A Novel Approach of Removing Externally Attached Debris from Animal Carcass to Ensure Meat Safety and Byproduct Quality

by
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Abstract

In this study, a formulation and technique are developed to be used for cattle carcass decontamination prior to removal of hide in a commercially preferred time-frame to ensure meat safety and byproduct quality. This formulation offers deep cleaning on carcass surface by removing debris including manure/mud balls which are firmly attached to the hair of animal hide harboring pathogens like *Salmonella* and *Escherichia coli*. Survival of such pathogens can facilitate cross-contamination of the underlying meat and meat-processing equipment in the packing plant posing a challenge to the meat industry as well as public-health. Also, the attached adobe type mud/manure balls have potential to create holes on the hide during leather processing which degrades byproduct's quality. Formulation was sprayed on cattle's hide and the attached debris were brushed off from the surface. The formulation was found very efficient in cleaning the hide surface both at 5 and 8 min treatments. The highest of aerobic, *Escherichia coli* and *Salmonella* populations were reduced by 8.71, 3.63 and 3.19 Log CFU/50 in^2, respectively when compared to water-wash. The efficacy of formulation can be optimized by adjusting its concentration and treatment time. Post-leather analysis showed no detrimental impact on byproduct caused by the formulation.

Introduction

Animal meat and hides are the main product and byproduct, respectively of the meat industries. In the meat processing facility (e.g., beef and pork), animals undergo a process where they are stunned, bled, flayed, eviscerated, and assembled into small pieces of meat that are packaged for public consumption or restaurant trade. Separately, after skinning, hides are processed into leather, a valuable commodity. Prior to entering the meat processing facility, animals are externally tarnished with various foreign materials such as dirt, manure, mud, and plant materials that adhere and entangle on their hair as well as microbial contamination. These debris, in particular hardened manure and mud balls (e.g., adobe-type), not only hinder the proper cleaning process[1-5] of the animal surface posing the threat of microbial cross-contamination from hide to underlying meat during skinning but also often cause damage such as holes in the hide during leather processing and destroy this valuable byproduct of the meat industry.

Research studies[6-10] showed, usually the interior portion of a carcass containing the meat is sterile, however bacterial contamination occurs because of transfer from hide/skin onto the meat during the slaughter and the hide/skin removal processes. In many cases, this bacterial contamination contains microorganisms that are pathogenic to humans. Enteric pathogenic bacteria, for example, on cattle surfaces serve as significant hazard and pose a substantial challenge to the meat industry as well as to public health. Such pathogens may arise from environmental exposures including from soil and manure during the lifespan of cattle, which may become firmly lodged onto their hides and hair and thus limits cleaning and decontamination efficacy. Therefore, it is important to properly clean the carcass through the removal of external debris before opening up the hide/skin.

Incomplete decontamination of carcasses prior to hide removal serves as a prime source of pathogen transfer to meat during slaughterhouse processing that leads to numerous public health risks and substantial economic loss. Previous studies revealed that meat contamination with pathogens is strongly correlated to hide contamination.[11-12] Due to the pathogenic contamination, a handful number of meat products of different meat processing plants have been recalled in recent years. For instance, an outbreak of *Salmonella* Dublin was linked to ground beef which caused a recall of 35k pounds of ground beef. In this incident, thirteen people were infected from eight states, where nine individuals were hospitalized including one death reported from California.[13] Another outbreak in 2018 caused a huge recall of a total 12.1 million pounds of beef products that was contaminated with *Salmonella* Newport reporting 333 cases in 28 states causing 99 individuals to be hospitalized.[14] In 2013, *Salmonella* Typhimurium was linked to an outbreak in Arizona, Illinois, Iowa, Michigan, Pennsylvania, and Wisconsin, where, 22 illnesses were reported, which was tracked back to two potential companies.[15] Although many *E. coli* are benign and are commonly found in the digestive tracts of mammals, some *E. coli* can cause major health issues, including diarrhea, urinary tract infections, respiratory illness, and bloodstream infections. An outbreak of *E. coli* infections linked to ground beef happened in 2018, where 18 cases were reported in four states with one death and six hospitalizations.[16] In

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2016, veal, beef and cattle products contaminated with *E.coli* from an arbitrator in Massachusetts caused a multistate food related outbreak in Connecticut, Massachusetts, Pennsylvania, and West Virginia and a recall was enacted on meat products from the specific vendor.[17] In 2014, over 1.8 million pounds of ground beef from a packing facility in Michigan was recalled due to its association with an outbreak of cases in Massachusetts, Michigan, Missouri, and Ohio.[18] Investigations of such widespread outbreaks often conclud that, contamination likely occurred in meat processing facilities due to the transfer of pathogens to meat either from haired surface of animal or their environmental contaminants or processing equipment.

Accumulation of external debris on cattle/animal surface mainly happens during the cold season. The mechanism of adobe-type mud/manure ball formation, for example, is liken to a freeze-thaw process where the manure and/or mud are accumulated on the hide hair and hardens as the temperature approaches to freezing. As the cycle repeats, it creates hardened mud and/or manure that becomes exceedingly attached and entangled with the hide hair. If not removed, adobe-type mud/manure balls serve as safe harbor for the microorganism including pathogens and remain firmly attached to the hair when the hides are delivered to tanneries for leather processing. In the tannery, the mechanical (using fleshing machine) process of forcefully removing the mud balls from the hair causes damage to the hide by creating holes, which results in unusable hides/skin or poor-quality leather products.

There are a few methods that have been reported previously that had limited success to partially address this issue. For example, soaking the hides in solutions containing glycerol and sodium carbonate with/without surfactants, enzymatic formulations, and oxidative chemicals such as sodium percarbonate with or without an additional caustic agent.[19-20] First of all, these methods are time consuming in terms of industrial time management and more importantly those soaking methods can only deal with hide/skin not carcass therefore, not applicable in removing mud/manure from carcass prior to removal of hide/skin, thus offer no role in meat safety.

Debris on cattle surface including mud/manure balls serves as a prime source of microbial carcass contamination during animal slaughter and meat processing. Spray washing with water alone or with any washing formulation[21-25] which are currently standard in the industry, for example, have limited effectiveness to remove foreign materials and decontaminate the surface of the hide because washing solutions cannot reach under the debris. Therefore, it is important to remove mud/manure balls or any other external debris to decrease bacterial contamination on animal carcasses prior to hide removal to reduce the risk of human exposure to these microorganisms. Mechanical removal methods such as shaving is inefficient, cumbersome, and inadequate to fully remove the foreign materials and contamination.

There thus exists an ongoing industrial need to develop methods for efficiently cleaning foreign materials including mud/manure balls from animal hides as well as decontamination of animal carcasses. Under this study, a novel formulation and method have been developed for cleaning and decontaminating animal carcass prior to slaughter. More specifically, the invention relates to a complete protocol for removing foreign materials and microorganisms including pathogens from the surface of animal carcass. This will improve meat safety and prevent cross-contamination in meat packing facilities as well as to lower the likelihood of damage of hides delivered to tanners for leather processing.

Materials and Methods

Hide Preparation

De-fleshed bovine hides were collected from a local meat processing facility, JBS Packerland (Souderton, PA). For the experiments, the sections of the hide (from belly and butt areas) which contained most of the external debris including mud/manure balls were cut into pieces of approximately equal size of 12-inch × 12-inch.

Preparation of Decontamination Formulation

Aqueous solution (40-44%) of potassium thioglycolate (K-TG) was purchased from Across Organics. Sodium dichloroisocyanurate dehydrate (SDCC) and sodium hydroxide (NaOH) were purchased from Aldrich Chemical (Milwaukee, WI). Different decontamination formulations were prepared by dissolving/mixing the chemicals in tap water using the concentrations as described in Table I. All

Table I

Composition of carcass decontamination formulation

Formulations	Composition
F-A (control)	Tap water
F-B	2.5% NaOH (wt./v) + 2.5% K-TG (wt./v)
F-C	5% NaOH (wt./v) + 5% K-TG (wt./v)
F-D	7.5% NaOH (wt./v) + 7.5% K-TG (wt./v)
F-X	0.75% SDCC (wt./v)
F-BX	2.5% NaOH (wt./v) + 2.5% K-TG (wt./v) + 0.75% SDCC (wt./v)
F-CX	5% NaOH (wt./v) + 5% K-TG (wt./v) + 0.75% SDCC (wt./v)
F-DX	7.5% NaOH (wt./v) + 7.5% K-TG (wt./v) + 0.75% SDCC (wt./v)

concentrations were dissolved in tap water at room temperature (~22°C) and prepared ~24 h prior to the experimental spray applications on hides.

Spray Treatment on Bovine Hide Panels

Decontamination formulations and water (control) were dispensed from a hand-held 1 Lt. polyethylene spray bottle to the haired surface of individual hide panels containing firmly attached external debris. To cover the whole hide panel (approximately 12 in × 12 in surface area) adequately, a certain amount of 25 mL (25 puffs) of tap water and different formulations were sprayed on individual sample panels. The formulation was allowed to sit for 5 to 8 min before brushing to remove the debris and taking samples for microbial testing.

Manure/Mud Balls Removing Protocol

Picture of each hide panel (Figure 1) was taken before and after the mud/manure/debris removing experiment. After certain time of spray treatment, the hide pieces treated with water and formulation were brushed with a high heat resistant polymeric hand brush to wipe off the debris attached to the haired surface of the hide. Similar forces were applied to brush all the panels. In between the hide panels, the brush was disinfected dipping it in hot water to minimize cross-contamination.

Microbial Testing

After brushing the treated hide panels to get rid of debris, a 10 in × 5 in surface area was independently 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 ~2 min. Samples were serially diluted and spread-plated on Tryptic Soy Agar (TSA), Xylose-Lysine-Tergitol 4 (XLT-4) Agar, Sorbitol MacConkey Agar, with Cefixime and Tellurite (CT-SMAC) for aerobic bacteria, Salmonella and E. coli counts, respectively (all agar was obtained from Fisher Scientific, Pittsburg, PA). After spread plating, samples were incubated between 24 to 48 h at 37°C and bacterial colonies were enumerated for bacterial recovery with the lowest detection level at 1 CFU per 10 in × 5 in area.

Leather Processing and Quality Evaluation

After collecting the microbial samples, the treated hide panels were subjected to tanning to convert them into crust leather following the established USDA tanning protocol.[26-27] To evaluate the final impact of newly developed manure/mud/debris removing formulations on byproduct quality, each leather panel underwent a series of quality tests. These included organoleptic evaluation (break, handle, fullness, and color) and microscopic analysis (data is not included). In the

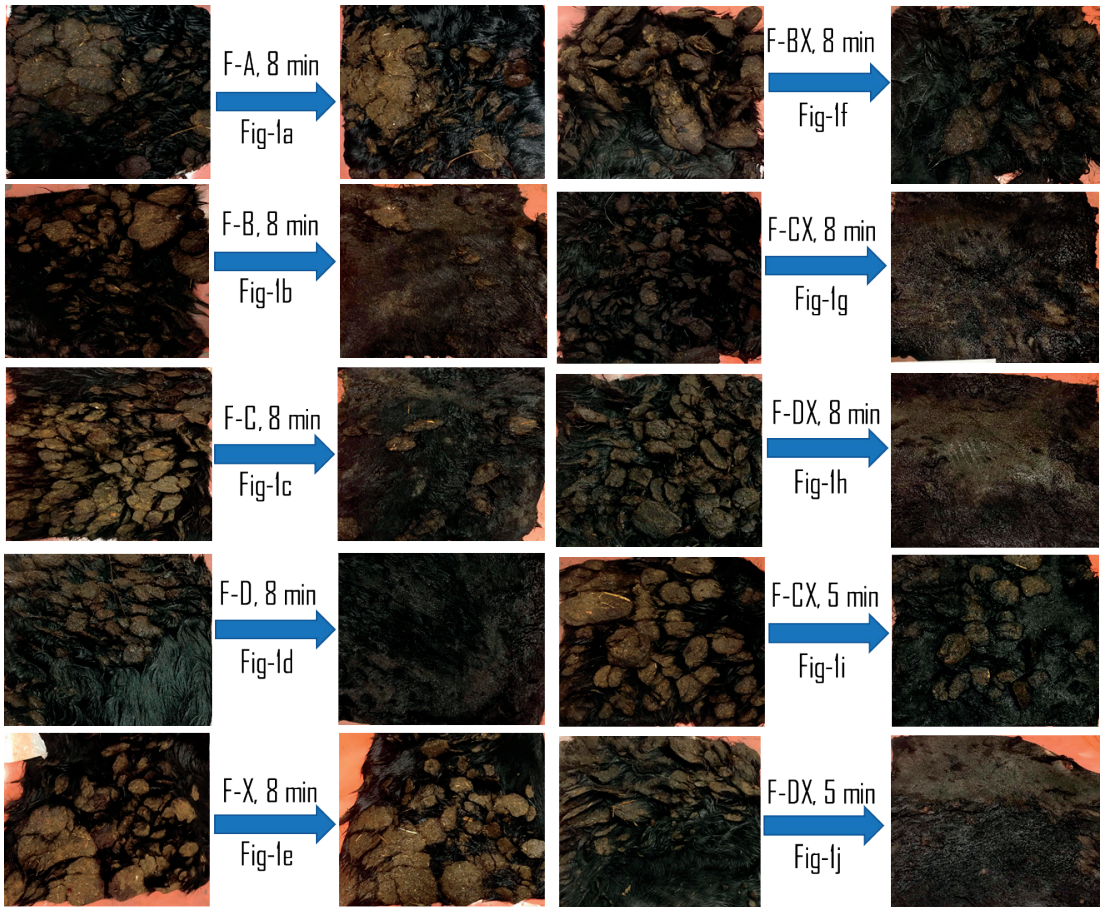

Figure 1. Efficacy of inventive formulations in removing debris from the haired surface of fresh bovine hide.

microscopic analysis, the leather samples were analyzed under a stereo microscope to determine any difference in the grain structures between the formulation treated samples (F-B, F-C, F-D, F-X, F-BX, F-CX, F-DX) and the control (F-A). The leather samples were also subjected to mechanical property analysis such as, tensile strength, elongation ("stretchability"), Young's Modulus ("stiffness") and fracture energy (energy required to fracture the leather sample). All the quality analyses of leather samples produced from the treated hide panels were carried out according to the published procedures.[2,21,28]

Statistical Analysis

All statistical analyses were carried out by using one-way analysis of variance (ANOVA) using Dunnett's comparison tests or unpaired t-tests. All calculations were carried out using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Significance was observed at $p < 0.05$.

Results and Discussion

Previous studies revealed that, meat contamination with pathogens is strongly correlated to hide contamination.[11-12] Therefore, it is important to remove the external debris from the cattle surface prior removal of hides to facilitate the proper cleaning of carcass. It offers not only better limit of surface microorganism including pathogens which pose threat to be migrated to the meat but also removing of debris ensures the quality assurance of byproduct.

As it is found that, those debris, especially adobe type manure/mud balls, are firmly attached to the hair therefore, in this investigation the developed formulation targets to weaken the hair to get off those debris. The inventive formulation combines two types of chemicals dissolved in aqueous solution, 1) manure/mud balls removing chemicals and 2) anti-microbial agent. Manure removing chemicals target to break down the hair by weakening the disulphide bonds of keratin. This formula consisted of a base such as sodium hydroxide (NaOH) and a salt of thioglycolic acid ($HSCH_2COOH$) for example potassium thioglycolate (K-TG). Thioglycolate dissolves the disulphide bonds in keratin where the base increases the pH which helps deliver thioglycolate in the hair. To strengthen anti-bacterial activity, an anti-microbial agent, *N*-halamine (R_1,R_2-N-X) was combined with manure removing chemicals to develop the final formulation. *N*-halamine represents a group of compounds with one or more nitrogen-halogen covalent bonds. They exhibit biocidal properties because of the +1 oxidation state of halide atoms in their molecule. *N*-halamine compounds are stable in aqueous solution and effective in limiting a broad spectrum of microorganism. They are cheap, weakly toxic, less corrosive than bleach, safe to humans and environmentally friendly.[29-32] For this research experiment, Sodium dichloroisocyanurate dehydrate (SDCC) was chosen because of its aqueous solubility. All the chemicals chosen for the formulation impose no/less toxicity as they are being used for human consumption in other applications.[33-34]

Efficacy of Formulation in Removing Debris from Hide Surface

As shown in Figure 1, the brushing after rinsing with water (control, Fig 1a) had no effect on removing debris from the hide surface. Similarly, treating with only SDCC (Fig 1e) solution resulted no removal of mud/manure balls. It was also shown that, the removal of debris including mud/manure balls depended both on the concentration and treatment time. At 8 min treatment, the highest concentration of formulation, F-D and F-DX (Fig 1d and Fig 1h) resulted in better performance in compare to F-B and F-C (Fig1b and Fig 1c) or F-BX and F-CX (Fig 1f and Fig 1g) by removing all the debris from hide surface. Also, F-C (Fig 1c) and F-CX (Fig 1g) worked better than F-B (Fig 1b) and F-BX (Fig 1f), respectively. In time variable experiments, 8 min treatment of F-CX (Fig 1g) worked better that the 5 min treatment (Fig 1i). However, the highest concentration of formulation F-DX showed equal level of efficiency in removing debris as it completely cleaned the hide surface both in 8 min (Fig 1h) and also in 5 min (Fig 1j). For such a short-time effectiveness, this formulation can be used in rapid industrial settings. Also, concentration and time variables can be adjusted based on the need of individual meat processing plants to establish an economically feasible setup.

Efficacy of Formulation in Reducing Bacterial Population from Hide Surface

Generally, underlying meat surface of carcass is sterile, but it can be contaminated as a result of pathogen transfer from hides onto the meat during slaughter and the hide removal process. Microbial decontamination of carcass prior to removal of hide is essential to minimize the risk of pathogen cross-contamination.

Aerobic Bacteria Colony Count

According to Table II, the treatment with every single formulation resulted in more reduction of aerobic bacteria comparing to the control. The formulations even without containing SDIC (F-B, F-C and F-D) also showed significantly better reduction than the control (F-A). This is because of removing debris which harbor microorganisms. It is also noteworthy to mention that, only antimicrobial solution (F-X) did not show the effectiveness in reducing aerobic bacteria as such as other formulations. This is due to the fact that, the formulation spray could not reach many places of the hide panel covered by the external debris to effectively kill the bacteria. Aerobic bacteria counts were reduced with the increased concentration of formulation meaning less debris on hide offers better decontamination. Formulation containing SDIC, F-BX, F-CX and F-DX resulted in 1.54, 2.32 and 3.28 Log CFU more reduction in compare to F-B, F-C and F-D, respectively. Comparing the treatments between 8 min and 5 min, results showed better reduction at longer time. Formulations F-CX and F-DX reduced 2.26 and 2.88 Log CFU/50 in^2 more of aerobic bacteria. respectively at 8 min in compare to their 5 min's treatments.

Table II

Survival of nature-borne bacteria (*Aerobic*, *Escherichia coli* and *Salmonella*) on bovine hide panels following the debris removing treatment.

Time of Treatment	Formulation	Bacterial populations recovered from haired surface of hide panels after treating with formulations (Log CFU/50 in^2)*		
		Aerobic bacteria colony count	*Escherichia coli* colony count	*Salmonella* colony count
8 min	F-A (control)	9.27±0.01^a	4.06±0.52^a	3.34±0.08^a
	F-B	7.70±0.21^b	3.70±0.12^a	0.15±0.27^{d,e}
	F-C	6.32±0.37^c	2.68±0.29^c	1.74±0.39^c
	F-D	3.84±0.06^a	2.90±0.09^c	1.25±0.24^c
	F-X	8.58±0.05^a	2.01±1.73^c	2.10±1.82^b
	F-BX	6.16±0.22^c	3.93±0.11^b	1.88±0.03^c
	F-CX	4.00±0.06^d	1.75±0.04^d	0.33±0.33^{d,e}
	F-DX	0.56±0.05^{e,f}	0.43±0.43^{e,f}	1.05±0.11^c
5 min	F-A (control)	9.11±0.01^a	4.19±0.52^a	3.46±0.08^a
	F-CX	6.26±0.33^{b,c}	3.71±0.04^b	2.11±0.06^{b,c}
	F-DX	3.44±0.42^d	2.63±0.13^c	0.33±0.33^d

*Results presented are a representation of triplicate calculation of bacterial population per sample.
The Dunnett's test was to evaluate the significance with confidence level was set to 95%; different letters within the same column indicate significant differences ($p < 0.05$).

Escherichia Coli Colony Count

In comparison with the control (F-A), inventive formulation resulted in maximum reduction of *Escherichia Coli* (*E. coli*) from the hide panels by 3.63 Log CFU/in^2 and minimum reduction of *E. Coli* by 0.13 Log CFU/in^2. The lowest *E. coli* population was counted from the hide panel treated with F-DX formulation at 8 min as expected. The addition of SDIC in formulation helped in further reduction of *E. coli* by 0.93 and 2.47 Log CFU/in^2 for F-CX and F-DX, in compare to F-C and F-D, respectively. However, hide panels were cut from a freshly flayed bovine hide collected from a local slaughter house and used for the experiments without any pretreatment to capture the real problem to be identified, therefore each piece of hide was loaded with different initial concentration of microorganism. Possibly for this reason, F-BX resulted in slightly higher colony count of *E. coli* than F-B. Treatment at 5 min with F-CX and F-DX also reduced *E.coli* population by 0.48 and 1.56 Log CFU/in^2, respectively in comparison to the 5 min-control. The bacterial population was reduced further by 1.96 and 2.2 Log CFU/in^2 when the hide panels were treated for 8 min instead of 5 min with F-CX and F-DX, respectively.

Salmonella Colony Count

As shown in Table II, similar results were obtained in recovery of *Salmonella* from the treated hide panels. All the formulations with/without SDIC were able to reduce *Salmonella* populations significantly. At 8 min treatment, F-B, F-C and F-D resulted in reduction of 3.19, 1.6 and 2.09 Log CFU/in^2, respectively in compare to the control (F-A). Treatment only with SDIC (F-X) was not effective as found in other cases. It only reduced 1.24 Log CFU/in^2 when compared to the water treatment (F-A). Formulations containing SDIC, F-BX, F-CX and F-DX offered reduction of *Salmonella* populations by 1.46, 3.01 and 2.29 Log CFU/in^2 respectively when compared to the control. Treatment with F-CX at 8 min reduced more *Salmonella* than its 5 min treatment as expected, however F-DX in 5 min showed better result over the 8 min treatment, was probably accounted for the difference in initial load of *Salmonella* on naturally collected hide surface.

Post-Leather Analysis

Bovine hide is a valuable byproduct as it produces leather which is a popular commodity. Therefore, it is important to evaluate that, any treatment on raw hides does not create any detrimental impact when the treated hide is converted into leather. From our previous experiment It was proven that the usage of SDIC alone on hide surface had no detrimental effect on leather quality (Sarker *et al*. 2020). For this study, all the leather panels produced from either formulation treated or control (water treated) hide samples were subjected under microscopic, organoleptic and mechanical property analysis for a side-by-side comparison. Microscopic analysis carryout out with a stereo microscope revealed (data is not included) no distinguishable difference on grain structures among

the leather pieces. Additionally, the leather panels were folded, and a stereo microscopic image was taken (data is not included) at the crease to observe if there was any sueding (fraying) from any of the samples. Again, there was no discernable difference between the experimental samples and the control. In Organoleptic analysis, crust leathers from formulation treated hides were assessed for softness, fullness, grain tightness (break), color and general appearance by hand and visual examination. This evaluation done by an USDA tanner exhibited similar subjective properties of all kind in comparison with the control. All this analysis suggests that the inventive formulations have no detrimental impact on subjective properties of finished leather.

Evaluation of leather panels for mechanical properties (Table III) revealed that, there was little to no difference on leather quality. Mechanical properties including tensile strength, elongation, Young's Modulus, and fracture energy of the leather samples produced from formulation treated hide samples (F-B, F-C, F-D, F-X, F-BX, F-CX and F-DX) were comparable to that produced from water washed hide panel (F-A, 8 min treated control was only evaluated). The little deviations in numbers can be attributed to naturally occurred uneven thickness of the bovine hide.

Conclusion

The removal of external debris from live cattle surface or at pre-eviscerated state of animal is a huge challenge for the meat industry. To clean the mud/manure debris is an essential task before meat processing as it relates to meat safety and also byproduct quality. The inventive formulation has been proven for its efficacy to remove external debris in such a short time that it has the potential to be used in industrial scale. The removal of attached debris from the haired surface facilitates the cleaning process of the animal carcass prior to removal of hide as it is shown in this study. Microbial populations, including nature-borne pathogens, were significantly reduced from the hide surface with the removal of debris when compared with water and also only antimicrobial solution treated hides. Therefore, the chance of cross-contamination of pathogens from hide to underlying meat will be minimized during meat processing. Also, the formulation treatment protects the hide's quality from being reduced during processing through the removal of adobe type of mud/manure balls which are firmly attached to the hair. The usage of formulation for such a short time on carcass surface has been demonstrated as non-detrimental for the valuable byproduct. This developed technology can potentially replace the current tedious and inefficient shaving or other conventional methods of removing attached debris from the cattle carcass which will save labor cost, utility cost and the most importantly reduce cross contamination during meat processing.

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Table III

Mechanical properties of crushed leather panels produced from water (control) and formulation treated hide pieces

Time of Treatment	Formulation	Tensile Strength (MPa)	Elongation, %	Young's Modulus (MPa)	Fracture Energy J/cm^3
8 min	F-A (control)	13.61 ± 2.32	45.26 ± 6.09	19.88 ± 5.15	1.58 ± 0.48
	F-B	15.154 ± 2.28	44.52 ± 7.25	14.99 ± 6.24	2.19 ± 0.70
	F-C	12.82 ± 2.00	45.03 ± 6.68	19.86 ± 2.7	2.37 ± 0.92
	F-D	15.35 ± 4.57	49.11 ± 6.25	18.50 ± 4.77	3.59 ± 1.49
	F-X	14.37 ± 2.13	46.23 ± 6.62	14.35 ± 7.19	2.08 ± 0.35
	F-BX	13.03 ± 1.28	46.48 ± 2.47	14.79 ± 0.98	1.40 ± 0.21
	F-CX	15.25 ± 6.15	38.72 ± 5.54	13.45 ± 2.24	1.86 ± 0.84
	F-DX	16.87 ± 1.31	42.43 ± 3.07	17.50 ± 8.52	2.32 ± 0.28
5 min	F-CX	14.65 ± 2.31	40.54 ± 5.75	13.62 ± 5.6	2.84 ± 0.53
	F-DX	15.39 ± 6.81	42.11 ± 7.65	11.87 ± 6.50	2.07 ± 1.00

Conflict of Interest

The authors declare that there is no conflict of interests on the work published in this paper.

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