

Effect of Surface Decontamination Using Antimicrobial Agents on Microbiological Quality of Beef Steaks

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Story in Brief

Beef steaks were inoculated with *Escherichia coli* (EC) and *Salmonella* Typhimurium (ST) and then decontaminated using 3% potassium lactate, 4% sodium metasilicate, 0.5% cetylpyridinium chloride, or 10% trisodium phosphate as a single microbial intervention. The packaged steaks were sampled on days 0, 1, 2, 3, and 7 of retail display for coliform (CO), EC, aerobic plate count (APC) and ST. All treatments showed significantly lower ($P < 0.05$) counts for all the bacteria monitored compared to an untreated control. The results indicate that incorporation of any of these antimicrobial agents in a meat product system would result in improved product safety.

Introduction

Even though the muscles of healthy animals are significantly sterile (Harris et al., 2006), meat products become contaminated during raw material handling and processing in harvest and processing plants. According to Koochmaraie et al. (2005), *Escherichia coli* O157:H7 and non-O157 are mostly associated with raw beef products, whereas *Salmonella* may be found in a variety of food products including raw meat. The microbial contamination influences shelf-life of refrigerated red meat (Acuff et al., 1987). Although cool temperature slows the growth of pathogens and spoilage organisms in meat, it will not eliminate them unless validated interventions are practiced on raw material (Harris et al., 2006). Therefore, the meat industry continues to seek new and improved techniques to produce raw products that have low levels of spoilage bacteria and non-pathogenic bacteria.

Many decontamination strategies are available that reduce both pathogen and spoilage microorganism loads in meat products. However, a decontamination technique applied near the end of the production line will be advantageous to supply a product with increased shelf-life and decreased or no risk for food born illnesses. Therefore, the purpose of this study was to determine the microbiological quality of beef steaks decontaminated using different antimicrobial agents and stored under simulated retail display conditions.

Experimental Procedures

Bacterial preparation and inoculation. Frozen stock cultures (-112°F) of *Escherichia coli* (ATCC # 11775; EC) and nalidixic acid resistant *Salmonella* Typhimurium (ATCC # 1769NR; ST) were used for inoculating steaks. The pure cultures were obtained from Biomass Laboratory (University of Arkansas, Fayetteville, Ark.). After thawing, 0.1 ml of *E. coli* and *S. Typhimurium* suspensions were added to 40 separate 40 ml aliquots of brain heart infusion (BHI; DIFCO Laboratories, Detroit, Mich.) and BHI with nalidixic acid, respectively. Following 18 hr incubation at 99 °F, bacteria were

harvested by centrifugation (3,649xg for 20 min at 99°F) (Beckman GS-6 series; Fullerton, Calif.). Then, the bacteria were re-suspended in 40 ml 0.1% buffered peptone water (Difco Laboratories, Detroit, Mich.). Finally, 1,600 ml of *E. coli* and 1,600 ml of *S. Typhimurium* were combined to make a bacterial cocktail (3,600 ml; log 10⁷ CFU *E. coli* and log 10⁷ CFU *S. Typhimurium*). After cooling the cocktail to 39°F, it was placed in a sterile bag with steaks 1-inch thick, obtained from top sirloin butts (IMPS#184). A total of 30 steaks were obtained from 15 top sirloin butts and the steaks were mixed well with the bacterial cocktail by shaking manually. This procedure was repeated 2 more times and a total of 90 inoculated steaks were produced. Following inoculation, the steaks were drained and separated into 3 batches and placed in a 39°F cooler for 12 to 14 h to allow further microbial attachment.

Antimicrobial treatment and processing. The antimicrobial treatments included 0.5% (w/v) cetylpyridinium chloride (CPC; Zeeland, Inc., Zeeland, Mich.), 0.3% (v/v) potassium lactate (KL; Purasal®, Purac America Inc., Lincolnshire, Ill.), 4% sodium metasilicate (NMS; Avgard®, Rhodia Inc., Cranbury, N.J.), and 10% (w/v) trisodium phosphate (TSP; Rhone Poulenc, Cranbury, N.J.). For antimicrobial application, 5 steaks from each batch of inoculated steaks were placed into a meat tumbler (Model 4Q, Lyco Inc. Janesville, Wis.). Then, the selected volume of antimicrobial agent was added and tumbled at 60 rpm for 3 min. Likewise, each antimicrobial treatment was repeated 3 times. Next, steaks were placed on styrofoam trays with absorbent pads and over-wrapped with polyvinyl chloride film (O₂ transmission rate = 14,000 cc/mm²/24 h/1 atm; Koch Supplies, Inc., Kansas City, Mo.). The steaks were stored at 39°F under 1,630 lux of deluxe warm white fluorescent lighting (Phillips Inc., Somerset, N.J.).

Microbiological analysis. On day 0, 1, 2, 3, and 7 of simulated retail display, ST counts on *Salmonella* shigella agar (DIFCO Laboratories, Detroit, Mich.) containing nalidixic acid, aerobic plate count (APC), and *E. coli* (EC) / coliform (CO) counts on Petrifilm® (3M Corporation, St. Paul, Minn.) were determined for steaks from all treatments. The microbial enumeration for each steak was carried out each day by aseptically removing 25 g from the surface using a sterile scalpel and forceps as described by Venturini et al. (2006). The 25-g samples of steak from each antimicrobial treatment were placed in sterile whirlpack bags (Nasco, Ft Atkinson,

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Wis.) separately and 225 ml of 0.1% buffered peptone water was added and samples were homogenized for 2 min in a stomacher (Model 400 Lab Stomacher; Seward, London, UK). Subsequently, serial 10-fold dilutions were made and spread plating was done in duplicates. The EC, APC and ST counts were read after 48 h, whereas coliform plates were read after 24 h. All the counts were recorded as colony forming units per gram (CFU/g).

Analysis of data. The bacterial values were transformed to log₁₀ CFU/g values and then analyzed for the main effects of antimicrobial treatment, day of display, and treatment by day of display interaction using the GLM procedure of SAS (SAS Inst., Inc., Cary, N.C.). Least squares means were generated for all variables and were separated using the PDIFF option of GLM.

Results and Discussion

Tables 1, 2, 3, and 4 show the antimicrobial treatment by days of display interaction for CO, EC, APC and ST counts, respectively. All treatments, except TSP, reduced ($P < 0.05$) CO, EC and ST counts by greater than 1 log CFU/g compared with the control on day 0. Nevertheless, the TSP treatment achieved a reduction ($P < 0.05$) of 1.4 log CFU/g for ST on day 0. The NMS-treated steaks showed the largest reduction ($P < 0.05$) for APC on day 0 compared to all other treatments.

On days 1 and 2, all treated samples showed a greater than 1 log CFU/g reduction ($P < 0.05$) for CO, EC, and ST compared with the control. The NMS treated steaks continued to have the largest reduction ($P < 0.05$) for APC on days 1, 2 and 3; however, TSP and NMS treatments had similar results ($P > 0.05$) for APC on day 2, 3, and 7.

Steaks from NMS and TSP treatments showed a greater than 1.5 log CFU/g reduction for CO and EC and a greater than 1 log CFU/g reduction for APC on day 3. Furthermore, KL, TSP and NMS treatments obtained a greater than 2 log CFU/g reduction for ST on day 3.

The NMS and TSP treatments were more successful ($P < 0.05$) in controlling CO and EC counts compared to all the other treatments on day 7, with a greater than 2 log CFU reductions, whereas the CPC and KL treated samples achieved approximately 1 and 1.5 log CFU/g decreases, respectively. All treatments had a greater than 1 log CFU/g less ($P < 0.05$) APC compared with the control on day 7. Steaks from KL, NMS, and TSP treatments had a greater than 2 log CFU/g reduction ($P < 0.05$) in ST count on day 7 in relation to the control. The CPC treatment was less efficient compared to the other antimicrobial treatments on day 7, still producing a 1 log CFU/g reduction for ST.

These results are in agreement with the previous studies that recognized successful application of 0.5% CPC, 10% TSP (Pohlman et al., 2004) and 3% KL, and 4% NMS (Pohlman et al., 2002) on beef trimmings to reduce similar bacterial counts of ground beef under retail display.

Implications

The ability of 3% potassium lactate, 4% sodium metasilicate, 0.5% cetylpyridinium chloride, and 10% trisodium phosphate to reduce bacterial counts on inoculated steaks indicates that decontamination of steaks using those antimicrobial agents prior to packing may improve the product safety and extend the product shelf life.

Literature Cited

- Acuff, G.R., et al. 1987. Meat Sci. 19:217.
 Harris, K., et al. 2006. J. Food Prot. 69:1802.
 Koohmaraie, M., et al. 2005. Meat Sci. 71:79.
 Pohlman, F.W., et al. 2002. Meat Sci. 60:349.
 Pohlman, F.W., et al. 2004. AAES. Res. 535:142.
 Venturini, A.C., et al. 2006. J. Food Sci. 71:560.

Table 1. Effect of antimicrobial treatment by day of display interaction on least squares mean (\pm SE) log CFU¹/g coliform counts of beef steaks.

Treatment ²	Days of display				
	0	1	2	3	7
INCON	7.53 ^a \pm 0.13	7.51 ^a \pm 0.13	7.54 ^a \pm 0.04	7.44 ^a \pm 0.42	7.46 ^a \pm 0.02
CPC	6.19 ^e \pm 0.13	5.98 ^d \pm 0.13	6.17 ^{bc} \pm 0.04	7.41 ^a \pm 0.42	6.66 ^b \pm 0.02
KL	6.27 ^e \pm 0.13	6.33 ^b \pm 0.13	6.33 ^b \pm 0.04	6.65 ^b \pm 0.42	5.91 ^e \pm 0.02
NMS	6.10 ^e \pm 0.13	5.65 ^e \pm 0.13	6.09 ^{bc} \pm 0.04	5.64 ^e \pm 0.42	5.40 ^d \pm 0.02
TSP	6.85 ^b \pm 0.13	6.28 ^b \pm 0.13	5.92 ^c \pm 0.04	5.48 ^d \pm 0.42	5.33 ^e \pm 0.02

¹Colony forming units.

²Treatments: INCON = untreated inoculated control, CPC = 0.5% cetylpyridinium chloride, K-L = 3% potassium lactate, NMS = 4% sodium metasilicate, TSP = 10% trisodium phosphate.

^{a-e} Least squares means within a column with different superscripts are different ($P < 0.05$).

Table 2. Effect of antimicrobial treatment by day of display interaction on least squares mean (\pm SE) log CFU¹/g *Escherichia coli* counts of beef steaks.

Treatment ²	Days of display				
	0	1	2	3	7
INCON	7.58 ^a \pm 0.13	7.52 ^a \pm 0.06	7.55 ^a \pm 0.13	7.43 ^a \pm 0.04	7.47 ^a \pm 0.09
CPC	6.21 ^c \pm 0.13	5.98 ^b \pm 0.06	6.17 ^{bc} \pm 0.13	7.41 ^a \pm 0.04	6.50 ^b \pm 0.09
KL	6.27 ^c \pm 0.13	6.33 ^c \pm 0.06	6.32 ^b \pm 0.13	6.65 ^b \pm 0.04	5.91 ^c \pm 0.09
NMS	6.10 ^c \pm 0.13	5.65 ^d \pm 0.06	6.09 ^{bc} \pm 0.13	5.65 ^c \pm 0.04	5.41 ^d \pm 0.09
TSP	6.85 ^b \pm 0.13	6.28 ^c \pm 0.06	5.93 ^c \pm 0.13	5.49 ^d \pm 0.04	5.34 ^d \pm 0.09

¹Colony forming units.²Treatments: INCON = untreated inoculated control, CPC = 0.5% cetylpyridinium chloride, K-L = 3% potassium lactate, NMS = 4 % sodium metasilicate, TSP = 10% trisodium phosphate.^{a-d} Least squares means within a column with different superscripts are different ($P < 0.05$).**Table 3. Effect of antimicrobial treatment by day of display interaction on least squares mean (\pm SE) log CFU¹/g Aerobic Plate counts (APC) of beef steaks.**

Treatment ²	Days of display				
	0	1	2	3	7
INCON	7.91 ^a \pm 0.97	7.92 ^a \pm 0.11	7.89 ^a \pm 0.06	7.54 ^b \pm 0.02	7.89 ^a \pm 0.12
CPC	7.04 ^b \pm 0.97	7.00 ^b \pm 0.11	6.35 ^c \pm 0.06	8.11 ^a \pm 0.02	6.67 ^b \pm 0.12
KL	7.14 ^b \pm 0.97	7.05 ^b \pm 0.11	7.19 ^b \pm 0.06	6.82 ^c \pm 0.02	6.23 ^c \pm 0.12
NMS	6.22 ^c \pm 0.97	6.07 ^c \pm 0.11	6.10 ^d \pm 0.06	6.39 ^d \pm 0.02	6.09 ^c \pm 0.12
TSP	7.17 ^b \pm 0.97	7.17 ^b \pm 0.11	6.02 ^d \pm 0.06	6.35 ^d \pm 0.02	6.04 ^c \pm 0.12

¹Colony forming units.²Treatments: INCON = untreated inoculated control, CPC = 0.5% cetylpyridinium chloride, K-L = 3% potassium lactate, NMS = 4 % sodium metasilicate, TSP = 10% trisodium phosphate.^{a-d} Least squares means within a column with different superscripts are different ($P < 0.05$).**Table 4. Effect of antimicrobial treatment by day of display interaction on least squares mean (\pm SE) log CFU¹/g *Salmonella* counts of beef steaks.**

Treatment ²	Days of display				
	0	1	2	3	7
INCON	7.72 ^a \pm 0.08	7.59 ^a \pm 0.04	7.96 ^a \pm 0.02	7.97 ^a \pm 0.03	6.82 ^a \pm 0.02
CPC	5.85 ^c \pm 0.08	5.97 ^c \pm 0.04	6.04 ^b \pm 0.02	6.81 ^b \pm 0.03	5.82 ^b \pm 0.02
KL	6.50 ^b \pm 0.08	6.43 ^b \pm 0.04	5.87 ^c \pm 0.02	5.88 ^c \pm 0.03	4.53 ^c \pm 0.02
NMS	5.93 ^c \pm 0.08	5.83 ^d \pm 0.04	5.47 ^d \pm 0.02	5.33 ^e \pm 0.03	4.30 ^e \pm 0.02
TSP	6.32 ^b \pm 0.08	6.02 ^c \pm 0.04	5.51 ^d \pm 0.02	5.56 ^d \pm 0.03	4.42 ^d \pm 0.02

¹Colony forming units.²Treatments: INCON = untreated inoculated control, CPC = 0.5% cetylpyridinium chloride, K-L = 3% potassium lactate, NMS = 4 % sodium metasilicate, TSP = 10% trisodium phosphate.^{a-e} Least squares means within a column with different superscripts are different ($P < 0.05$).