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Full Length Research Paper

Effectiveness of trisodium phosphate, lactic acid, and acetic acid in reduction of *E .coli* and microbial load on chicken surfaces

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Procedures for dipping of chicken carcasses in trisodium phosphate (TSP), lactic acid (LA) and acetic acid (AA) were evaluated to determine their effectiveness for reducing *E. coli* NCTC 10538 and aerobic total counts on the chicken meat surfaces. Chicken portions were dipped in a suspension of *E. coli* (7 log CFU/ml) for 20 s and kept for 90 min to allow *E. coli* to get attached to the chicken surface. The chicken portions were then dipped in 8, 10, and 12% concentration of TSP, 1, 2, and 3% concentration of LA, and 0.5, 1, and 1.5% concentration of AA for 20 s each followed by dipping in tap water for 20 s. A sterile template 4 x 4 cm was placed on the chicken surface and then swabbed by swab cotton. The number of *E. coli* and aerobic total counts were enumerated. The reduction of *E. coli* on chicken meat surfaces dipped in 8, 10 and 12% of TSP decreased *E. coli* by 0.5, 1.2 and 1.6 log CFU/ cm^2, respectively. The reduction of *E. coli* on chicken meat surfaces dipped in 1, 2 and 3% LA was 0.5, 1.8 and 2.1 log CFU/cm^2 respectively. The reduction of *E. coli* on chicken meat surface dipped in 0.5, 1.0 and 1.5% of AA had decreased the *E. coli* counts of 0.7, 1.1 and 1.4 log CFU/ cm^2, respectively. The results showed that LA was more effective against *E. coli* and aerobic total counts than TSP and AA.

Key words: Trisodium phosphate, Lactic acid, Acetic acid, *E. coli*, Aerobic count, Quality, Chicken, Poultry.

INTRODUCTION

Microbial loads in meat depend on the way the animal is slaughtered and eviscerated and the way by which the meat is generally handled and stored in terms of time and temperature. They also depend on hygienic conditions in the slaughterhouses (Brown and Baird-Parker, 1982). Shelf lives of fresh red meat, poultry, fish, and milk are limited due to the growth of meat spoiling micro-organisms, which cause the off-odours and slime. The spoilage of refrigerated chicken is due to microbial growth and metabolic activities of bacteria (Jackson et al., 1997). Psychrotrophic bacteria on chicken carcasses generally consist of *Flavobacterium* spp., *Shewanella putrefaciens*, *Acintebacter* spp. and *Pseudomonas* spp. *Pseudomonas* spp. is most abundant in fresh and spoiled chicken (Arnaut-Rollier et al., 1999), and 80% of the total microbial flora on chicken carcasses was found to consist of *Pseudomonas* spp. and *B. thermosphancta* (Gallo et al., 1988)

Chemicals, salt, low temperature, heat, and irradiation processes have been used to eliminate, inhibit or reduce pathogens in food and food products. The inhibition activities of antimicrobials are due to the undissociated form of the molecules (Eklund, 1983 and 1985). Antimicrobials such as trisodium phosphate (TSP), lactic acid (LA), and acetic acid (AA) have been used as food preservatives and are generally recognised as safe (GRAS) (Branen et al., 1990). Lactic and acetic acids are commonly used in the beef meat industry to reduce the microbial loads in the carcasses (Berry and Cutter, 2000).

TSP has been used as an effective sanitizer in controlling the growth of *Salmonella* spp. and *E. coli* (Giese, 1992), *Staphylococcus aureus* (Lee et al., 1999), *Camplybacter*, *Listeria monocytogenes* and psychrotrophs (Slavic et al., 1994). However, it is less effective against total aerobic microorganisms (Lillard, 1994) and may cause brown discoloration of meat when used in high concentrations (Bhide et al., 2001). Several investigators have evaluated the effects of TSP against pathogens attached to chicken carcasses. Their results have indicated that dipping or spraying the fresh chicken with TSP

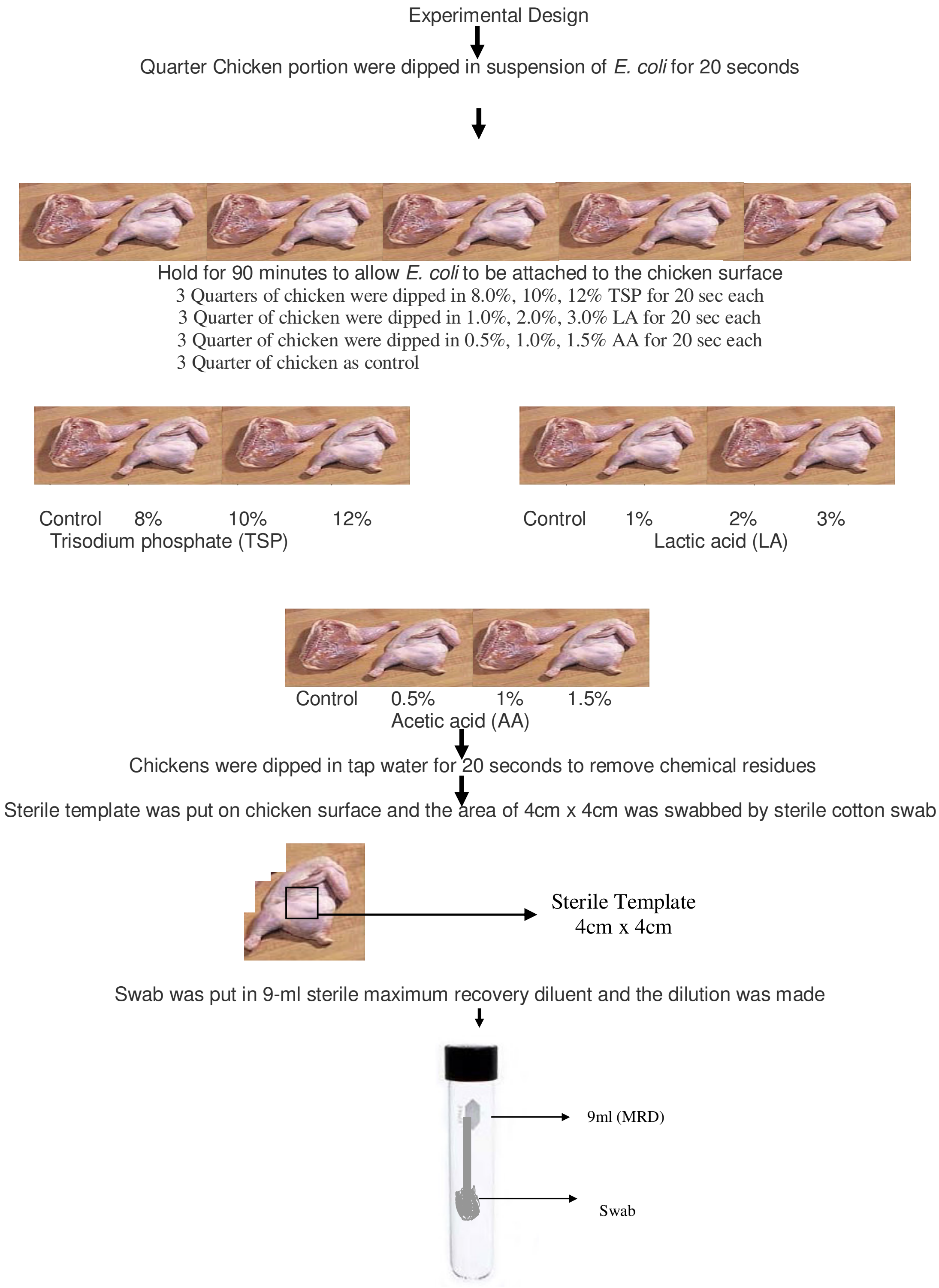

Figure 1. Illustration of chicken treatments with TSP, LA, and AA.

concentrations reduced *Salmonella* and *Listeria monocytogenes* on chicken carcasses (Hwang and Beuchat, 1995). TSP was also found to be very active against gram-negative bacteria such as *E. coli*, *Salmonella, Campylobacter,* coliforms, and pseudomonas (Fratamico et al., 1996). Ramirez et al. (2001) carried out a sensory evaluation of poultry meat dipped in 8 to 12% concentration of TSP for 30 s and the results indicated that the sensory quality was not affected by TSP treatment.

Lactic acid is an antimicrobial agent used to eliminate pathogens and reduce the microbial loads in meat carcasses. The advantage of using lactic acid is that it does not require the use of high pressure spraying to eliminate the pathogens from meat carcasses. In addition, it remains on the meat carcasses, and works as antimicrobial agent on carcass surfaces (Ramirez et al., 2001).

Acetic acid has been used to reduce or inhibit the bacterial and yeast growth in food or food products. It is also used directly in the pickling of vegetables and other foodstuffs. Acetic acid is generally recognized as safe (GRAS) as a food ingredient (Anderson et al., 1980). Bell et al. (1986) found that acetic acid reduced the aerobic total counts in raw meat. When chicken carcasses were washed with 0.5% acetic acid, total counts were reduced by 0.76 log CFU/ cm^2 (Sakhare et al., 1999).

The main objective of the present study was to evaluate and compare the effectiveness of trisodium phosphate, lactic acid, and acetic acid treatment in reducing *E. coli* and aerobic bacteria load on chicken meat surfaces.

MATERIALS AND METHODS

Culture details

E. coli NCTC 10538 is present in the intestinal tract of most of the warm-blood animals and is used as an indicator of faecal contamination. It was obtained from the microbiology lab at Queen's University, Belfast.

Antimicrobial dipping compounds

Trisodium phosphate (sodium phosphate tribasic dodecahydrate), lactic acid (2-Hydroxpropionic acid), and acetic acid were used as antimicrobial compounds.

Culture preparation

Tryptic soy broth (TSB) was inoculated by a loopful of culture from triple sugar iron agar slope (TSA). The broth was incubated for 18 h at 37°C to reach the stationary phase. After 18 h, serial decimal dilutions were prepared up to 10^{-4} from the stationary phase culture. 1 ml of the 10^{-4} dilution was transferred to 10 ml of TSB and incubated at 37°C for 18 h. A 10 ml portion of the resulting station-nary phase culture was transferred to a 50 ml centrifuge tube and centrifuged at 4000 rpm for 20 min. The supernatant was removed by using a sterile plastic pastette. Sterile phosphate saline buffer (PBS) (10 ml) was added to the pellet. PBS was suspended in the pellet by withdrawing liquid into the pipette tip and was repeatedly ejected until a uniform suspension was obtained. The suspension was poured in sterile PBS and made up to 50 ml, and centrifuged again at 4000 rpm for 20 min. The supernatant was then removed and 10 ml of PBS was added to the pellet and mixed until a uniform suspension was obtained. Sterile PBS was added to make it up to 50 ml. Decimal dilution series were prepared and were spread plated (0.1 ml) in duplicate plates on the MacConkey agar for enumeration of *E. coli*. Plates were incubated at 37°C for 24 h. The suspension was found to contain 10^7 CFU/ml.

Dipping procedures

For each treatment, 12 chicken quarters with skin, obtained from a local supermarket, were used. These samples were dipped into the suspension of *E. coli* (10^7 CFU/ ml) for 20 s and put in a tray for 90 min to allow *E. coli* to be attached to the skin surface. The samples were subjected to one of the following treatments: 8, 10 and 12% TSP concentrations for 20 s; 1, 2 and 3% LA concentrations for 20 s; and 0.5, 1.0 and 1.5% concentrations AA for 20 s. All samples were also dipped in tap water for 20 s. Each treatment was repeated four times. The details are shown in Figure 1.

Surface swabbing

A sterile template (4 x 4 cm) was put on chicken skin, and the area was swabbed by sterile swab cotton. The swab was put in 9 ml sterile maximum recovery diluent (MRD) (peptone saline diluent). Decimal dilution series were prepared and were spread plated (0.1 ml) in duplicate on the TSA for total counts and on MacConkey agar for enumeration of *E. coli*. Plates were incubated at 37°C for 24 h for *E. coli* and at 32°C for 48 h for aerobic total counts.

RESULTS AND DISCUSSION

Trisodium phosphate

The results have indicated that dipping chickens in the TSP concentrations lowered the *E. coli* and aerobic total counts on chicken carcasses as compared to the control. Dipping chickens in 8, 10 and 12% concentration of TSP reduced *E. coli* by 0.5, 1.2 and 1.6 log CFU/ cm^2, respectively, whereas 0.5, 1.0, and 1.7 log CFU/ cm^2 reduction was observed in aerobic total counts, respectively. The effect of TSP may be due to its high pH (pH 10), which affects the cell wall and the adherence of bacteria. TSP may also repress enzyme synthesis and inhibit enzyme activity of bacteria (Wagner and Busta, 1986). Figure 2 shows the corresponding reduction curve. It shows decreased numbers of *E. coli* and aerobic total counts on chicken dipped in 8, 10 and 12% of TSP. The reduction of *E. coli* and aerobic total counts in the 8, 10 and 12% concentrations of TSP was statistically significant at $P > 0.001$ level. The least significant differences (LSD) of means were 0.095 for *E. coli* and 0.15 for aerobic total counts. The decreased survival of *E. coli* and aerobic total count in 12% concentrations was highest compared with 8 and 10% concentrations.

The results obtained from this experiment were similar with those obtained in previous studies. Wang et al. (1997) studied the effects of 10% TSP spraying on *S. Typhimurium* and found that the reduction was 1.5 - 2.3 log CFU/ cm^2. Aerobic bacteria were reduced by 1.21 log CFU/ cm^2 when chicken wings were treated by dipping in 8% trisodium phosphate (Ismail et al., 2001). Lillard (1994) found that the reduction of *S. Typhimurium* on chicken carcasses was 2 log CFU/ cm^2 when chicken was treated with 10% TSP concentration. Kim et al. (1994) reported that the treatment of chickens by dipping them in 10% TSP solution reduced the bacteria by 1.6 to 1.8 logs CFU/ cm^2 as compared to control samples. Slavik et al. (1994) evaluated the effectiveness of 10% TSP solution on the chicken carcasses inoculated with *Campylobacter jejuna* and reported that the reduction was 1.5 log CFU/cm^2.

Lactic acid

The reduction of *E. coli* on chicken surface dipped in 1, 2 and 3% LA was 0.5, 1.8 and 2.1 log CFU/cm^2 respectively. The reduction of aerobic total counts was 0.9, 1.4 and 2.1 log CFU/cm^2, respectively. The effectiveness of lactic acid against microorganisms is due to the pH decrease and metabolic inhibition by the undissociated acid

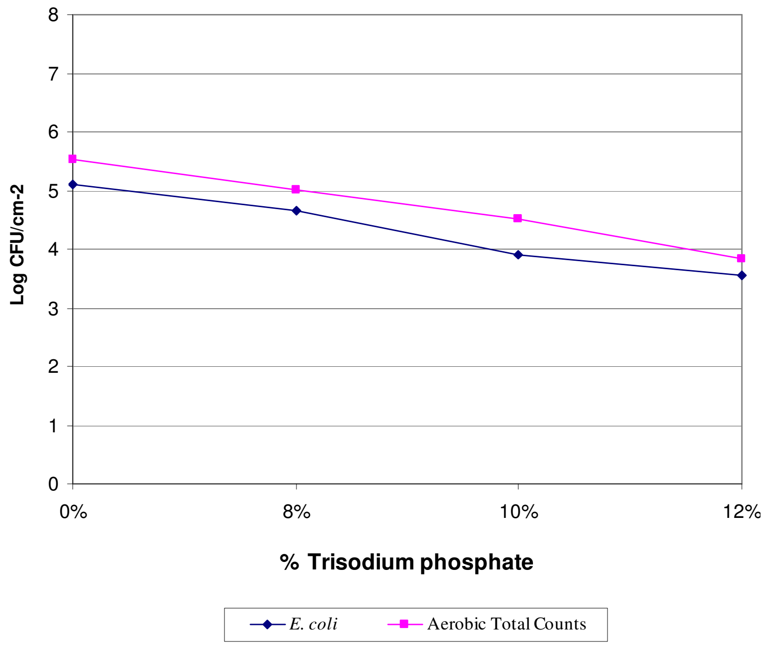

Figure 2. Reduction of *E. coli* and aerobic total counts on chicken carcasses dipped in different concentrations of trisodium phosphate solution for 20 s.

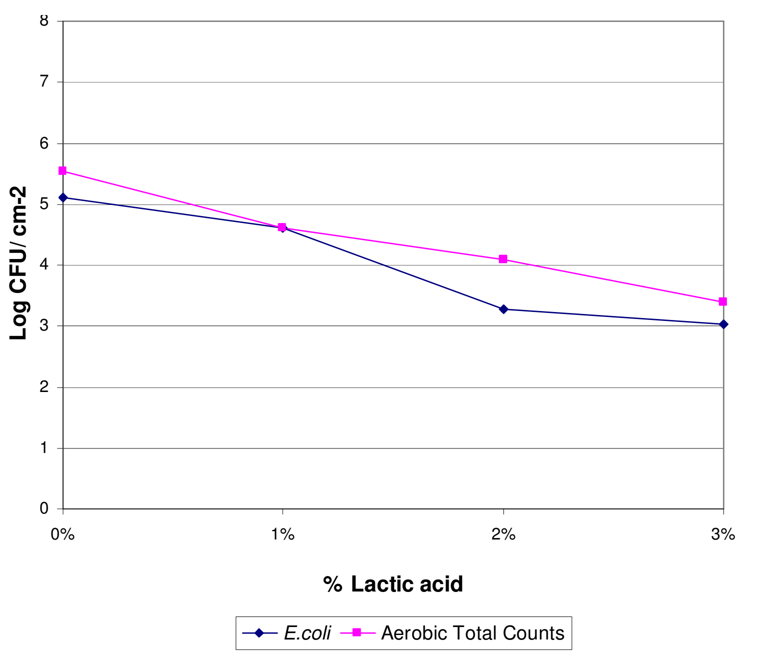

Figure 3. Reduction of *E. coli* and aerobic total counts on chicken carcasses dipped in different concentration of lactic acid solutions for 20 s.

molecules (Jay 1992). Figure 3 shows the corresponding reduction curve. Results showed low numbers of *E. coli* and aerobic total counts as compared to control samples. The reduction of *E. coli* and aerobic total counts in 1, 2 and 3% lactic acid solutions was found to be significantly different from control samples. However, the reduction of *E. coli* was higher when the 3% concentration of LA was used. The LSD of means was 0.095 for *E. coli* and 0.15 for aerobic total counts. Ramirez et al. (2001) dipped lamb breast carcass in 2% lactic acid and *E .coli* and aerobic total counts were found to be reduced by 1.6 and 1.6 log CFU/ cm^2, respectively. Also, a 2.5 log reduction was observed when the calf carcass surface was treated with 2% lactic acid (Woolthuis and Smulders, 1985). Davidson and Juneja (1990) observed that the treatment with 1-2% lactic acid reduced *Enterobacteriaceae*, and aerobic

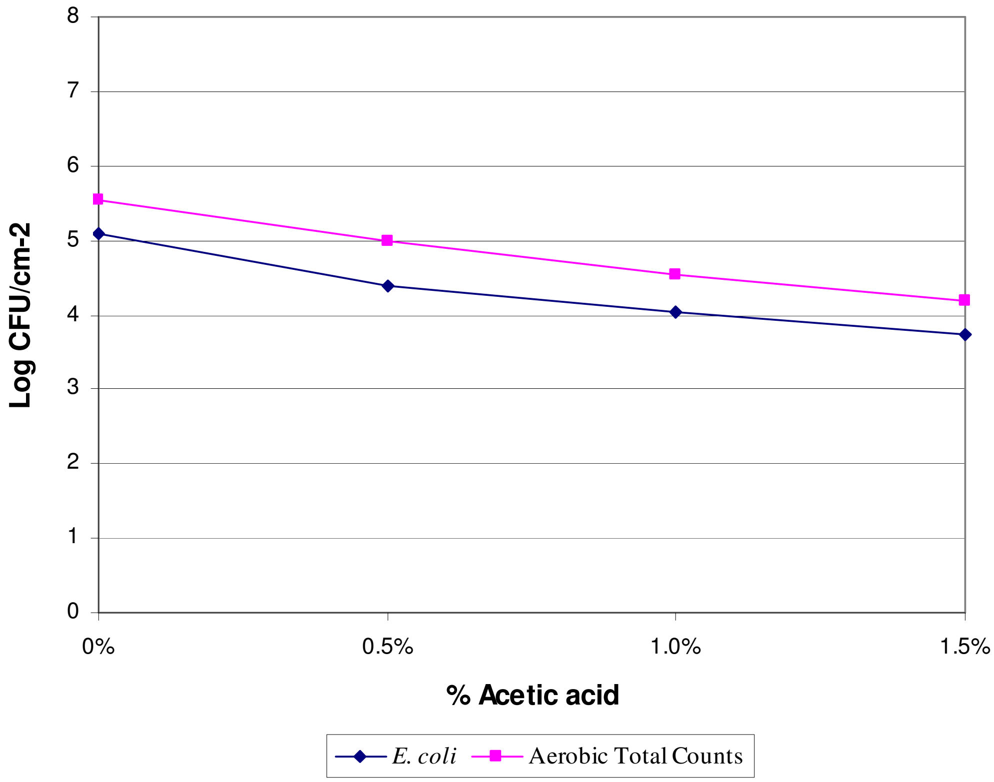

Figure 4. Reduction of *E. coli* and aerobic total counts on chicken carcasses dipped in different concentrations of acetic acid for 20 s.

plate counts by log 0.3 - 2.7 CFU/ cm^2 on beef, veal, pork, and poultry. Aerobic bacteria were reduced by 2.6 log CFU/ cm^2 when chicken wings were treated by dipping in 2% lactic acid (Ismail et al., 2001). Chicken carcasses were washed with 0.25% lactic acid and total counts were reduced by 0.76 log CFU/ cm^2 (Sakhare et al., 1999).

Acetic acid

Dipping chicken portions in 0.5, 1 and 1.5% acetic acid solutions decreased the *E. coli* counts by 0.7, 1.1 and 1.4 log CFU/ cm^2, respectively. Figure 4 shows the corresponding reduction curve. Significant decrease of *E. coli* and aerobic total count as compared to control was noticed at 0.5 1.0 and 1.5% AA solutions. The least significant differences (LSD) of means were 0.095 for *E. coli* and 0.15 for aerobic total counts. Chicken portion which were dipped in 0.5, 1.0 and 1.5% concentrations of acetic acid had lower the *E. coli* counts by 0.7, 1.1 and 1.4 log CFU/ cm^2, respectively. Also, the reduction of *E. coli* in 1% and 1.5% was higher than the 0.5% treatment. Aerobic total count was also reduced by 0.5, 1.0 and 1.6 log CFU/ cm^2, respectively.

Chicken carcasses were washed with 0.5% acetic acid and total counts were reduced by 0.76 log CFU/ cm^2 (Sakhare et al., 1999). Anderson et al. (1980) observed that the total count was reduced by 1.49 log when 3% of acetic acid was sprayed on the beef carcass. When beef portions were dipped in 1.2% acetic acid for 10 s, the populations of *S*. Typhimurium, *Shigella sonnei, Yersinia enterocolytica, E. coli*, *Pseudomonas aeruginosa*, and *Streptococcus faecalis* were reduced by 65% and the colour and flavour of the beef remained similar to the control samples (Bell et al., 1986).

Conclusion

Comparing the effects of the three treatments, lactic acid was found to be more effective against *E. coli* than acetic acid and trisodium phosphate. The reduction of aerobic total counts on chicken surfaces treated with lactic acid was highest as compared to those obtained from trisodium phosphate and acetic acid treatments. The reducetion of *E. coli* by trisodium phosphate and acetic acid were similar. However, the reduction of total counts by trisodium phosphate was higher than that of acetic acid. Lactic acid showed better antimicrobial effects against *E. coli* and aerobic total counts than trisodium phosphate and acetic acid. Lowering of *E. coli* and aerobic total counts by the use of trisodium phosphate required higher concentrations of trisodium phosphate. However, high concentration of TSP can adversely affect the meat quailty due to presence of its residues in food.

Finally, the use of trisodium phosphate, lactic acid and acetic acid to wash or sanitize meat carcasses (beef, veal, lamb and poultry) will not eliminate the pathogen aerobic total count completely, but will reduce the number of most harmful pathogens and microbial loads on meat carcasses, which will increase the shelf life and meat quality. The major benefit of the use of organic acid to wash meat carcasses is that they can reduce the dose of irradiation necessary to eliminate the pathogens and reducing microbial load in meat.

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REFERENCES

Anderson ME, Marshall RT, Stringer WC, Naumann HD (1980). In-plant evaluation of a prototype carcass cleaning and sanitizing unit. J. Food Prot. 40: 568-570.

Arnaut-Rollier I, De Zutter L, Van Hoof J (1999). Identities of the *Pseudomonas* spp. in flora from m chilled chicken. Int. J. Food Microbiol. 48: 87-96.

Bell MF, Marshal RT, Anderson ME (1986). Microbiological and sensory tests of beef treated with acetic and formic acid. J. Food Prot. 49: 207-210.

Berry ED, Cutter C (2000). Effects of acid adaptation of *Escherichia coli* O157:H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. J. Appl. Environ. Microbiol. 66: 1493-1498.

Bhide MR, Paturkar AM, Sherikar AT, Waskar VS (2001). Presensitization of microorganisms by acid treatment to low dose gamma irradiation with special reference to *Bacillus cereus*. Meat Sci. 58: 253-258.

Branen AL, Davidson PM, Salminen S (1990). Food Additives. Marcel Dekker, Inc., New York, NY.

Brown MH, Baird-Parker AC (1982). The microbial examination of meat. In Brown, M. H. (Ed.). Meat Microbiology. London, Applied Science. pp. 423-520.

Davidson M, Juneja VK (1990). Antimicrobial Agent. In: Branen AL, Davidson PM, Salminen S (Eds). Food Additives,. Marcel Dekker Inc, New York, USA. pp. 83-137

Eklund T (1983). The antimicrobial effect of dissociation and undissociation organic acid at different levels. J. Appl. Bacteriol. 54:383-389.

Eklund T (1985). Inhibition of microbial growth different pH levels by benzoic and propionic acids and esters of p-hydroxybenzoic acid. Int. J. Food Microbiol. 2:159-167.

Fratamico PM, Schultz FJ, Benedict RC, Buchanan RL, Cooke PH (1996). Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissue and removal using selected sanitizing rinses. J. Food Prot. 59: 453-459.

Gallo L, Schmidt RE, Schmidt-Lorenz W (1988). Microbial spoilage of refrigerated fresh broilers. I. Lebensmittel-Wissenschaft und-Technologie. 21: 216-223.

Giese J (1992). Experimental process reduces *Salmonella* on poultry. Food Technol. 46: 110-112.

Hwang CA, Beuchat LR (1995). Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. J. Food Prot. 64: 13-19.

Ismail SAS, Deak T, Abd El-Rahman HA, Yassien, MAM, Beuchat LR (2001). Effectiveness of immersion treatment with acids, trisodium phosphate, and herb decoction in reducing population of *Yarrowia lipolytica* and naturally occurring aerobic microorganisms on raw chicken. Int. J. Food Microbiol. 64: 13-19.

Jackson TC, Acuff GR, Dickson JS (1997). Meat, poultry, and seafood. In: Doyle MP, Beuchat LR, Montville TJ (Eds.). Food Microbiology: Fundamentals and Frontiers. pp. 83-100. American Society for Microbiology, Washington, DC.

Jay, JM (1992). Modern Food Microbiology. (4^{th} Ed.). Chapman & Hall, New York, NY.

Kim JW, Slavik MF, Pharr MD, Raben P, Lobsinger CM, Tsai S (1994). Reduction of *Salmonella* on post-chill chicken carcasses by trisodium phosphate ($Na_3 PO_4$) treatment. J. Food Safety. 14: 9-17.

Lee RM, Hartman PA., Olson DG, Williams FD (1999). Bactericidal and bacteriolytic effects of selected food grade phosphates, using *Staphylococcus aureus* as a model system. J. Food Prot. 57: 276-283.

Lillard HS (1994). Effect of trisodium phosphate on *Salmonella* attached to chicken skin. J. Food Prot. 57: 465-469.

Ramirez AJ, Acuff GR, Lucia LM, Savell JW (2001). Research Note: Lactic acid and trisodium phosphate treatment of lamb breast to reduce bacterial contamination. J. Food Prot. 64: 1439-1441.

Sakhare PZ, Sachindra NM, Yashoda KP, Narasimha Rao D (1999). Efficacy of intermittent decontamination treatments during processing in reducing the microbial load on broiler chicken carcass. Food Control. 10: 189-194.

Slavic MF, Kim, JW, Pharr MD, Raben DP, Tsai S, Lobsinger CM (1994). Effect of trisodium phosphate on *Campylobacter* attached to post-chill chicken carcasses.. J. Food Prot. 57: 324-326.

Wagner MK, Busta FF (1986). Association of {^{32}P} with *Clostridium botulinum* 52A vegetative cells following growth in a medium containing sodium dihydrogen {^{32}P}-pyrophosphate. J. Food Prot. 49: 353-354.

Wang W, Li Y, Slavik MF, Xiong H (1997). Trisodium phosphate and cetylpyridinium chloride spraying on chicken skin to reduced attached *salmonella typhimurium*. J. Food Prot. 60: 992-994.

Woolthuis CHJ, Smulders FJM (1985). Microbial decontamination of calf carcasses by lactic acid sprays. J. Food Prot. 48:832-837.