

# Cinnamaldehyde/Lactic Acid Spray Wash Treatment for Meat Safety and Byproduct Quality Assurance

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**Abstract** This study evaluated the effectiveness of spray washing with aqueous based solution of cinnamaldehyde (CA) and CA plus lactic acid (LA) in reducing aerobic bacteria, *Enterobacteriaceae*, *E.coli* and *Salmonella* from the grain surface of bovine hide so that the developed solutions can potentially be used to decontaminate the cattle carcasses to ensure meat safety. This study also examined the application impacts of the developed formulations on leather produced from the treated hides recognizing the value of cattle byproducts. Two concentrations (0.5% and 0.75%) of CA and LA were used to develop the formulations for 2 to 5 minutes treatment. Research data revealed the fresh cattle hides washed with water alone (control) resulted in recovery of aerobic bacteria of 7.39 and 9.30; *Enterobacteriaceae* of 5.43 and 5.42; *E.coli* of 4.88 and 5.50 and *Salmonella* of 3.81 and 4.65 log CFU per leather panel at 2 and 5 minutes of treatment respectively. Comparing to control, hides treated with CA solution alone resulted in the highest reduction of aerobic bacteria, *Enterobacteriaceae*, *E.coli* and *Salmonella* up to 2.22, 0.42, 0.72 and 1.61 Log CFU respectively for 2 to 5 minutes treatment. The treatment with the formulations of CA plus LA resulted in the highest reduction of aerobic bacteria, *Enterobacteriaceae*, *E.coli* and *Salmonella* up to 2.12, 3.12, 2.33 and 2.28 log CFU respectively for 2-5 minutes of treatments. From microscopic analysis, mechanical and subjective examinations, it was revealed that the leather produced from the formulation treated hides were comparable to the control in terms of structural integrity.

**Keywords:** meat safety, salmonella, E.coli, Enterobacteriaceae, hides, decontamination

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## 1. Introduction

Enteric pathogenic bacteria serve as significant hazards and pose a substantial challenge to the meat industry as well as public health. Such pathogens may incorporate into and onto surfaces of cattle to include skin, hair, attached manure and mixed biofilms, thus limiting cleaning and decontamination efficacy [1-5]. Survival of such harbored bacteria can facilitate cross-contamination of the underlying meat and meat processing equipment in an arbitrator [2,6,7]. Furthermore, washing, cleaning and decontamination with water only has been evaluated and shown to have minimal effects on bacterial populations [4]. In order to intensify the efficacy of current antimicrobial treatments to overcome contaminations, a concentrated antimicrobial treatments may be adopted but could adversely affect the quality of the outer grain surface of cattle, a valuable byproduct and commodity of the meat industry.

Microbial contamination of meat carcasses occurs during the conversion of live animals to meat [8,9]. Muscles within the animal have been noted to be essentially sterile

[10], however, workers and equipment can spread microbial contamination from the hide to the carcasses meat surface during processing [11,12,13]. Thus, it is important to decrease pathogens on cattle hides to reduce the risk of human exposure to these pathogens from meat carcasses. Cattle hide contamination with pathogens such as *Salmonella*, *Escherichia coli* and other *Enterobacteriaceae* (ENT), and related gram negative bacilli (gnEB), may arise from environmental exposures including adherent soil, manure and straw during their lifespan where they are continuously exposed to potentially harmful microorganisms, which may become firmly lodged onto their hides and hair [1,2,4]. The major food-borne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* colonize the gastrointestinal tract of cattle and are shed in the manure, thereby leading to pathogen contamination and persistence on hides for long time [14]. While investigating antimicrobial applications on carcass prior removal of hides for the meat industry to ensure food safety it is also important to take into account that the novel formulations have no detrimental impact on the outer grained hide/skin surfaces. The damage on outer grained surface of hide/skin during meat processing may lead to detrimental financial losses

because animal hides/skins are value added byproducts for the meat industry which are used to produce items such as leather.

Currently, limited research has been conducted under either experimental or commercial conditions on the antimicrobial effectiveness of hide decontamination treatment although some researchers recognize that hide interventions are the most effective means to reduce pathogens on meat. The reported techniques for decontamination of bovine hides include, pre-slaughter animal washing, [15], steam condensation [16], hot water rinse/chemical dehairing [17], hair removal with wax [18], antimicrobial washing, [19], ozonated and electrolyzed oxidizing water [20], hair shaving [21] and application of bacteriophages [22]. The ultimate goal of all hide treatment techniques is to stop microorganisms transferring from hide surface to the underlying meat.

This study was designed to evaluate the effectiveness of low concentrations of cinnamaldehyde (CA)/ Lactic acid (LA) spray washing formulations for food safety by reducing pathogens on the haired surface of cattle hides while evaluating the effects on value added byproducts of the meat industry. Lactic acid is generally recognized as safe (GRAS) by the Food and Drug Administration and has been approved by the Food Safety and Inspection Services (FSIS) for usage in a hazard analysis and critical control points (HACCP) design plan up to 5% as an antimicrobial to decrease pathogenic contamination on surfaces of meat including whole or cut meat, carcasses, parts, trim, and organs, as a wash, spray, rinse, dip, chiller water or scald water, pre and post chill [23]. CA is a natural oil phenolic aldehyde antimicrobial obtained from cinnamon bark that can interfere with biological processes. The carbonyl bond in CA is highly polar and due to the polarity of this bond carbon atom becomes electrophilic and reactive toward nucleophiles such as nitrogen containing structures (DNA, proteins) through their amine groups and thus disrupting the metabolic function of microorganism [24]. Such natural oils reduce activity and replication rates, changes in the cell morphology, motility and extrusion of intracellular material of bacteria. Furthermore, CA has shown to inhibit the formation of biofilms at below minimum inhibitory concentrations [24,25,26].

## 2. Materials and Methods

### 2.1. Hide Preparation

Fresh de-fleshed cattle hides were acquired from a local meat packing and processing facility, JBS Packerland (Souderton, PA). For the experiments, hide pieces were cut into 12 in x12 in panels from backbone area of a whole bovine hide for subsequent spray wash treatment with the individual formulations. For bacterial recovery from treated hides, random 10 in x 5 in surfaces of the hide panels were designated and swab samples were collected after 2 min and 5 min of treatment. To illustrate structural characteristics which facilitate bacterial survival and attachment a metal stamp was used to punch out a 0.5in stamps from cattle hides. One set of stamps was non-inoculated while one set was inoculated with 1 microliter

of a  $10^8$  Log/ml cocktail of 3 *Salmonella* spp. (*S. anatum*, *S. saint-paul* and *S. Typhimurium*) and allowed to sit for 20 mins.

### 2.2. Antimicrobial Formulation Preparation

Commercial grade chemicals used for all testing formulations. cinnamaldehyde  $\geq 95\%$  and LA  $\geq 85\%$ , were purchased from Aldrich Chemical (Milwaukee, WI). All additional reagents used for preparations of formulations were of the highest purity available from commercial suppliers. Tap water was used as a control in addition for formulations where prepared of 0.50% CA in 0.20% Tween-20 aq. solution brought to a pH of 6.89; 0.75% CA in 0.20% Tween-20 aq. solution brought to a pH of 6.75; 0.50% CA + 0.50% LA in 0.20% Tween-20 aq. solution brought to a pH of 2.77; and 0.75% CA + 0.75% LA in 0.20% Tween-20 aq. solution brought to a pH of 2.77. All formulations where dissolved in tap water at room temperature ( $\sim 21^\circ\text{C}$ ) and prepared  $\sim 24$  hours prior to experimental spray applications on hides.

### 2.3. Spray Treatment

For antimicrobial testing the following formulations, and (control) tap water, (Solution A) of 0.50% CA in 0.20% Tween-20 aq.; (Solution B) 0.75% CA in 0.20% Twee-20 aq.; (Solution C) 0.50% CA + 0.50% LA in 0.20% Twee-20 aq.; (Solution D) 0.75% CA + 0.75% LA in 0.20% Twee-20 aq. were applied to the haired surface of individual hide panel using a hand held 1000 ml polyethylene spray bottle. A certain amount of 20 mL (20 puffs) of individual formulation was sprayed on each panel, adequate enough to cover the 12 in x 12 in surface area (panel size). The individual formulation was allowed to sit for  $\sim 5$ min before two separate 10 cm x 5 cm surface areas were swabbed from the same panel at 2 and 5 min respectively for microbial testing. After treatment, all hide panels were washed separately in a 6-in-1 drum set-up (Dose Maschinenbau GmbH, Lichtenau, Germany) for 2h using the USDA hide washing protocol (100% water, 0.15% Boron TS, and 0.10% Proxel) as a first step of leather processing.

### 2.4. Microbial Testing

The independently 10cm x 5cm spray washed areas treated with the selected formulations were aseptically swabbed with a sterile sponge and placed into a corresponding sampling bag with 25ml of buffered peptone water for analysis (Nasco Meat and Turkey Carcass Sampling Kit, Salida, California). The sample bags were then hand massaged for  $\sim 2$  min. Samples were serially diluted and spread-plated on Tryptic Soy Agar (TSA), Xylose-Lysine-Tergitol 4 (XLT-4) Agar, MaConkey Agar (MAC), Sorbitol MacConkey Agar, with Cefixime and Tellurite (CT-SMAC) for aerobic bacteria, *Enterobacteriaceae* (ENT) and related gram-negative bacilli (gnEB), *Salmonella* and *E. coli* counts, respectively (all agar was obtained from Fisher Scientific, Pittsburg, PA). After spread plating, samples were incubated between 24-48 h at  $37^\circ\text{C}$  and bacterial colonies were enumerated for bacterial recovery with the lowest detection level at 1 CFU per 10cm x 5cm area.

## 2.5. Cattle Byproduct Preparation

After washing separately, the 12in x 12in formulation treated panels were placed in a dehairing drum and the control panel was placed into a separate individual drum. All hide panels were de-haired per the USDA tanning protocol [27]. Subsequent to dehairing, all panels were combined into one drum for pickling, tanning, re-tanning, coloring, and fat liquoring steps. The samples were tanned into crust upper shoe leather following the standard USDA tanning procedures [27]. The resulting panels were remained at temperature (21°C) and humidity (50% relative humidity) in a controlled environmental chamber (Caron Environmental Chamber, Marietta, OH) until further subjective and mechanical testing and stereo microscopy analyses were conducted.

## 2.6. Evaluation of the Antimicrobial Treatment on Byproduct Quality

To assess the effects of the developed formulations on byproduct quality, the mechanical properties of the processed leather were measured. The mechanical properties including tensile strength, Young's Modulus ("stiffness"), elongation ("stretchability"), and fracture energy ("energy required to open unit area of crack surface") were conditioned and tested as per the ASTM methods D1610 and D2209. Five dumbbell shaped leather samples were cut from each leather panel following the protocol in ASTM D2209 parallel to the backbone. The average thickness of the leather sample ranged from 2.0 mm to 2.7 mm. An Insight-5 test frame and Testworks-4 data acquisition software (MTS Systems Corp., Minneapolis, MN) were used to evaluate the mechanical properties of the leather samples. The strain rate and the grip distance for this study were set to 24.5 cm/min and 10.16 cm respectively. Samples were tested in a room set at 23±3 °C and 50±5 % relative humidity. Leather subjective tests (break, handle, fullness, color and general appearance) were conducted by an experienced tanner in our labs. Each value was graded from 1 to 5 for each testing parameter, with 1 being the lowest quality and 5 being the highest quality. From these ratings, an overall evaluation (general appearance) was determined from 1 to 5.

Representative leather panels subjected to spray treatment with individual formulation were inspected under a stereo microscope (Nikon Digital Microscope SMZ-2T, Melville, NY) to determine any detectible changes in the hide grain structure from the treatment. Scanning electron microscope (SEM) images were taken to identify potential finer structural changes in the surface and fibers of the leather. For SEM images, samples were mounted on stubs and sputter gold coated for 1 minute (EMS 150R ES, EM Sciences, Hatfield, PA). Samples were viewed with a FEI Quanta 200 F Scanning Electron Microscope (SEM), (Hillsboro, OR, USA) with an accelerating voltage of 10KV in high vacuum mode.

## 2.7. Statistical Analysis

Based on a minimum of three replications per treatment, log-values of microbial populations were analyzed by

One-way analysis of variance, using SPSS software (version 14.0, SPSS Inc., Chicago, IL). To compare treatment group differences against the control group (water treatment alone), Dunnett's post-hoc analyses were conducted.

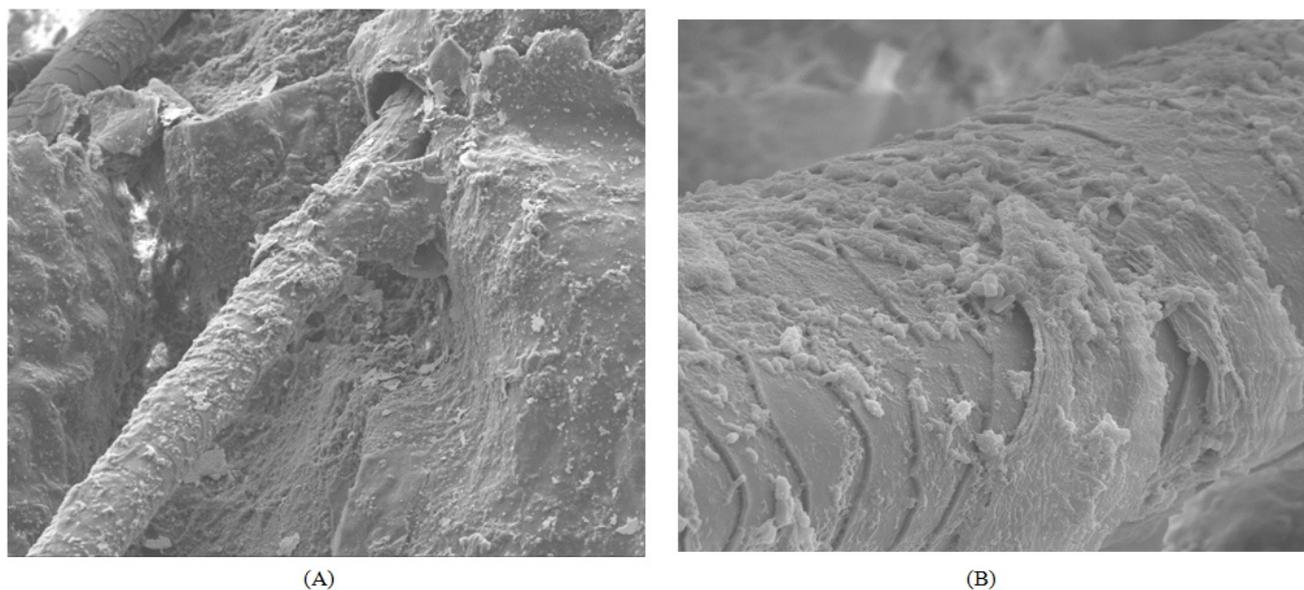
## 3. Results and Discussion

### 3.1. Evaluation of Surface Structures of Cattle for Bacterial Survival

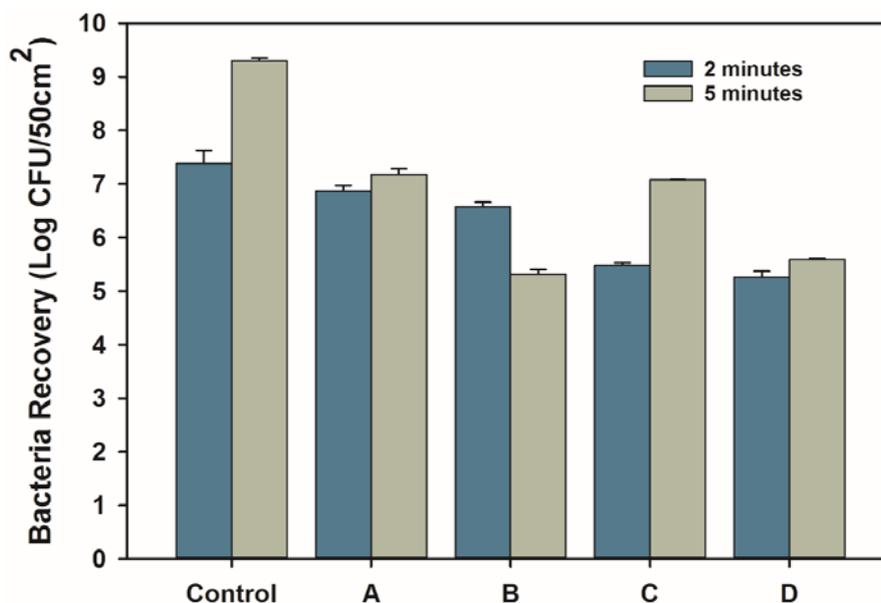
Figure 1A illustrates an SEM image of an un-inoculated stamp of the outer grained hide of cattle. The image displays rough jagged edges and biofilm formulations on the surface and hair of the cattle. Further, Figure 1B shows the outer grained hide of cattle inoculated with a cocktail of *Salmonella* spp. This image illustrates the capability of pathogens such as *Salmonella* ability to attach to hide surfaces and incorporating into preexisting biofilms. Hygiene of the haired surface of cattle will continue to challenge the effort for meat safety. As meat carcasses are essentially sterile it is understood that meat-borne illness cases are highly associated with hide contamination where the pathogens can transfer from the hide surface to the underlying meat during processing. Bacteria are known to attach to various topographies, which may include hides, may be riddled with filth which maybe harboring pathogens [2,28,29]. To increase the effectiveness of antimicrobials and overcome the protective nature of the environmental materials which harbor the bacteria, concentrations of antimicrobials may be increased but need to be properly evaluated. Such increases may cause damage to the hide and result in high levels of antimicrobial residuals.

### 3.2. Reduction of Aerobic Bacteria

Aerobic bacterial recovery of  $7.38 \pm 0.43$  and  $9.30 \pm$  Log/CFU was enumerated from 50 cm<sup>2</sup> surface area when un-inoculated hide panels were spray washed with tap water alone (control) at 2 and 5 minutes, respectively (Figure 2). On hide panels treated with 0.50 % CA alone aerobic bacterial recovery was enumerated at  $6.87 \pm 0.17$  and  $7.17 \pm 0.18$  log/CFU at 2 and 5 mins of treatments respectively. When CA treatment was increased to 0.75%, aerobic bacterial recovery reduced to  $6.57 \pm 0.15$  and  $5.32 \pm 0.15$  respectively at 2 and 5 mins of treatment (Figure 2). In a spray wash combined formulation of 0.50% CA and 0.50% LA aerobic bacteria recovery was  $5.48 \pm 0.08$ ,  $7.08 \pm 0.01$  log/ CFU. Furthermore, when hide panels were spray washed with a combined formulation of 0.75% CA and 0.75% LA bacterial recovery further reduced to  $5.26 \pm 0.16$  and  $5.59 \pm 0.04$  log/ CFU at 2 and 5 minutes (Figure 2). Within each treatment, time was a factor on bacterial recovery except at the 0.50% CA ( $P \leq 0.001$ ). At a concentrations of 0.50 % CA, time was not a significant factor ( $P = 0.60$ ). However, at 2 and 5 mins of treatment, each formulation were significantly different than its corresponding control ( $P \leq 0.003$ ). Results indicate that CA and CA + LA formulations have the ability to effectively reduce the overall number of aerobic bacteria on hide surfaces.



**Figure 1.** (A) Image of a non-inoculated surface of a cattle fresh hide depicting hide and skin and biofilm formation. (B) Displays a hair on the surface of a cattle hide with inoculated *Salmonella* spp cocktail firmly incorporated into bacterial biofilms after 20 mins of inoculation

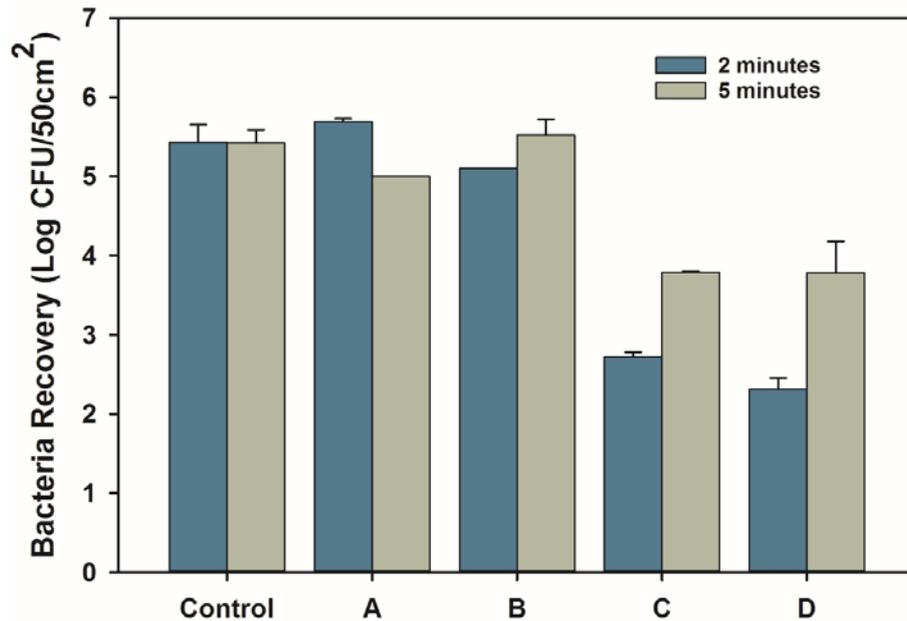


**Figure 2.** Effects of (control) water, (A) 0.50% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments on total bacteria recovery. Data represents means SD (n = 3)

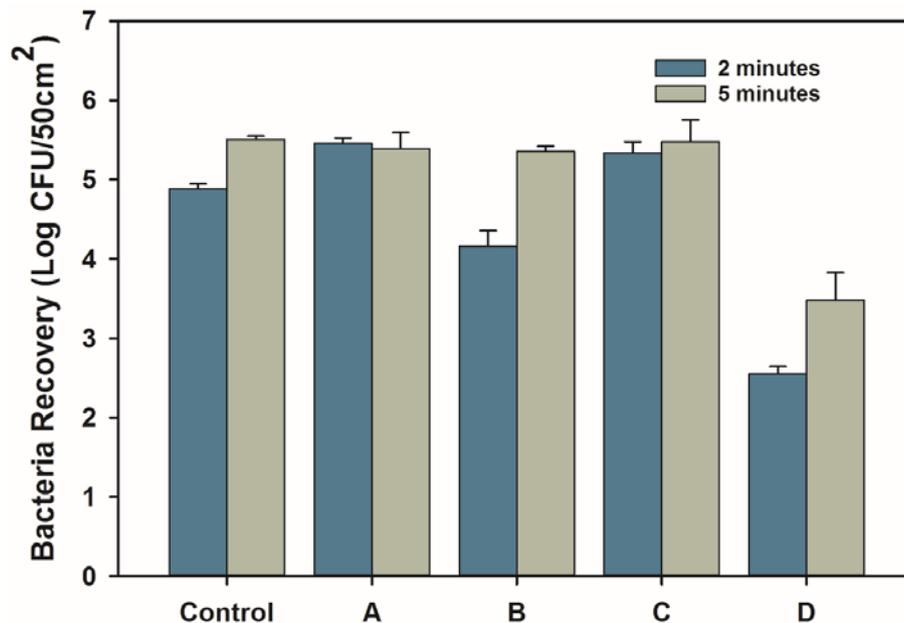
### 3.3. Reduction of *Enterobacteriaceae* and Related Gram Negative Bacteria

This test revealed that spray washing with tap water alone resulted in ENT/gnEB recovery of  $5.43 \pm 0.38$  and  $5.43 \pm 0.29$  Log/CFU from un-inoculated hide at 2 and 5 minutes, respectively (Figure 3). Panels treated with 0.50% CA alone ENT/gnEB recovery was enumerated at  $5.69 \pm 0.07$  and  $5.00 \pm 0.00$  log/ CFU at 2 and 5 minutes of treatments respectively. CA treatment of 0.75% alone resulted in recovery ENT/gnEB of  $5.10 \pm 0.17$  and  $5.53 \pm 0.34$  respectively at 2 and 5 mins of treatment (Figure 3). For a combined formulation of 0.50% CA and 0.50% LA, ENT/gnEB recovery was enumerated at  $2.71 \pm 0.12$  and  $3.79 \pm 0.69$  Log/CFU at 2 and 5 mins respectively. When 0.75% CA and 0.75% LA combination formulation was used bacterial recovery was

enumerated at  $2.31 \pm 0.25$  and  $3.78 \pm 0.69$  Log/ CFU at 2 and 5 minutes respectively (Figure 3). When water alone was used time was not a significant factor in bacterial recovery ( $P = 0.68$ ). At 2 minute of treatment no significant difference was observed between the control and treatments of 0.50% CA and 0.75% CA ( $P \geq 0.54$ ). Similarly, at 5 minutes of treatment no significant difference in ENT/gnEB recovery was observed between the control and 0.75% CA formulation ( $P \geq 0.58$ ). However, both combined formulations of 0.50% and 0.75% CA + LA resulted significant reduction in bacterial recovery in compare to control. It was interesting to see that in some cases, at 5 minutes treatment higher recovery was observed than at 2 minutes of treatment. This is probably either due to the individuality of naturally collected hide panels or reducing action of antimicrobials with time.



**Figure 3.** Effects of (control) water, (A) 0.50% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments on Enterobacteriaceae and related gram negative bacilli recovery. Data represents means SD (n = 3)



**Figure 4.** Effects of (control) water, (A) 0.5% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments on *E. coli* recovery. Data represents means SD (n = 3)

### 3.4. Reduction of *E. coli*

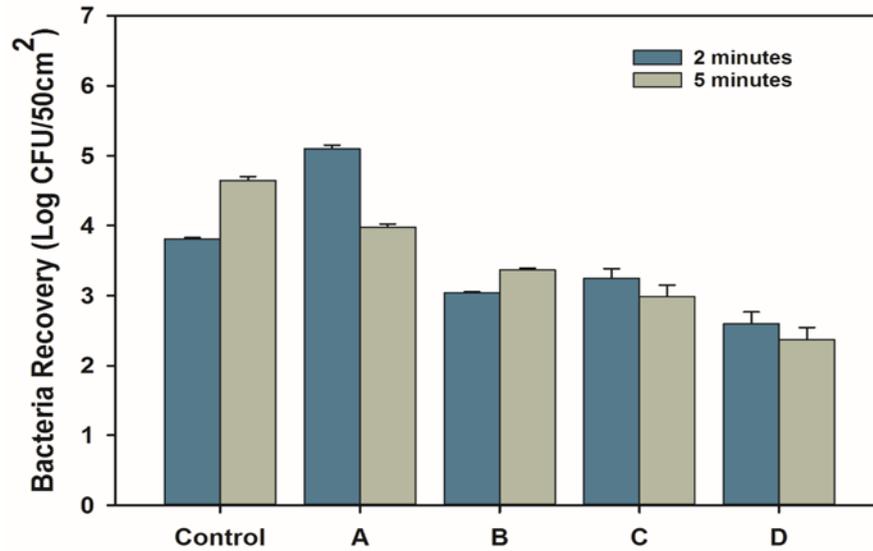
In this study spray washing with tap water alone resulted in *E. coli* recovery of  $4.88 \pm 0.10$  and  $5.50 \pm 0.08$  Log/CFU from un-inoculated hide at 2 and 5 minutes, respectively (Figure 4). Panels treated with 0.50% CA alone *E. coli* recovery was enumerated at  $5.46 \pm 0.11$  and  $5.39 \pm 0.34$  Log/CFU at 2 and 5 mins of treatments respectively. CA treatment of 0.75% alone resulted in *E. coli* recovery of  $4.16 \pm 0.34$  and  $5.36 \pm 0.10$  respectively at 2 and 5 mins of treatment (Figure 4). For a combined formulation of 0.50% CA and LA, *E. coli* recovery was enumerated at  $5.34 \pm 0.24$  and  $5.48 \pm 0.48$  log/ CFU at 2 and 5 mins respectively. When 0.75% CA and 0.75% LA combination formulation

was used bacterial recovery was enumerated at  $2.55 \pm 0.16$  and  $3.48 \pm 0.60$  log/ CFU at 2 and 5 minutes respectively (Figure 4). Additionally, results show that treatment with the concentrations of 0.50% CA and 0.50% CA + 0.50% LA, exposure time did not result in significant change in reduction ( $P \geq 5.90$ ) of *E. coli*. A significant difference in reduction of *E. coli* was observed between the control and formulation treatments of 0.75% CA + 0.75% LA ( $P \leq 0.001$ ) at both time frames. The overall results indicate CA alone may have limited ability to reduce *E. coli*. However, the formulation of CA combining LA has an increased ability to suppress *E. coli* growth this can be further improved by increasing the concentration of the formulation.

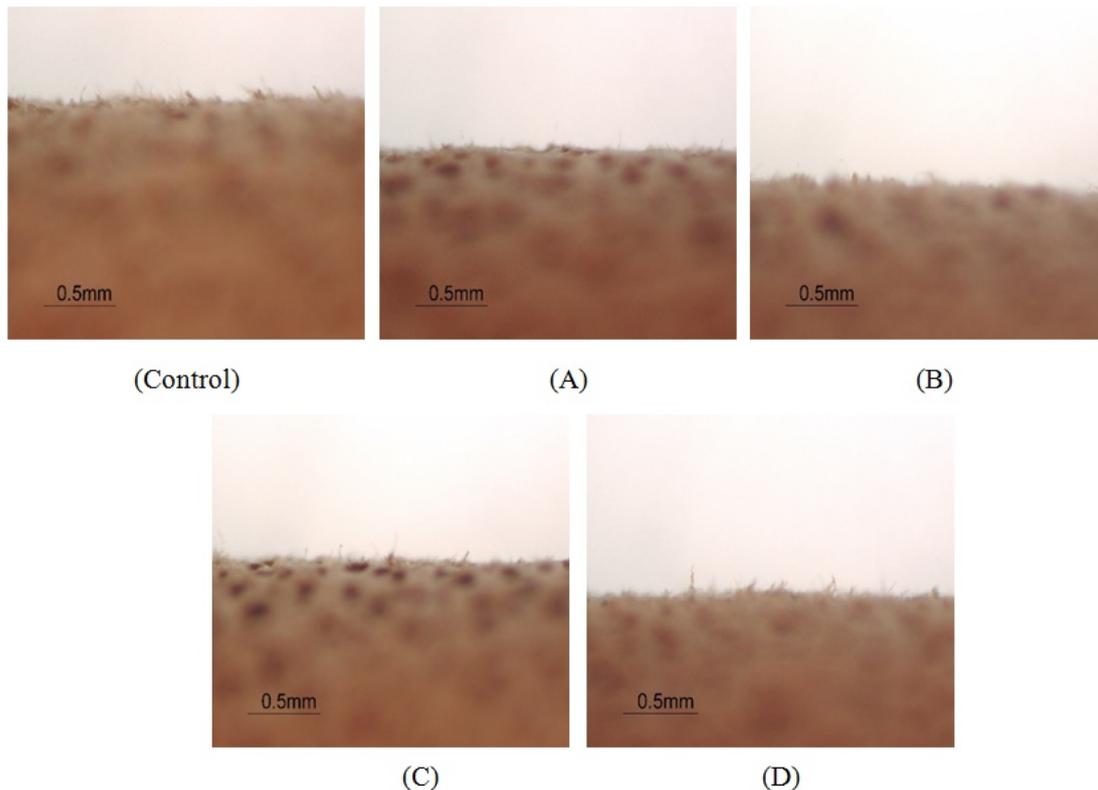
### 3.5. Reduction of *Salmonella*

Spray washing with tap water alone resulted in *Salmonella* recovery of  $3.81 \pm 0.02$  and  $4.65 \pm 0.10$  Log/CFU from un-inoculated hide at 2 and 5 minutes, respectively (Figure 5). Hide panels treated with 0.50% CA alone *Salmonella* recovery was enumerated at  $5.10 \pm 0.09$  and  $3.97 \pm 0.08$  log/ CFU at 2 and 5 mins of treatments respectively. CA treatment of 0.75% alone resulted in *Salmonella* recovery of  $3.04 \pm 0.02$  and  $3.37 \pm 0.04$  respectively at 2 and 5 mins of treatment (Figure 5). At combined formulation of 0.50% CA and 0.50% LA, *Salmonella*, recovery was enumerated at  $3.24 \pm 0.24$

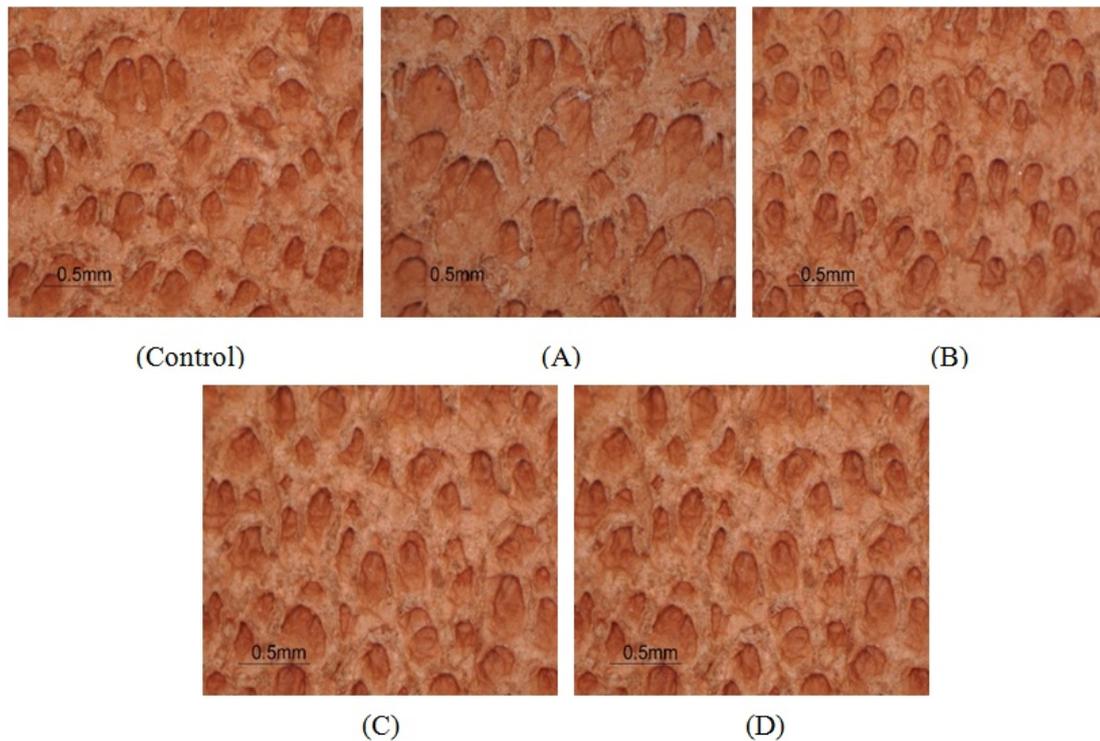
and  $2.98 \pm 0.28$  Log/CFU at 2 and 5 mins respectively. When 0.75% CA and 0.75% LA combined formulation was used bacterial recovery was enumerated at  $2.60 \pm 0.30$  and  $2.37 \pm 0.30$  log/ CFU at 2 and 5 minutes respectively (Figure 5). In compare to control a significant difference in reduction of *Salmonella* from hide surface was observed for 0.75% of CA and combined formulation of CA + LA. This trend indicates further reduction of the pathogen can be potentially achieved by washing with the developed formulation at a concentration over 0.75% and also combined formulation has stronger ability than CA alone to reduce *Salmonella* on hide surfaces.



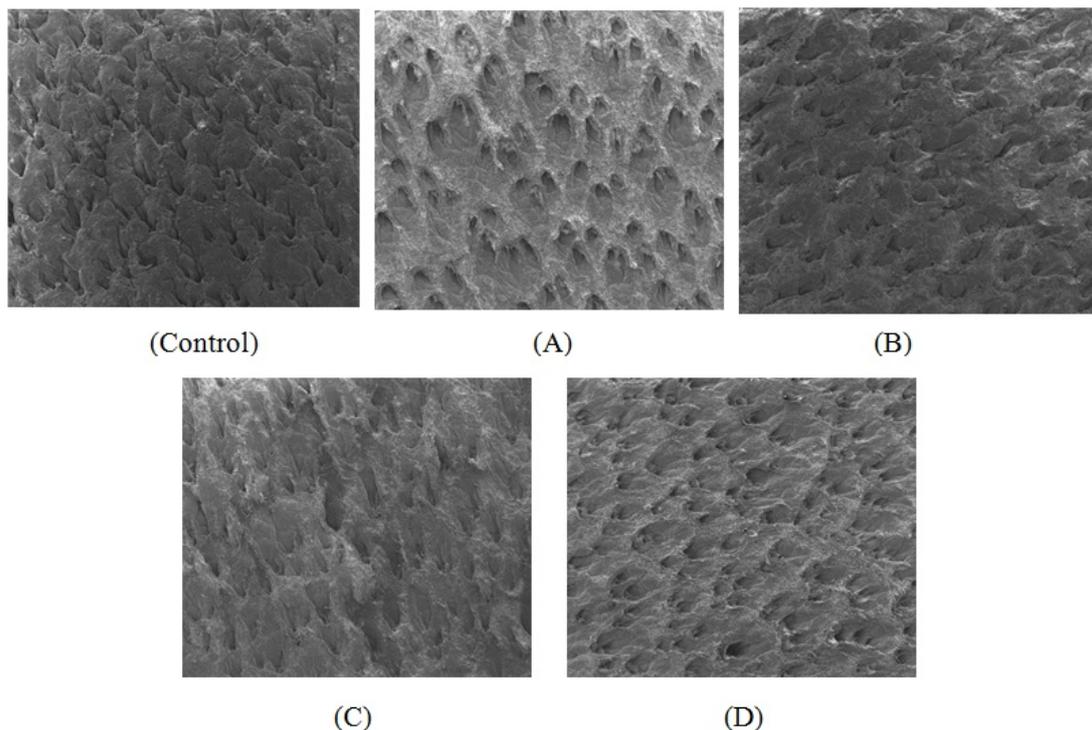
**Figure 5.** Effects of (control) water, (A) 0.50% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments on *Salmonella* recovery. Data represents means SD (n = 3)



**Figure 6.** Effects of (control) water, (A) 0.50% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments on hide sueding. Bars represent 0.5 mm



**Figure 7.** Stereo microscopic image of the leather made from hides treated with: (control) water, (A) 0.50% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments



**Figure 8.** Scanning Electron Micrograph (SEM) images of the surface of leather made from hides treated with: (control) water, (A) 0.5% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments

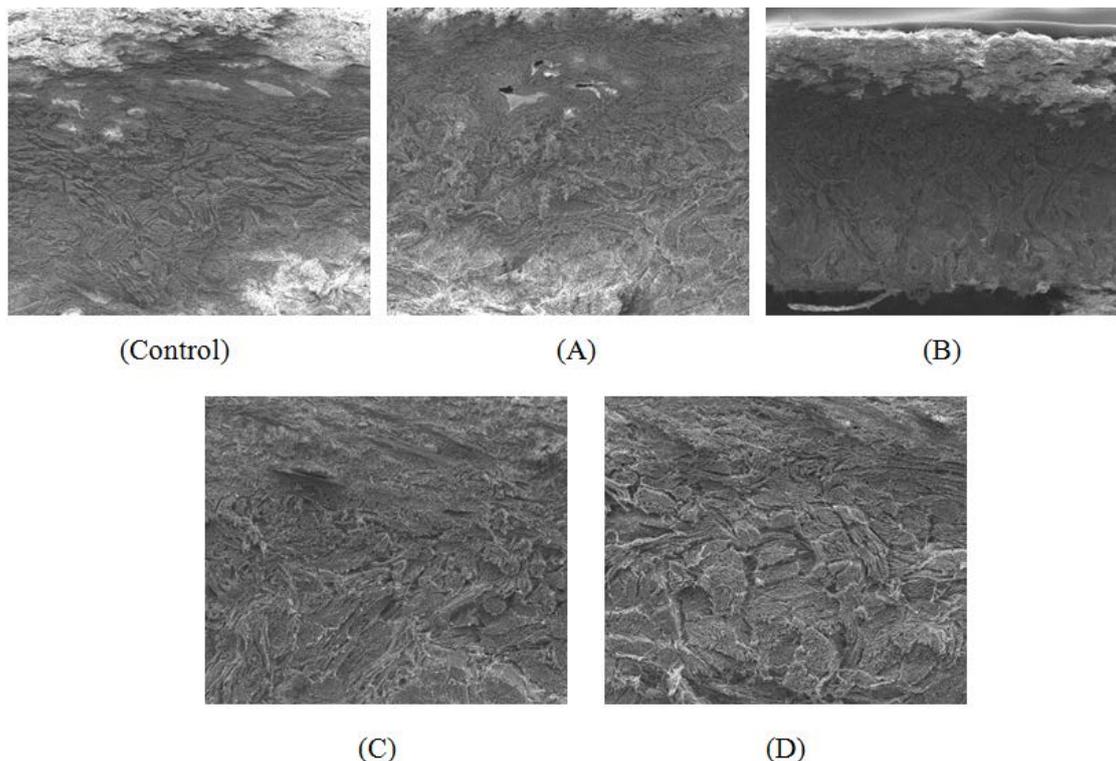
### 3.6. Evaluation of the Antimicrobial Treatment on Byproduct Quality

Chemical treatments of hides may lead to microscopic defects in produced leather quality such as sueding which is resulted from losing of fibers on the grain surface of the prepared leather or abrasions of the grain surface. Such defects reduce the tightness and quality of the prepared leather. To assess the quality of leather produced from the

formulation treated hides, leather panels were examined using a stereo microscope (Figure 6). Produced leather panels were folded, and a stereo microscopic image was taken at the crease to enhance the surface features. Leather samples 6A and 6B (Figure 6) which were produced from 0.5% CA and 0.75% CA treated hides respectively revealed less sueding than the control. Figure 6C and Figure 6D had similar visual amount of sueding with no discernable difference from the control. Surface stereo

microscope images (Figure 7) revealed that there was no discernable difference between the grain structure of leather made from control and formulation treated hides. SEM images of the surface of crust leather produced from hides treated with water (Figure 8, Control) revealed uneven or rough surface, whereas the leather from formulations treated hides (Figure 8A - Figure 8D) appeared to have smoother and homogeneous surfaces. Transactional SEM images revealed no noticeable unraveling of bundle fibers with no apparent difference between the control and the treated panels (Figure 9, Control - 9D).

Leathers produced from formulation treated hides were assessed for softness, fullness, grain tightness (break), color and general appearance by hand and visual examination and found at similar quality to the control (Table 1). In addition, the overall mechanical properties (tensile strength, elongation, Young's Modulus, and fracture energy) of the resulting leather products from the formulation treated hides were comparable to that produced from the control (Table 2). This indicates that the application of the solutions did not have any detrimental effect on the properties of the final produced leather.



**Figure 9.** Cross sectional SEM images of leather made from hides treated with: (control) water, (A) 0.5% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments

**Table 1.** Subjective properties of leather made from hides treated with (control) water, (A) 0.5% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments

Formulation	Handle	Fullness	Grain Tightness (Break)	Color	General Appearance
Water (control)	3	3	4	4	4
A	4	3	4	4	4
B	3	2	4	4	3.5
C	3	4	4	4	4
D	3	3	4	4	4

**Table 2.** Mechanical properties of leather made from hides treated with (control) water, (A) 0.5% CA, (B) 0.75% CA, (C) 0.50% CA and 0.50% LA and (D) 0.75% CA and 0.75% LA spray wash treatments

Formulation	Tensile Strength (Mpa)	Elongation %	Young's Modulus Mpa	Fracture Energy J/cm <sup>3</sup>	Toughness Index
Water (control)	9.53±2.13	32.45±2.03	20.55±1.82	1.19±0.31	0.46±0.06
A	11.18±1.49	39.44±2.52	19.75±4.10	1.65±0.24	0.57±0.06
B	10.2±2.81	48.02±4.35	10.97±4.86	1.67±0.55	0.98±0.19
C	11.03±2.67	43.37±4.52	15.97±7.03	1.68±0.51	0.75±0.17
D	10.37±1.88	49.05±2.99	11.23±2.69	1.75±0.47	0.94±0.10

## 4. Conclusion

Bacterial contamination is a major issue in the meat industry which may lead to food related illnesses. In this study, two natural products have been tested for their antimicrobial efficacy in reducing microorganisms including pathogens from the haired surface of cattle hides. Formulation of CA alone is found effective in reducing swab recoveries of aerobic bacteria and *Salmonella* from treated hides with better results for increased concentration. The limited effectiveness of CA on Ent/gnEB and *E.coli* reduction can be overcome by using a combined formulation of CA and LA. In most cases for this study, the higher concentration of combined formulation works more effectively in limiting microorganism than the lower concentration as expected. Additionally, at the concentrations used no detrimental impacts on final leather was observed due to the spray formulation treatments. In future research, increased concentrations and time frames may be implemented to further increase reduction of pathogens on hide surfaces.

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## References

- Dickson, J.S., Macneil, M.D. Contamination of Beef Tissue Surfaces by Cattle Manure Inoculated with *Salmonella typhimurium* and *Listeria monocytogenes*. *Journal of Food Protection*. 54(2): 102-104. 1991.
- Long III, W., Sarker, M.I., Liu, C.-K. Evaluation of Novel Pre-Slaughter Cattle Wash Formulations for Meat and Byproduct Safety and Quality. *Advanced Journal of Food Science and Technology*. 14(2): 33-41. 2018.
- Manyi-Loh, C.E., Mamphweli, S.N., Meyer, E.L., Makaka, G., Simon, M., Okoh, A.I. An Overview of the Control of Bacterial Pathogens in Cattle Manure. *International Journal of Environmental Research and Public Health*. 13 (9): 1-27. 2016.
- Mies, P.D., Covington, B.R., Harris, K.B., Lucia, L.M., Acuff, G.R., Savell, J.W. Decontamination of Cattle Hides Prior to Slaughter Using Washes with and without Antimicrobial Agents. *Journal of Food Protection*. 67 (3): 579-582. 2004.
- Terrance, A.M., Bosilevac, J.M., Brichta-Harhay, D.M., Kalchayanand, N., Shackelford, S.D., Wheeler, T.L., Mohammad, K. Effects of a Minimal Hide Wash Cabinet on the Levels and Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the Hides of Beef Cattle at Slaughter. *Journal of Food Protection*. 70(5): 1076-1079. 2007.
- Long III, W., Sarker, M.I., Marsico, R., Lindsey, U., Latona, N.P., Muir, Z., Liu, C.-K. Efficacy of Citrilow and Cecure spray wash on prevalence of aerobic and Enterobacteriaceae bacteria/gram - negative enteric bacilli and cattle hide quality. *Journal of Food Safety*. 38 (3): 1-7. 2018.
- McEvoy, J.M., Doherty, A.M., Finnerty, M., Sheridan, J.J., McGuire, L., Blair, I.S., McDowell, D.A., Harrington, D. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*. 30(5): 390-395. 2000.
- Conner, D.E., Kotrola, J.S., Mikel, W.B., Tamblyn, K.C. Effects of acetic-lactic acid treatments applied to beef trim on populations of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in ground beef. *Journal of Food Protection*. 60: 1560-1563. 1997.
- Dickson, J.S., Anderson, M.E. Microbial decontamination of food animal carcasses by washing and sanitizing systems: a review. *Journal of Food Protection*. 55: 133-140. 1992.
- M.E. Anderson, R.T. Marshall, W.C. Stringer, H.D. Naumann, Efficacies of the three sanitizers under six conditions of application to surfaces of beef. *Advanced Journal of Food Science and Technology* 42 (1977) 326-329.
- Johnston, R.W., Harris, M.E., Moran, A.B., Krumm, G.W., Lee, W.H. A comparative study of the microbiology of commercial vacuum-packaged and hanging beef. *Journal of Food Protection*. 45: 223-228. 1982.
- Kotula, A.W., Lusby, W.R., Crouse, J.D. Variability in microbiological counts on beef. *Journal of Animal Science*. 35: 204. 1972.
- McEwen, S.A., Martin, S.W., Clarke, R.C., Tamblyn, S.E., McDermott, J.J. The prevalence, incidence, geographical distribution, antimicrobial sensitivity patterns and plasmid profiles of milk filter *Salmonella* isolates from Ontario dairy farms. *Canadian Journal of Veterinary Research*. 52: 18-22. 1988.
- Wesley, I.V. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl. Environ. Microbiol.* 66: 1994-2000, 2000.
- Byrne, C.M., Bolton, D.J., Sheridan, J.J., McDowell, D.A., Blair, I.S. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*. 30: 142-145. 2000.
- McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Blair, I.S., McDowell, D.A. Use of steam condensing at subatmospheric pressures to reduce *Escherichia coli* O157:H7 numbers on bovine hide. *Journal of Food Protection*. 64: 1655-1660. 2001.
- Castillo, A., Dickson, J.S., Clayton, R.P., Lucia, L.M., Acuff, G.R. Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. *Journal of Food Protection*. 61: 623-625. 1998.
- Purnell, G., James, S., Wilkin, C.-A., Fisher, A., Corry, J., Howell, M., Brown, T., James, C. Microbial studies of carcass hygiene issues, International Symposium on Food and Agricultural Products: *Processing and Innovations Naples, Italy*. 24-26. 2007.
- Bosilevac, J.M., Arthur, T.M., Wheeler, T.L., Shackelford, S.D., Rossman, M., Reagan, J.O. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and Enterobacteriaceae are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *Journal of Food Protection*. 67: 646-650. 2004.
- Bosilevac, J.M., Shackelford, S.D., Brichta, D.M., Koohmaraie, M. Efficacy of ozonated and electrolyzed oxidative waters to decontaminate hides of cattle before slaughter. *Journal of Food Protection*. 68: 1393-1398. 2005.
- Van Donkersgoed, J., Jericho, K.W.F., Grogan, H., Thorlakson, B. Preslaughter hide status of cattle and the microbiology of carcasses, *Journal of Food Protection*. 60: 1502-1508. 1997.
- O'Flynn, G., Ross, R.P., Fitzgerald, G.F., Coffey, A. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*. 70: 3417-3424. 2004.
- F.S.I.S.U.S.D.O. Agriculture, FSIS Directive SAFE AND SUITABLE INGREDIENTS USED IN THE PRODUCTION OF MEAT POULTRY AND EGG PRODUCTS, <https://www.fsis.usda.gov>, 2018.
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V. Effect of Essential Oils on Pathogenic Bacteria, *Pharmaceuticals*. 6. 1451-1474. 2013.

- [25] Jia, P., Xue, Y.J., Duan, X.J., Shao, S.H. Effect of cinnamaldehyde on biofilm formation and sarA expression by methicillin-resistant *Staphylococcus aureus*. *Letters in Applied Microbiology*. 53(4): 409-416. 2011.
- [26] Zhang, H., Zhou, W., Zhang, W., Yang, A., Liu, Y., Jiang, Y., Huang, S., Su, J. Inhibitory effects of citral, cinnamaldehyde, and tea polyphenols on mixed biofilm formation by foodborne *Staphylococcus aureus* and *Salmonella enteritidis*. *Journal of Food Protection*. 77(6): 927-933. 2014.
- [27] Cabeza, L., Taylor, M.M., DiMaio, G.L., Cot, J. Processing of Leather Waste: Pilot Scale Studies on Chrome Shavings. Part II. Purification of Chrome Cake and Tanning Trials. *Journal of American Leather Chemists Association*. 93(3): 83-98. 1998.
- [28] Giaouris, E., Heir, E., Hebraud, M., Chorianopoulos, N., Langsrud, S., Moretro, T., Habimana, O., Desvaux, M., Renier, S., Nychas, G.-J. Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Science*. 97(3): 298-309. 2014.
- [29] Yuan, Y., Hays, M.P., Hardwidge, P.R., Kim, J. Surface characteristics influencing bacterial adhesion to polymeric substrates. *RSC Advances*. 7(23): 14254-14261, 2017.