

Original Research Article

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## Effect of Chemical Decontaminants on Quality of Buffalo Heart meat

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### ABSTRACT

In this experiment, the effect of dipping buffalo heart meat in 2% Lactic Acid (LA) for 10 sec., 150 ppm Chlorinated water (CW) for 5 sec and 10% Trisodium Phosphate (TSP) for 5 min., on its quality characteristics were assessed. Tap water washed heart meat samples were used as control. The control and treated samples were stored at refrigerated temperature ( $4\pm 1^\circ\text{C}$ ) until visible signs of spoilage observed as indicated by color and odor changes. The treated and control heart meat samples were analyzed for the effect of decontaminants on various inherent microorganisms, inoculated pathogens, sensory and physio-chemical characteristics, at regular intervals. Result revealed that 2% LA and 10% TSP treatments were equally and significantly effective ( $p\leq 0.01$ ) in reducing total viable bacteria, coliforms, staphylococcal and *E. coli* counts on heart meat samples immediately after the treatment compared to control and 150 ppm CW. Inoculation studies revealed that 10% TSP was significantly effective in reducing *Listeria monocytogenes* and Verotoxigenic *E. coli* counts, 2% LA was effective in reducing *Campylobacter* count whereas both 2% LA and 10% TSP were equally and significantly effective in reducing *Salmonella* and *Aeromonas* counts on buffalo heart meat samples immediately after the treatment. Sensory scores for odour and colour were significantly lower ( $p\leq 0.01$ ) for all treated samples than control. Among treatments, odour and colour scores of 150 PPM chlorinated water and 10% TSP treated samples were equally and significantly higher than that of 2% LA treated samples.

#### Keywords

Buffalo heart meat;  
lactic acid;  
chlorinated water;  
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### Introduction

Organ meats are defined as animal products obtained from food animals and poultry and are not a part of dressed carcasses. The nutritionally rich meats often described as

‘edible byproducts’, ‘offal’, ‘variety meats’ and sometimes as ‘super food’ due to dense source of some of the vitamins and minerals. The yield of byproducts (both edible and inedible) as a whole ranges from 20 to 30 percent of live weight for beef, pork and lamb

(Ockerman and Hansen, 1988) and contributes to 7 to 12 percent of the total income in meat industry (Bengtsson and Holmqvist, 1984). Naik (2002) reported the average yields of edible and inedible offal from buffaloes as 10.61 and 23.31 percent of live weight, respectively. Ever increasing demand for animal proteins and high cost of meat have directed emphasis on maximum utilization of different edible meat byproducts. Several studies on buffalo offal (Kondaiah *et al.*, 1986; Krishnan and Sharma, 1991; Anna Anandh, 2001) have indicated that edible meat byproducts could replace a significant portion of lean meat in processed meat products, if proper technology is developed.

Variety meat includes such as heart, tripe, tongue, brains, liver, kidneys and sweetbreads. Heart is an edible muscular organ, rich in folate, iron, zinc, selenium and B complex vitamins. Miller (2004) recorded the mean weight of heart of buffaloes as 1175 gm whereas Panhwar *et al.*, (2007) reported that the average weight of heart from ox is about 2.5 kg or ranging 0.4 to 0.5% of the total body weight.

During slaughter and processing of food animals, all potentially edible tissues including organ meats are subjected to contamination from a variety of sources within and outside the animal (Ayres, 1955). Due to readily available nutrients and poor hygienic conditions during handling, collection and processing, offal generally spoil faster than meat from skeletal muscles. Further, pathogenic microorganisms are the major safety concern for the meat industry. In general, microbial contamination of offal results in economic loss and may lead to public health concerns when consumed or used as ingredient in processed meat products. If microbes on the surface of meat and offal could be eliminated or substantially reduced immediately after slaughter, the risk of cross

contamination during processing would be substantially reduced.

Several methods have been developed for bacterial reduction on carcasses which include trimming (Prasai *et al.*, 1995a,b), water washing (Hardin *et al.*, 1995; Reagan *et al.*, 1996), hot water spraying (Smith and Graham, 1978), steam pasteurizing (Phebus *et al.*, 1997), as well as sanitizing by chemicals such as organic acids (Dorsa *et al.*, 1997b), choline compounds (Kotula *et al.*, 1974), polyphosphates (Dickson *et al.*, 1994) and disinfectants. Although there are several studies on decontamination of carcasses, only limited reports are available on decontamination of offal (Patterson and Gibbs, 1979; Woolthuis *et al.*, 1984; Delmore *et al.*, 2000). These studies on carcasses and offal indicate that an efficient method of surface decontamination provides an additional barrier protection beyond low temperature control and, thereby offers substantial advantages in terms of food safety, spoilage and economics. Considering these points, in the present study, lactic acid, sodium hypochlorite and trisodium phosphate were used to evaluate their efficacy as a decontaminant by determining their effect on intrinsic microbial flora, sensory and physiochemical characteristics of buffalo heart. Apart from this, the effects of these decontaminants were also tested on various food borne pathogens of public health importance by artificial spiking studies.

## **Materials and Methods**

### **Collection and packaging of buffalo heart samples**

Buffalo heart samples were collected, on separate days, from offal market of Bareilly (India) within 3 to 4 hours of slaughter. Collected samples were placed immediately into sterile polyethylene bags and held on ice during their transport to the laboratory.

## Decontaminants used

Immersion treatments applied to reduce contamination of the heart samples included Lactic acid (LA) (2% vol/vol, pH 2.5, 37°C prepared from LA, Himedia), chlorine (Sodium hypochlorite, SHC, 0.015% vol/vol or 150 ppm, 37°C, pH adjusted to 6.5, Ranbaxy) and Trisodium phosphate (TSP) (10% wt/vol, pH 12.65, 45°C, prepared from trisodium phosphate dodecahydrate, Merck). Tap water (sterile) washed heart samples were maintained as control. Concentration-contact time combination for each decontaminant utilised in the present study was based on the results obtained through preliminary experiments (Selvan *et al.*, 2007a, b & c).

## Bacterial strains and culture conditions

*Listeria monocytogenes* MTCC 1143 4(b), *Aeromonas hydrophila* MTCC 646, *Salmonella Typhimurium*, field isolates of Verotoxigenic *E. coli* and *Campylobacter jejuni* were obtained from the Division of Veterinary Public Health, Indian Veterinary Research Institute (IVRI, India). Except for *C. jejuni*, stock cultures of each pathogen were grown aerobically in Brain Heart Infusion broth (BHI) at 37 °C for 24 h (*Salmonella Typhimurium* and *L. monocytogenes*) or at 35 °C for 24 h (Verotoxigenic *E. coli*) or at 30 °C for 24 h (*Aeromonas hydrophila*) for the preparation of inoculum whereas stock cultures of *C. jejuni* were grown in BHI broth supplemented with 6% defibrinated horse blood and incubated at 42 °C for 48 h under microaerophilic conditions. 10 ml of each overnight grown culture (pathogen) was individually centrifuged at 8000 rpm for 10 min at 4 °C to harvest cells. The harvested cells were washed twice with Phosphate Buffer Saline (PBS) by centrifugation at 5000 g for 10 min. Final cell pellet was suspended in the same buffer and the concentrations of cells were adjusted to 10<sup>4</sup> cells/ml using

Mcfarland's turbidity method. Viable cell counts from individual dilutions were confirmed by retrospective spread plating onto BHI agar plates.

## Effect of decontaminants on intrinsic microbial flora, sensory and physio-chemical characteristics

### Decontamination procedure

On the day of collection, heart samples obtained from each animal was hygienically portioned into pieces of 100 g and the pieces were divided into four groups each containing four. Heart pieces from the three groups were separately immersed in solutions of 2% LA, 0.015% SHC and 10% TSP for 10 s, 5 s and 5 min, respectively, whereas heart pieces from fourth group were washed with tap water and maintained as control(s). The ratio of heart pieces and dipping/washing solutions was 1:4 (wt/vol). Treated and control pieces were drained and packed separately in sterile polyethylene bags. Then, they were stored at refrigerated, aerobic (4±1°C) conditions until discernible signs of spoilage appeared as indicated by odor and odour changes. The control and treated pieces were analysed 45 min after the treatments as well as on day 4, 8 and 12 of refrigerated storage for certain microbial, sensory and physio-chemical characteristics. One piece from each group was drawn for analysis on each occasion.

### Microbiological analysis

Microbiological quality of control and treated heart pieces was evaluated based on the counts of total viable bacteria (Total viable count), Coliforms, *Staphylococcus* and *Escherichia coli*. All microbial groups except *Escherichia coli* were determined by pour plate method, following the procedures of APHA (1984) whereas *E. coli* counts were determined using spread plate method. Five grams from each

piece was aseptically blended with 45 ml of 0.1% sterile peptone water in a pre-sterilised mortar. Decimal dilutions were prepared from the blended samples using sterile 0.1% peptone water. For the counts, one ml from each of the serially diluted homogenate was inoculated in duplicate, using appropriate growth media, in sterile petri dishes by pour plate method. Inocula on plate count agar were incubated at  $37\pm 1^{\circ}\text{C}$  for 48 h under aerobic conditions to assess the total viable counts. Enumeration of coliforms was carried out on Violet Red Bile Agar incubated at  $37\pm 1^{\circ}\text{C}$  for 24 h aerobically. Staphylococci were enumerated on Baird Parker agar supplemented with egg yolk tellurite emulsion incubated at  $37\pm 1^{\circ}\text{C}$  for 48 h under aerobic condition. A quantity of 0.1 ml of appropriate decimal dilutions plated on Mac Conkey's agar using spread plate technique were incubated at  $35\pm 1^{\circ}\text{C}$  for 48 h to enumerate *E. coli* colonies. The presumptive colonies were determined by counting the number of pinkish sharp colonies with about 0.5 mm diameter. Colonies judged to be borderline cases were also counted. The average numbers of colonies were expressed as  $\log_{10}$  cfu/g of heart samples.

### **Sensory analysis**

The effect of treatments on the colour and odour of heart pieces were noted during the refrigerated, aerobic ( $4\pm 1^{\circ}\text{C}$ ) storage by a sensory evaluation panel comprising post graduate students and scientists of Livestock Products Technology division (IVRI, India). The six point sensory scale as described by Anna Anandh (2001) was used for scoring colour and odour of heart pieces with modifications.

### **Physio chemical analysis**

Control and treated heart pieces were evaluated for physiochemical characteristics

like pH, Extract Release Volume (ERV) and Titratable acidity (only for 2% LA treated samples) during refrigerated storage at ( $4\pm 1^{\circ}\text{C}$ ). The pH-value of the buffalo heart pieces were determined by homogenising 10 g of sample with 50ml distilled water in an Ultra Turrex (IKA, Model T-25, Germany) homogeniser for one min at 3000 rpm. pH of the suspension was recorded by immersing the combined glass electrode of digital pH merer (Model CP-901, Century Instruments Ltd., India). The method described by Konecko (1979) was used with some modifications for estimation of titratable acidity. Similarly, Extract Release Volume (ERV) was estimated using the procedure described by Jay, 1964.

### **Effect of decontaminants on inoculated pathogens**

Experimental inoculation studies were carried out to compare the effect of decontaminants on pathogens (*Listeria monocytogenes*, *Aeromonas hydrophila*, *Salmonella Typhimurium*, verotoxigenic *E. coli* and *Campylobacter jejuni*) inoculated in buffalo heart samples. For this purpose, randomly collected buffalo heart samples were screened for the presence of above mentioned pathogens and the negative samples were used for respective experimental inoculation studies. To compare the effect of decontaminants on Verotoxigenic *E. coli*, heart pieces were steamed for 10 min at  $100^{\circ}\text{C}$ , prior to use in the study.

### **Inoculation of samples**

Buffalo heart samples negative for *L. monocytogenes* was portioned into pieces of five gram and were divided into four groups each containing five. Then, the pieces were separately placed in sterile polypropylene sachets/HiDispo bags (Hi media). Each piece of heart in all groups was individually inoculated with *L. monocytogenes* at a

concentration of  $10^4$  cells per gram of sample. After inoculation, the pieces were kept for 30 min at room temperature to allow the bacteria to attach. All the procedures were carried out aseptically to avoid any contamination.

### **Decontamination procedure**

Listeria inoculated heart pieces from the three groups were separately immersed in solutions of 2% LA, 0.015% SHC and 10% TSP for 10 s, 5 s and 5 min, respectively whereas heart pieces from fourth group were washed with tap water and maintained as control(s).

After immersion, pieces (both control and treated) were drained and packed independently in sterile polyethylene bags. Then, they were stored under refrigerated, aerobic ( $4\pm 1^\circ\text{C}$ ) conditions. The control and treated samples were evaluated 45 min after the treatment as well as on day 3, 6, 9 and 12 of refrigerated storage for *L. monocytogenes* count. One sample from each group was drawn for analysis on each occasion.

### **Microbiological analysis**

Each inoculated heart pieces was aseptically blended with 45 ml of 0.1% sterile peptone water in a pre-sterilised mortar. Decimal dilutions in sterile 0.1% peptone water were prepared from the blended samples and 0.1 ml volumes were spread in duplicate onto Dominguez-Rodriguez agar and incubated at  $37^\circ\text{C}$  for 48 h. The typical colonies of *L. monocytogenes* (appeared as greenish yellow, glistening, iridescent and pointed colonies of about 0.5 mm surrounded by diffused black zone of aesculin by hydrolysis) were counted and expressed as  $\log_{10}$  cfu/g of sample.

Similar procedure was followed in duplicate using other pathogens with the inoculation

level of  $10^4$  cells/g of sample. Then, they were enumerated in appropriate growth media using spread plate technique. Enumeration of *Salmonella Typhimurium*, verotoxigenic *E. coli* and *Aeromonas hydrophila* were carried out on Bismuth Sulphite agar at  $37^\circ\text{C}$  for 24 h, Mac Conkey agar at  $35^\circ\text{C}$  for 24 h and Ampicillin Dextrin agar at  $37^\circ\text{C}$  for 24 h, respectively. Colonies of *C. jejuni* were enumerated in modified Park and Sanders agar at  $43^\circ\text{C}$  for 48 h under microaerophilic conditions. Minimum five trials were conducted using each pathogen.

### **Statistical analysis**

The data obtained were analysed using standard statistical procedures (Snedecor and Cochran, 1994). Analysis of variance (ANOVA) procedure was used to determine the significant difference ( $p\leq 0.01$ ) among means obtained for different treatments.

## **Results and Discussion**

### **Effect on intrinsic microbial flora**

Total viable count (TVC), *Coliforms*, *Staphylococcal* and *E. coli* counts of treatment groups were significantly lower ( $p\leq 0.01\%$ ) than that of tap water washed control (Table. 1). At day zero, 2% LA and 10% TSP treatments elicited greatest ( $p\leq 0.01\%$ ) reduction in all the microbial groups studied compared to 150 ppm CW and was maintained throughout the storage period studied. The level of reduction did not significantly differ between 2% LA and 10% TSP. The results of the present study is in accordance with Delmore *et al.*, (2000) who also observed similar reduction in aerobic plate, coliforms and *E. coli* counts of beef cheek meats immersed in 2% LA for 10 s as well as that immersed in 12% TSP for 10 s.

**Table.1** Effect of different decontaminants on microbial quality of buffalo heart during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
<b>Total viable count</b>					
0 <sup>th</sup> day	5.38±0.08 <sup>dA</sup>	4.24±0.07 <sup>dC</sup>	4.55±0.07 <sup>dB</sup>	4.31±0.05 <sup>dC</sup>	<b>4.62±0.11<sup>d</sup></b>
4 <sup>th</sup> day	6.15±0.08 <sup>cA</sup>	5.16±0.04 <sup>cC</sup>	5.50±0.06 <sup>cB</sup>	5.30±0.10 <sup>cBC</sup>	<b>5.53±0.09<sup>c</sup></b>
8 <sup>th</sup> day	7.02±0.06 <sup>bA</sup>	5.81±0.04 <sup>bC</sup>	6.24±0.02 <sup>bB</sup>	6.06±0.06 <sup>bC</sup>	<b>6.29±0.11<sup>b</sup></b>
12 <sup>th</sup> day	7.93±0.06 <sup>aA</sup>	6.75±0.03 <sup>aC</sup>	7.03±0.06 <sup>aB</sup>	6.83±0.06 <sup>aC</sup>	<b>7.13±0.11<sup>a</sup></b>
<b>Treatment Mean±SE</b>	<b>6.62±0.22<sup>A</sup></b>	<b>5.49±0.21<sup>D</sup></b>	<b>5.83±0.21<sup>B</sup></b>	<b>5.62±0.22<sup>C</sup></b>	
<b>Coliform count</b>					
0 <sup>th</sup> day	4.95±0.23 <sup>cA</sup>	3.49±0.23 <sup>cC</sup>	4.33±0.23 <sup>cAB</sup>	3.68±0.24 <sup>cBC</sup>	<b>4.11±0.17<sup>d</sup></b>
4 <sup>th</sup> day	5.41±0.21 <sup>bcA</sup>	3.97±0.27 <sup>bcB</sup>	4.96±0.24 <sup>bcA</sup>	4.11±0.24 <sup>bcB</sup>	<b>4.61±0.18<sup>c</sup></b>
8 <sup>th</sup> day	5.89±0.20 <sup>abA</sup>	4.41±0.28 <sup>abB</sup>	5.49±0.24 <sup>abA</sup>	4.61±0.24 <sup>abB</sup>	<b>5.10±0.18<sup>b</sup></b>
12 <sup>th</sup> day	6.33±0.20 <sup>aA</sup>	4.87±0.27 <sup>aB</sup>	5.94±0.23 <sup>aA</sup>	5.08±0.26 <sup>aB</sup>	<b>5.56±0.18<sup>a</sup></b>
<b>Treatment Mean±SE</b>	<b>5.64±0.15<sup>A</sup></b>	<b>4.19±0.17<sup>C</sup></b>	<b>5.18±0.10<sup>B</sup></b>	<b>4.37±0.17<sup>C</sup></b>	
<b>Staphylococcal count</b>					
0 <sup>th</sup> day	5.18±0.09 <sup>bA</sup>	4.24±0.08 <sup>dC</sup>	4.59±0.07 <sup>dB</sup>	4.29±0.05 <sup>dC</sup>	<b>4.58±0.09<sup>d</sup></b>
4 <sup>th</sup> day	5.52±0.09 <sup>bA</sup>	4.69±0.12 <sup>cC</sup>	5.11±0.08 <sup>cB</sup>	4.64±0.03 <sup>cC</sup>	<b>4.99±0.09<sup>c</sup></b>
8 <sup>th</sup> day	5.98±0.09 <sup>aA</sup>	5.16±0.13 <sup>bC</sup>	5.59±0.11 <sup>bB</sup>	5.12±0.04 <sup>bC</sup>	<b>5.46±0.09<sup>b</sup></b>
12 <sup>th</sup> day	6.23±0.24 <sup>aA</sup>	5.63±0.12 <sup>aB</sup>	6.07±0.11 <sup>aAB</sup>	5.67±0.07 <sup>aB</sup>	<b>5.90±0.09<sup>a</sup></b>
<b>Treatment Mean±SE</b>	<b>5.73±0.11<sup>A</sup></b>	<b>4.93±0.13<sup>C</sup></b>	<b>5.34±0.10<sup>B</sup></b>	<b>4.93±0.12<sup>C</sup></b>	
<b>E. coli count</b>					
0 <sup>th</sup> day	2.93±0.08 <sup>bA</sup>	2.20±0.07 <sup>cC</sup>	2.53±0.06 <sup>cB</sup>	2.13±0.05 <sup>cC</sup>	<b>2.45±0.08<sup>d</sup></b>
4 <sup>th</sup> day	3.11±0.08 <sup>bA</sup>	2.35±0.07 <sup>bcC</sup>	2.67±0.05 <sup>bcB</sup>	2.29±0.05 <sup>bcC</sup>	<b>2.61±0.08<sup>c</sup></b>
8 <sup>th</sup> day	3.37±0.08 <sup>aA</sup>	2.50±0.07 <sup>abC</sup>	2.83±0.06 <sup>abB</sup>	2.43±0.06 <sup>abC</sup>	<b>2.78±0.09<sup>b</sup></b>
12 <sup>th</sup> day	3.51±0.06 <sup>aA</sup>	2.67±0.06 <sup>aC</sup>	2.99±0.06 <sup>aB</sup>	2.57±0.05 <sup>aC</sup>	<b>2.94±0.09<sup>a</sup></b>
<b>Treatment Mean±SE</b>	<b>3.23±0.06<sup>A</sup></b>	<b>2.43±0.05<sup>C</sup></b>	<b>2.76±0.05<sup>B</sup></b>	<b>2.36±0.04<sup>C</sup></b>	

Means with common superscripts in a row (capital alphabets) and in a column (small alphabets) for a given organism do not differ significantly (p<0.01).

### Effect on sensory characteristics

The effect of treatments on the colour and odour of heart samples were noted 45 min after the treatment and on day 4 of refrigerated, aerobic (4±1 °C) storage (Table 2). As the control(s) have spoiled, sensory evaluation could not be conducted on day 8

and 12 of storage. In general, treatments resulted in significantly lower colour and odour scores than that of control which may be attributed to the effect of respective decontaminants. S150 ppm chlorinated water and 10% TSP treated samples had significantly higher odour and colour scores compared to 2% LA treated samples. Similar

findings were also reported by various researchers. Ravindranath (1994) also observed reduction in odour scores of buffalo meat treated with 2% LA. Saoji *et al.*, (1990) also observed reduction in odour scores of buffalo meat streaks prepared from 4% LA treated buffalo carcasses. Snijders *et al.*, (1979) also observed slight reversible discolouration in beef carcasses sprayed with 1% LA. Delmore *et al.*, (2000) also reported that immersion of variety meats in decontaminants had greater effect on the colour than spraying. Similarly, Capita *et al.*, (2000) observed reduction in odour, colour and overall acceptability scores of chicken thighs treated with 12% TSP for 15 min.

**Effect on pH, Extract Release Volume (ERV) and titratable acidity**

The effect of treatments on pH, weight loss/gain, ERV and titratable acidity (for LA treated samples only) are presented in Table 3. pH values of treatments differed significantly ( $p \leq 0.01$ ) from that of control (Tab. 3). Among treatments, 2% LA and 10% TSP treatments resulted in significantly lower and higher

( $p \leq 0.01$ ) pH-values, respectively. Dorsa *et al.*, (1998) also observed similar patterns of lower and higher surface pH values of beef carcass tissues (BCT) washed with 2% LA and 12% TSP, respectively. Similarly, King *et al.*, (2012) also observed the lower pH-values in all the pig variety meats immediately after spraying with LA. Extract release volume of treatments were significantly ( $p \leq 0.01$ ) different from that of control. Among treatments, 2% LA and 10% TSP treatments elicited significantly ( $p \leq 0.01$ ) higher and lower ERV, respectively. The reduction of pH by LA treatment might have contributed to increase in the ERV by reducing hydration of proteins. Conversely, TSP would have favoured the hydration of proteins by increasing the pH of treated samples thereby led to decrease in ERV. Titratable acidity values (ml of 0.01N NaOH/g of sample) for control and 2% LA treated samples were 0.81 and 1.03, respectively; However, the values did not significantly differ among them. Factors that were responsible for lowering the pH would have contributed to the increase in titratable acidity.

**Table.2** Effect of different decontaminants on sensory characteristics of buffalo heart during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
<b>Odour score</b>					
0 <sup>th</sup> day	5.00±0.00 <sup>A</sup>	4.10±0.12 <sup>C</sup>	4.52±0.11 <sup>B</sup>	4.29±0.12 <sup>BC</sup>	<b>4.48±0.06</b>
4 <sup>th</sup> day	5.00±0.00 <sup>A</sup>	4.43±0.11 <sup>C</sup>	4.79±0.07 <sup>AB</sup>	4.62±0.10 <sup>BC</sup>	<b>4.71±0.05</b>
<b>Treatment Mean±SE</b>	<b>5.00±0.00<sup>A</sup></b>	<b>4.26±0.08<sup>D</sup></b>	<b>4.65±0.07<sup>B</sup></b>	<b>4.45±0.08<sup>C</sup></b>	
<b>Colour score</b>					
0 <sup>th</sup> day	5.00±0.00 <sup>A</sup>	4.24±0.14 <sup>C</sup>	4.57±0.15 <sup>BC</sup>	4.67±0.14 <sup>AB</sup>	<b>4.62±0.07</b>
4 <sup>th</sup> day	5.00±0.00 <sup>A</sup>	4.50±0.12 <sup>B</sup>	4.69±0.12 <sup>AB</sup>	4.71±0.14 <sup>AB</sup>	<b>4.73±0.06</b>
<b>Treatment Mean±SE</b>	<b>5.00±0.00<sup>A</sup></b>	<b>4.37±0.09<sup>C</sup></b>	<b>4.63±0.09<sup>B</sup></b>	<b>4.69±0.10<sup>B</sup></b>	

Means with common superscripts in a row (capital alphabets) do not differ significantly ( $p < 0.01$ ).

**Table.3** Effect of different decontaminants on physicochemical parameters of buffalo heart during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
<b>pH</b>					
0 <sup>th</sup> day	5.83±0.03 <sup>dC</sup>	4.81±0.05 <sup>dD</sup>	6.24±0.04 <sup>dB</sup>	8.86±0.04 <sup>dA</sup>	<b>6.44±0.34<sup>d</sup></b>
4 <sup>th</sup> day	6.17±0.03 <sup>cA</sup>	5.93±0.03 <sup>cB</sup>	6.01±0.04 <sup>cB</sup>	6.00±0.04 <sup>cB</sup>	<b>6.03±0.03<sup>c</sup></b>
8 <sup>th</sup> day	6.52±0.03 <sup>bA</sup>	6.25±0.04 <sup>bC</sup>	6.42±0.04 <sup>bAB</sup>	6.32±0.03 <sup>bBC</sup>	<b>6.38±0.03<sup>b</sup></b>
12 <sup>th</sup> day	6.87±0.03 <sup>aA</sup>	6.64±0.04 <sup>aC</sup>	6.77±0.03 <sup>aAB</sup>	6.69±0.03 <sup>aBC</sup>	<b>6.74±0.03<sup>a</sup></b>
<b>Treatment Mean±SE</b>	<b>6.35±0.09<sup>B</sup></b>	<b>5.91±0.16<sup>C</sup></b>	<b>6.36±0.07<sup>B</sup></b>	<b>6.97±0.26<sup>A</sup></b>	
<b>Weight loss/gain</b>					
0 <sup>th</sup> day	100.80±0.24 <sup>aB</sup>	100.46±0.26 <sup>aB</sup>	100.94±0.29 <sup>aB</sup>	103.03±0.45 <sup>aA</sup>	<b>101.31±0.27<sup>a</sup></b>
4 <sup>th</sup> day	98.80±0.23 <sup>bB</sup>	98.67±0.20 <sup>bB</sup>	98.27±0.28 <sup>bB</sup>	101.27±0.26 <sup>bA</sup>	<b>99.25±0.29<sup>b</sup></b>
8 <sup>th</sup> day	96.32±0.21 <sup>cB</sup>	96.87±0.32 <sup>cB</sup>	96.29±0.37 <sup>cB</sup>	99.01±0.21 <sup>cA</sup>	<b>97.12±0.29<sup>c</sup></b>
12 <sup>th</sup> day	95.29±0.20 <sup>dB</sup>	95.68±0.31 <sup>dB</sup>	95.06±0.41 <sup>dB</sup>	97.27±0.27 <sup>dA</sup>	<b>95.82±0.24<sup>d</sup></b>
<b>Treatment Mean±SE</b>	<b>97.80±0.50<sup>B</sup></b>	<b>97.92±0.43<sup>B</sup></b>	<b>97.64±0.53<sup>B</sup></b>	<b>100.15±0.52<sup>A</sup></b>	
<b>Extract Release Volume (ERV)</b>					
0 <sup>th</sup> day	44.14±0.54 <sup>aB</sup>	52.40±1.53 <sup>aA</sup>	42.90±0.48 <sup>aB</sup>	31.80±0.51 <sup>bC</sup>	<b>42.81±1.73<sup>a</sup></b>
4 <sup>th</sup> day	33.04±0.44 <sup>bC</sup>	42.14±0.50 <sup>bA</sup>	37.72±0.79 <sup>bB</sup>	37.02±0.51 <sup>aB</sup>	<b>37.48±0.79<sup>b</sup></b>
8 <sup>th</sup> day	20.66±0.51 <sup>cD</sup>	33.42±0.55 <sup>cA</sup>	23.18±0.43 <sup>cC</sup>	28.38±0.60 <sup>cB</sup>	<b>26.41±1.15<sup>c</sup></b>
12 <sup>th</sup> day	08.84±0.70 <sup>dC</sup>	22.08±1.31 <sup>dA</sup>	09.80±0.49 <sup>dC</sup>	18.94±0.87 <sup>dB</sup>	<b>14.91±1.37<sup>d</sup></b>
<b>Treatment Mean±SE</b>	<b>26.67±3.05<sup>C</sup></b>	<b>37.51±2.61<sup>A</sup></b>	<b>28.40±2.98<sup>B</sup></b>	<b>29.04±1.54<sup>B</sup></b>	
<b>Titrateable acidity</b>					
0 <sup>th</sup> day	0.88±0.03 <sup>a</sup>	1.33±0.04 <sup>a</sup>	-	-	<b>1.10±0.08<sup>a</sup></b>
4 <sup>th</sup> day	0.86±0.02 <sup>a</sup>	1.08±0.04 <sup>b</sup>	-	-	<b>0.97±0.04<sup>b</sup></b>
8 <sup>th</sup> day	0.81±0.02 <sup>ab</sup>	1.03±0.03 <sup>b</sup>	-	-	<b>0.92±0.04<sup>bc</sup></b>
12 <sup>th</sup> day	0.78±0.02 <sup>b</sup>	0.99±0.03 <sup>b</sup>	-	-	<b>0.88±0.04<sup>c</sup></b>
<b>Treatment Mean±SE</b>	<b>0.83±0.01</b>	<b>1.11±0.03</b>	<b>-</b>	<b>-</b>	

Means with common superscripts in a row (capital alphabets) and in a column (small alphabets) do not differ significantly (p<0.01).

**Table.4** Effect of different decontaminants on pathogenic organisms inoculated in buffalo heart meat samples during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
<b><i>Listeria monocytogenes</i> count</b>					
0 <sup>th</sup> day	4.09±0.04 <sup>eA</sup>	3.72±0.03 <sup>eB</sup>	3.17±0.04 <sup>eC</sup>	2.46±0.05 <sup>eD</sup>	3.36±0.14 <sup>e</sup>
3 <sup>rd</sup> day	4.60±0.05 <sup>dA</sup>	4.26±0.02 <sup>dB</sup>	3.64±0.07 <sup>dC</sup>	3.09±0.05 <sup>dD</sup>	3.90±0.13 <sup>d</sup>
6 <sup>th</sup> day	5.26±0.02 <sup>cA</sup>	4.92±0.04 <sup>cB</sup>	4.12±0.06 <sup>cC</sup>	3.73±0.06 <sup>cD</sup>	4.51±0.14 <sup>c</sup>
9 <sup>th</sup> day	5.75±0.07 <sup>bA</sup>	5.59±0.04 <sup>bA</sup>	4.56±0.03 <sup>bB</sup>	4.36±0.06 <sup>bC</sup>	5.07±0.14 <sup>b</sup>
12 <sup>th</sup> day	6.41±0.05 <sup>aA</sup>	6.37±0.04 <sup>aA</sup>	5.03±0.07 <sup>aB</sup>	4.97±0.04 <sup>aB</sup>	5.69±0.16 <sup>a</sup>
Treatment Mean±SE	5.22±0.17 <sup>A</sup>	4.97±0.19 <sup>B</sup>	4.10±0.14 <sup>C</sup>	3.72±0.18 <sup>D</sup>	
<b><i>Salmonella</i> count</b>					
0 <sup>th</sup> day	3.33±0.07 <sup>aA</sup>	2.77±0.07 <sup>aB</sup>	3.23±0.06 <sup>aA</sup>	2.67±0.04 <sup>aB</sup>	3.00±0.07 <sup>a</sup>
3 <sup>rd</sup> day	2.91±0.05 <sup>bA</sup>	2.54±0.03 <sup>bB</sup>	2.95±0.02 <sup>bA</sup>	2.58±0.05 <sup>abB</sup>	2.75±0.05 <sup>b</sup>
6 <sup>th</sup> day	2.70±0.05 <sup>cA</sup>	2.46±0.03 <sup>bcB</sup>	2.78±0.05 <sup>cA</sup>	2.53±0.05 <sup>bcB</sup>	2.62±0.04 <sup>c</sup>
9 <sup>th</sup> day	2.56±0.03 <sup>cdB</sup>	2.41±0.03 <sup>cC</sup>	2.70±0.06 <sup>cdA</sup>	2.47±0.05 <sup>bcBC</sup>	2.53±0.03 <sup>d</sup>
12 <sup>th</sup> day	2.48±0.04 <sup>dB</sup>	2.38±0.03 <sup>dB</sup>	2.62±0.05 <sup>dA</sup>	2.42±0.04 <sup>dB</sup>	2.48±0.03 <sup>d</sup>
Treatment Mean±SE	2.80±0.06 <sup>B</sup>	2.51±0.03 <sup>C</sup>	2.86±0.05 <sup>A</sup>	2.54±0.03 <sup>C</sup>	
<b><i>Aeromonas</i> count</b>					
0 <sup>th</sup> day	3.73±0.03 <sup>cA</sup>	2.44±0.03 <sup>eB</sup>	3.72±0.04 <sup>eA</sup>	2.33±0.06 <sup>eB</sup>	3.05±0.16 <sup>c</sup>
3 <sup>rd</sup> day	4.11±0.05 <sup>dA</sup>	2.55±0.03 <sup>dC</sup>	4.01±0.04 <sup>dA</sup>	2.75±0.04 <sup>dB</sup>	3.36±0.16 <sup>d</sup>
6 <sup>th</sup> day	4.42±0.05 <sup>cA</sup>	2.82±0.02 <sup>cC</sup>	4.36±0.03 <sup>cA</sup>	3.06±0.04 <sup>dB</sup>	3.67±0.17 <sup>c</sup>
9 <sup>th</sup> day	4.75±0.05 <sup>bA</sup>	3.15±0.04 <sup>bc</sup>	4.69±0.04 <sup>bA</sup>	3.37±0.04 <sup>bB</sup>	3.99±0.17 <sup>b</sup>
12 <sup>th</sup> day	5.10±0.05 <sup>aA</sup>	3.47±0.05 <sup>aC</sup>	5.02±0.03 <sup>aA</sup>	3.65±0.03 <sup>aB</sup>	4.31±0.17 <sup>a</sup>
Treatment Mean±SE	4.42±0.10 <sup>A</sup>	2.89±0.08 <sup>D</sup>	4.36±0.10 <sup>B</sup>	3.03±0.10 <sup>C</sup>	
<b><i>Verotoxigenic E.coli</i> count</b>					
0 <sup>th</sup> day	3.21±0.04 <sup>A</sup>	2.60±0.04 <sup>C</sup>	2.95±0.06 <sup>B</sup>	2.34±0.06 <sup>D</sup>	2.78±0.08
3 <sup>rd</sup> day	2.59±0.04	<2	<2	<2	-
6 <sup>th</sup> day	<2	<2	<2	<2	<2
9 <sup>th</sup> day	<2	<2	<2	<2	<2
12 <sup>th</sup> day	<2	<2	<2	<2	<2
Treatment Mean±SE	-	-	-	-	-
<b><i>Campylobacter</i> count</b>					
0 <sup>th</sup> day	2.82±0.04 <sup>A</sup>	2.42±0.04 <sup>C</sup>	2.65±0.06 <sup>B</sup>	2.79±0.05 <sup>AB</sup>	2.67±0.04
3 <sup>rd</sup> day	<2	<2	<2	<2	<2
6 <sup>th</sup> day	<2	<2	<2	<2	<2
9 <sup>th</sup> day	<2	<2	<2	<2	<2
12 <sup>th</sup> day	<2	<2	<2	<2	<2
Treatment Mean±SE	-	-	-	-	-

### Effect on inoculated pathogens

Table 4 shows counts of *L. monocytogenes*, *Salmonella Typhimurium*, *Aeromonas hydrophila*, verotoxigenic *E. coli* and *C. jejuni* recovered from inoculated buffalo heart meat samples that had undergone tap water (control) washing or other treatments prior to refrigerated aerobic storage at  $4\pm 1^\circ\text{C}$ .

Compared to control, all the treatments resulted in significant reduction in the population of *L. monocytogenes* inoculated in buffalo heart meat samples. On day zero, 10% TSP brought out significantly higher reduction ( $p\leq 0.01$ ) followed by 150 ppm CW and 2% LA and the trend was maintained until day nine of the refrigerated storage.

When *S. Typhimurium* inoculated buffalo heart meat samples was washed and/ treated, significant difference was observed between control and treatments as well as among treatments with respect to reduction in the population of *Salmonella*. Of the compounds studied, 10% TSP and 2% LA were equally and significantly effective compared to 150 ppm CW and the trend was maintained throughout the storage period. During the storage period, the *Salmonella* count had reduced for all the treatment studied including tap water washed control which would be due to the effect of refrigerated storage ( $4\pm 1^\circ\text{C}$ ) and or treatments. Similar findings were observed in beef carcass tissue (Dickson *et al.*, 1994) and fresh pork (Fabrizio and Cutter, 2004).

At day 0, populations of *Aeromonas hydrophila* were significantly reduced by 2% LA and 10% TSP compared to control and the level of reduction made by the treatments were maintained throughout the storage period. Interestingly, 150 ppm CW treatment did not result in significant reduction of *Aeromonas* population compared to control. Uyttendaele *et al.*, (2004) also observed that

decontamination with LA solutions (1% and 2%; wt/vol) showed the most potential to reduce the number of *Aeromonas* count and to guarantee prolonged shelf-lives of fresh-cut vegetables without affecting sensory properties.

At day 0, 10% TSP resulted in significantly lower verotoxigenic *E. coli* count followed by 2% LA and 150 ppm CW treatments whereas 2% LA treatment resulted in significantly higher reduction of *Campylobacter* count followed by both 150 ppm CW and 10% TSP. Upon storage, both these inoculated pathogens were not detected in any of the treated samples by normal plating procedures. However, enrichment and selective plating of stored samples confirmed the presence of these pathogens. Hence, it can be concluded that treatments could not eliminate the organisms and the reduction observed during storage would be due to the effect of low temperature storage and or treatment employed. Dorsa *et al.*, (1998) also observed that the populations of *S. Typhimurium* and *E. coli* O157:H7 inoculated on beef surface tissues were significantly reduced or brought to below detectable levels by hot water and other antimicrobial treatments including 2% LA and 12% TSP. LA treatment immediately reduced levels of all microorganisms similar to previous reports (Delmore *et al.*, 2000; King *et al.*, 2012, Selvan *et al.*, 2014). Similarly, Wagenaar *et al.*, (2006) stated that *Campylobacter* are much more fragile and the highest concentration of *Campylobacter* is found on meat directly after processing. In all subsequent steps of the food chain, the concentration may stabilise but is more likely to decrease, due to death of bacteria which is analogous to the results of present study.

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