

ORIGINAL ARTICLE

Effects of acidified sodium chlorite, cetylpyridinium chloride and hot water on populations of *Listeria monocytogenes* and *Staphylococcus aureus* on beef

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Keywords

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Abstract

Aims: The present study was designed to determine the individual and combined effects of acidified sodium chlorite (ASC, 0.1%, 24 ± 1°C), cetylpyridinium chloride (CPC, 0.5%, 24 ± 1°C) and hot water (HW, 93 ± 1°C) treatments on the survival of *Listeria monocytogenes* and *Staphylococcus aureus*.

Methods and Results: Beef samples inoculated with *L. monocytogenes* and *S. aureus* were treated with nine different applications singly or in combination. Treatment groups comprised (i) untreated control; (ii) sterile tap water; (iii) 0.1% ASC; (iv) 0.5% CPC; (v) HW; (vi) HW followed by 0.1% ASC; (vii) HW followed by 0.5% CPC; (viii) 0.1% ASC followed by HW; (ix) 0.5% CPC followed by HW. Compared with the untreated control group, the reductions in *L. monocytogenes* populations were 1.14–2.31 log CFU g⁻¹, while the reductions in *S. aureus* populations were 0.83–2.74 log CFU g⁻¹ on day 0.

Conclusion: The reduction effect that occurred after combined treatment with ASC followed by HW, HW followed by ASC, CPC followed by HW and HW followed by CPC was found to be significantly greater ($P < 0.05$) than after treatment with ASC and CPC alone on days 0, 2 and 4 of storage.

Significance and Impact of the Study: ASC, CPC and HW treatments can be used to reduce *L. monocytogenes* and *S. aureus*, which would provide an additional measure of safety on the production line.

Introduction

Listeria monocytogenes is a food-borne pathogen of significant public health concern because of its virulence in susceptible individuals; as a consequence, it has received a presidential mandate for reduction to decrease the incidence of food-borne illness (Slutsker and Schuchat 1999). *Listeria monocytogenes* can proliferate under refrigeration temperatures, tolerate freezing and resist moderate heat treatment better than most vegetative bacteria (Doyle 1988). *Listeria monocytogenes* has been frequently isolated from beef processing equipment, red meat and meat products (Wendlandt and Bergann 1994). *Staphylococcus aureus* outbreaks are typically a result of contamination from food handlers and the production of a heat-stable toxin in the

contaminated food (Jay 2000). Dehiding and evisceration are the most important steps in slaughtering as regards the contamination of beef carcasses with pathogens (Vanderlinde *et al.* 1998; Gracey *et al.* 1999; Phillips *et al.* 2001).

The beef industry reduces the likelihood of pathogens on meat using a number of chemical or physical methods, including lactic acid, hot water (HW), steam, trisodium phosphate, acidified sodium chlorite (ASC) and cetylpyridinium chloride (CPC) (Anon 2000; Huffman 2002). One or multiple treatments can be used for the purpose of decontamination, the multiple hurdle concept has been shown to be practical and effective. Researchers reported that the order of application of antimicrobial treatments is important when using multiple intervention technologies (Pohlman *et al.* 2002).

Acidified sodium chlorite, which is a combination of any acid generally recognized as safe (GRAS) and sodium chlorite in aqueous solution, is approved by the US Department of Agriculture and the Food and Drug Administration as a direct food additive for decontamination of poultry and red meat carcasses (Code of Federal Regulations: CFR 1998; Anon 2000). When used in a spray or dip, sodium chlorite concentrations should be between 500 and 1200 ppm with GRAS acid levels high enough to produce a solution pH of 2.3–2.9 (United States Department of Agriculture: USDA 1982). 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, and has also been shown to be an effective microbial control agent on fruit and vegetables. CPC is effective against many pathogens, including *Salmonella*, *L. monocytogenes*, *Campylobacter* and *Escherichia coli* O157:H7 and does not adversely affect the flavour, texture, appearance or odour of foods. Its pH is near neutral and CPC is non-volatile and soluble in water (Anon 2000). HW treatments are commonly used and USDA, Food Safety and Inspection Service (FSIS) have approved the use of HW as antimicrobial treatments for beef carcasses (FSIS 1996). At present, the use of these substances in the European Union is still under discussion and has not yet been approved.

The aim of this study was to determine the effects of ASC, CPC and HW treatments on the reduction of *L. monocytogenes* and *S. aureus* in contaminated beef during refrigerated storage.

Materials and methods

Bacterial inoculum preparation

Listeria monocytogenes (Refik Saydam Laboratory Culture Collection RSKK 472-1/2b, Turkey) and *S. aureus* (Refik Saydam Laboratory Culture Collection RSKK 96090, Turkey) lyophilized stock cultures were transferred to 10-ml volumes of Brain Heart Infusion (BHI) broth and incubated at 37°C for 18–20 h in aerobic conditions. *Listeria monocytogenes* and *S. aureus* were subcultured by loop inoculation of 10-ml volumes of BHI broth and incubated at 37°C for 18–20 h in aerobic conditions. The broth cultures of *L. monocytogenes* and *S. aureus* yielded counts of approx. 6.4×10^8 and 7.2×10^8 CFU ml⁻¹ respectively, and each culture was diluted 10-folds in 90 ml of sterile peptone (0.1%) water to obtain a 1 log reduction from the initial bacterial count.

Sample inoculation

Beef (*m. longissimus dorsi* muscle) was purchased from a local supermarket and transported to the laboratory

under refrigerated conditions within 30 min. The surface of the muscle was aseptically trimmed to minimize the initial numbers of microflora. The trimmed meat was cut into slices of $5 \times 5 \times 0.2$ – 0.3 cm (about 20–25 g) using sterile scissors and scalpel. The samples were immersed for 5 min in a sterile bottle with freshly prepared suspensions of *L. monocytogenes* or *S. aureus* at room temperature. After inoculation, samples were kept in sterile bags at room temperature for 30 min to allow the attachment of bacteria.

Antimicrobial treatment and sample processing

All antimicrobial solutions were made fresh prior to experiments. ASC solution (pH 2.38) was made by mixing sodium chlorite (Merck 8.14815; Merck, Darmstadt, Germany) and citric acid (Merck 242.1000) to a final concentration of 0.1% sodium chlorite and 0.9% citric acid. CPC (Merck 1.02340) was prepared in sterilized distilled water at a concentration of 0.5% (pH 7.10, w/v). The inoculated muscle tissue samples were immersed in solutions of ASC ($24 \pm 1^\circ\text{C}$), CPC ($24 \pm 1^\circ\text{C}$) and HW ($93 \pm 1^\circ\text{C}$) for 15 s. The inoculated samples were divided into nine groups, each containing 10 muscle tissue samples and were treated with nine different applications alone or in combination. Treatment groups were: (i) untreated control; (ii) sterile tap water; (iii) 0.1% ASC; (iv) 0.5% CPC; (v) HW; (vi) HW followed by 0.1% ASC; (vii) HW followed by 0.5% CPC; (viii) 0.1% ASC followed by HW; (ix) 0.5% CPC followed by HW. After treatment, the samples were kept at room temperature for 10 min, and then stored at $4 \pm 1^\circ\text{C}$ in the sterile bottles. All samples were subjected to microbiological analysis and pH measurement on days 0, 2, 4, 6 and 8 of storage at $4 \pm 1^\circ\text{C}$. Day 0 samples were tested immediately after antimicrobial treatment. Antimicrobial treatments and sample processing were carried out for both *L. monocytogenes* and *S. aureus*. Each experiment was repeated three times on different days and a total of 540 meat samples were used.

Microbiological analysis and pH determination

For each treatment, half the muscle tissue samples were used for microbiological analysis and the other half for pH determination. For microbiological analysis, each sample was prepared by excising 10 g of meat with a sterile knife blade. The samples were placed in a stomacher bag containing 90 ml of buffered peptone water (pH 7.0) with 0.1% sodium thiosulfate (for ASC neutralization) and homogenized in a stomacher (Lab-Blender 400; Seward, London, UK) for 2 min. After homogenization, decimal dilutions were prepared from the meat homogenates in buffered peptone water. For *L. monocytogenes*

enumeration, 0.1-ml volumes were surface plated onto PALCAM agar (Merck, 1.11755, supplement Merck 1.12122), while for *S. aureus* enumeration, samples were plated onto BP agar (Baird–Parker agar; Merck 1.05406, supplement Merck 1.03785). All plates were incubated at 35°C for 24–48 h and enumerated by standard methods (Baumgart 1997). For pH analysis, 5 g of meat sample were weighed in a stomacher bag containing 15 ml of sterile deionized water and homogenized in a stomacher for 2 min (Capita *et al.* 2002). The pH of the samples was measured using an electronic pH meter (Inlab 427; Mettler Toledo, Urdorf, Switzerland).

Statistical analysis

All bacterial counts were converted to \log_{10} CFU g^{-1} values and data were analysed using analysis of variance (GLM) by the SPSS 10.0 (Ref. No: 651544; SPSS Inc., Chicago, IL, USA) statistical package programme.

Results

The effects of antimicrobial treatments in *L. monocytogenes* populations are summarized in Table 1. Compared with the control (untreated) group, ASC, CPC and HW treatments resulted in 1.14, 1.50 and 2.06 log reductions in *L. monocytogenes* respectively on day 0 of storage. HW followed by ASC caused a 1.99 log reduction, HW followed by CPC a 2.31 log reduction, ASC followed by HW a 1.87 log reduction and CPC followed by HW a 2.31 log reduction in *L. monocytogenes* on day 0 of storage. No reduction ($P > 0.05$) in *L. monocytogenes* was observed in the group treated with tap water from immediately after the treatment (day 0) until the end of storage period. The effect of ASC on *L. monocytogenes* increased on days 2 and 4 of the storage period. The reduction effect occur-

ring after combined treatment with ASC followed by HW and CPC followed by HW was found to be generally higher ($P < 0.05$) than treatment with ASC and CPC alone on days 0, 2 and 4 of storage. These results emphasize the importance of multiple treatments on decontamination.

Mean counts of *S. aureus* populations on control and treated samples during storage are shown in Table 2. The microbiological analyses on day 0 of storage showed that compared with the control group, *S. aureus* reductions were 0.05 log for tap water, 0.83 log for ASC alone, 2.43 log for CPC alone, 2.48 log for HW alone, 2.74 log for HW followed by ASC, 2.50 log for HW followed by CPC, 1.76 log for ASC followed by HW and 2.40 log for CPC followed by HW.

The maximum reductions in *S. aureus* levels obtained on day 0 of storage were thus produced by HW followed by ASC, HW followed by CPC, CPC followed by HW and ASC followed by HW treatment groups in that order. In addition, ASC treatments alone resulted in lower reductions on *S. aureus* than HW followed by ASC and ASC followed by HW treatment groups on day 0 of storage. However, the reduction effect of ASC increased during the storage period of the study and a maximum reduction of 1.50 log CFU g^{-1} was observed by day 8.

Discussion

Compared with day 0 of storage, *L. monocytogenes* populations increased in all groups during refrigerated storage, perhaps because of the psychrotrophic nature of microorganisms. This property of the pathogenic agent, as indicated by Capita *et al.* (2002) and Ikeda *et al.* (2003) emphasizes the importance of *L. monocytogenes* contaminated food in food-borne infections. Lim and Mustapha (2004) reported that 0.5% CPC treatment of fresh beef

Table 1 Effect of antimicrobial treatments on the numbers (\log_{10} CFU g^{-1}) of *Listeria monocytogenes* on beef stored at 4°C for 0, 2, 4, 6 and 8 days

Groups	Treatment	Days of storage (at 4°C)				
		0	2	4	6	8
1	Untreated (control)	6.69 ± 0.09 ^e	7.62 ± 0.07 ^d	7.87 ± 0.03 ^e	8.45 ± 0.15 ^e	8.74 ± 0.04 ^d
2	Tap water	6.53 ± 0.06 ^e	7.61 ± 0.08 ^d	7.84 ± 0.06 ^e	8.58 ± 0.11 ^e	8.75 ± 0.04 ^d
3	0.1% ASC	5.55 ± 0.05 ^d	5.38 ± 0.08 ^b	5.81 ± 0.03 ^c	6.66 ± 0.04 ^b	7.27 ± 0.20 ^b
4	0.5% CPC	5.19 ± 0.19 ^c	5.74 ± 0.04 ^c	5.87 ± 0.03 ^c	6.82 ± 0.02 ^{bc}	7.65 ± 0.05 ^c
5	HW	4.63 ± 0.16 ^{ab}	5.38 ± 0.08 ^b	6.81 ± 0.02 ^d	7.38 ± 0.08 ^d	7.65 ± 0.05 ^c
6	HW followed by 0.1% ASC	4.70 ± 0.08 ^{ab}	4.69 ± 0.09 ^a	4.77 ± 0.07 ^a	5.84 ± 0.06 ^a	6.73 ± 0.11 ^a
7	HW followed by 0.5% CPC	4.38 ± 0.08 ^a	4.65 ± 0.06 ^a	4.84 ± 0.06 ^a	5.84 ± 0.06 ^a	6.58 ± 0.12 ^a
8	0.1% ASC followed by HW	4.82 ± 0.12 ^b	4.84 ± 0.06 ^a	6.69 ± 0.09 ^d	7.15 ± 0.15 ^{cd}	7.53 ± 0.06 ^{bc}
9	0.5% CPC followed by HW	4.38 ± 0.08 ^a	4.82 ± 0.04 ^a	5.53 ± 0.06 ^b	7.33 ± 0.26 ^d	7.74 ± 0.04 ^c

Results are reported as a mean ($n = 3$ replicates). ASC, acidified sodium chlorite; CPC, cetylpyridinium chloride; HW, hot water.

^{a–e}Different letters within same column indicate significant difference ($P < 0.05$).

Table 2 Effect of antimicrobial treatments on the numbers (\log_{10} CFU g^{-1}) of *Staphylococcus aureus* on beef stored at 4°C for 0, 2, 4, 6 and 8 days

Groups	Treatment	Days of storage (at 4°C)				
		0	2	4	6	8
1	Untreated (control)	7.10 ± 0.03 ^e	7.09 ± 0.02 ^f	7.17 ± 0.03 ^f	7.25 ± 0.05 ^e	7.31 ± 0.06 ^d
2	Tap water	7.05 ± 0.01 ^e	7.28 ± 0.01 ^f	7.15 ± 0.01 ^f	7.18 ± 0.11 ^e	7.37 ± 0.23 ^d
3	0.1% ASC	6.27 ± 0.02 ^d	6.45 ± 0.15 ^e	5.69 ± 0.09 ^e	5.84 ± 0.05 ^d	5.81 ± 0.03 ^c
4	0.5% CPC	4.67 ± 0.07 ^b	4.77 ± 0.07 ^{cd}	4.64 ± 0.17 ^c	4.67 ± 0.05 ^b	4.95 ± 0.11 ^b
5	HW	4.62 ± 0.15 ^b	3.81 ± 0.03 ^a	3.82 ± 0.02 ^a	4.18 ± 0.11 ^a	4.46 ± 0.16 ^a
6	HW followed by 0.1% ASC	4.36 ± 0.02 ^a	4.48 ± 0.18 ^{bc}	4.69 ± 0.09 ^c	4.27 ± 0.02 ^a	4.51 ± 0.04 ^a
7	HW followed by 0.5% CPC	4.60 ± 0.05 ^b	4.59 ± 0.12 ^{bc}	4.74 ± 0.10 ^c	4.75 ± 0.02 ^b	4.49 ± 0.15 ^a
8	0.1% ASC followed by HW	5.34 ± 0.04 ^c	4.98 ± 0.08 ^d	5.22 ± 0.08 ^d	5.22 ± 0.08 ^c	5.65 ± 0.06 ^c
9	0.5% CPC followed by HW	4.70 ± 0.02 ^b	4.65 ± 0.08 ^b	4.48 ± 0.08 ^b	5.03 ± 0.04 ^c	5.84 ± 0.06 ^c

Results are reported as a mean ($n = 3$ replicates). ASC, acidified sodium chlorite; CPC, cetylpyridinium chloride; HW, hot water.

^{a-d}Different letters within same column indicate significant difference ($P < 0.05$).

reduced *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* by 2.78, 2.90 and 4.01 \log CFU cm^{-2} respectively on day 14 of storage. In the present study, maximum reduction levels were achieved by 0.5% CPC treatments, which reduced *L. monocytogenes* and *S. aureus* by 2.0 and 2.58 \log respectively, although these reductions were not as large as those reported by Lim and Mustapha (2004). This difference may be due to the different decontamination treatments and sampling methods used. Cutter *et al.* (2000) reported that 1% CPC reduced *E. coli* O157:H7 by 5–6 \log and *Salm.* Typhimurium to virtually undetectable levels (0 \log_{10} CFU cm^{-2}), immediately after treatment and that this effect persisted, after 35 days of refrigerated (4°C), vacuum-packaged storage.

However Breen *et al.* (1997) reported that CPC caused a 4.87 \log reduction in *Salm.* Typhimurium from poultry tissues at a concentration of 4 mg ml^{-1} for 3 min. In our study, 0.5% CPC treatment resulted in 1.09 and 2.36 \log reductions in *L. monocytogenes* and *S. aureus* respectively on day 8 of storage. These reduction levels are lower than those reported by Breen *et al.* (1997) and Cutter *et al.* (2000). The difference may be due to the different CPC concentration and different treatment and sampling methods used. Kim and Slavik (1996) investigated the mechanism of how CPC reduced the number of *Salmonella* on chicken skins via scanning electronic microscopy. These authors noted that although CPC did not detach cells from chicken skin, the treatment might cause metabolic malfunctions resulting from morphological and structural damage to *Salmonella* cells because indentations were observed on cells treated with 0.1% CPC solutions for 5 min.

Our results are similar to those obtained by Lim and Mustapha (2004) who treated fresh beef samples with 0.12% ASC and reported 1.81 \log CFU cm^{-2} reduction in *L. monocytogenes* by day 14 of storage. The reduction

effects of ASC on *S. aureus* on days 0 and 6 of storage reported by these authors are also in agreement with those in our study. Castillo *et al.* (1999) reported that phosphoric acid activated acidified sodium chloride and citric acid-activated acidified sodium chloride spray treatment on beef carcass surfaces resulted in 3.8–3.9 and 4.5–4.6 \log reductions in *E. coli* O157:H7 and *Salm.* Typhimurium, respectively. Likewise reduction effects of ASC on *E. coli* O157:H7, *S. aureus*, aerobic bacteria and coliform groups were also reported by Gill and Badoni (2004) and Hajmeer *et al.* (2004).

In addition, CPC treatment alone resulted in greater reductions on *Staph. aureus* population than ASC treatment alone. Furthermore, the same reduction effect was observed on *L. monocytogenes* population on day 0. This difference may be due to unneutralization of CPC treated samples.

Our research using HW (93 ± 1°C, 15 s) treatments reduced *L. monocytogenes* and *S. aureus* by 2.06 and 2.48 \log respectively on day 0 of storage. These results are similar to the results obtained by Castillo *et al.* (1998), who treated beef carcasses with HW spray (95°C) and reported 3.7–3.8 \log reductions in *E. coli* O157:H7 and *Salm.* Typhimurium, respectively. However, Ellebracht *et al.* (1999) studied the effects of using HW and lactic acid to reduce pathogens on beef trimmings prior to grinding. These authors reported that HW treatment of 95°C for 3 s reduced *E. coli* O157:H7 and *Salm.* Typhimurium by 0.5 and 0.7 \log CFU g^{-1} , respectively.

The reduction levels of *L. monocytogenes* and *Staph. aureus* on day 2, 4, 6 and 8 were greater ($P < 0.05$) in groups treated with HW followed by ASC and HW followed by CPC, than the groups treated with ASC followed by HW and CPC followed by HW. Pohlman *et al.* (2002) supported that the treatment order is the most important factor during multiple decontamination

applications. Likewise, Gorman *et al.* (1995) reported the loss of activity of antimicrobial agents when followed by plain water spray washing, possibly due to the physical removal or dilution of the sanitizing agents.

No significant differences were observed between the samples contaminated with *L. monocytogenes* and *S. aureus* in terms of the pH value during storage (data not shown). The pH values of samples were affected by the pH values of treatment solutions. pH values were determined for the untreated (control) groups (5.43–6.70), tap water (5.48–6.48), ASC (5.25–5.42), CPC (5.58–5.78), HW (5.58–5.95), HW followed by ASC (5.35–5.60), HW followed by CPC (5.54–5.68), ASC followed by HW (5.38–5.64) and CPC followed by HW (5.52–5.65) groups. As expected, ASC treatments lowered meat pH values more than the other treatments. This may be due to the presence of citric acid used for the acidification of sodium chlorite. The pH value of CPC solution used in this study was found to be 7.10, which is also similar to Lim and Mustapha's (2004) results.

The results of this study indicated that ASC, CPC and HW treatments on beef showed significant reduction effects on *L. monocytogenes* and *S. aureus*. The reduction effect that occurred after combined treatment with ASC followed by HW, HW followed by ASC, CPC followed by HW and HW followed by CPC was found to be significantly greater ($P < 0.05$) than that after treatment with ASC and CPC alone on days 0, 2 and 4 of storage. Higher reduction levels were observed in groups previously treated with HW (treatments 6 and 7) than in groups to which HW treatments were applied secondarily (treatments 8 and 9). As a result, antimicrobials tested here are applicable to beef as an additional measure in production line safety. Such antimicrobials do not pose a hazard for public health safety and do not cause changes in physical or organoleptic characteristics of the product.

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