Research Article

Citric and Lactic Acid Effects on the Growth Inhibition of *E. coli* and *S. typhymurium* on Beef during Storage

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Abstract | Preservative and antibacterial effects of citric acid and lactic acid treated beef were studied previously on *Escherichia coli* O157: H7 and *Salmonella typhymurium*. This was aimed to investigate the effects of these acids on the total viable count (TVC) of these bacteria. Three diluted concentrations (1.0, 3.0 and 5.0%) of both citric acid and lactic acid were used. A total of 3024 meat samples were collected from cattle slaughtered at Peshawar, Pakistan. Samples were processed and analyzed for TVC of *E. coli* and *S. typhymurium* at 0, 48, 144 and 288hrs during 12 days of meat samples refrigeration storage at 4±1 °C. The pH of meat samples were checked and recorded on similar intervals. During 12 days storage, significant reductions (i.e. P <0.05) were found in both *E. coli* O157: H7 and *S. typhymurium* growth when exposed to these treatments. Results revealed that levels of citric and lactic acid concentrations effect were maximum for 5.0%, followed by 3.0% and least for 1.0% on *E. coli* and Salmonella load on beef surfaces. In addition, citric acid was more effective than lactic acid at all concentrations (1.0, 3.0 and 5.0%) in reducing *E. coli* and Salmonella growth. The citric acid at 5.0% was found significantly effective treatment in reducing *E. coli* O157: H7 and *S. typhymurium* population on beef stored samples.

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Introduction

Pakistan beef production increased from 1665 to 1887 tons out of total meat production 3531 tons (Pak Econ Survey, 2015). However, Pakistan is lowest rank in the export of meat as compared to the developed countries. Post slaughter contaminations are due to unhygienic slaughter house floor, poor and almost completely unhygienic means of transportation, open meat cuts display and meat storage (Siraj et al., 2015; Aftab et al., 2011). The main constrains are poor hygiene, lack of meat processing and inefficient meat preservation hurdle technologies (HT) application. In order to enhance beef export potential of Pakistan several HTs can be applied for meat preservation and processing (Lawrie and Ledward, 2006).

Elsewhere scientists have developed preventive measures to decrease microbial load on meat surfaces such as hot water (Gorman et al.,1995) or chlorinated wa-

ter treatments, use of food grade organic acids and salt treatments. These technologies are helpful in reduction in microbial load. Presently scientists focus is on reducing microbial load without having undesirable changes in meat sensory properties (Lawrie and Ledward, 2006). The use of organic acids for meat preservation is important because it is simple, cheap, fast and efficient meat preservation hurdle technology (Corry and Hinton, 1995). Earlier study has proved that the dilute organic acid solutions have no undesirable effects on meat (Greer and Dilts, 1995).

E. coli and Salmonella are present in the animal gastrointestinal tract of the cattle. Poor slaughtering practices normally contaminate the meat in the early stages of slaughtering process. Therefore in present study lactic acid and citric acid was used against two types of highly pathogenic bacteria i.e. *Escherichia coli* O157: H7 and *Salmonella typhymuriumon* beef surface. In this research it was studied that whether the organic acid such as citric acid and lactic acid do reduce the residual growth of bacteria on meat surface. This study helped to understand the concentration of organic acid effects on meat against pathogens like *E. coli* O157: H7 and *S. typhymurium.*

Materials and Methods

Pre-Sampling Steps

Three dilute concentrations (i.e. 1.0, 3.0 and 5.0%) of lactic and citric acid were used. Both citric acid and lactic acid were prepared in sterile distilled water (DW). The pH of solution was determined using a pH meter (Barloworld Scientific Ltd., U.K) while for determination of titrable acidity, titration procedures were performed. Titerable acidity and pH readings of both citric acid and lactic acid were calculated using the protocol described by Cutter and Siragusa (1994) and Yang et al. (2013). *E. coli* O157: H7 and *S. typhymurium* cultures were obtained from laboratory of RMI (Rahman Medical Institute), Peshawar.

Meat Collection

Beef of freshly slaughtered cattle was collected from the local butchers shop in Peshawar. Meat was transported in sterile bags in a cool box to the laboratory of Veterinary Microbiology, Department of Animal Health, The University of Agricultural University Peshawar. A total of 3024 samples (each 10 g) were prepared from 42 beef large size cuts obtained from Peshawar slaughterhouse. Samples were processed and analyzed for total viable count (TVC) of *E. coli* O157: H7 and *S. typhymurium*. Only samples having 75.0 to 80.0% lean meat was processed following the Raftari-et al. (2009). The following procedures were used: All the glassware were disinfected and properly labeled. Bacterial suspensions were prepared for both bacteria i.e. *E. coli* O157:H7 and *S. typhymurium* in distilled water (DW). The cell concentration was adjusted to about 10^3 cells/mL via serial dilution procedure (Benson, 2001). Then meat pieces weighing 10 g each were prepared. Samples were then decontaminated by suspending in hot sterile distilled water at 80°C for about 30 sec (Chowdhury et al., 2006). After decontamination meat samples were immediately packed in sterile plastic bags and kept for a few minutes to reach room temperature. After reaching room temperature samples were individually suspended in respective bacterial suspension for a few seconds (Dorsa et al., 1997). The inoculated samples were then immediately packed in sterile plastic bags and 20 min incubation time was given to individual sample. Certain samples were kept as inoculation control (Dubal et al., 2004). After 20 min attachment time the inoculated samples with selected bacterium was dipped in the respective treatment (acid solution or sour orange juice) for about 15s individually (Bell et al., 1997). As the acid juice treatment completed, all meat samples were packed in sterile plastic bags and stored in refrigerator at 4±1°C. Other replication was also prepared at the same time for both bacteria *E. coli* and *S. typhymurium* (Raftari et al., 2009).

Microbiological Analyses

Microbiological analyses were carried out immediately after acid and or juice treatment at 0, 48, 144 and 288 hrs of 12 days storage. After acid treatment the surface pH of the samples was measured by using the flat probe pH meter at 0, 48, 144 and 288 h of 12 days storage. Each 10 g meat sample was aseptically blended with 90 mL sterile peptone water in a laboratory blender at 0, 48, 144 and 288 h of 12 days refrigeration storage. Performed pour plate culturing for each blended sample. Transferred 1 mL of each blended sample onto empty petri dish and poured standard plate count agar at 45 to 48°C temperature. Another 1 mL of the same blended sample was cultured as a duplicate. All cultured Petri dishes were then incubated for 24 h at 37°C. After 24 h incubation colony count were carried out via colony counter in each sample. *E. coli* was grown as red/pink colonies, while Salmonella exhibited white/colorless

colonies. The difference in color is due to the fact that *E. coli* is a lactose-fermenter while Salmonella is not.

Statistical Design

A 2x3x2 factorial design (CRD) was used for ANOVA and statistical analysis of experimental results through Genstat Discovery edition-3. Where treatments were at two levels and concentrations were at three levels, and two replication of each microorganism were used in the study. The bacterial population obtained was log_{10} transformed. Difference between the effects of control and treated samples was calculated as percent mean *log* reduction (Bjornsdottir et al., 2006; Bell et al., 1997). Statistical analysis compared the concentration effect, on microorganism's decontamination.

Results and Discussion

Lactic acid and citric acid effects on *E. coli* O157: H7 load during storage

Effect of different concentrations of citric and lactic acid on the *E. coli* load on meat surfaces during storage is revealed in Table 1. The various concentrations of citric and lactic acids on the growth of *E. coli* were observed at 0, 48, 144 and 288 h in 12 days of beef samples storage. The bacterial load was highest at 0 h, while lowest on 288 h post treatment for all three concentrations of both the acids. Mean *log* values increased in *E. coli* in 12 days storage. Citric and lactic acid (1.0%) reduced the *E. coli* load on meat surface by 20.93% and 17.44%, respectively, in 288 hours (Table 1). Result revealed that citric acid was 3.5% more effective than lactic acid at 1.0% concentration in reducing *E. coli* on beef samples. Citric acid (3.0%) was about 2.03% more effective than lactic acid 3.0% inhibiting *E. coli* on beef. This study further revealed that both citric acid and lactic acid 5.0% treated meat samples reduced more *E. coli* load at the rate (i.e. 24.42% and 22.38%, respectively) on meat surfaces in 288 hours post inoculation. Citric acid was about 2.04% more effective than lactic acid at 5.0% concentration in reducing *E. coli* on beef samples. In control group no organic acid was applied to the meat surfaces and mean *log* increased noted in *E. coli* during 12 days storage (Table 1).

Citric acid concentrations (1.0, 3.0 and 5.0%) were plotted against controlled meat samples in Figure 1. All citric acid concentrations significantly reduced *E. coli* load in 288 h time (interval). The concentration

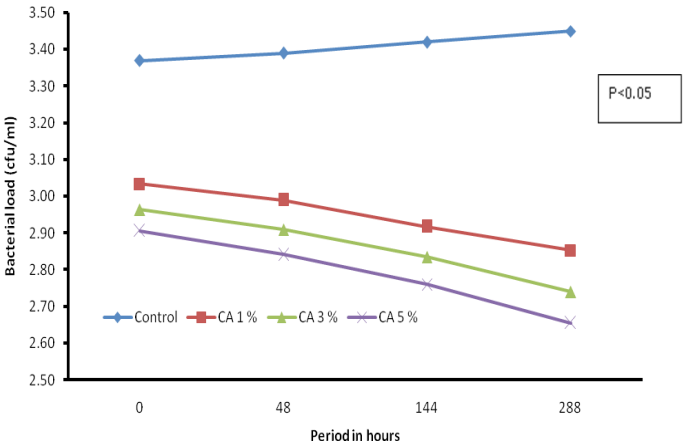

Figure 1: *Decrease in E. coli O157: H7 load in different period at concentration of 1, 3 and 5 % of citric acid on meat samples*

Table 1: *Citric and lactic acid various concentrations effects on mean E. coli O157: H7 load on beef samples at 0, 48, 144 and 288 hours of storage*

Conc.	O.A	Period 00-1.0HR	48HR	144HR	288HR	P- Value
		E. coli Mean* ± S.E	*E. coli* Mean* ± S.E	*E. coli* Mean* ± S.E	*E. coli* Mean* ± S.E	
1.0%	CA	$2.99 \pm 0.01^{a,1}$	$2.92 \pm 0.01^{a,2}$	$2.81 \pm 0.01^{a,3}$	$2.72 \pm 0.02^{a,4}$	
	LA	$3.06 \pm 0.01^{b,1}$	$3.01 \pm 0.01^{b,2}$	$2.92 \pm 0.02^{b,3}$	$2.84 \pm 0.02^{b,4}$	
3.0%	CA	$2.91 \pm 0.01^{c,1}$	$2.86 \pm 0.01^{c,2}$	$2.76 \pm 0.01^{c,3}$	$2.67 \pm 0.02^{c,4}$	
	LA	$2.97 \pm 0.01^{d,1}$	$2.92 \pm 0.01^{a,2}$	$2.83 \pm 0.01^{d,3}$	$2.74 \pm 0.01^{d,4}$	< 0.05
5.0%	CA	$2.84 \pm 0.01^{e,1}$	$2.78 \pm 0.01^{d,2}$	$2.71 \pm 0.01^{e,3}$	$2.60 \pm 0.02^{e,4}$	
	LA	$2.89 \pm 0.01^{f,1}$	$2.84 \pm 0.01^{e,2}$	$2.75 \pm 0.02^{f,3}$	$2.67 \pm 0.01^{f,4}$	
control	NIL	3.34 ± 0.01	3.36 ± 0.01	3.40 ± 0.01	3.44 ± 0.01	
P- Value			< 0.05			

Conc. = *concentration;* **LA** = *lactic acid;* **CA** = *citric acid;* **O. A** = *Organic Acid;* **S.E** = *Standard Error; P- Value in column compared the concentration effect on E. coli O157: H7 load on meat surface, whereas the row p- value explains the significance difference in term of time interval effect on E. coli O157: H7 load; The values within one column if having different superscript means the value is significantly different at α=0.05; The 2nd superscript across the column if different means the value is significantly different at α=0.05; * The Average bacterial load on meat surface was to log_{10} transformed.*

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effects of citric acid were 5.0% higher than 3.0% and of 3.0% higher than 1.0% concentration in reducing *E. coli* population in beef samples Figure 1.

Lactic acid different concentrations (1.0, 3.0 and 5.0%) reduced *E. coli* in 288 h (time interval) in stored beef samples (Figure 2). It is further revealed that 5.0% lactic acid concentration reduced more *E. coli* followed by 3% lactic acid in 288 h time (interval).

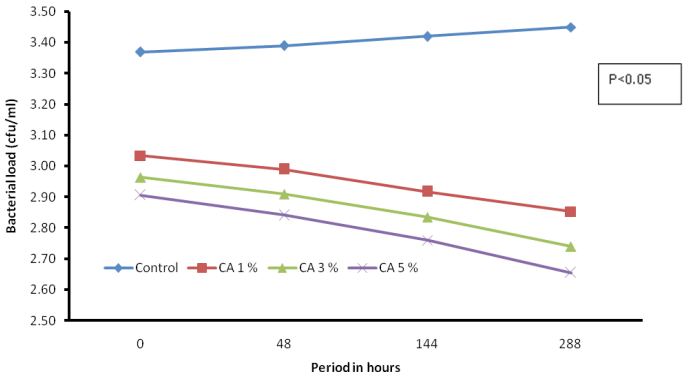

Figure 2: *Decrease in E. coli O157: H7 load in different period at concentration 1, 3 and 5 % of lactic acid on meat samples*

Citric and lactic acid concentration level effects on *S. typhymurium* contamination in beef stored samples

Effects of different concentration of citric and lactic acid on the *log* mean *S. typhymurium* load on surfaces of stored beef samples. The bacterial load was highest on 0 h while lowest on 288 h for all three concentrations of both the acids, with the exception of control samples where no organic acid was applied to the meat surfaces and mean *log* load showed an increase trend in *Salmonella* population during 12 days storage. Citric and lactic acid (1.0%) concentration reduced 17.39 and 15.65% respectively the *S. typhymurium* load on meat surface in 288 h (Table 2). Citric acid was about 1.74% more effective than lactic acid at 1.0% concentration in reducing *Salmonella* population on beef samples. Citric and lactic acid (3.0 %) concentration application on the meat surfaces cut the *S. typhymurium* load by 20.58 and 17.97% respectively. Citric acid was about 2.61% more effective than lactic acid at 3.0% concentration in reducing *Salmonella* population on beef. Increasing the concentration of both citric and lactic acid up to 5.0% added shrink of more *S. typhymurium* load at the rate (i.e. 22.90% and 20.87% respectively) on meat surfaces with the passage of time from 0 to 288 hours. Citric acid was about 2.03% more effective than lactic acid at 5.0% concentration in reducing Salmonella. In control group no organic acid or juice was applied to the meat surfaces and mean *log* increase was noted in *Salmonella* population during 12 days storage (Table 2). The most effective concentration was citric acid (5.0%) in reducing *E. coli* and *Salmonella* population in beef samples. Over all citric acid was more effective than lactic acid in reducing *E. coli* and *Salmonella* load in beef samples. Generally, *E. coli* O157: H7 showed more sensitivity and less resistant to all concentrations (1.0, 3.0 and 5.0%) of citric and lactic acid as compared to *S. typhymurium* in beef samples. Citric acid concentrations (1.0, 3.0 and 5.0%) were observed against controlled samples in Figure 3. All citric acid concentrations (1.0, 3.0 and 5.0%) significantly reduced *Salmonella* population in 288 h time (interval) in beef samples.

Figure 4 revealed that there was a significant difference between treated and untreated (controlled) beef samples. The treatment lactic acid concentrations (1.0, 3.0 and 5.0%) significantly reduced *Salmonella* in 288 h, whereas the controlled meat samples the *Salmonella* load increased at 2.32% rate.

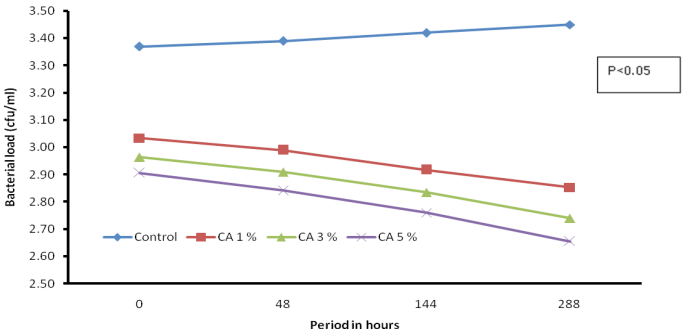

Figure 3: *Decrease in S.typhymurium load in different period at concentration 1, 3 and 5 % of citric acid on meat samples*

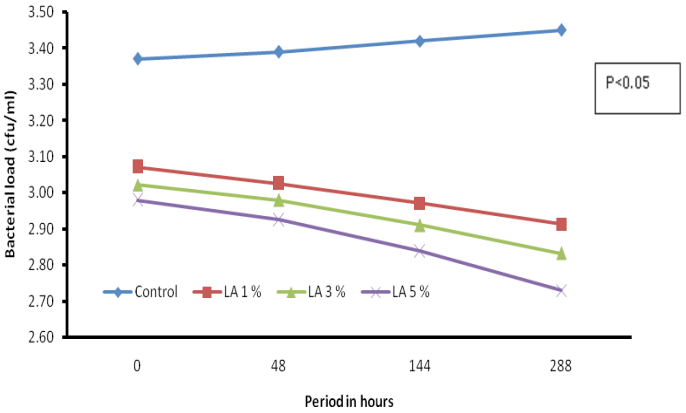

Figure 4: *Decrease in S. typhymurium load in different period at concentration 1, 3 and 5 % of lactic acid on meat samples*

Efficacies of organic acids such as citric acid, lactic acid, acetic acid, propionic acid and ascorbic acid have been

Table 2: *Citric and lactic acid various concentrations effects on mean Salmonella typhymurium load on beef samples at 0, 48, 144 and 288 hours of storage*

Conc.	O.A	Period 0-1.0HR	48HR	144HR	288HR	P-Value
		Salmonella Mean* ±S.E	*Salmonella* Mean* ±S.E	*Salmonella* Mean* ± S.E	*Salmonella* Mean* ± S.E	
1.0%	CA	$3.03 \pm 0.01^{a,1}$	$2.99 \pm 0.01^{a,2}$	$2.92 \pm 0.01^{a,3}$	$2.85 \pm 0.01^{a,4}$	
	LA	$3.07 \pm 0.01^{b,1}$	$3.02 \pm 0.01^{b,2}$	$2.97 \pm 0.01^{b,3}$	$2.91 \pm 0.01^{b,4}$	
3.0%	CA	$2.96 \pm 0.01^{c,1}$	$2.91 \pm 0.01^{c,2}$	$2.83 \pm 0.01^{c,3}$	$2.74 \pm 0.01^{c,4}$	< 0.05
	LA	$3.02 \pm 0.01^{d,1}$	$2.98 \pm 0.01^{d,2}$	$2.91 \pm 0.01^{d,3}$	$2.83 \pm 0.01^{d,4}$	
5.0%	CA	$2.91 \pm 0.01^{e,1}$	$2.84 \pm 0.01^{e,2}$	$2.76 \pm 0.01^{e,3}$	$2.66 \pm 0.01^{e,4}$	
	LA	$2.98 \pm 0.01^{f,1}$	$2.92 \pm 0.01^{f,2}$	$2.84 \pm 0.01^{f,3}$	$2.73 \pm 0.01^{f,4}$	
control	NIL	3.37 ± 0.01	3.39 ± 0.01	3.42 ± 0.01	3.45 ± 0.01	
P-Value			< 0.05			

Conc. = *concentration;* **LA** = *lactic acid;* **CA** = *citric acid;* **O. A** = *Organic Acid;* **S.E** = *Standard Error; P- Value in column compared the concentration effect on E. coli O157: H7 load on meat surface, whereas the row p- value explains the significance difference in term of time interval effect on E. coli O157: H7 load; The values within one column if having different superscript means the value is significantly different at α=0.05; The 2nd superscript across the column if different means the value is significantly different at α=0.05; * The Average bacterial load on meat surface was to log_{10} transformed.*

evaluated by several researchers (Raftari et al., 2009; Castillo et al., 2001; Dorsa, 1996; Cutter et al., 1994 and Anderson and Marshall, 1990). Elsewhere studied have evaluated 0.1 to 24% concentrations of organic acids for their efficacies on red meat. The use of organic acids such as citric, lactic and acetic acid at 1.5 to 2.5% concentrations has been approved (USDA–FSIS, 1996). Bacterial reductions were directly proportional to higher acids concentrations, acids combinations, elevated acid temperature and if the acids applied to adipose tissues (Dickson et al., 1992). The (1.0 to 5.0%) concentrations of organic acids are typically used in reducing microbial load on meat surface (James et al., 1997).

Greer and Dilts (1992) revealed that the organic acid efficacy is proportional to microbial initial load, species of microorganism and type of organic acid. The efficacy of lactic acid inhibition of *E. coli* O157: H7 from meat surface depends upon lactic acid concentration, methods of preparing acid concentration (w/v or w/w), and strains of *E. coli* O157: H7 investigated, times of exposure and acid temperature. Dipping the inoculated meat in hot lactic acid (1.0%) solution at 55°C for a period of 5.0s reduced 0.75 log_{10} of *E. coli O157: H7* load (Podolak et al., 1996). Dipping meat for 5.0s in lactic acid 2.0% concentration at (25.0, 55.0 and 60.0°C) reduced 0.30, 1.00 and 1.00 log_{10}*cfu*/cm^2 (Anderson and Marshall, 1989), respectively. The lactic acid 1.0 to 3.0% concentration reduced *E. coli* O157: H7 load on fresh beef (vacuum packed) stored at 5.0°C to or bellow (0.80 log_{10}*cfu*/cm^2) in 21 days storage (Dorsa et al., 1997). While hot (55°C) lactic acid 1.0% solution decreased *E. coli* O157: H7 load (0.75 log_{10}) on meat surface (vacuum packed) stored at 4.0 °C in 21 days (Podolak et al., 1996). In the present study *E. coli* O157: H7 reduced on beef (vacuum packed at 4±1°C) 0.60, 0.70 and 0.77 *log* 10 *cfu* per ml (Table 1) in 12 days storage, when exposed to lactic acid at 1.0, 3.0 and 5.0% concentrations respectively. While *S. typhymurium* reduced on beef (vacuum packed at 4±1°C) 0.54, 0.62 and 0.72 *log* 10 *cfu* per ml (Table 2) in 12 days storage, when exposed to lactic acid at 1.0, 3.0 and 5.0% concentrations respectively. The beef samples were inoculated in 10^3 cells/mL (i.e. 3.0log_{10} per ml).

Suspensions of *E. coli* O157: H7 or *S. typhymurium* and inoculated meat were then dipped in lactic acid solutions at room temperature (25 °C) in present study. In a research (Castillo et al., 2001) studied the antibacterial effects of 2.0 and 4.0% lactic acid hot solution (i.e. heated at 55 °C) against *E. coli* O157: H7 and *S. typhymurium* on warm and cold carcasses respectively. The results data of above research showed that the lactic acid was effective on cold carcass when applied at 55°C (i.e. at higher temperatures). In the study the pre-chilled beef carcasses were spray washed with water alone and washed with water followed by lactic acid hot solution (55.0 °C) wash for 15.0 s time (interval). While the post-chilled beef carcasses were spray washed with hot lactic acid solution (55.0 °C) for 30.0 s time period. In the pre-chilled beef carcasses both pathogens, *E. coli* O157: H7 and *S. typhymurium* were reduced 3.30 to 3.40 *log* (water washed alone) and 5.20 *log* (water & lactic acid wash). In the

post-chilled beef carcasses *E. coli* O157: H7 reduced (2.0 to 2.40 *log*) while *S. typhymurium* reduced (1.60 to 1.90 *log*). The above research revealed that organic acid could be efficiently used on post-chilled carcasses. In a study conducted by (Cutter et al., 1994) the LRF (*log* reduction factor) of *E. coli* O157: H7 on lean BCT (beef carcass tissue) showed 1.21, 1.77 and 1.88 *cfu* per cm^2, when exposed to citric acid at 1.0, 3.0 and 5.0% concentration respectively, and showed 1.00, 1.76 and 2.60*cfu* per cm^2, when exposed to lactic acid at 1.0, 3.0 and 5.0% concentration respectively. The study explained that, for *E. coli* O157: H7 a greater than 1 log_{10} reduction can only be expected when concentration of citric and lactic acid was (5.0%). In current study the mean *log* reductions of *E. coli* O157: H7 on beef showed 0.72, 0.77 and 0.84 *log* 10 *cfu* per ml (Table 1), when exposed to citric acid at 1.0, 3.0 and 5.0% concentrations respectively. While the mean *log* reductions of *E. coli* O157: H7 on beef showed 0.60, 0.70 and 0.77 *log* 10 *cfu* per ml (Table 1), when exposed to lactic acid at 1.0, 3.0 and 5.0% concentrations respectively. The results of present study were in agreement with the above mentioned study in the sense that there was significant ($p<0.05$) difference in concentration (i.e. 1.0, 3.0 and 5.0%) effects of citric and lactic acid on mean *log* reductions of *E. coli* O157: H7 population. Citric and lactic acid concentration effects were 5.0% > 3.0% >1.0% in reducing *E. coli* O157: H7population on beef. In an another study recently conducted by (Raftari et al., 2009) the mean *log* reduction of *E. coli* O157: H7 on beef surface was 1.08 *log* 10 *cfu* per mL, when exposed to lactic acid at (1.0%) concentrations at room temperature (25˚C). In current study the mean *log* of *E. coli* O157: H7 reduced by lactic acid (1.0%) concentration on beef, was 0.60 log_{10}*cfu* per mL (Table 1) at room temperature (25˚C). In the present study the *E. coli* O157: H7 load reduction of lactic acid (1.0%) concentration was about 0.48 log_{10}units lower than *E. coli* O157: H7 reduction in the mentioned research (Raftari et al., 2009). In a study conducted by Anderson and Marshall (1990) reported that the application of dilute organic acid solution on meat is the additional defense against microbes along with good hygienic measures, must be observed strictly at meat slaughtering and processing. The researchers further found that the application of hot (70˚C) lactic acid solution (3.0%) for meat preservation could reduce 1.10 and 1.80 log_{10}units of *E. coli* O157: H7 and APC (Aerobic Plate Count) on beef respectively. While the application of lactic acid (3.0%) solution at room temperature (25˚C) could reduce 0.40 and 1.20 log_{10}units of *E. coli* O157: H7 and APC (Aerobic Plate Count) on beef respectively. In current study the mean *log* of *E. coli* O157: H7 reduced by lactic acid (3.0%) concentration on beef, was 0.70 log_{10}*cfu* per ml (Table 1) at room temperature (25˚C). In present study the *E. coli* O157: H7 load reduction of lactic acid (3.0%) concentration was about 0.30 log_{10}units higher than *E. coli* O157: H7 reduction in the mentioned research (Siragusa, 1995; Anderson and Marshall, 1990).

Conclusions

Both citric acid and lactic acid has reduced the growth/viability of *E. coli* and S. typhymurium load on beef surface (22.60%, 20.10%) and (20.30%, 18.20%), respectively. The inhibitive effects of 5 % concentration were highest, followed by 3 % concord least for 1 % conc. of both citric and lactic acid. The citric acid effects were significantly higher ($p<0.05$) than lactic acid at all concentration (1.0, 3.0 and 5.0%). It is recommended that citric acid 5% should be used in Pakistani slaughtering environment.

Authors' Contribution

Gultaj Hussain and Abdur Rahman contributed equally and carried out the execution of research activity such as samples collection, and writing initial draft, while T. Hussain helped in apparatus setting and facilitation in preparing the 1, 3 and 5 percent solutions and inoculation of samples. Siraj Uddin helped in preparation of 10 folds dilution and also helped in the identification of *E. coli*, whereas Tariq Ali confirmed the growth *S. typhymurium* colonies in the studied beef samples. He also helped in analysing the results and in writing and revising the manuscript.

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