

Effect of Different Durations of Exposure to Food-Grade Organic Lactic Acid on the Growth of *S. aureus* and *E. coli* in Meat from Persian Sheep (Afshari Breed) Stored at Refrigeration Temperature

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Abstract

Moghareei M.A., Ahari H., Sefekordi A.A., Haghighat Khajavi S. (2018): Effect of different durations of exposure to food-grade organic lactic acid on the growth of *S. aureus* and *E. coli* in meat from Persian sheep (Afshari breed) stored at refrigeration temperature. Czech J. Food Sci., 36: 44–50.

The effect of lactic acid in extending the shelf life of sheep carcasses stored at 4°C was evaluated. Carcasses were assigned to four testing groups: T1 (control group), T2 (1% lactic acid spray), T3 (2% lactic acid spray) and T4 (3% lactic acid spray). There were statistically significant differences among the groups in the colour score for each minced meat sample at 48, 72, and 96 h ($X_2 = 9.9$, $P = 0.019$; $X_2 = 9.7$, $P = 0.021$, respectively). The minimum mean rank of the odour score was found using 1% lactic acid at 72 h (3.0), which was significantly different ($P < 0.05$) when compared to the other groups. The MANOVA test showed no significant difference ($P > 0.05$) in the percentage of protein during the days of the experiment. The interaction effect between the sampling time and acid concentration levels on the measurement of *S. aureus* log₁₀ CFU showed that the marginal mean of that measurement was significant ($P < 0.05$) at 48, 72, and 96 hours. The minimum value for *E. coli* log₁₀ CFU was observed at the time point of 96 h in groups 2 and 4 (3.3 ± 0.03) with no significant difference ($P > 0.05$) between them. In conclusion, 1% lactic acid is more effective in extending the shelf life of sheep meat in comparison to 2 and 3% lactic acid.

Keywords: *Escherichia coli*; lactic acid; shelf life; sheep meat; *Staphylococcus aureus*

The Iranian Afshari breed of sheep is characterised by good weight and daily weight gain, low FCR (feed conversion ratio) and remarkable shelf life when butchered, in comparison to other Iranian breeds (MOGHADDAM & HASSANEYN 1983). Higher bacterial counts found in some meat retailers could be due to poor facilities for drainage, lack of appropriate hygienic conditions, unnecessary handling of carcasses, rupturing the viscera and releasing the gut contents, removal of the anal sphincter from the body in abattoirs and ultimately cross-contamination between the slaughterhouse, workers, transport vehicles, or other facilities and the retail outlet (RISTIC 1997; BHANDARE *et al.* 2007). Iran

has a few structured slaughterhouses, but they mostly supply the newly slaughtered meat to retail outlets, whose hygienic conditions are sometimes questionable (MOGHADDAM & HASSANEYN 1983). The poor sanitary standards in the slaughterhouse and in the retail shops enhance bacterial growth (BHANDARE *et al.* 2007).

Pathogenic microorganisms such as *Salmonella*, *S. aureus* and *E. coli* can be poisonous for those who eat sheep meat. Several studies have evaluated the efficiency of organic acids in extending meat shelf life (SMULDERS *et al.* 1986; BOSILEVAC *et al.* 2006; GONZÁLEZ & FANDOS *et al.* 2009). Bacterial pathogens are found on hides and easily migrate to

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clean carcass tissue situated right under the hides during the hide peeling process (BAIRD *et al.* 2006).

E. coli has been of concern in the processing and packaging of meat for several decades (BOSILEVAC *et al.* 2006). The recent onset of food poisoning due to red meat products contaminated with *E. coli* and *S. aureus* has resulted in research focused on reducing bacteria in sheep meat using organic acids (GREER & DILTS 1995). In beef slaughterhouses, carcass washing with lactic acid is a common step in preventing microbial growth during the post-evisceration phase. Many slaughterhouses also employ 2% lactic acid washes during the pre-evisceration step (BOSILEVAC *et al.* 2006). For example, MARSHALL and KIM (1996) studied the efficacy of 1–4% lactic acid in the reduction of bacterial contamination in refrigerated catfish fillets.

The objectives of this study were to evaluate the effect of 1–3% lactic acid in extending the shelf life of Persian (Afshari breed) sheep carcasses through decreasing bacterial (*S. aureus* and *E. coli*) growth, inhibiting large changes in pH, reducing discoloration and preventing an off-putting odour in meat stored at a refrigeration temperature of 4°C over a 96-h period.

MATERIAL AND METHODS

Sheep meat. Approximately 2 kg of boneless sheep (Afshari breed) haunches in sealed PVC trays were transferred to the food laboratory at the Science and Research Branch of the Islamic Azad University within 45 min of purchase. The meat was bought from a local butcher who slaughters sheep every morning in Tehran province (2015).

Treatments of sheep haunch meat. Twenty-four pieces were cut from sheep haunches and each piece was separately homogenised using a Stomacher Lab Blender (model BA6021; Seward Laboratory, UK) for 1 minute. Small glass plates ($n = 24$) were selected to preserve the blended pieces with AW (water activity) of 50 g forming group 1 (T1, control group), group 2 (T2, 1% lactic acid spray), group 3 (T3, 2% lactic acid spray) and group 4 (T4, 3% lactic acid spray). Lactic acid at the appropriate percentage (Kirsch Pharma, Germany) was sprayed onto the surface of all samples before blending. A sterile glass thin-layer chromatography atomising sprayer was used to spray the acid solution. The nozzle was connected to a 200-ml flask. To increase the efficacy

of the method, the nozzle was kept 5 cm away from the meat surface and visual tests were conducted to ensure that whole surface of the meat was sprayed. The plates were kept in a refrigerator at 4°C for up to 24 h from the point of meat delivery to the lab, after which sampling was then performed at 0, 24, 48, 72, and 96 hours.

Sampling schedule. In total, 24 samples were taken for each treatment or lactic acid concentration, and the replicates for *S. aureus*, *E. coli* and total viable counts (TVC) were harvested four times: at 0, 24, 48, 72, and 96 hours.

Total protein measurement. According to the method of HORWITZ and GEORGE (AOAC 2005), weighed 1 g samples from each treated group and their replicates were transferred into a Kjeldhal digestion flask prefilled with 20 ml of sulphuric acid and 8 g of a catalyst mixture, including 96% potassium sulphate, 3.5% copper sulphate, and 0.5 g of selenium oxide. The mixture was heated gently until skim ceased. The solution was then boiled until the organic materials became digested and a pale liquid appeared. Particles attached to the side of flask were then washed with distilled water so as to collect them at the bottom of the flask. The heating process continued until all of the organic materials were digested. Washing the flask was repeated three times to ensure all the digested materials were collected in the condenser flask, which was connected to a distillation system. When the incorporated flask became cool, an anti-bump granule was added together with a sodium hydroxide solution (1 : 1) to the flask (about 75 ml). Then, 5 ml of methyl red was added and titrated with 0.1 N of NaOH. Ultimately, 1 ml of 0.1 N sulphuric acid is equivalent to 0.0014 of nitrogen (total protein = $N \times 6.25$; where: N – nitrogen).

Sensory and pH assessment of the meat samples. Sensory assessment was carried out to evaluate the quality of the meat by four co-workers using a four-point scale for colour (1 – normal; 2 – light dark; 3 – dark; 4 – heavy dark) and a four-point scale for odour (1 – normal; 2 – mildly rancid; 3 – rancid; 4 – severely rancid), according to ACUFF *et al.* (1987). Five grams from each treatment and its replicates were homogenised with 20 ml of deionized water to determine pH using an AZ pH meter (model 86502, multi-chemical detector; AZ, Taiwan).

Differential counts. Differential counts for the two organisms were made using media selective for each of them with 0.1 ml inoculum (initial suspension) from 10^{-1} dilutions. Briefly, 1 g of sample was care-

fully weighed, transferred into a test tube containing 9 ml of sterile Ringer's solution (Pasteur; Iran), homogenised and mixed well. This initial suspension was then used for subsequent sample dilution.

Staphylococcus spp. One gram of sample was carefully weighed, transferred into a test tube containing 9 ml of sterile Ringer's solution (Pasteur, Iran) homogenised and mixed well. This initial suspension (0.1 ml) was added to two plates containing sterile Baird Parker Agar 11705 (Sigma-Aldrich, USA) and spread quickly with a glass rod on the surface of the plates, which subsequently were incubated at 37°C for 24–48 hours. Specific colonies were marked at the end of the incubation period. Colonies appeared transparently black or grey, shiny and convex with a diameter of 1–1.5 mm (after 24 h of incubation) or 1.5–2.5 mm (after 48 h of incubation). Inocula from the surface of each selected colony from each tube were inoculated into test tubes containing brain-heart infusion (BHI) broth (53286; Sigma-Aldrich, USA), using a sterile wire and incubated at 35–37°C for 24 hours. According to ISO 6888-1, 0.1 ml of each culture were added to 0.3 ml of rabbit plasma in a sterile tube, and incubated at 35–37°C. Ultimately, the coagulase plasma was examined by inclining the tube after incubation for 4–6 hours. The coagulase test was considered positive if the clot volume was observed to be more than half of the original liquid volume.

E. coli. Firstly, 0.1 ml of initial suspension were inoculated in tubes containing selective enrichment broth (lauryl sulfate broth) and incubated at 37°C for 48 hours. The final product was evaluated if the suspension produced gas. In positive cases, an inoculum of that product was incubated in EC broth (CM0853; Oxid, UK) at 44°C for 48 h, and gas production was again monitored. If the gas result was again positive, an inoculum was incubated with 1% non-indole peptone. This was incubated at 44°C for 48 h and indole production was monitored. If the results of three incubations were positive, this proved the presence of *E. coli* in the sample (ISIRI No. 2946, 2012).

Total viable count (TVC). Total viable count was enumerated according to the standard pour plate (P6351; Sigma-Aldrich, USA) technique (BHANDARE *et al.* 2007). In brief, 1 ml of the initial suspension was added to a sterile plate containing 12–15 ml of autoclaved culture medium at 44–47°C. The plate was allowed to cool until quite stiff by gentle rotation on a lab bench surface. The plates were incubated at 37°C for 24 h and counts were expressed as log₁₀ CFU/g.

Statistical analysis. To analyse the effect of the interaction between the subject and exposure time, and between the subject and lactic acid concentrations on *Staphylococcus* spp. log₁₀ CFU/g, *E. coli* log₁₀ CFU/g and TVC log₁₀ CFU/g in a mixed model, repeated ANOVA measures were used. The one-way multivariate analysis of variance (MANOVA) was applied as an alternative test, when Mauchly's test was significant for protein percentage and pH analysis. Kruskal-Wallis nonparametric analysis was used to test the significance of differences for each minced-meat sample sprayed with different levels of lactic acid, and for different scores given by participants for colour and odour sensory criteria at each exposure time point of the experiment. Subsequently, for post-hoc analysis, a Mann-Whitney U test was used to determine pair-wise differences.

RESULTS AND DISCUSSION

Sensory analysis. The sensory scores for the sheep meat were evaluated according to the sampling schedule; for the treatment groups these significantly decreased ($P < 0.05$) compared with the control, particularly after 24 h (Table 1). A Kruskal-Wallis H test indicated that there were statistically significant differences between the colour scores of each minced meat sample at 48, 72, and 96 h ($X_2 = 9.9$, $P = 0.019$; $X_2 = 9.7$, $P = 0.021$), respectively. In contrast, no significant differences ($P > 0.05$) were observed among the colour scores at the time points of 0 h and 24 h. The minimum mean ranks of black discolora-

Table 1. Sensory criteria ranks (colour and odour) for each lactic acid percentage during 4 days of storage at 4°C ($n = 6$).

	Lactic acid (%)	Exposure (h)				
		0	24	48	72	96
Colour	control	6.5	8.0	9.8 ^a	11.0 ^a	10.0 ^a
	1	6.5	6.0	3.1 ^b	2.3 ^b	2.0 ^b
	2	6.5	6.0	6.5 ^c	7.1 ^c	7.5 ^c
	3	6.5	6.0	6.5 ^c	6.5 ^c	6.5 ^c
Odour	control	6.5 ^a	6.0 ^a	9.6 ^a	10.3 ^a	11.0 ^a
	1	6.5 ^a	6.0 ^a	4.8 ^a	3.0 ^b	4.5 ^b
	2	6.5 ^a	6.0 ^a	6.6 ^a	6.3 ^a	6.0 ^b
	3	6.5 ^a	6.0 ^a	4.8 ^a	6.3 ^a	6.0 ^b

Data are presented as a mean ± SE; dissimilar letters for each column significantly difference ($P < 0.05$).

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tion scores (2.0) were observed in the 1% lactic acid group (Table 1) at 96 h, followed by the mean rank (2.3) at 72 h (Table 1). The maximum mean rank of colour score (11.0) was detected in the 1% lactic acid group (Table 1) at 72 h, followed by 96 h with a mean rank of 10.0. No significant differences ($P > 0.05$) were observed between the T3 group and T4 group at the 24–72-h time points. The Kruskal-Wallis H test showed a significant difference in the odour scores for each minced sample at 72 and 96 h ($X_2 = 7.27$, $P = 0.049$; $X_2 = 7.37$, $P = 0.048$), respectively. The results for the odour mean ranks at 0 h, 24 h and 48 h implied an insignificant difference ($P > 0.05$). The minimum mean rank of the odour score was achieved in the 1% lactic acid group (Table 1) at 72 h (3.0), which was significantly different ($P < 0.05$) when compared to other treatments and the controls. This was followed by a mean rank of 4.5 for the 1% lactic acid group at 96 h, with no significant difference ($P > 0.05$) against other treatments, except with the control group ($P > 0.05$).

pH of the meat samples. According to the results of the MANOVA test (Table 2), no significant difference was observed in the protein percentages within the experiment samples treated with different levels of lactic acid ($F_{12, 21} = 2.42$, $P < 0.05$; Wilk's $\Lambda = 0.04$, partial $\eta^2 = 0.66$). There were no significant differences ($P > 0.05$) in the pH values between the groups at 0, 24, and 48 h (Table 2). The marginal means of the pH showed no significant differences ($P > 0.05$) between the treatments at 72 h, but they were significantly different ($P < 0.05$) in comparison to the control group. An insignificant difference in this mean value ($P > 0.05$) was observed between groups 2 and 4, and also groups 1 and 3 at 96 hours.

Total protein of meat samples. According to the result of MANOVA, there were no significant dif-

Table 2. The post-hoc multi-comparison showing the differences between the pH of each paired lactic acid level ($n = 6$)

Lactic acid (%)	Exposure (h)				
	0	24	48	72	96
0	5.5 ± 0.0 ^a	5.5 ± 0.0 ^a	5.7 ± 0.0 ^a	5.8 ± 0.0 ^a	5.8 ± 0.2 ^a
1	5.5 ± 0.0 ^a	5.5 ± 0.0 ^a	5.5 ± 0.0 ^a	5.5 ± 0.0 ^b	5.3 ± 0.2 ^b
2	5.4 ± 0.0 ^a	5.5 ± 0.0 ^a	5.7 ± 0.0 ^a	5.6 ± 0.0 ^b	5.7 ± 0.2 ^a
3	5.5 ± 0.0 ^a	5.5 ± 0.0 ^a	5.5 ± 0.1 ^a	5.4 ± 0.0 ^b	5.1 ± 0.2 ^b

Data are presented as a mean ± SE; dissimilar letters for each column significantly difference ($P < 0.05$)

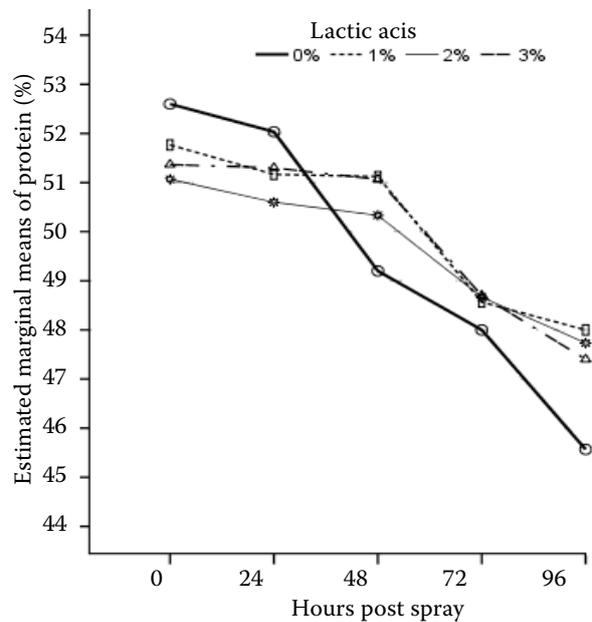


Figure 1. Reduction the protein content of the meat samples sprayed with different doses of lactic acid during the experiment stored in a refrigerator at 4°C ($n = 6$)

ferences between the different lactic acid levels in terms of protein percentage [$F(15, 11) = 1.68$, $P > 0.05$; Wilk's $\Lambda = 0.04$, partial $\eta^2 = 0.65$]. The protein content decreased ($P < 0.05$) over the course of the experiment regardless of whether lactic acid was sprayed on the surface of samples.

Post-hoc tests, using the Bonferroni correction, revealed that over time there was a slight decrease in the protein levels from before the start of the experiment up to the 96-h time point, at 2 and 3% lactic acid (51 ± 0.1 and 47.7 ± 0.1 versus 51.3 ± 0.1 and 47.4 ± 0.1 , respectively), which was statistically significant ($P < 0.05$). For protein percentage, no significant differences were observed ($P > 0.05$) among the different lactic acid percentages for all the sampling days, with the exception of the control group, which showed a percentage of 45.5 ± 0.1 at 96 h.

S. aureus. Mauchly's sphericity test showed that the main effect of time did not significantly violate the sphericity assumption for *S. aureus* \log_{10} CFU ($W = 0.117$, $X_2 = 13.77$, $P = 0.14$). The interaction effect between the sampling time and acid concentration on the measurement of *S. aureus* \log_{10} CFU showed that the marginal mean was significant ($P < 0.05$) at 48, 72, and 96 hours. These values are listed in Table 3. At 48 h, the minimum marginal mean for *S. aureus* \log_{10} CFU (1.9 ± 0.13) was observed for 1% lactic acid (T2) which was significantly different ($P < 0.05$) compared with the other treatments and

Table 3. The estimated marginal means (\log_{10} CFU/g) of *S. aureus* and *E. coli* grown and TVC isolated in minced-meat samples sprayed with different concentrations of lactic acid at different hours during the experiment ($n = 6$)

Hours	Lactic acid (%)	<i>S. aureus</i>			<i>E. coli</i>			TVC		
		mean \pm SE	95% confidence interval		mean \pm SE	95% confidence interval		mean \pm SE	95% confidence interval	
			lower bound	upper bound		lower bound	upper bound		lower bound	upper bound
0	0	2.0 \pm 0.07	1.836	2.164	3.4 \pm 0.02	3.365	3.365	6.9 \pm 0.02	6.880	7.013
	1	2.1 \pm 0.07	1.936	2.264	3.4 \pm 0.02	3.353	3.353	6.9 \pm 0.02	6.843	6.977
	2	1.9 \pm 0.07	1.752	2.081	3.4 \pm 0.02	3.396	3.396	6.8 \pm 0.02	6.797	6.930
	3	2.1 \pm 0.07	1.936	2.264	3.4 \pm 0.02	3.380	3.380	6.8 \pm 0.02	6.820	6.953
24	0	2.3 \pm 0.22	1.808	2.858	3.5 \pm 0.22	3.497	3.497	6.8 \pm 0.01 ^a	6.848	6.912
	1	1.8 \pm 0.22	1.338	2.388	3.4 \pm 0.22	3.401	3.401	6.4 \pm 0.01 ^b	6.445	6.509
	2	1.9 \pm 0.22	1.408	2.458	3.4 \pm 0.22	3.403	3.403	6.4 \pm 0.01 ^b	6.415	6.479
	3	1.9 \pm 0.22	1.442	2.492	3.4 \pm 0.22	3.386	3.386	6.4 \pm 0.01 ^b	6.411	6.475
48	0	3.2 \pm 0.13 ^a	2.916	3.550	3.5 \pm 0.02	3.484	3.484	6.8 \pm 0.02 ^a	6.811	6.909
	1	1.9 \pm 0.13 ^b	1.583	2.217	3.5 \pm 0.02	3.452	3.452	6.4 \pm 0.02 ^b	6.415	6.512
	2	2.6 \pm 0.13 ^c	2.283	2.917	3.5 \pm 0.02	3.472	3.472	6.4 \pm 0.02 ^b	6.371	6.469
	3	2.4 \pm 0.13 ^c	2.150	2.784	3.4 \pm 0.02	3.375	3.375	6.4 \pm 0.02 ^b	6.361	6.459
72	0	3.4 \pm 0.18 ^a	3.046	3.888	3.7 \pm 0.02 ^a	3.665	3.665	6.8 \pm 0.02 ^a	6.797	6.896
	1	1.5 \pm 0.18 ^b	1.146	1.988	3.4 \pm 0.02 ^b	3.404	3.404	6.4 \pm 0.02 ^b	6.364	6.463
	2	2.4 \pm 0.18 ^c	2.046	2.888	3.5 \pm 0.02 ^b	3.504	3.504	6.3 \pm 0.02 ^b	6.320	6.420
	3	1.8 \pm 0.18 ^b	1.379	2.221	3.4 \pm 0.02 ^b	3.432	3.432	6.3 \pm 0.02 ^b	6.314	6.413
96	0	3.6 \pm 0.09 ^a	3.443	3.891	3.6 \pm 0.03 ^a	3.610	3.610	6.9 \pm 0.04 ^a	6.817	7.003
	1	1.5 \pm 0.09 ^b	2.709	3.157	3.3 \pm 0.03 ^b	3.253	3.253	6.3 \pm 0.04 ^b	6.287	6.473
	2	2.4 \pm 0.09 ^c	3.209	3.657	3.5 \pm 0.03 ^b	3.421	3.421	6.3 \pm 0.04 ^b	6.260	6.446
	3	1.5 \pm 0.09 ^b	1.343	1.791	3.3 \pm 0.03 ^b	3.294	3.294	6.4 \pm 0.04 ^b	6.374	6.560

data were presented as a mean \pm SE; different letters indicate a significant difference ($P < 0.05$)

the control group. This group consistently exhibited the lowest mean value among the groups (Table 3) even at 72 h (1.5 ± 0.18) and 96 h (1.5 ± 0.09), with no significant difference ($P > 0.05$) compared with group T4; however, values were significantly different than those for T1 and T3. On the other hand, the marginal mean value \log_{10} CFU in group T1 started at 2.0 ± 0.07 at the time point of 0 h, with no significant difference ($P > 0.05$) compared with the other groups, and reached 3.6 ± 0.09 at 96 h, which was significantly higher ($P < 0.05$) compared with the other groups.

E. coli. Mauchly’s sphericity test showed that the main effect of time did not significantly violate the sphericity assumption for *E. coli* \log_{10} CFU ($W = 0.413$, $X_2 = 5.67$, $P = 0.78$). The interaction effect between time and acid concentrations on the marginal mean of *E. coli* \log_{10} CFU indicated that this value of *E. coli* was not significant ($P > 0.05$) at the time points of

0, 24, and 48 hours. These values are listed in Table 3. The minimum value for *E. coli* \log_{10} CFU was observed at the time point of 96 h in groups 2 and 4 (3.3 ± 0.03), with no significant difference ($P > 0.05$) between them and between them and T3, but with a significant difference with T1. A similar pattern of significant differences was observed among the groups at the point time of 72 h (Table 3).

Total viable count. Mauchly’s sphericity test showed that the within-subjects effect of time did not significantly violate the sphericity assumption for TVC \log_{10} CFU ($W = 0.277$, $X_2 = 8.22$, $P = 0.52$). The interaction effect between the duration of exposure to lactic acid and its concentration on the TVC \log_{10} CFU showed that the marginal mean was significant ($P < 0.05$) at 48, 72, and 96 h (Table 3). There was a significant difference ($P < 0.05$) between the treatments and the control group at every time point of the experiment, with the exception of the first

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samples. From 24 h to the end of the experiment, no significant differences were observed among the treatments (Table 3).

The results show that black discoloration occurred in the control group at 48 h, versus the less intensity treatment groups. On the other hand, the lowest off-odour intensity was observed in the T2 group between 46 and 96 hours. Normally, the pH of meat decreases from 7.2 (physiological) to 5.5–5.8 during the course of 24 h after butchering (BREWER *et al.* 2001), which was similar to the value measured in this study (5.5) at 0 hour. Using a 2% lactic acid spray as a beef hide antimicrobial resulted in a reduction of about $1.3 \log_{10}$ CFU/100-cm² *E. coli* (BAIRD *et al.* 2006).

The amount of *S. aureus* naturally isolated showed approximately a two-fold \log_{10} CFU reduction in response to the spraying of 1% lactic acid on the meat samples compared with the control group at 48–96 h, with a considerably decreased score in discoloration and slight off-odour intensity. This *S. aureus* count for 2% lactic acid showed an approximately $0.5 \log_{10}$ CFU decrease against the control and compared with the 1% lactic acid group. This finding is not in line with DUBAL *et al.* (2004) who showed that 2% lactic acid inhibited the growth of *S. aureus* experimentally inoculated in sheep meat. In accordance with our results, SMULDERS *et al.* (1986) showed that discoloration of meat samples still occurred in response to 1% lactic acid treatment.

BOSILEVAC *et al.* (2006) reported that the bacterial count for *E. coli* was $3 \log_{10}$ CFU/100 cm² in response to the spraying of 2% lactic acid on carcass tissue, a reduction of $1.0 \log$ CFU/100 cm² compared to the control group. However, the results reported here show that the efficacy of 1–3% lactic acid in reducing *E. coli* was lower than in this previous study; 2% lactic acid reduced *E. coli* numbers by $0.1 \log_{10}$ CFU/g in sheep meat at 96 h. In some studies in which inocula were experimentally added to meat samples (CASTILLO *et al.* 1998, 1999, 2001), colony counts showed large reductions of greater than $4 \log_{10}$ CFU/g. Similar to this study, levels were reduced to less than $1 \log_{10}$ CFU/g when natural contaminants were evaluated (DORMEDY *et al.* 2000; GILL & BADONI 2004).

MARSHALL and KIM (1996) found that dipping (60 s) was more effective in reducing bacterial growth compared with the spraying of acid onto refrigerated catfish fillets. They showed that food-grade lactic acid was capable of suppressing bacterial growth in a dose-dependent manner, but due to black discoloration and inappropriate odour a lower concentration of acid was recommended. Those findings are in

accordance with those reported here, which showed that although 3% lactic acid was markedly more effective than smaller doses in suppressing the growth of *S. aureus*, the off-odour intensity and black discoloration of the sheep meat kept at a refrigeration temperature of 4°C precludes the use of 3% lactic acid as an antimicrobial. These results are not in accordance with MENDONCA *et al.* (1989), who revealed that a mixture of 1% acetic acid and 1% lactic acid damaged the colour of vacuum-packed pork chops.

GILL AND BAKER (1998) reported that sheep meat had a mean count of $4.6 \log_{10}$ TVC CFU/cm² in different parts of the carcass during the post-washing phase in the abattoir, a value which is close to the results of BHANDARE *et al.* (2007) who reported $5.13 \pm 0.58 \log$ CFU/cm². Those results are similar to those reported here, which were calculated to be 6.9 ± 0.02 . On the other hand, DUFFY *et al.* (2001) found that chilled sheep carcasses (5042) had mean \log TVC CFU/cm² of 4.23 and 4.61, respectively, during the spring and winter, and a general prevalence of *E. coli* of 66.2%. DUFFY *et al.* (2001) findings are similar to those of this study that showed mean counts of 6.9 ± 0.02 and $3.4 \pm 0.02 \log_{10}$ TVC and *E. coli* CFU/g, respectively.

In fact, taking into account that widespread protein denaturation (loss of functionality) takes place, the T1 (control group) exhibited significant differences ($P < 0.05$) versus the other groups that were not related to the pH, which showed a normal range. The loss of protein could be due to the growth of *S. aureus*, which showed a significant reduction of about $2 \log_{10}$ CFU/g against the control group after 96 h at the refrigeration temperature. No differences were found in the TVC and *E. coli* counts due to the bacteriostatic behaviour of lactic acid in all treatments; both colony counts showed no significant differences ($P > 0.05$) compared with the control group.

In conclusion, this study shows that the cross-contamination of sheep carcasses is unavoidable. However, food-grade organic acids, in particular 1% lactic acid, can be used to extend the shelf life of sheep carcasses stored at 4°C, and such substances exert bacteriocidal and bacteriostatic effects on *S. aureus* and *E. coli*, respectively. The odour and colour intensities are less pronounced, and pH is more consistent after 96 h at the refrigeration temperature of 4°C, in comparison to the control and the 2 and 3% lactic acid groups.

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