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Effects of spray volume, type of surface tissue and inoculum level on the survival of *Escherichia coli* on beef sprayed with 5% lactic acid

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ABSTRACT

Membrane, fat and cut muscle surfaces of beef were inoculated with *Escherichia coli* at numbers about 4, 1 or −1 log cfu/cm^2. The inoculated meat was sprayed with water or 5% lactic acid at volumes of 0.5, 0.1 or 0.02 ml/cm^2. Spraying with water reduced the numbers of *E. coli* on membrane surfaces by up to 1 log unit, but had little effect on the numbers of *E. coli* on fat or cut muscle surfaces. Spraying with 5% lactic acid reduced the highest numbers of *E. coli* on membrane surfaces by up to 4 log units; but those numbers on fat or cut muscle surfaces were reduced by ≤1.5 log unit, and the reductions declined with decreasing volumes of 5% lactic acid. With inocula of 1 log cfu/cm^2, spraying lactic acid in any volume reduced the numbers of *E. coli* on membrane or fat surfaces by about 1 log unit, and the numbers on cut muscle surfaces by between 0.8 and 0.2 log unit. *E. coli* were detected in enrichment cultures of samples from all surfaces inoculated with *E. coli* at −1 log cfu/cm^2 and sprayed with 5% lactic acid at 0.5 ml/cm^2. The findings indicate that spraying relatively heavily contaminated cuts or trimmings with 5% lactic acid at ≥0.1 ml/cm^2 can be expected to reduce numbers of *E. coli* and, presumably, associated pathogens by between 0.5 and 1 log unit. However, such a treatment is likely to be at best marginally effective for reduce the numbers of these organisms on lightly contaminated product.

1. Introduction

Carcasses at many North American beef packing plants are sprayed with solutions of lactic acid as a decontaminating treatment (Gill, 2009). Lactic acid sprays may also be applied to beef cuts and trimmings, to control contamination with pathogens, particularly *Escherichia coli* O157:H7 (Harris, Miller, Loneragan, & Brashears, 2006; Heller et al., 2007). The effects of lactic acid solutions on *E. coli* O157:H7 and/or *Salmonella* inoculated at high numbers on portions of beef carcass surface have been extensively studied. The general findings of such studies have been that the numbers of *E. coli* O157:H7 are substantially reduced by spraying inoculated tissues with or dipping them in solutions of lactic acid at concentrations between 2% and 5% (Huffman, 2002; King et al., 2005). Even so, in some studies with inoculated steaks or smaller portions of beef the reductions in numbers of *E. coli* O157:H7 obtained by spraying with lactic acid solutions were small (Echeverry et al., 2009). Moreover, in studies of the effects of lactic acid solutions applied to beef carcasses or cuts in commercial practice the treatments were found to have little decontaminating effect (Bacon, Sofos, Belk, & Smith, 2002; Gill & Landers, 2003).

The disparate findings suggest that the decontaminating effects of lactic acid sprays used on beef are modified by a number of factors. These could include the lactic acid concentration, the nature of the treated surface, the volume of solution applied to the surface, and the degree of contamination. Some studies have shown that decontamination is enhanced by concentrations of lactic acid > 2% in the applied solution (Castillo et al., 2001; Stopforth et al., 2005), as might be expected. However, no such effect was evident in other studies (Heller et al., 2007), and the effects of the other factors do not appear to have been the subject of systematic study. Therefore, the effects of solution volume, type of surface and inoculation level on the survival of *E. coli* on beef was investigated. The work was carried out using a strain of generic *E. coli* as the study was intended for investigation of the relative effects of the various factors rather than to determine the responses of a specific pathogen. Pieces of meat were tumbled together when they were inoculated, to simulate the handling of meat during cut preparation; and to ensure that the inoculated bacteria were distributed approximately log normally over meat surfaces (Gill, 2006). A solution of 5% lactic acid was applied to inoculated beef surfaces using equipment designed to apply controlled volumes of solution evenly over meat surfaces.

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Treatment with 5% lactic acid was adopted because that is now the maximum concentration of lactic acid permitted in solutions used for the decontamination of raw meats at North American plants (U.S. Department of Agriculture, 2008).

2. Materials and methods

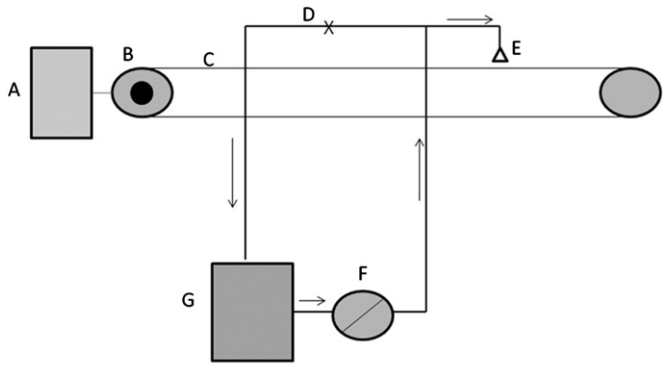
2.1. Equipment used for spraying meat

The equipment used for spraying meat consisted of a stainless steel wire mesh conveyor belt, driven by a variable speed DC motor (Leeson Electric Corp., Grafton, MI, USA) connected to a speed controller (Penta-Drive Model NEMA-4X/IP 65; KB Electronics Inc., FL, USA) that carried the meat through a fan spray that delivered fluid across the width of the belt (Fig. 1). The line to the spray nozzle (Maxx 1/8 KSJB.50; John Brooks Company Ltd., Winnipeg, Manitoba, Canada) was connected to a line of plastic tubing (MasterFlex Tygon tubing; Cole-Parmer, Vernon Hills, IL, USA) which ran from a fluid reservoir through a peristaltic pump head (Easy-Load Model No. 7529-10; Cole-Parmer) driven at 1725 rpm by a 372 W, constant speed motor (Model M6C17FC1F; Leeson Electric Corp.). A line fitted with an adjustable ball valve (Model RC4-1000WOG; Green Line Hose & Fittings, Delta, BC, Canada) returned fluid from the spray nozzle line to the reservoir. The fluid pressure at the spray nozzle was varied as required by adjustment of the valve.

The meat to be treated was packed in a layer 1 cm deep, into plastic trays with internal measurements of $23 \times 15 \times 1.5$ cm. The height of the spray head above the meat surfaces was fixed at 10 cm. The volume of fluid sprayed onto meat surfaces was controlled by adjustment of the valve in the line to the spray nozzle and the speed of the conveyor belt. The valve and speed settings used for the required volumes were determined by running empty trays through the spray operated with water, and weighing the water collected in trays after drying of the outer surfaces.

2.2. Preparation of inoculated meat

Beef for inoculation was obtained from chilled carcasses at a local, federally inspected abattoir. Portions of meat were cut into slices 1 cm thick and of a size to fit within a tray. Slices with surfaces of cut muscle, fat with intact fascia, and membrane overlying muscle tissue were prepared from sirloin tip primal cuts, the outer parts of briskets and rounds, and the medial surfaces of flanks, respectively.



A Speed control for motor.
B Variable speed motor.
C Open mesh conveyer belt.
D Adjustable ball valve connected to 3.1 mm ID tubing.
E Fan spray nozzle; delivers spray across the belt.
F Constant speed pump connected to 6.2 mm ID tubing.
G Reservoir.

Fig. 1. Schematic diagram of the equipment used for spraying meat slices with controlled volumes of water or 5% lactic acid. A: Speed control for motor. B: Variable speed motor. C: Open mesh conveyer belt. D: Adjustable ball valve connected to 3.1 mm ID tubing. E: Fan spray nozzle; delivers spray across the belt. F: Constant speed pump connected to 6.2 mm ID tubing. G: Reservoir.

Four slices of meat with one or both surface of a type that was to be treated were inoculated as a batch. The meat was inoculated with a suspension of a strain of *E. coli* isolated from a beef packing plant. Each suspension was prepared by growing the organism overnight at 35 °C, to the stationary phase, in half strength Brain Heart Infusion (BHI; Oxoid, Mississauga, Ontario, Canada), and diluting the culture with 0.1% (w/v) peptone water (Difco; Becton Dickinson, Sparks, MD, USA) to numbers of about 7, 4 or 1 log cfu/ml. Each slice of meat was inoculated on each major surface with 2 ml of the suspension as the meat was placed in a plastic bag. The slices of meat in the bag were tumbled together for 3 min to distribute the inoculated bacteria over all surfaces of the meat, at final numbers of about 4, 1 or −1 log cfu/cm^2. The meat was then loaded into four trays with all the exposed surfaces being of a single tissue type.

2.3. Treatment of inoculated meat

For treatment of the inoculated meat from each batch, the rate at which fluid was delivered to the spray head and the speed of the conveyor belt were adjusted to deliver fluid onto meat surfaces at volumes of 0.5, 0.1 or 0.02 ml of fluid per cm^2 of meat surface. The reservoir of the spraying equipment was filled with water; then one of the four trays filled with meat and an empty tray were passed through the spray. The tray that had been empty was weighed, after drying the outside, to verify that fluid was delivered in the intended volume. The water was then replaced with a 5% (w/v) solution of L + lactic acid (Sigma–Aldrich, Mississauga, Ontario, Canada) and two trays of meat were passed through the spray. The remaining tray of meat was not treated. The times between inoculating of meat and spraying with water or 5% lactic acid were between 15 and 20 min and between 30 and 40 min, respectively.

2.4. Enumeration of E. coli on meat

Trays containing meat treated with water or lactic acid solution were held for 15 min before samples were removed from the meat. The meat in each tray containing meat that had been inoculated with *E. coli* at about 4 log cfu/cm^2 was sampled by cutting five strips of meat, each of which measured 5×2 cm and were 0.2 cm thick, from the exposed meat surface. The strips were cut from each corner and from the centre of each slice.

Each strip was placed in a separate stomacher bag with 10 ml of 0.1 M phosphate buffer, pH 7, and the bag was pummelled for 2 min in a stomacher. Two 1 ml portions of the stomacher fluid were used to prepare two series of ten-fold dilutions to 10^{-4} or 10^{-2}, and the whole 9 or 10 ml of each dilution was each filtered through a hydrophobic grid membrane filter (Oxoid). One filter used for each dilution was placed on a plate of lactose monensin glucuronate agar (LMG; Acumedia, Lansing, MI, USA). The other filter used for each dilution was placed on a plate of LMG supplemented with bile salts (Sigma–Aldrich) at 1.5 g/l (LMGB).

The meat in each tray containing meat that had been inoculated with *E. coli* at 1 log cfu/cm^2 was sampled in the same manner as meat inoculated with *E. coli* at 4 log cfu/cm^2. However, only one series of ten-fold dilutions was prepared from each strip of meat; the remaining undiluted stomacher fluid as well as each dilution was filtered through a hydrophobic grid membrane filter; and all filters were placed on plates of LMG.

All LMG and LMGB plates were incubated at 35 °C for 24 h. The filters were then transferred to plates of buffered 4-methyl-umbelliferyl-β-D-glucuronide agar (BMA; Acumedia), which were