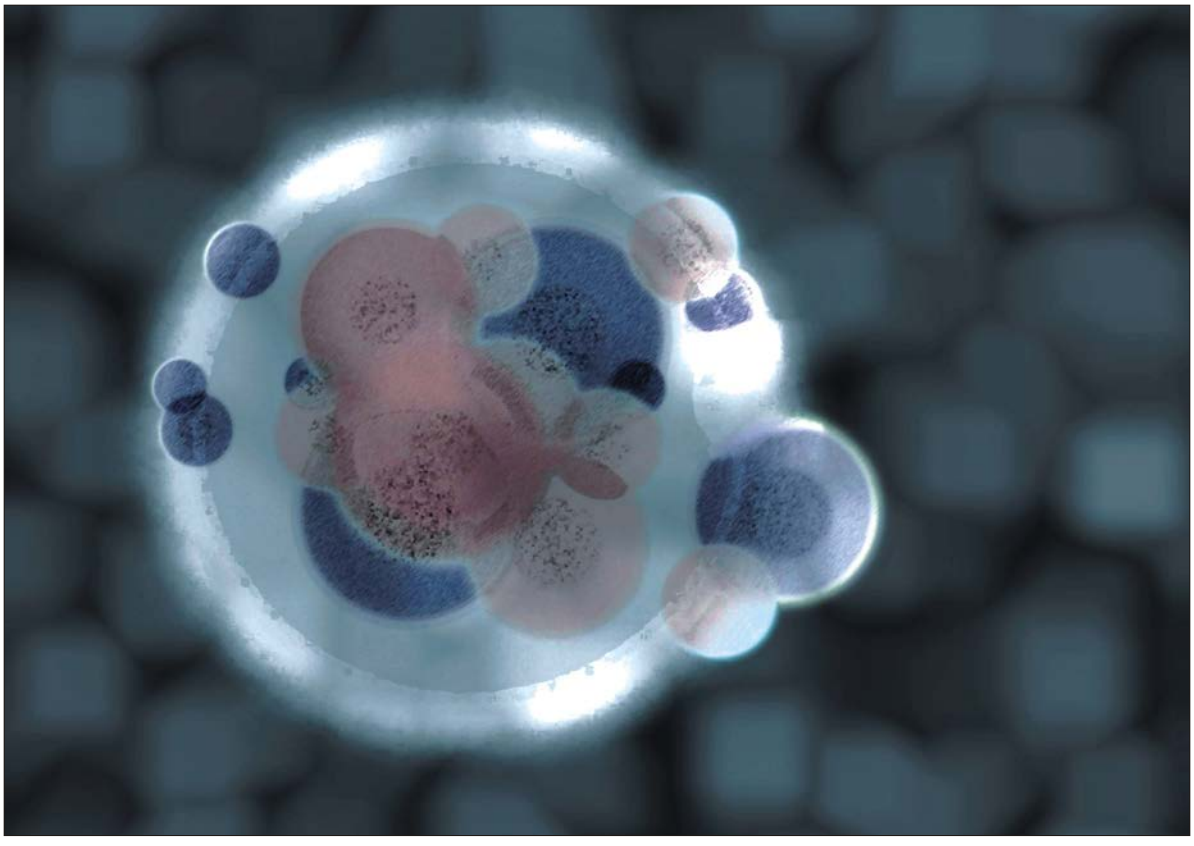

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 5 (2): 181-185, 2005
ISSN 1727-3048

Efficacy of Neem Oil in Pretanning Operations to Control Green Hide Deterioration

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Abstract: The study was carried out to determine the efficacy of neem oil and common salt as curing agent to control biodegradation of green hides. A total number of nine intact hides were obtained from slaughtered cattle of 4 and 4½ years of age. Three types of curing treatments were employed using various concentrations of neem oil and common salts. T_1 provides 40% neem oil and 10% common salt, T_2 includes 10% neem oil and 20% common salt and T_3 contains 10% neem oil and 10% common salt. The hides of each treatment were kept at 10, 20 and 30 days storage periods. After every 10 days of storage hides were subjected to bacteriological examinations. Storage properties of hides were also determined by the evidence of spot, color changes and sliminess of the skin. The mean value of total bacterial count was recorded lowest 6.96±0.30 in T_2. Total coliform counts were found almost nil in T_2. None of the treatment was found effective to control the growth of staphylococci. No signs of bacterial spoilage in hides were evident in T_1 upto 10 days of storage. However indication of some sliminess could be noticed in the regions of neck and butt after storage of 20 and 30 days. T_2 exhibited more or less changes in color on the 10th day of storage and sliminess was noticed fairly distributed on the neck and belly regions on the 30th day of storage. T_3 exhibited some noticeable changes in the butt region. Putrefactive action started and there was indication of tissue changes, which could be marked, from the 10th day of storage. At the butt region foul odor was perceptible and few hair slip was evidenced on the 30th day of storage. It is concluded that the treatment using combination of neem oil and common salt showed antibacterial activity against the growing contaminating putrefactive organisms but less effective against halophiles.

Key words: Efficacy, neem-oil, pretanning, green-hide, deterioration

INTRODUCTION

In Bangladesh the tanners usually operate through collection agents who accumulate hides and skin and preserve them through wet salting or salting and drying. It has been established that sodium chloride used in the curing of raw stock is one of the major pollutants in the tanning industry, which affects the biofauna of the environment. In earlier researches considerable success has been achieved with antibiotics or chemicals having antimicrobial property in preservation of hides and skin[1-3].Although antibiotics showed pronounced antimicrobial activity against common putrefactive organisms, but these proved much less effective against halophilic bacteria[4-7].

Current approach for overcoming this problem of deterioration consists in finding suitable alternative materials for curing and generates technology for developing improved curing agents. In this regard antibiotic mixture with some antiseptics, possessing antibacterial activity against halophilic organisms, in admixture with common salt may considerably improve the curing efficiency of the salt[8]. Moreover many researchers emphasized the feasibility of an adequate cure with a conventional simple application of salt plus bactericidal additives, because this would lower the total salt consumption between 30 and 40% of the green weight and thus reduce national foreign exchange costs and minimize tannery effluents problems[6,9,10]. The present study with this end in view encompasses a modified vegetable pretanning method in which neem oil has been incorporated. The study was conducted to find out the efficacy of neem oil as bacteriostatic or bactericidal agent to control bio-deterioration and enhance the storage property of hides.

MATERIALS AND METHODS

A total number of nine intact hides were obtained from slaughtered cattle of 4 and 4½ years of age. After the

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usual traditional method of flaying these hides were properly washed prior to curing process. Three types of curing treatments were employed using various concentrations of neem oil and common salt. T_1 provides 40% neem oil and 10% common salt; T_2 includes 10% neem oil and 20% common salt and T_3 contains neem oil 10% and 10% common salt. Raw or green hide without subjected to curing treatment process was used as control. Three hides of each treatment were kept at three different storage periods (10, 20 and 30 days) in such a way that after every 10 days of storage each of three hides could be brought to scheduled examination. From each treated and stored hide sample portions were taken from neck, shoulder, belly, butt and hind shank regions. These are subsequently subjected to bacteriological examination and determination of storage property having indications of spots, color changes, and sliminess development in skin during prescribed storage periods. The minimization of bacterial load and survivability of selected bacterial attributes will be considered as criteria for capability of ensuing damage of skin evidencing the efficacy of neem oil to control bio-deterioration. The application of treatments with curing agent and storage conditions as well as the bacteriological examination were as per recommendation and instruction of Samad *et al.*[1], Samad *et al.*[2,3], Kirtikar and Basu[11], Rahman[12].

RESULTS AND DISCUSSION

The microbes contaminating green hides, the basic raw material for the leather industry, have attracted attentions of investigators from various biological disciplines, as because the deterioration in quality of such products causes rejection and this eventually leads to heavy economic loss. The result of the present research dictates the magnitude of microflora associated with contamination of green hide after flaying, their influence on storage conditions and interrelatedness with curing processes. The counts of total viable bacteria in hide samples were found in millions (Table 1).

It appears from the Table 1 that the bacterial population may vary more or less in different skin regions. The mean value of total bacterial count in raw skin after flaying was log 7.45±0.25. The result also represented the counts of coliforms and staphylococci recovered from hide samples. It is interesting to note that coliforms were not found in belly and shank regions. On the contrary the hide samples exhibited coliforms in the regions of shoulder, neck and butt. The coliform counts (TCC) obtained were in the range from log 6.14 to log 7.16, with the mean value of

Table 1: Extent of bacterial contamination on different regions of green hides prior to curing process*

	Extent of bacterial contamination per gram of green hide sample		
Skin regions	TVC	TCC	TSC
Shoulder	7.51	6.47	6.43
Neck	7.37	6.41	7.26
Belly	7.23	Nil	7.20
Butt	7.86	7.16	7.46
Hind shank	7.29	Nil	7.28
Mean±SD	7.45± 0.25	6.68± 0.41	7.13±0.40

*All counts are expressed in logarithms

Table 2: Survivability of viable bacteria per gram of samples of hide cured by employing neem oil and stored at three different periods

	Survivability of viable bacteria in hide samples cured by using								
	Neem oil 40% +common salt 10% Kept after storage period of			Neem oil 10% +common salt 20% Kept after storage period of			Neem oil 10% +common salt 10% Kept after storage period of		
Skin region	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30days
Shoulder	6.94	7.01	7.25	6.71	6.89	6.87	7.20	7.25	7.17
Neck	7.01	7.37	7.64	7.13	7.32	6.69	7.17	7.59	7.64
Belly	6.56	7.20	7.63	7.09	7.04	7.25	7.25	7.49	7.48
Butt	7.73	7.41	7.53	7.41	7.54	7.33	7.44	7.09	7.17
Shank	7.03	7.00	7.17	6.69	6.71	6.70	6.54	6.67	6.85
Mean±SD	7.05±0.42	7.19±0.19	7.44±0.22	7.00±0.30	7.1±0.33	6.96±0.30	7.12±0.34	7.21±0.36	7.23±0.30

Table 2a: Survivability of Coliform bacteria per gram of samples of hide cured by employing neem oil and stored at three different periods

	Survivability of coliform bacteria in hide samples cured by using								
	Neem oil 40% +common salt 10% Kept after storage period of			Neem oil 10% +common salt 20% Kept after storage period of			Neem oil 10% +common salt 10% Kept after storage period of		
Skin region	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30days
Shoulder	5.25	Nil	Nil	Nil	Nil	Nil	5.80	Nil	Nil
Neck	4.70	Nil	Nil	Nil	Nil	Nil	4.53	Nil	Nil
Belly	5.81	4.25	Nil	4.30	Nil	Nil	Nil	Nil	Nil
Butt	4.60	4.50	Nil	Nil	Nil	Nil	5.50	Nil	Nil
Shank	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 2b: Survivability of Staphylococci per gram of samples of hide cured by employing neem oil and stored at three different periods

	Survivability of Staphylococci per gram in hide samples cured by using								
	Neem oil 40% +common salt 10% kept after storage period of			Neem oil 10% +common salt 20% Kept after storage period of			Neem oil 10% +common salt 10% Kept after storage period of		
Skin region	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30days
Shoulder	6.72	6.71	7.04	7.32	7.20	7.25	7.33	7.24	7.32
Neck	6.70	7.13	7.45	6.89	7.41	7.49	7.21	7.26	7.52
Belly	6.53	7.09	7.21	7.04	7.10	7.49	7.15	7.18	7.35
Butt	7.54	7.41	7.30	6.87	6.95	7.63	6.89	7.02	7.64
Shank	6.70	6.69	7.30	6.69	7.24	7.09	6.92	7.18	7.28
Mean±SD	6.84±0.39	7.00±0.30	7.26±0.15	6.96±0.24	7.18±0.17	7.39±0.22	7.1±0.19	7.18±0.09	7.42±0.15

Table 3: Storage property of hide samples cured with neem oil and common salt of different concentrations and kept for different storage periods

		Bacterial growth evidencing spots/color changes/ sliminess at different storage periods			Observation on changes occurring in skin properties after specific storage time of 30 days		
Curing agents used	Skin regions	10 days	20 days	30 days	Hair slip	Smell/odor	Tensile strength
T_1: Neem oil	Shoulder	-	-	-	-	-	Marginal elasticity
40% and	Neck	-	+	+	-	+	Strongly bounded
common	Belly	-	-	-	-	-	Strongly bounded
salt%	Butt	-	+	+	-	+	Marginal elasticity
	Hind shank	-	-	-	-	-	Strongly bounded
T_2: Neem oil	Shoulder	-	-	-	-	-	Strongly bounded
10% and	Neck	-	-	+	+	++	Strongly bounded
common	Belly	-	-	+	+	++	Strongly bounded
salt 20%	Butt	+	+	++	+	++	Loosely bounded
	Hind shank	-	-	-	-	-	Strongly bounded
T_3: Neem oil	Shoulder	-	-	-	-	-	Strongly bounded
10% and	Neck	-	+	+	+	+	Marginal elasticity
common	Belly	-	+	++	+	++	Marginal elasticity
salt 10%	Butt	+	-	++	+	++	Marginal elasticity
	Hind shank	-	-	-	-	-	Strongly bounded

Signs: - = no changes; + = putrefaction begins; ++ = putrefaction continues emitting foul odor; +++ = degeneration starts

log 6.68±0.41. In the butt coliforms were enumerated in the highest number. The mean value of staphylococcal counts (TSC) in green hides was found to be log 7.13±0.40.

A freshly flayed skin when subjected to curing operation prior to tanning undergoes change in respect to their bacterial population. The antibacterial activity of certain chemical gent, particularly neem oil in curing mixture has been studied. Table 2, 2a and 2b clearly evidenced that the antibacterial activity of treatments against contaminating microorganisms was not consistently recognized. The vegetable or herbal chemical present in neem was not found considerably effective to inhibit propagation of microbes. Even after storage for 30 days the total viable bacteria survived quite unacceptably high in neem oil cured hide. The survivability of bacteria in these samples were between mean value ranges of log 6.96±0.30 to log 7.44±0.22. This indicated that none of the three treatments used possessed acceptably limited antimicrobial activity.

The survivability of coliforms in neem oil cured hide exhibited remarkable phenomenon (Table 2a). It is interesting to note that the use of neem oil and common salt in curing process could minimize the coliform contamination and the range of inhibitory concentration is quite comparable to lower concentration. Neem oil 10% with common salt 20% was found to possess the maximum anticoliform property.

In comparison to coliform counts, staphylococcal count however presented an alarming situation. It is revealed from Table 2b that staphylococci in salted hides were not susceptible to curing agents. The results claimed that the salt concentration curing or herbal agent used (neem oil) had no effect either on the growth of staphylococci or on the skin regions where these organisms inhabited. However the maximum number of staphylococci was recovered from samples of neck and butt regions (log 7.52 and log 7.64).

The changes in storage properties of hides cured with neem oil and common salt and later kept at different storage periods were observed. It is revealed from the study of neem oil 40% and common salt 10% treated hide that no sign of bacterial spoilage was perceptible on the 10th day of storage (Table 3). However indication of some sliminess could be noticed in the regions of neck and butt after storage of 20 and 30 days. Although there was no hair slip present throughout the storage period, but very negligible hair slip is encountered at inspection in the neck and butt regions after 20th day of storage. Strongly bounded tensile strength was found in the neck, belly and

hind shank regions, while marginal strength was found in other regions of the hide during the subsequent storage period of the study.

The hide when treated with neem oil 10% and common salt 20% exhibited more or less changes in color on the 10th day of storage. Moreover the sliminess was noticed fairly distributed on the neck and belly regions on the 30th day of storage. There were few hair slips and offensive odor emitting from the neck and belly regions. The tensile strength although remained strongly bounded in the shoulder, butt and shank regions, but there was marginal elasticity recorded in the neck and belly regions.

The hide cured with neem oil 10% and common salt 10% exhibited some noticeable changes in the butt region. Putrefactive action started and there was indication of tissue changes, which could be marked, from the 10th day of storage. At the butt region foul odor was perceptible and few hair slip was evidenced on the 30th day of storage. Most of the regions of the hide were strongly bounded, except the butt region, where it was found loosely bounded after 20th day of storage. On the 30th day of storage sliminess on the belly and neck region, considerable hair slips, and slight repulsive odor emitted from neck and belly regions were recorded. The occurrence of marginal elasticity was found in the neck and belly regions.

The detachment of the epidermis is more commonly referred to as "hair slip", since the hair, that can be pulled off a spoiled hide is much more easily seen than fragments of epidermis. During storage the storage property of cured hide revealed that the bacterial spoilage was not shown on the 10th day of storage. Hair slip is the most obvious sign of spoilage in hides and skin, but the contaminating bacteria also react themselves in other ways[6]. The offensive smell and the deterioration in spoiled materials are usually attributed to waste products of bacterial activity. Similarly a slippery or slimy texture may be associated with the same waste product. Moreover oils or fats may be released or exuded onto the surface from subsurface cells, which have been damaged by bacteria. In the present study indication of incipient spoilage like sliminess could be noticed in the regions of neck and butt after 20 days of storage and progressively onward. It is evident from the studies about the course of spoilage of skin that as bacteria move through the dermis, they cause a steady deterioration in physical structure of collagen fibers and appearance of holes, since some bacteria present secrete proteolytic enzymes and even collagenases[13].

Hair slip was not encountered considerably throughout the storage period; only slight or negligible hair slip in the butt, neck, and belly regions was observed. It could be postulated that the neem oil provided protection to bacteria and acted against inherent bactericidal property. Moreover the lipolytic action was very much pronounced. The development of off-odor was found positively correlated with hair slip. These indications were also observed to be associated with texture or tensile strength of skins. The present findings evidenced strongly bind tensile strength in the neck, belly and hind shank regions, while marginal structural strength was found in other regions of the skin during the entire period of storage conditions excepting 30 days of storage. In prolonged storage of 30 days the tensile strength became weak, particularly in the region of hind shank. Thus it should be realized that if a hide or skin is exhibiting hair slip, then bacteria have penetrated and affected the whole thickness of the dermis. Accordingly the hair slip should be considered as the sign of advanced rather than beginning of spoilage. Hides and skin exhibiting hair slip should be discarded and no attempts should be made to process them[6,14]

It is well recognized that for better curing and preservation of hides and skins, additives to curing salt are to function in two ways:

- Prevent or check the growth of putrefactive organisms
- Inhibit the growth of halophilic organisms

It has been observed from the present study though the treatment using combination neem oil and common salt showed antibacterial activity against the growing contaminating putrefactive organisms somewhat considerably as because neem oil contains antibacterial component margosic acid[11] but they were much less effective against halophiles. To maintain the quality of the skin Samad *et al.*[2,3] suggested adding chlortetracycline to sodium chloride even in case it is preserved under unsuitable conditions. However the research findings of other investigators advocated for the use of combination of treatment (antibiotic + antiseptic + salt). It has been emphasized that not only in the case of antibiotics, but even in the case of antiseptics, a mixture of two antiseptics, one active against non-halophilic putrefactive organisms and the other against the halophilic organisms, may be a better additive to salt than any others[15,16]. To generate technology for developing improved curing method the promotion of introducing combination method treatment will be fruitful and useful. In this regard antibiotics mixture with certain antiseptics possessing strong antibacterial activity against halophilic organisms in admixture with common salt may considerably improve the curing efficiency of the salt. But this warrants a careful selection of antibiotics and the

antiseptics because of antagonistic action may result in simple combination with some antibiotics. Further research is therefore needed to find out better curing agents that can improve the curing efficiency of the salt, lower total salt consumption by about 30-40% of green weight of hide, reduce national foreign exchange costs and minimize tannery effluent problem.

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