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Short Communication

Inhibition of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* on sliced roast beef by cetylpyridinium chloride and acidified sodium chlorite

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Abstract

The effects of 0.5% cetylpyridinium chloride (CPC), 0.12% acidified sodium chlorite (ASC) and a mix of equal volume of the two (0.25% CPC-0.06% ASC) on *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* were evaluated on inoculated sliced roast beef. The antimicrobial agents were, respectively, sprayed on the beef surfaces and tray absorbent pads, and samples were stored at 4 °C for 10 days (d). At 0 d, *L. monocytogenes* and *S. aureus* were reduced to undetectable levels in 2 h after spraying with CPC. CPC–ASC treatment reduced *E. coli* O157:H7, *L. monocytogenes* and *S. aureus* by 4.07, 6.37 and 4.32 log cfu/cm^2, respectively, at 0 d. ASC treatment reduced the population of *E. coli* O157:H7 by 6.09 log cfu/cm^2 at 10 d. CPC treatment caused a slight discoloration and ASC-treated beef surfaces demonstrated the lowest redness and highest lightness. The grey colour and off-odour were significant in the ASC-treated beef samples, while CPC-treated samples demonstrated less off-odor and brown colour from 0 to 4 d. Based on our results, it appears that the application of CPC on sliced roast beef can extend the shelf-life of the product without impairing its quality.

Keywords: Cetylpyridinium chloride; Acidified sodium chlorite; *Escherichia coli* O157:H7; *Listeria monocytogenes*; *Staphylococcus aureus*; Sliced roast beef

1. Introduction

Ready-to-eat (RTE) meats have a potential to become contaminated with pathogens due to the multiple handlings involved in their manufacture, coupled with the possibility of post-processing contamination (Anderson et al., 1989; Bryan, 1990).

Acidified sodium chlorite (ASC) is an FDA-approved antimicrobial food additive for poultry and red meat. The way ASC works is by converting chlorite ions into chlorous acid that inhibits cellular protein synthesis (Schmidt et al., 1984) by attacking sulphide and disulphide linkages and making non-specific attacks on the amino acid components of bacterial proteins (Kross, 1984). Another antimicrobial that is FDA-approved for decontaminating raw poultry is cetylpyridinium chloride (CPC) (Anonymous, 2004). The CPC mode of action is based on electrostatic interactions. Negative charges of bacterial cell wall constituents favor the binding of the positively charged cetyl radicals of CPC, thus allowing CPC to permeate and destroy cell walls and cell membranes sequentially (Huyck, 1944; Kourai et al., 1985).

The use of an antimicrobial-containing tray absorbent pad that is in direct contact with the underside of a food, combined with a treated film overwrap that has contact with the top surface of the food, may help to increase product shelf-life and reduce the potential for foodborne illness from RTE food consumption (Natrajan and Sheldon, 2000). Thus, the objective of this study was to determine the effects of spraying CPC, ASC and an equal volume mixture of the two on sliced roast beef and tray absorbent pads at inhibiting *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* growth.

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2. Materials and methods

2.1. Bacterial strains and microbial inocula

Fresh overnight cultures (18 h at 37 °C) of *E. coli* O157:H7 505B, *L. monocytogenes* Scott A and *S. aureus* FR1 (Food Science Program, University of Missouri, Columbia, Missouri, USA) were individually grown in tryptic soy broth supplemented with 0.1% yeast extract (TSBYE, Difco Labs, BD Diagnostic Systems, Sparks, Maryland, USA) at 37 °C for 9 h. Cells were pelleted by centrifugation at 10,000*g* for 10 min, washed twice in 0.1% peptone-water (w/v) and the pellets resuspended in sterile 0.1% peptone-water. The cell suspension was used as the beef inocula to obtain an initial viable cell density of approximately 7 log cfu/ml.

2.2. Preparation of antimicrobial solutions

CPC (ICN Biomedicals Inc., Aurora, Ohio, USA) was prepared in sterilized distilled water at a concentration of 0.5% (w/v). ASC was made by mixing sodium chlorite (Alfa Aesar, Ward Hill, Massachusetts, USA) and citric acid (Acros Organics, Geel, Belgium) to a final concentration of 0.12% and 0.9%, respectively. CPC–ASC was prepared by mixing an equal volume of 0.5% CPC and 0.12% ASC. In addition to an untreated control, sterile distilled water was also used as a positive control.

2.3. Preparation of sliced roast beef

Less than 1-d old freshly thin sliced deli-style roast beef was purchased from a local grocery store. For pH, colour and microbial population measurements, a $6 \times 6\,cm^2$, 0.2 mm thick piece, was aseptically cut out from each slice. Using a sterile glass spreader, a 0.2-ml peptone-water suspension containing approximately 7 log cfu/ml of *E. coli* O157:H7, *L. monocytogenes* or *S. aureus* was spread on the top surface of each beef square. The beef squares were air-dried for 5 min to allow for bacterial attachment. Using a hand-held, sterilized plastic sprayer, the beef squares were then sprayed with 2.5 ml of one of the three antimicrobial formulations so that each pathogen was exposed to each antimicrobial treatment, respectively, and air-dried for 5 min. Then, two pieces of beef squares (one piece for pH and colour, the other for microbial counts) were foam tray-packed and over-wrapped with polyvinyl chloride (PVC) film. Six trays for each treatment were prepared for 0, 2, 4, 6, 8, and 10 d analyses (total 90 trays = 5 treatments × 3 pathogens × 6 sampling days). Trays were sorted by treatment and stacked one on top of another under fluorescent lighting for 10 d at 4 °C. At each sampling time, one tray was removed from the top of each stack for pH, colour and microbial population measurements.

For sensory analysis, beef slices were not cut or inoculated. Five millilitres of a single antimicrobial solution were sprayed on the top of one whole piece (approximately 26–30 g) of sliced roast beef surface. Tray foams that contained an absorbent tray pad sprayed with a single antimicrobial solution and one piece of sliced roast beef were overwrapped with PVC film. Then, samples were sorted by treatment and stored by stacking (total 165 trays = 5 treatments × 11 panellists × 3 sampling days) for 4 d at 4 °C.

2.4. Preparation of antimicrobial packaging materials

The top sides of Dri-Loc® AC-50 absorbent pads (10 × 18 cm size, 50–55 g absorbency, Cryovac, Saddle Brook, New Jersey, USA) were sprayed with 5 ml of a single antimicrobial solution and placed in a laminar flow hood for 5 min. Prepared beef slices were placed on the treated pads in foam tray packs (W2S, 20.32 × 14.61 × 1.74 cm, Cryovac, Saddle Brook, New Jersey, USA). The trays were over-wrapped with PVC film on a heat-sealing station (Model HS 625A, Cleveland, Ohio, USA). The PVC film (ClearView™ stretch wrap film, Hobart, Troy, Ohio, USA; O_2 transmission rate of 236.22 cc/100 cm^2/24 h@1 atm, 23 °C, 0% relative humidity (RH) and a moisture transmission rate of 1.57 g/100 cm^2/24 h@90% RH) was aseptically cut into 38 cm pieces by the heat-sealing station. Another set of inoculated and water-sprayed roast beef slices, as controls, were similarly wrapped after placing the samples on water-treated tray pads in the foam tray packs. Untreated controls were prepared with inoculated roast beef slices, which were not sprayed with any antimicrobial agent and which were placed on untreated tray pads.

2.5. pH determination and microbial enumeration

pH values were determined from the top spray-treated surfaces of each roast beef square. Two areas on each roast beef surface were examined using a surface pH probe and the numbers averaged. A beef square in 20 ml of 0.1% sterile peptone-water (pH 7.0) was homogenized for 2 min. Homogenates were diluted in peptone-water and plated on MacConkey Sorbitol agar for *E. coli* O157:H7 enumeration, Modified Oxford agar for *L. monocytogenes* enumeration and Baird-Parker agar for *S. aureus* enumeration. A baseline total aerobic count (APC) was also obtained by plating, prior to inoculating with respective pathogens, on Plate Count agar. All agar media were procured from Difco Labs. and all plates were aerobically incubated at 37 °C for 2–3 d.

2.6. Instrumental colour evaluation

The colour of the roast beef squares was measured using a Hunter D25L Optical Sensor (Hunter Associates Lab., Reston, Virginia, USA) standardized with black and white tiles. Hunter colour criteria for lightness ('*L*'), redness ('*a*') and yellowness ('*b*') values were recorded through the PVC