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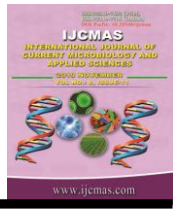

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Effect of Chemical Decontamination on Quality of Buffalo liver

P. Selvan[1]* and S. K. Mendiratta[2]

[1]Department of Poultry Technology, College of Poultry Production and Management, Tamil Nadu Veterinary and Animal Sciences University, Hosur, Tamil nadu – 635 110, India
[2]Department of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar – 243 122, Bareilly, Uttar Pradesh, India

**Corresponding author*

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A B S T R A C T

A study was conducted to assess the effect of dipping buffalo liver in 2% Lactic Acid (LA) for 10 sec., 0.015% Sodium hypochlorite (SHC) solution for 5 sec and 10% Trisodium Phosphate (TSP) for 5 min., on its various quality characteristics. Tap water washed liver pieces were used as control. Both the control and treated liver samples were stored at refrigerated temperature (4±1°C) until visible signs of spoilage observed as indicated by colour and odour changes. The treated and control liver 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 ($p<0.01$) in reducing all the inherent microbial groups on liver samples studied compared to 0.015% SHC treatment. Inoculation studies revealed that 10% TSP was significantly effective($p<0.01$) in reducing *Listeria monocytogenes* count followed by 0.015% SHC and 2% LA on day zero of refrigerated storage and was maintained throughout the storage period studied. *Salmonella* count on liver samples was significantly reduced ($p<0.01$) by 10% TSP followed by 2% LA and 0.015% SHC. 2% LA and 10% TSP were equally and significantly effective ($p< 0.01$) in reducing *Aeromonas* count on day zero of refrigerated storage; however, upon subsequent storage, 2% LA was significantly more effective than 10% TSP in reducing the *Aeromonas* count.10% TSP was significantly effective in reducing Verotoxigenic *E. coli* counts whereas 2% LA was significantly effective in reducing *Campylobacter* count on buffalo liver samples immediately after the treatment. Sensory scores for odour and colour were significantly lower ($p<0.01$) for all treated samples than control. However, 0.015% SHC treatment resulted in significantly higher sensory score than that of 2% LA and 10% TSP treatments.

Introduction

'Offal' is nutritionally rich meats often described as 'edible byproducts', 'variety meats' and sometimes as 'super food' due to dense source of some of the vitamins and minerals. The average yields of edible and inedible offal from buffaloes as 10.61 and 23.31 percent of live weight, respectively (Naik, 2002). The edible meat byproducts could replace a significant portion of lean meat in processed meat products, if proper technology is developed (Krishnan and Sharma, 1991; Anna Anandh, 2001).

These variety meats includes such as liver, tripe, tongue, brains, liver, kidneys and sweetbreads. Liver is an edible muscular organ, rich in folate, iron, zinc, selenium and B complex vitamins. Further, this organ meat is also a great source of coenzyme Q10 which is an antioxidant that can help treat and prevent certain diseases, particularly liver disease. CoQ10 has been shown to slow down the aging process and to improve energy levels. Miller (2004) recorded the mean weight of liver of buffaloes as 1175 gm whereas Panhwar *et al.,* (2007) reported that the average weight of liver 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. Further, pathogenic microorganisms are the major safety concern for the meat industry. Several methods have been developed for bacterial reduction on carcasses which include trimming (Prasai *et al.*, 1995a,b), water washing (Hardin *et al.*, 1995), hot water spraying (Smith and Graham, 1978), as well as sanitizing by chemicals such as organic acids (Dorsa *et al.*, 1997b), chroline compounds (Kotula *et al.*, 1974), polyphosphates (Dickson *et al.*, 1994) and disinfectants. 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 liver.

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 liver samples

Buffalo liver 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 under refrigerated condition during their transport to the laboratory.

Decontaminants used

Immersion treatments applied to reduce contamination of the liver samples included Lactic acid (LA) (2% vol/vol, pH 2.5, 37 °C prepared from LA, Himedia), chlorine (Sodium hyphochlorite, SHC, 0.015% vol/vol, 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 liver samples were maintained as control.

Concentration-contact time combination for each decontaminant utilized in the present study was based on the results obtained through preliminary experiments conducted by 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 Liver 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 10^4 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, liver samples obtained from each animal were hygienically portioned into pieces of 100 g and the pieces were divided into four groups each containing four. Liver 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 liver pieces from fourth group were washed with tap water and maintained as control(s). The ratio of liver 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 appearance 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 liver 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±1°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±1°C for 24h aerobically. Staphylococci were enumerated on Baird Parker agar supplemented with egg yolk tellurite emulsion incubated at 37±1°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±1°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 log10 cfu/g of liver samples.

Sensory analysis

The effect of treatments on the colour and odour of liver pieces were noted during the refrigerated, aerobic (4±1°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 liver pieces with modifications.

Physio chemical analysis

Control and treated liver pieces were evaluated for physiochemical characteristics like pH, weight loss/gain, Extract Release Volume (ERV) and Titratable acidity (only for 2% LA treated samples) during refrigerated storage at (4±1°C). The pH-value of the buffalo liver pieces were determined by homogenizing 10 g of sample with 50ml distilled water in an Ultra Turrex (IKA, Model T-25, Germany) homogenizer 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 liver samples. For this purpose, randomly collected buffalo liver 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*, liver pieces were steamed for 10 min at 100°C, prior to use in the study.

Inoculation of samples

Buffalo liver 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 liver 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 liver pieces from the three groups were separately immersed in solutions of 2% LA, 0.015% SHC and 10% TSP for 10 sec, 5 sec and 5 min, respectively whereas liver 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±1°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 liver 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 Dominiguez-Rodriguez agar and incubated at 37 °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 log10 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 °C for 24 h, Mac Conkey agar at 35 °C for 24 h and Ampicillin Dextrin agar at 37°C for 24 h, respectively. Colonies of *C. jejuni* were enumerated in modified Park and Sanders agar at 43°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

Inherent microbial quality

The mean values for total viable, coliforms, staphylococcal and *E.coli* counts of liver treated with 2% LA/10 sec, 0.015% SHC/5 sec and 10% TSP/5 min as affected by refrigeration storage are presented in Table 1.

Total viable count

ANOVA of data revealed highly significant effect ($p<0.01$) of both treatments and storage days on total viable count of liver. However, interactions between treatments and storage days were non-significant.

Overall treatments mean values of TVC (log10cfu/g) for control, 2% LA, 0.015% SHC and 10% TSP treated samples were 6.77, 5.64, 5.96 and 5.69, respectively. Treatments resulted in significantly ($p<0.01$) lower TVC than control. Among treatments, 2% LA and 10% TSP treatments ($p<0.01$) elicited significantly lower TVC than 0.015% SHC treatment whereas, mean TVCs of 2% LA and 10% TSP treatments did not differ significantly.

Coliforms count

ANOVA of data revealed highly significant effect ($p<0.01$) of both treatments and storage days on coliforms count of liver. However, interactions between treatments and storage days were non-significant.

Overall treatments mean values of coliforms count (log10cfu/g) for control, 2% LA, 0.015% SHC and 10% TSP treated samples were 5.92, 4.90, 5.21 and 5.00, respectively. Treatments resulted in significantly ($p<0.01$) lower coliforms count than control. Among treatments, 2% LA and 10% TSP treatments elicited significantly ($p<0.01$) lower coliforms count than 0.015% SHC treatment whereas, mean coliforms counts of 2% LA and 10% TSP treatments did not differ significantly.

Stapylococcal count

ANOVA of data revealed highly significant effect ($p<0.01$) of both treatments and storage days on staphylococcal count of liver. However, interactions between treatments and storage days were non-significant.

Overall treatments mean values of Staphylococcal count (log cfu/g) for control, 2% LA, 0.015% SHC and 10% TSP treated samples were 5.89, 5.05, 5.49 and 5.30, respectively. Treatments resulted in significantly ($p<0.01$) lower staphylococcal count than control. Among treatments, 2% LA treatment elicited significantly ($p<0.01$) lower staphylococcal count followed by 10% TSP and 0.015% SHC CW treatments.

E. coli count

ANOVA of data revealed highly significant effect ($p<0.01$) of both treatments and storage days on *E.coli* count of liver. However, interactions between treatments and storage days were non-significant.

Overall treatments mean values of *E.coli* count (log cfu/g) for control and 2% LA, 0.015% SHC and 10% TSP treated samples were 3.17, 2.59, 2.74 and 2.41, respectively. Treatments resulted in significantly ($p<0.01$) lower *E.coli* count than control. Among treatments, 10% TSP treatment elicited significantly lower *E.coli* count followed by 2% LA and 0.015% SHC treatments.

In general, 2% LA and 10% TSP treatments resulted in significantly higher reduction of TVC and coliforms count. 2% LA treatment resulted in significantly lower ($p<0.01$) staphylococcal count whereas 10% TSP treatment resulted in significantly ($p<0.01$) lower *E. coli* counts. Delmore *et al.,* (2000) also observed significant reduction in aerobic plate, coliforms and *E. coli* counts in beef liver treated with 2% LA or 12% TSP for 10 sec. Dorsa *et al.,* (1997b) also observed significant reduction in mesophilic aerobic bacteria count of beef carcass surface tissues washed with 2% LA or 12% TSP. Woolthuis *et al.,* (1984) also found immersion of porcine livers in 0.2% LA solution for 5 minutes resulted in significant reduction of total bacterial count.

Sensory characteristics

The mean odour and colour scores of liver treated with 2% LA/10 sec, 0.015% SHC/5 sec and 10% TSP/5 min as affected by refrigeration storage are presented in Table 2.

Effect on odour

ANOVA of data revealed highly significant effect ($p<0.01$) of treatments and significant ($p<0.05$) effect of storage days on odour score of liver. Interactions between treatments and storage days were also non-significant.

Overall treatment mean values of odour score for control, 2% LA, 0.015% SHC and 10% TSP treated samples were 5.00, 4.20, 4.56 and

4.24, respectively. Treatments resulted in significantly ($p<0.01$) lower odour scores than control. Among treatments, 0.015% SHC treatment elicited significantly ($p<0.01$) higher odour scores. Odour scores did not differ significantly between 2% LA and 10% TSP treatments.

Effect on colour

ANOVA of data revealed highly significant effect ($p<0.01$) of treatments and non-significant effect of storage days on colour score of liver. Interactions between treatments and storage days were also non-significant. Overall treatment mean values of colour score for control, 2% LA, 0.015% SHC and 10% TSP treated samples were 5.00, 4.25, 4.56 and 4.35, respectively. Treatments resulted in significantly ($p<0.01$) lower colour scores than control. Among treatments, 0.015% SHC treatment elicited significantly ($p<0.01$) higher colour scores. Colour scores did not differ significantly between 2% LA and 10% TSP treatments.

In general, treatments resulted in significantly ($p<0.01$) lower sensory scores of odour and colour than that of control which may be due to the effect of respective decontaminants. Similar findings were also reported by various researchers (Saoji *et al.,* 1990; Anna Anandh, 2001; Morris *et al.,* 1997; Capita *et al.,* 2000).

Physicochemical parameters

The mean values for pH, weight loss/gain, ERV and titratable acidity of liver treated with 2% LA/10 sec, 0.015% SHC/5 sec and 10% TSP/5 min as affected by refrigeration storage are presented in Table 3.

pH

ANOVA of data revealed highly significant effect ($p<0.01$) of treatments, storage days and interactions on pH values of liver. Overall treatment mean pH values of control, 2% LA, 0.015% SHC and 10% TSP treated samples were 5.92, 5.85, 5.93 and 6.92, respectively. pH values of treatments were differed significantly ($p<0.01$) from that of control. Among treatments, 2% LA and 10% TSP treatments resulted in significantly ($p<0.01$) lower and higher pH values, respectively.

Weight loss/gain

ANOVA of data revealed highly significant effect ($p<0.01$) of both treatments and storage days on weight of liver samples. However, interactions between treatments and storage days were non-significant.

Overall treatment mean weights (in gm) of control, 2% LA, 0.015% SHC and 10% TSP treated samples were 97.92, 97.68, 97.67 and 100.02, respectively. Weight values of 2% LA and 0.015% SHC treatments did not differ significantly from that of control whereas 10% TSP treatment significantly ($p<0.01$) increased the weight of samples.

Extract Release Volume (ERV)

ANOVA of data revealed highly significant effect ($p<0.01$) of treatments, storage days and interactions on ERV values of liver.

Overall treatment mean ERV (in ml) values of control, 2% LA, 0.015% SHC and 10% TSP treated samples were 8.80, 12.89, 6.41 and 4.67 respectively. ERV values of treatments differed significantly ($p<0.01$) from that of control. Among treatments, 2% LA treatment resulted in significantly ($p<0.01$) higher ERV.

Higher ERV obtained by 2% LA treatment may be due reduction of pH. Shelf (1975) also observed higher ERV from fresh beef liver treated with dilute hydrochloric acid for the reduction of pH.

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

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	Total viable count				
0th day	5.72±0.07^{dA}	4.59±0.08^{dC}	4.89±0.05^{dB}	4.63±0.03^{dC}	**4.96±0.11^{d}**
4th day	6.28±0.04^{cA}	5.16±0.08^{cC}	5.46±0.06^{cB}	5.22±0.03^{cC}	**5.53±0.11^{c}**
8th day	7.11±0.06^{bA}	5.99±0.09^{bC}	6.32±0.08^{bB}	6.05±0.05^{bC}	**6.36±0.11^{b}**
12th day	7.97±0.07^{aA}	6.84±0.09^{aC}	7.18±0.07^{aB}	6.86±0.06^{aC}	**7.21±0.11^{a}**
Treatment Mean±SE	**6.77±0.20^{A}**	**5.64±0.20^{C}**	**5.96±0.20^{B}**	**5.69±0.19^{C}**	
	Coliform count				
0th day	5.30±0.08^{dA}	4.28±0.06^{dC}	4.60±0.10^{dB}	4.33±0.08^{dC}	**4.63±0.10^{d}**
4th day	5.70±0.07^{cA}	4.66±0.06^{cC}	4.95±0.07^{cB}	4.76±0.08^{cBC}	**5.02±0.10^{c}**
8th day	6.11±0.07^{bA}	5.13±0.07^{bC}	5.43±0.06^{bB}	5.21±0.08^{bC}	**5.47±0.09^{b}**
12th day	6.55±0.07^{aA}	5.54±0.07^{aC}	5.88±0.05^{aB}	5.69±0.09^{aBC}	**5.92±0.09^{a}**
Treatment Mean±SE	**5.92±0.11^{A}**	**4.90±0.11^{C}**	**5.21±0.12^{B}**	**5.00±0.12^{C}**	
	Staphylococcal count				
0th day	5.41±0.09^{cA}	4.52±0.05^{dC}	4.82±0.05^{dB}	4.70±0.06^{dBC}	**4.86±0.08^{d}**
4th day	5.61±0.09^{cA}	4.80±0.05^{cD}	5.29±0.08^{cB}	5.07±0.05^{cC}	**5.19±0.08^{c}**
8th day	6.07±0.08^{bA}	5.26±0.04^{bD}	5.74±0.08^{bB}	5.51±0.04^{bC}	**5.64±0.07^{b}**
12th day	6.48±0.08^{aA}	5.64±0.04^{aD}	6.10±0.04^{aB}	5.91±0.02^{aC}	**6.03±0.07^{a}**
Treatment Mean±SE	**5.89±0.10^{A}**	**5.05±0.10^{D}**	**5.49±0.11^{B}**	**5.30±0.11^{C}**	
	***E. coli* count**				
0th day	2.88±0.11^{cA}	2.36±0.05^{dBC}	2.52±0.05^{dB}	2.20±0.05^{cC}	**2.49±0.07^{d}**
4th day	3.08±0.10^{bcA}	2.51±0.04^{cBC}	2.67±0.05^{cB}	2.36±0.05^{bcC}	**2.66±0.07^{c}**
8th day	3.29±0.10^{abA}	2.67±0.04^{bBC}	2.82±0.05^{bB}	2.47±0.08^{abC}	**2.81±0.08^{b}**
12th day	3.45±0.10^{aA}	2.81±0.04^{aBC}	2.97±0.05^{aB}	2.62±0.08^{aC}	**2.96±0.08^{a}**
Treatment Mean±SE	**3.17±0.07^{A}**	**2.59±0.04^{C}**	**2.74±0.05^{B}**	**2.41±0.05^{D}**	

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

Titratable acidity

ANOVA of data revealed highly significant effect ($p<0.01$) of treatments and a significant effect of interactions ($p<0.05$) on titratable acidity values of liver samples. However, effect of storage days was non-significant.

Overall treatment mean titratable acidity (ml of 0.01N NaoH/g of sample) values for control and 2% LA treated samples were 5.35 and 5.69, respectively. The titratable acidity value of 2% LA treatment was significantly ($p<0.01$) higher than that of control.

These results revealed that 2% LA treatment for 10 sec and 10% TSP treatment for 5 min were equally effective in reducing TVC and coliforms count whereas 2% LA treatment was highly effective in reduction of staphylococcal count and 10% TSP treatments was highly effective in reduction of *E. coli* count. In general, sensory scores for odour and colour did not differ significantly between 2% LA and 10% TSP treatments. Thus, 2% LA or 10% TSP treatment could be effective for decontamination of buffalo liver.

Effect on inoculated pathogens

Table 4 shows effect of different decontaminants on pathogenic microorganisms (log cfu/g) inoculated in buffalo liver during refrigerated storage at 4±1°C. Inoculation studies revealed that 10% TSP was significantly effective ($p<0.01$) in reducing *Listeria monocytogenes* count followed by 0.015% SHC and 2% LA on day zero of refrigerated storage and was maintained throughout the storage period studied. Capita *et al.*, (2003) also observed that immersion of *L. monocytogenes* inoculated whole chicken legs in 8%, 10% and 12% TSP for 15 min resulted in significant reduction of the organisms. Fabrizio and Cutter (2004) also observed that sprayin of *L. monocytogenes* inoculated pork bellies with 2% LA or chlorinated water (20 ppm) for 15 sec resulted in significant reduction of the organisms than untreated control. Salmonella count on liver samples was significantly reduced ($p<0.01$) by 10% TSP followed by 2% LA and 0.015% SHC.

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

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	Odour score				
0th day	5.00±0.00^{A}	4.00±0.17^{C}	4.48±0.14^{B}	4.12±0.12^{C}	**4.40±0.07**
4th day	5.00±0.00^{A}	4.40±0.10^{BC}	4.64±0.12^{B}	4.36±0.10^{C}	**4.60±0.05**
Treatment Mean±SE	**5.00±0.00^{A}**	**4.20±0.10^{C}**	**4.56±0.09^{B}**	**4.24±0.08^{C}**	
	Colour score				
0th day	5.00±0.00^{A}	4.17±0.13^{C}	4.52±0.13^{B}	4.31±0.14^{BC}	**4.50±0.07**
4th day	5.00±0.00^{A}	4.33±0.10^{B}	4.60±0.12^{B}	4.38±0.12^{B}	**4.58±0.06**
Treatment Mean±SE	**5.00±0.00^{A}**	**4.25±0.08^{C}**	**4.56±0.09^{B}**	**4.35±0.09^{C}**	

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

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

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	pH				
0th day	6.37±0.05^{aB}	5.25±0.05^{bD}	6.23±0.03^{aC}	9.38±0.05^{aA}	**6.81±0.36^{a}**
4th day	6.07±0.03^{bC}	6.25±0.04^{aB}	6.02±0.02^{bC}	6.36±0.05^{bA}	**6.18±0.04^{b}**
8th day	5.78±0.03^{cC}	6.06±0.03^{cA}	5.92±0.05^{cB}	6.09±0.06^{cA}	**5.96±0.03^{c}**
12th day	5.46±0.03^{dB}	5.84±0.04^{dA}	5.53±0.03^{dB}	5.83±0.05^{dA}	**5.67±0.04^{d}**
Treatment Mean±SE	**5.92±0.08^{B}**	**5.85±0.09^{C}**	**5.93±0.06^{B}**	**6.92±0.33^{A}**	
	Weight loss/gain				
0th day	100.50±0.32^{aB}	100.62±0.26^{aB}	101.04±0.62^{aB}	102.27±0.28^{aA}	**101.11±0.24^{a}**
4th day	97.98±0.51^{bB}	98.00±0.35^{bB}	97.51±0.29^{bB}	100.63±0.25^{bA}	**98.53±0.33^{b}**
8th day	97.23±0.34^{bB}	96.43±0.23^{cC}	96.58±0.28^{bcBC}	99.35±0.12^{cA}	**97.40±0.29^{c}**
12th day	95.96±0.32^{cB}	95.69±0.18^{cB}	95.55±0.28^{cB}	97.84±0.36^{dA}	**96.26±0.25^{d}**
Treatment Mean±SE	**97.92±0.42^{B}**	**97.68±0.45^{B}**	**97.67±0.51^{B}**	**100.02±0.39^{A}**	
	Extract Release Volume (ERV)				
0th day	1.40±0.07^{dB}	33.28±1.62^{aA}	1.54±0.05^{dB}	0.56±0.15^{dB}	**9.19±3.21^{d}**
4th day	3.62±0.06^{c}	3.52±0.16^{d}	3.80±0.10^{c}	3.48±0.06^{c}	**8.60±0.05^{c}**
8th day	5.84±0.11^{bA}	5.16±0.08^{cB}	5.70±0.12^{bA}	5.00±0.07^{bB}	**5.43±0.09^{b}**
12th day	24.32±0.46^{aA}	9.58±0.21^{bC}	14.60±0.34^{aB}	9.62±0.21^{aC}	**14.53±1.89^{a}**
Treatment Mean±SE	**8.80±2.00^{B}**	**12.89±2.77^{A}**	**6.41±1.14^{C}**	**4.67±0.75^{D}**	
	Titratable acidity				
0th day	5.32±0.08	5.86±0.03^{a}	-	-	**5.59±0.10^{a}**
4th day	5.33±0.02	5.60±0.06^{b}	-	-	**5.47±0.06^{b}**
8th day	5.38±0.03	5.62±0.05^{b}	-	-	**5.50±0.05^{ab}**
12th day	5.38±0.03	5.67±0.05^{b}	-	-	**5.52±0.03^{ab}**
Treatment Mean±SE	**5.35±0.03**	**5.69±0.03**	-	-	

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

Table.4 Effect of different decontaminants on pathogenic organisms inoculated in buffalo liver during refrigerated storage

Day of storage	Treatment				Days mean±SE
	Control	2% LA	150 ppm CW	10% TSP	
	***Listeria monocytogenes* count**				
0th day	4.11±0.07^{eA}	3.75±0.07^{eB}	3.26±0.06^{eC}	2.23±0.06^{eD}	**5.34±0.16^{e}**
3rd day	4.62±0.07^{dA}	4.37±0.07^{dB}	3.75±0.07^{dC}	2.81±0.08^{dD}	**3.89±0.16^{d}**
6th day	5.30±0.07^{cA}	4.81±0.04^{cB}	4.24±0.07^{cC}	3.45±0.08^{cD}	**4.45±0.16^{c}**
9th day	5.89±0.07^{bA}	5.28±0.06^{bB}	4.77±0.07^{bC}	4.04±0.07^{bD}	**5.00±0.16^{b}**
12th day	6.62±0.07^{aA}	5.86±0.07^{aB}	5.23±0.07^{aC}	4.65±0.08^{aD}	**5.59±0.17^{a}**
Treatment Mean±SE	**5.31±0.18^{A}**	**4.82±0.15^{B}**	**4.25±0.15^{C}**	**3.44±0.18^{D}**	
	***Salmonella* count**				
0th day	3.40±0.06^{aA}	2.77±0.03^{aC}	3.16±0.03^{aB}	2.56±0.06^{aD}	**2.97±0.08^{a}**
3rd day	3.02±0.07^{bA}	2.47±0.03^{bC}	2.85±0.02^{bB}	2.49±0.04^{abC}	**2.70±0.06^{b}**
6th day	2.95±0.08^{bcA}	2.35±0.04^{cC}	2.71±0.05^{cB}	2.42±0.03^{bcC}	**2.61±0.06^{c}**
9th day	2.80±0.08^{bcA}	2.29±0.04^{cC}	2.62±0.03^{cdB}	2.38±0.03^{bcC}	**2.52±0.05^{d}**
12th day	2.71±0.09^{cA}	2.25±0.04^{cB}	2.57±0.03^{dA}	2.34±0.03^{cB}	**2.47±0.05^{d}**
Treatment Mean±SE	**2.98±0.06^{A}**	**2.43±0.04^{C}**	**2.78±0.05^{B}**	**2.44±0.02^{C}**	
	***Aeromonas* count**				
0th day	3.87±0.04^{eA}	2.26±0.08^{dC}	3.66±0.06^{eB}	2.42±0.07^{eC}	**3.05±0.17^{e}**
3rd day	4.22±0.06^{dA}	2.37±0.08^{dD}	3.94±0.06^{dB}	2.74±0.07^{dC}	**3.32±0.18^{d}**
6th day	4.58±0.06^{cA}	2.67±0.08^{cD}	4.27±0.07^{cB}	3.08±0.08^{cC}	**3.65±0.19^{c}**
9th day	4.89±0.04^{bA}	3.04±0.10^{bD}	4.60±0.07^{bB}	3.41±0.09^{bC}	**3.99±0.18^{b}**
12th day	5.21±0.03^{aA}	3.39±0.10^{aD}	4.91±0.06^{aB}	3.71±0.08^{aC}	**4.31±0.18^{a}**
Treatment Mean±SE	**4.55±0.10^{A}**	**2.75±0.09^{D}**	***4.27±0.10B***	**3.07±0.10^{C}**	
	Verotoxigenic E.coli count				
0th day	3.24±0.07^{A}	2.66±0.08^{C}	2.89±0.05^{B}	2.36±0.06^{D}	**2.79±0.08**
3rd day	2.62±0.06	<2	<2	<2	-
6th day	<2	<2	<2	<2	**<2**
9th day	<2	<2	<2	<2	**<2**
12th day	<2	<2	<2	<2	**<2**
Treatment Mean±SE	-	-	-	-	-
	***Campylobacter* count**				
0th day	2.93±0.03^{A}	2.60±0.04^{B}	2.79±0.06^{A}	2.80±0.06^{A}	**2.78±0.04**
3rd day	<2	<2	<2	<2	**<2**
6th day	<2	<2	<2	<2	**<2**
9th day	<2	<2	<2	<2	**<2**
12th day	<2	<2	<2	<2	**<2**
Treatment Mean±SE	-	-	-	-	-

Salvat *et al.,* (1997) also observed that immersion of poultry carcasses in 10% TSP for 15 sec resulted in significant reduction of Salmonella count than control. It has been documented that some amount of bacteria is removed by virtue of washing (Gorman *et al.,* 1995a; Hardin *et al.,* 1995; Prasai *et al.,* 1995b). Similar reduction associated with tap water washed control were also noted in this study.2% LA and 10% TSP were equally and significantly effective ($p < 0.01$) in reducing *Aeromonas* count on day zero of refrigerated storage; however, upon subsequent storage, 2% LA was significantly more effective than 10% TSP in reducing the *Aeromonas* count and was maintained throughout the storage period. Uyttendaele *et al.,* (2004) also reported that *Aeromonas* was resistant towards 0.5 ppm chlorinated water, but was susceptible to 1% and 2% LA when applied for decontamination of chopped bell peppers. 10% TSP was significantly effective in reducing Verotoxigenic *E. coli* counts whereas 2% LA was significantly effective in reducing Campylobacter count on buffalo liver samples immediately after the treatment. On day three of storage and onwards, both Verotoxigenic *E. coli* and *Campylobacter* organisms were not enumerable in control and treatments. However, enrichment and selective plating of stored samples confirmed the presence of organisms. Hence, it can be concluded that the treatments resulted in significant reduction of these organisms but could not eliminate them. Dorsa *et al.,* (1998) also observed similar reduction in *E. coli* O157:H7 count of ground beef (stored at 4°C) made from experimentally contaminated beef carcasses that have been washed with 2% LA or 12% TSP. Van Netten *et al.,* (1996) also reported that lactic acid decontamination replaced the fluid film on the surface of the carcasses by a film containing lactic acid. The bactericidal activity of this film at low pH results in a rapid 0.5 to 3.0 log reduction in number of mesophilic Gram negative pathogens such as *C. jejuni, Salmonella Typhimurium* and *E. coli* O157:H7. Fabrizio and Cutter (2004) also observed that spraying of *Campylobacter coli* inoculated pork bellies with 2% LA or chlorinated water (20 ppm) for 15 sec resulted in significant reduction of the organisms than untreated control. Similarly, Slavik *et al.,* (1994) observed that dipping of chicken carcasses in 10% TSP for 15 sec resulted in 1.2 to 1.5 log reductions in *Campylobacter* count.

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