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The Effect of Lactic Acid Spray Application on the Microbiological Quality of Sheep Carcasses

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Abstract: This study was conducted to decontaminate sheep carcasses by spraying lactic acid solutions in two different concentrations (1 and 2%). The microbiological quality of carcasses and the effects of lactic acid spraying after slaughter and one day cold storage were determined. Commercial lactic acid solutions (1 and 2%) were sprayed to the sheep carcasses for 30 sec just before cold storage in a commercial slaughterhouse belonged to a private company located in Bursa. Sampling was carried out 30 min after spraying and after 24 h cold storage. A total of 400 samples were examined for Total Viable Count (TVC), the number of coliforms and *Escherichia coli*. A total of 1.57, 2.69 and 2.06 log cfu cm^{-2} reductions in the numbers of TVC, the number of coliforms and *E. coli* were obtained when 1% lactic acid concentration was applied. The reduction rates for these microorganisms were 1.77, 2.98 and 2.23 log cfu cm^{-2}, respectively when 2% lactic acid was applied. Following a 24 h cold storage the TVC, the number of coliforms and *E. coli* numbers reduced 1.30, 2.16 and 1.59 log cfu cm^{-2} in the 1% lactic acid treated samples when compared with untreated control samples. The reductions in the numbers of TVC, the number of coliforms and *E. coli* in 2% lactic acid treated samples after 24 h of cold storage were 1.67, 2.31 and 1.76 log cfu cm^{-2}, respectively. As a result, application of 2% lactic acid was more effective than 1% lactic acid application on the microorganisms investigated. It could be suggested that 2% lactic acid application with proper hygiene and handling procedures could provide safer meat/meat products.

Key words: Carcass, sheep, decontamination, microbiological quality, lactic acid, investigated

INTRODUCTION

Gradual increase in world population and change in lifestyles has resulted in demands for quality oriented foods of animal origin. Meanwhile, the number of incidences of food poisoning cases is increasing through out the world and many of these outbreaks have been associated with red meats and poultry (Goksoy *et al*., 2000). The deep tissues of meat carcasses are instrincally sterile (Gill, 1979, 1980) with the majority of microorganisms being found on the skin or any surfaces exposed during slughter processing. Meat carcasses may become contaminated from fecal material, paunch contents and the hide according to Lahr (1996). Additional sources of cross contamination exist in the slaughter process such as processing tools and equipment, astructural components of the facility, human contact and carcass-to-carcass contact. Fortunately, the majority of microflora transferred to carcass surfaces, while aesthetically undesirable are non-pathogenic (Institute of Food Technologists, 2002). Microbial contamination of meat starts up with the arrival of microorganisms to the carcass surface from where they penetrate into deeper layers of the meat. Reducing this primal surface contamination and avoiding or limiting the microbial growth, the shelf life of carcasses might be considerably extended.

Reducing surface contamination would also improve food safety (James *et al*., 2000). Several intervention strategie have been developed to reduce the level of bacteria on animal carcass surfaces such as washing and sanitizing with chilled water, hot water, chlorinated water, food grade acids and salts, alone and in combination (Dubal *et al*., 2004). Decontamination techniques for carcasses are targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage. According to Kotula and Kotula (2000) the bacteria of most concern for meat spoilage include Pseudomonas, Acinetobacter/Moraxella, Aeromonas, *Alteromonas putrefaciens*, *Lactobacillus*

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and Brochothrix thermosphacta. The pathogenic bacteria of most concern include *Escherichia coli* O157:H7, *Salmonella* sp., *Listeria monocytogenes*, Campylobacter, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Bacillus cereus*.

The natural content of lactic acid in meat is approximately. About 10 g kg^{-1}; it contributes to the flavour of meat and owing to its antimicrobial effects affects keeping quality. A variety of organic acids applied as a spray or dips for decontamination purposes have been studied extensively and appear to constitute an effective bactericidal or bacteriostatic surface treatment which also effectively prevents the attachment microorganisms (Dickson and Anderson, 1992; Bolder, 1997; Huffman, 2002; Pipek *et al*., 2006; Hardin *et al*., 1995). Moreover, the lactate anion slows down the growth of surviving microbes during storage (Kotula and Thelappurath, 1994; Siragusa, 1995; Dincer and Baysa, 2004; Mead, 1994). Antimicrobial effect of the organic acids is due to reduction of pH below the growth range and metabolic inhibition by the undissociated molecules (Levine and Fellers, 1940).

Undissociated weak acids are 10-600 times as effective inhibiting and killing microorganisms as dissociated forms (Eklund, 1983). Castillo *et al*. (2002) in a book chapter on reduction of microbial contaminants on carcasses provide a comprehensive review of organic acid sprays. Of the organic acids evaluated in the literature, acetic and lactic acids have been most widely accepted as carcass decontamination rinses. Additionally, it has become widely accepted that the effectiveness of organic acids is best achieved shortly after hide removal when the carcass is still warm (Huffman, 2002). The application of lactic acid is generally known and was effective also in industrial conditions in previous trials, e.g. (Staruch *et al*., 2001).

The implementation of a HACCP system has forced meat producers to study their production process and find, monitor and control the critical points (Bolder, 1997). Organic acids are legally allowed as a surface (including meat) decontaminant in the USA; the US Department of Agriculture permits the use of lactic acid for preevisceration rinsing of carcasses (Smulders, 1987). This study presented here was conducted to demonstrate the effectiveness of lactic acid decontamination of sheep carcasses by spraying 2 different concentrations (1 and 2%) immediately after traeatments and after 1 day of storage at 2±1°C.

MATERIALS AND METHODS

This study was conducted in March and April 2006 and a total of 100 sheep carcasses were used. Right thoracal sites of carcasses (50 carcasses for each treatment) were sprayed with commercial lactic acid solutions (1 and 2%) for 30 sec. After 30 min of exposure sampling was carried out by on the same region. The left sides of carcasses were used as controls and sampling was carried out at the same period with treated carcasses. Similar sampling procedures were conducted 24 h later after cold storage at 2±1°C.

Sampling: Sampling was carried out swabbing with sterile swabs on 100 cm^2 area by using templates. Swabs then put into sterile container containing 10 mL of 0.1% sterile pepton water and brought to laboratory in an insulated box at 4°C within 2 h. Samples were examined for TVC and the numbers of coliforms and *E. coli*. Following homogenization by using vortex (MSI minishaker, IKA) serial dilutions were carried out in sterile pepton water with a concentration of 0.1%, then plating out was conducted from appropriate dilutions on Plate Count Agar (PCA, OXOID CM 325) and Violet Red Bile Agar (VRB, OXOID CM 107) incubated at 37°C' de 24-48 h and 37°C'de 24 h, respectively. To be able to count *E. coli* colonies 5 typical colonies with a dark pink precipitation picked and put in to Lactose Broth (OXOID, CM 137) and incubated at 37°C for 48 h. Following incubation, inoculation on to Eosine Methylene Blue Agar (EMB, OXOID CM 69) from gas producing samples was carried out. After 24 h incubation at 37°C colonies with reflectance were subjected to Indol, Metil Red, Voges Proskauer and Citrate (IMVIC) tests (Turkish Standard Enstitute, 1990; Bridson, 1998; ICMSF, 1982; Lab, 2002).

Statistical analysis: Statistical analyses were carried out by using Student's test in SPSS (1999) 10.0 program.

RESULTS AND DISCUSSION

It was shown that there was a statistically significant difference between control and 30 sec lactic acid spray-treated groups for the microorganisms investigated. TVC and the numbers of coliform and *E. coli* in control and treated groups after 30 min of treatment are given at Table 1 ($p<0.001$). While the TVC of control group was 4.99±0.08 log cfu cm^{-2}, 30 sec lactic acid sprayed samples had this value 3.42±0.12 log cfu cm^{-2}. Table 2 shows the difference between microbial load of 1% lactic acid-sprayed carcasses 30 min and 24 h after treatment. It was shown that although, the increase in the TVC of treated samples was found to be significant ($p<0.05$) the increase in the numbers of coliforms and *E. coli* was not significant ($p>0.05$). Following a 24 h of storage at 2±1°C, the difference between control and 1% lactic acid spray-treated samples for TVC was found to be as 1.30 log cfu cm^{-2}. The differences between control and treated group were statistically different for the

Table 1: The numbers of microorganisms (log cfu cm^{-2}) examined on the control (n = 50) and treated (1% lactic acid) groups (n = 50) 30 min after application

Types	Control (30 min) $X \pm S_x$	Treated (30 min) $X \pm S_x$	Significance
TVC	4.99±0.08	3.42±0.12	***
Coliforms	3.08±0.13	0.39±0.11	***
E. coli	2.36±0.14	0.30±0.09	***

***p<0.001

Table 2: The effects of 1% lactic acid application on the microorganisms (log cfu cm^{-2}) examined on sheep carcasses (n = 50) after 24 h of cold storage

Types	Treated (30 min) $X \pm S_x$	Treated (24 h) $X \pm S_x$	Significance
TVC	3.42±0.12	3.75±0.09	*
Coliforms	0.39±0.11	0.51±0.12	ND
E. coli	0.30±0.09	0.31±0.10	ND

*p<0.05; ND: Not Determined (p>0.05)

Table 3: The numbers of microorganisms (log cfu cm^{-2}) examined on the control (n = 50) and treated (1% lactic acid) groups (n = 50) following 24 h of cold storage

Types	Control (24 h) $X \pm S_x$	Treated (24 h) $X \pm S_x$	Significance
TVC	5.05±0.06	3.75±0.09	***
Coliforms	2.67±0.12	0.51±0.12	***
E. coli	1.90±0.17	0.31±0.10	***

***p<0.001

Table 4: The numbers of microorganisms (log cfu cm^{-2}) examined on the control (n = 50) and treated (2% lactic acid) groups (n = 50) 30 min after application

Types	Control (30 min) $X \pm S_x$	Treated (30 min) $X \pm S_x$	Significance
TVC	5.03±0.09	3.26±0.10	***
Coliforms	2.98±0.12	0.00±0.00	***
E. coli	2.23±0.12	0.00±0.00	***

***p<0.001

Table 5: The effects of 2% lactic acid application on the microorganisms (log cfu cm^{-2}) examined on sheep carcasses (n = 50) after 24 h of cold storage

Types	Treated (30 min) $X \pm S_x$	Treated (24 h) $X \pm S_x$	Significance
TVC	3.26±0.10	3.57±0.11	ND
Coliforms	0.00±0.00	0.15±0.07	ND
E. coli	0.00±0.00	0.09±0.05	ND

ND: Not Determined (p>0.05)

Table 6: The numbers of microorganisms (log cfu cm^{-2}) examined on the control (n = 50) and treated (2% lactic acid) groups (n = 50) following 24 h of cold storage

Types	Control (24 h) $X \pm S_x$	Treated (24 h) $X \pm S_x$	Significance
TVC	5.24±0.09	3.57±0.11	***
Coliforms	2.46±0.14	0.15±0.07	***
E. coli	1.85±0.16	0.09±0.05	***

***p<0.001

microorganisms examined (p<0.001) (Table 3). The differences between the control and 2% spray-treated samples for TVC, the numbers of coliforms and *E. coli* were also significant (p<0.001) 30 min after application. The difference in TVC between control and 2% lactic acid spray-treated samples was found to be as 1.77 log cfu cm^{-2} 30 min after application (Table 4). Table 5 shows the difference between control and 2% lactic acid spray treated samples after 24 h of application. It was shown that no statistical significant differences (p>0.05) were observed between samples taken after 30 min and 24 h of application. Following a 24 h of cold storage the differences between control and 2% lactic acid spray-treated samples were 1.67, 2.31 and 1.76 log cfu cm^{-2} for TVC, coliforms and *E. coli*, respectively (Table 6).

Decontamination with organic acid solutions has been previously reported to reduce the number and prevalence of foodborne pathogens and the microbial load of meat a carcasses (Huffman, 2002; Smulders and Greer, 1998). For example, a commercial lactic acid spray cabinet applying 2% lactic acid at approximately, 42°C to beef carcasses (pre-evisceration) has been shown to reduce aerobic plate counts by 1.6 $\log_{10}$, Enterobacteriaceae counts by 1.0 $\log_{10}$ and *E. coli* O157:H7 prevalence by 35% (Bosilevac *et al*., 2006). Martinez *et al.* (2002) also reported that 1.5% lactic acid application reduced the level of total viable count 2 log cfu cm^{-2} and even more reductions were found in the numbers of coliforms and *E. coli*. In the study presented here, it was found that lactic acid applications with two different concentrations were found to have different efficiencies on the microorganisms investigated. TVC reduced 1.57 ve 1.77 log cfu cm^{-2} by using 1 ve 2% lactic acid solutions, respectively. The reductions in the numbers of coliforms and *E.coli* were found to be as 2.69 and 2.98 log cfu cm^{-2} and 2.06 and 2.23 log kob cm^{-2}, respectively. Similar results were observed by Woolthuis and Smulders (1985) and Castelo *et al.* (2001) that increased lactic acid concentration resulted in increased reduction rates in the numbers of microorganisms on the surface of carcasses.

Ramirez *et al.* (2001) also reported that either lactic acid alone or combination with TSP resulted in reductions in TVC and the numbers of *E. coli*. In another study, a stepwise increase in pH from 2.6-3.5 and 4.0 in an *in vitro* model resulted in a decrease of the bactericidal effect of lactic acid decontamination on meat-borne pathogens (Van Netten *et al*., 1994). In addition, it was also reported that a 2% solution of lactic acid at 37°C for 30-90 sec would eliminate Salmonella but not *L. monocytogenes* in pork skin suspensions. This is because Gram-positive bacteria are generally more resistant to lactic acid than Gram-negative bacteria. Combination of cold or hot water followed by lactic acid treatment resulted in lower populations of aerobic bacteria, psychotrophic bacteria, coliforms, *E. coli* and lactic acid bacteria on fat-covered pork trim tissue than on lean pork trim tissue (Castillo *et al*., 2001). It is also reported by other

researchers that Snijders *et al.* (1985) lactic acid application during the early stage of postmortem reduced the TVC 1.5 log. Similarly, Smulders (1995) was also observed a 1.5 log reduction in the TVC when 2% lactic acid applied.

Mesophilic enterobacteria are usually more sensitive to organic acid decontamination than other pathogens (Smulders and Greer, 1998). However, concerns have been raised whether changes in the natural microflora of meat caused by decontamination technologies will emerge risks such as higher acid tolerance of pathogens and increase of their growth due to possible reduction of microbial competition. For example, it was reported that the aerobic storage of fresh beef meat treated with 2% lactic acid at 55°C shifted the predominant spoilage association from Gram-negative-Gram-positive bacteria and yeasts (Koutsoumanis *et al.*, 2004).

The other important observation about organic acid decontamination is reported by Firstenberg-Eden (1981) that the action of organic acids is further complicated by the evidence of acid conditions may enhance the adesion of bacteria to surface. This is supported by El-Khateib *et al.* (1993) research which showed that lactic acid treatment of beef mucle caused a greater percentage of a *L. monocytogenes* population to attach to the meat surface than equivalent treatments with water and bacteriocins.

It is very certain that the efficacy of organic acid decontamination depends on the type of the meat tissue, the type and the load of initial microbial contamination as well as the pH, the concentration and the temperature of the organic acid solution (Koutsoumanis *et al.*, 2006; Sofos and Smith, 1998). Therefore the differences between other studies using 1 and 2% lactic acid solutions and the study presented here might be due to these factors.

The TVC of carcasses treated with 1% lactic acid solution incrased 0.33 log cfu cm^{-2} after 24 h of storage at 2±1°C. The increases in the numbers of coliforms and *E.coli* were found to be as 0.12 log cfu cm^{-2} and 0.01 log cfu cm^{-2}, respectively. When 2% lactic acid solution was applied the increases for these microorganisms were 0.31, 0.15 ve 0.09 log cfu cm^{-2} after 24 h of storage at 2±1°C. The numbers of coliform and *E. coli* increased insignificantly during the storage period ($p>0.05$). This might be due to the residuel effect of lactic acid on microorganism during the storage (Martinez *et al.*, 2002; Ramirez *et al.*, 2001).

CONCLUSION

As a result higher reduction was observed in the microorganism examined with 2% lactic acid usage in compare to 1%. It was very certain that this reduction was very significant in the numbers of coliform and *E.coli.* It was concluded that application of 2% lactic acid to the carcasses immediately after slaughter would increase shelf-life, reduce the pathogen microorganisms and therefore will aid public health.

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