkey meat. Similar to our results, McMeekin et al. *(18)* reported no direct effects of 5% PS on the reduction of *Alteromonas putrefaciens,* but a bactericidal effect of PS was seen when chill temperatures and vacuum packaging were used.

In this study, antimicrobial spraying of beef surfaces and absorbent tray pads had the potential to reduce bacterial contamination and prevent further cross-contamination on beef cuts during processing. Strain-to-strain variation, differences in inactivation rates, degree of injury, and time required for resuscitation among the bacteria might result in variable sensitivity to the antimicrobial agents used in this study *(20).* For example, in the case of *E. coli* O157:H7, ASC was the most effective but, in the case of *L. monocytogenes* or *S. aureus,* CPC was the most effective. This may be explained by the fact that hydrophobic molecules of CPC can easily interact with gram-positive bacteria, including *L. monocytogenes* and *S. aureus,* which have a more hydrophobic cell-surface structure than gram-negative bacteria such as *E. coli* O157:H7 *(13, 16).* Reductions in numbers of pathogens obtained in this study were similar to those found by other investigators for carcass treatments with ASC or CPC *(5, 7).*

Effect of treatments on color of beef surfaces. The lightness of the *E. coli* O157:H7-inoculated control, which showed the least brightness, was significantly different from those of the CPC-, water-, CPC-PS-, or ASC-treated samples at day 0, but it was not significantly different from PS-, CPC-ASC-, or ASC-PS-treated samples (data not shown). In addition, all treated samples had less redness than the control at day 0 for *E. coli* O157:H7-inoculated beef. However, at the end of storage, the control was significantly less bright than all other treatments but all treatments had similar redness and yellowness. In *L. monocytogenes*-inoculated samples, the ASC-PS treatments were lighter, less red, and less yellow than beef samples treated with ASC alone during storage, except for day 14 (data not shown). However, there were no significant differences in lightness, redness, and yellowness values between ASC- and ASC-PS-treated samples. Mean Hunter redness scores of raw beef surfaces inoculated with *S. aureus* were not significantly different among all treatments at day 14 (data not shown). During storage, yellowness of beef surfaces inoculated with *S. aureus* followed similar trends except for days 8 and 14. Loss of redness and light color of beef surfaces consistently coincided with decreases in pH for all ASC treatments including ASC, ASC-PS, and CPC-ASC when compared with the control. These results agreed with those of other studies *(13, 26).* Discoloration of chicken skin or a chemical odor was caused by high concentrations of organic acids or other chemical treatments *(13).* However, data from previous research did not match observations by Xiong et al. *(26),* who found that because CPC has neutral pH values, it did not cause discoloration of chicken skin. In general, even with an ideal oxygen-permeable film, meat color is acceptable during a 4-day storage, but after 5 days, a gray-brown discoloration and a sour odor are unavoidable *(14).*

This study demonstrated that spraying tray absorbent pads and beef surfaces with CPC or ASC resulted in the greatest reduction of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* populations on fresh beef surfaces under refrigerated storage. Although the equal mix of CPC-ASC, CPC-PS, or ASC-PS treatment was less effective than any single antimicrobial spray, spraying with these mixtures produced significant reductions in populations of *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* when compared with the control and water-treated samples.

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