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COMPLIANCE EFFICACY OF MODIFIED CURING METHODS TO CONTROL BLACK BENGAL GOAT SKIN DETERIORATION

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ABSTRACT

The study was conducted to determine microbial spoilage of skins of Black Bengal goats at various stages and to assess the efficacy of modified curing processes of that skins, during the period of July to November 2006. A total number of six intact skins from Black Bengal goat of two years of age were obtained from markets located at Bangladesh Agricultural University Campus and Mymensingh town. After the usual traditional method of flaying these skins were properly washed prior to curing process. Raw or green skin samples without subjected to treatment process were used as control. Two categories of samples were prepared, one immediately after the completion of flaying, i.e. considered as zero hour of sampling and the other after twenty-four hours of storage at the ambient temperature. The rest of the samples were treated with different preparations of Neem oil, Tolcide (30L), and Busan (40L) and common salt and kept for various periods. From each of the differently treated skins, bacteriological samples were taken aseptically from the regions of shoulder, belly and butt each sampling weighing about 10 grams. The bacteriological analysis of the samples was performed by determining total viable count in order to find out the extent of contamination and varying bacterial load of the three different regions. Moreover, the isolation and identification of various bacteria contaminating the skin samples was done and the efficacy of curing agents to minimize bacterial load and the survivability of microbes were studied. The results showed that *Staphylococci*, *E. coli*, *Bacillus*, *Pseudomonas* etc bacteria were isolated from raw and cured skin samples and Tolcide (30L) 0.04% and Busan (40L) 0.04% and Neem oil 10% and common salt 20% were the best curing agents from microbial quality point of view.

Key words: Goats skin, curing agent, bacterial load, TVC, TCC, TSC

INTRODUCTION

Hides and skins, the basic raw materials of leather industries, are obtained as by-products of livestock industries. The annual availability of hides and skins in Bangladesh is 15 million square meters. Unlike others, it is a constant source of export earnings and contributes about 10.7% of the total export earnings. However a large proportion of the materials is downgraded and rejected by their defects. An annual (1990-91) economic loss of Taka 818 crores or US $ 220.95 millions (cattle US $ 194.5 m., buffalo US $ 1.9 m., goat US $ 24.1 m., sheep US $ 0.5 m) was estimated to be associated with leather defects in Bangladesh (Dey and Nooruddin, 1993). In Bangladesh leather industries (about 214) are mostly concentrated in Hazaribagh area. About 50,000 tons of hides and skins are processed in this area yearly, and daily 15,000 m^3 waste water is discharged into the nearby flowing river Buriganga. The impact is that the river has now become mostly unsuitable for fish habitation (Rahman, 1996).

Most of the degenerative changes in the skins are brought about by some bacteria or fungi normally present on the skins. Some complex chemicals within the skins known as enzymes have also damaging effect on the flayed skin. The skin of live animal is resistant to putrefactive bacteria. But on death, warmth and moisture promote bacterial growth and penetration of them into skin tissue with their enzymes attacking interfibrillary proteins, cellular structures and then the protein fibers. Putrefaction causes weakening of reticular tissue, splitting of fiber bangles, and loss of cellular structure and breakdown of keratin (Kritzinger, 1946). The hot and humid monsoon season in a country like Bangladesh cure provides a special storage problem, which is aggravated by the condition of preparing, collecting and curing process of skins. Nandy and Sen (1962) and Shirayama *et al.* (1967) used antibiotics for preservation of hides and skins and obtained positive result to a great extent. Curing is the temporary preservation of hides and skins from the time of flaying to processing in the tannery. It is essentially an attempt to prevent or at least to curtail bacterial decomposition of the skin protein during the time elapsing between slaughtering, flaying and processing in the tannery (Kritzinger, 1950).

The period from flaying to initial treatment with salt to render hides and skins non-putrescible is generally called postmortem period. DeBeukelaer (1956) considered this period very critical from the standpoint of maximum conservation of the hides initial leather-making potential. An adequate cure should prove feasible with a conventional simple application of salt plus bactericidal additive. This would reduce total salt consumption between 30 and 40% of green weight and reduce national foreign exchange costs and minimize tannery effluent problem. In Bangladesh fresh hides and skins are traditionally cured by salting. In Britain and European countries, salt preservation is still used extensively due to its low cost, ease of application and the maintenance of resulting leather quality for extended storage periods (Berwick *et al.,* 1990). Correct methods of curing, the proper doses and the size of the grain of salt used as preservative are basic prerequisites for the production of good quality leather (Samad *et al.,* 1984). Samad *et al.,* (1984) observed that hides treated with (i) 40% common salt, (ii) salt + 2% sodium carbonate, (iii) salt + 2% zinc chloride, (iv) salt + 1% naphthalene had an inhibiting growth of predominant proteolytic bacteria like gram positive micrococci and bacilli and thus improved the condition of hide. So, the present research work was undertaken to assess the efficacy of the modified curing method of Black Bengal goat skins commensurating with the reduction of water pollution and to find out the effects of different treatments to be incorporated in the modified curing method.

MATERIALS AND METHODS

Experimental design

The whole study was conducted in the Department of Microbiology and Hygiene, during the periods of July to November 2006. A total number of six intact skins from Black Bengal goat of two years of age were obtained from markets located at Bangladesh Agricultural University (BAU) Campus and Mymensingh town. After the usual traditional method of flaying these skins were properly washed prior to curing process. Raw or green skin samples without subjected to treatment process were used as control. Two categories of samples were prepared, one immediately after the completion of flaying, i.e. considered as zero hour of sampling and the other after twenty-four hours of storage at the ambient temperature. The rest of the samples were treated with different preparations of Neem oil, Tolcide (30L), and Busan (40L) and common salt and kept for various periods as per recommendation of Samad *et al.* (1984) and Kirtikar and Basu (1995). From each of the differently treated skins, bacteriological samples were taken aseptically from the regions of shoulder, belly and butt each sampling weighing about 10 grams. The bacteriological analysis of the samples was performed by determining selected bacterial attributes of the three different regions. Moreover, the isolation and identification of various bacteria contaminating the skin samples was done and the efficacy of curing agents to minimize bacterial load and the survivability of microbes were studied.

Curing of skin samples

Curing of skin samples were subjected to curing process as per following schedule (Table 1).

Table 1. Curing agents and storage periods

Sample No.	Treatment given with curing agents	Duration of storage time
1	No treatment	As control
2	No treatment	0 to 24 hours
3	Tolcide (30L) 0.04% and Busan (40L) 0.04%	22 days
4	10% Neem oil and 10% common salt	06 days
5	10% Neem oil and 20% common salt	10 days
6	10% Neem oil and 20% common salt	30 days

Examination of skin samples for bacteriological studies

Samples collection, transportation and inoculum preparation: Skin portion were excised aseptically from shoulder, belly and butt regions of apparently healthy Black Bengal goat of one 1 year 9 month to 2 two years of age each weighing about 10 grams, using sterile instruments and transferred carefully to sterile containers. The samples were brought to the laboratory within 30-45 minutes for subsequent studies to determine the

bacteriological status. During transportation, the samples in sterile containers were kept in iceboxes using fragments of good quality ice. Sample weighing 10 grams was subjected to studies for bacteriological analysis. According to the procedure an amount of 90 ml Phosphate buffer saline was added to individual raw or cured samples and homogenized in Colworth stomacher as per standard method. Thus a 1:10 dilution of the sample was prepared. Subsequently using whirly mixture machine different serial dilutions ranging from 10^{-2} to 10^{-5} were prepared according to the instruction.

Enumeration of Total viable count (TVC), Total coliform count (TCC) and Total staphylococcal count (TSC): For the determination of TVC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate NA agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 35^0C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVC. For the determination of total coliform, TVC method was employed. For TCC method MC agar plates were used. For TSC, the procedures of sampling, dilution and streaking were similar to those followed in TVC of bacteria. Only in case of staphylococcal count, SM-110 was used. The calculation for TSC was similar to that of TVC (Carter, 1986).

Isolation and identification of bacteria from the skin samples

Isolation and identification of bacteria from the skin samples were based on the morphology (size, shape, arrangement and motility), colony characteristics, Gram's staining property, biochemical reaction, catalase test, IMViC reaction, DNAase test, oxidase test and nitrate reduction test as suggested by Wilson *et al.* (1979), Cowan (1985) and Carter (1986).

Isolation and identification of *Staphylococcus*: The colonies of *Staphylococcus* were round, glistering, convex, smooth and opaque. They were gram-positive cocci arranged in cluster. Most *Staphylococcus* was catalase positive. Beta (β) hemolysis was produced by most strains on BA. The coagulase test was performed for the identification of the pathogenic *Staphylococci aureus* from non-pathogenic ones. Coagulase negative *Staphylococci* did not produce β-hemolysis.

Isolation and identification of *E. coli* and other coliforms: Coliform organisms grew well on BA and MA media. The organisms were oxidase negative. To identify *E. coli* and other coliforms lactose fermenting red colonies from the MA were subcultured on EMB agar. Colonies on EMB agar with metallic sheen were suspected as positive for *E. coli* and were confirmed by the IMViC test. *E. coli* was positive to indole and MR tests but negative to VP and citrate tests.

Isolation and identification of *Pseudomonas* sp.: These were gram-negative rods and oxidase positive. The organisms grew well on BA but might be recovered form other media. They produced large grayish colonies with irregular spreading margins. Some cultures were markedly mucoid. On blood agar *Pseudomonas* frequently produced beta (β) hemolysis. On MA media, colonies were colorless. When the organisms were subcultured on NB at 37^0C for 24-48 hours, bluish green color appear in the broth culture and distinct ammoniacal odor was perceptible, indicating the presence of pigment producing organisms, i.e. *Pseudomonas*. Most *Pseudomonas* was motile and negative to indole test.

Isolation and identification of *Bacillus* spp.: For the isolation and identification of *Bacillus*, the samples were diluted, inoculated on BA and incubated at 37^0C for 24-48 hours. On Gram's staining the gram positive large sporulated rod shaped bacteria in chain form indicates *Bacillus.* Many rod shaped bacilli produced β-hemolysis

Isolation and identification of *Flavobacterium* and *Enterobacter*: *Flavobacterium* was the gram negative rod, and produced yellow colony. The organism was shown to be aerobic, catalase positive and exhibited oxidase positive reaction. *Enterobacter* were gram negative rod, motile, aerobic and facultatively anaerobic. They were catalase and VP positive, but oxidase, Indole and MR negative.

Maintenance of stock culture

For the maintenance of stock culture, nutrient agar (NA) slants were employed. One slant was used for individual isolate and was kept at room temperature. Finally sterile mineral oil was overlaid and the culture was kept at refrigeration temperature for further study.

Statistical analysis

The data on TVC, TCC and TSC obtained form the study of skin samples were analyzed in completely randomized design (CRD) using computer package MSTAT-C (Freed, 1992). Correlation between TVC, TCC and TSC were evaluated.

RESULTS AND DISCUSSION

Bacterial population in different locations of skin

The results as presented in Table 2 demonstrated that the bacterial load was more in skin staled after 24 hours than fresh skin. This explanation in favor of the higher count was postulated by other authors (Leach, 1995).

Table 2. Extent of bacterial contamination* on different skin regions prior to curing process

Skin regions	Extent of bacterial load per gram of raw skin sample											
	Total viable count				Total coliform count				Total staphylococcal count			
	BAU market		Town market		BAU market		Town market		BAU market		Town market	
	'0' H	24 H	'0' H	24 H	'0' H	24 H	'0' H	24 H	'0' H	24 H	'0' H	24 H
Shoulder	5.57	5.69	5.61	5.70	4.89	4.98	4.52	4.70	5.27	5.43	4.82	4.97
Butt	5.32	5.42	5.27	5.36	Nil	Nil	4.36	4.14	5.15	5.31	5.02	5.13
Belly	5.32	5.80	5.65	5.77	Nil	4.32	Nil	Nil	5.06	5.23	5.11	5.28
Mean± SD	5.47± 0.13	5.64± 0.20	5.51± 0.21	5.61± 0.22	1.63± 0.00	3.10± 2.70	2.96± 2.56	2.95± 2.57	5.16± 0.11	5.32± 0.10	4.98± 0.15	5.13± 0.16

*All counts are expressed in logarithm; SD = Standard deviation; H = Hour; BAU = Bangladesh Agricultural University.

Bacterial population in cured skin samples after different storage periods

If skin was not properly cured or proper dose of preservatives were not applied, the combined effects resulting from the presence of moisture in the skin and the proteolytic activity of bacteria would render putrefaction and ultimately cause destruction of the hide substance (Elliot, 1985). It was note worthy from tables 3 that, samples taken from cured skin at different storage periods exhibited variation of bacterial attributes as grown on different media. Further observation in Table 3 revealed that the salt tolerant organisms of the skin treated with four different curing agents gradually adapted themselves and grow even at high salt concentrations. The preservative effects of salt were often described in terms of its osmotic effect. For all these reasons the objective of preservation of skin was achieved a moisture content of 10-20%. If the water content was reduced, the bacterial activity decreased and eventually stopped when the moisture content was less than 35% (Leach, 1995).

Table 3. Selected bacterial attributes* per gram of skin samples of shoulder, butt and belly regions cured by employing different curing processes

Parameters studied	Survivability of viable bacteria in skin samples after curing			
	Busan (40L) and tolcide (30L)	Neem oil 10% and common salt 10%	Neem oil 10% and common salt 20%	Neem oil 10% and common salt 20%
	22 days storage Mean ± SD	6 days storage Mean ± SD	10 days storage Mean ± SD	30 days storage Mean ± SD
TVC	5.28±0.09	5.19±0.14	5.31±0.18	5.35±0.13
TCC	4.72±0.00	4.51±0.00	4.43±0.00	0.00±0.00
TSC	5.31±0.42	5.31±0.38	5.33±0.26	5.40±0.32

*All counts are expressed in logarithm; SD = Standard deviation; TVC = Total viable count; TCC = Total coliform count; TSC = Total staphylococcal count.

Storage property of skin treated with various curing agents

During storage condition the storage property of cured skins were noted and the observations were recorded in Table 4. It was revealed that Busan (40L) and Tolcide (30L) treatment given to cure skin did not show any bacterial spoilage on the 10th day of storage. Hair slip was the most obvious sign of spoilage in the skins, but the contaminating bacteria also reveal themselves in other ways. The offensive smell and the discoloration in spoiled materials are usually attributed to waste products of bacterial activity. Skins exhibiting hair slip should be discarded and no attempts be made to process them (Thorstensen, 1993 and Leach, 1995).

Table 4. Storage property of skin samples of different regions cured with different curing agents and kept for various periods

Skin regions	Bacterial growth evidencing spots/color changes/sliminess at different storage period												Observation on changes occurring in skin properties after specific storage time of 4 weeks											
	10 days				20 days				22 days				Hair slip				Smell				Tensile strength			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Shoulder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	MES	B	SB	SB
Butt	-	+	-	-	+	+	-	-	+	++	-	+	-	+	-	-	+	++	-	+	SB	LB	SB	SB
Belly	-	-	-	-	-	-	-	+	-	-	-	++	-	-	-	+	-	-	-	++	SB	SB	SB	ME

- = No change; + = Putrefaction begins; ++ = putrefaction continues emiting foul smell; +++ = Degeneration starts; T1 = Busan (40L) and tolcide (30L); T2 = Neem oil 10% and common salt 10%; T3 = Neem oil 10% and common salt 20%; T4 = Neem oil 10% and common salt 20%; ME = Marginal elasticity; SB = Strongly bound; LB= Loosely bound.

Percent distribution of selected bacterial isolates present in skin samples

Staphylococci showed the highest percentage of occurrence in all the raw and cured skin samples (Table 5). The presence of such high number of *Staphylococci* in the skin samples was alarming (Gianelli, 1985; Ruhrmann, 1987 and Larsen *et al.*, 2000). The presence of pathogenic *Bacillus* in the skin samples treated with treatment 1, 3 and 4 in skin sample must receive particular attention as these organisms are responsible for causing hazards to public health. Elder *et al.* (2000) made similar observations and found correlation of enterohemorrhagic *E. coli* prevalence in feces, hide and carcasses during processing. The prevalence of *Pseudomonas* sp. in skin samples signified the fact that the skins are contaminated with soil or water as these organisms are widely distributed in nature.

Table 5. Percent distribution of bacterial isolates obtained from skin samples, cured with various agents for different duration

S/N	Isolates	Percentage distribution of isolates in the samples				
		Raw skin samples	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	*Staphylococcus* spp.	81.32	61.08	63.72	70.21	85.57
2	*Escherichia coli*	5.78	11.87	31.16	-	-
3	*Pseudomonas* spp.	12.90	-	-	-	1.32
4	*Bacillus* spp.	-	27.05	-	19.72	13.11
5	*Enterobacter* spp.	-	-	5.12	-	-
6	*Flavobacterium* spp.	-	-	-	10.07	-

Treatment 1 = Busan (40L) and tolcide (30L); Treatment 2 = Neem oil 10% and common salt 10%; Treatment 3 = Neem oil 10% and common salt 20%; Treatment 4 = Neem oil 10% and common salt 20%.

The high number of *Staphylococci* as revealed in this study should receive potential attention as an occupational health hazard. Nagase *et al.* (2002) indicated the hazard occurring due to *Staphylococci.* These organisms were found to be highly resistant to common antibiotics; as a result the skin handlers, processors and tanners may acquire these organisms during processing which become health risk factors for them.

Correlation among TVC, TCC and TSC in cured skin and hide samples

A highly significant correlation ($p>0.01$) was found between TVC and TSC, which agreed with the report of Gianelli (1985) and Ruhrmann (1987) where they found the maximum abundance of the staphylococci among all the other microbes in the skin samples. But, no significant correlation was found between TVC and TCC ($p>0.01$) also between TCC and TSC ($p>0.01$). Correlation between TVC and TSC signified that staphylococcal count could be taken as an index of bacteriological quality of the hide and skin samples, but as there was no significant correlation between TVC and TCC, so counts of Coliforms could not be taken as a good index of bacteriological quality of skin samples, although high coliform counts present may cause alarms of potential hazards related to fecal contaminations.

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