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**BENZALKONIUM CHLORIDE AS A PRESERVATIVE
FOR HIDES AND SKINS***

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ABSTRACT

Benzalkonium chloride was tested for the short-time preservation of hides and skins and as an adjunct for use with salt in brine-curing and green-salting. It was found to help control microbial growth in each application. There was no difficulty in processing hides treated with this material and no adverse effect on the leather. However, leather from skins which had not been treated with salt was not uniform as to temper and appearance.



INTRODUCTION

Although the current methods of curing hides and skins with salt are adequate for most conditions, there are certain circumstances under which these methods are deficient. When hides and skins are collected from small operators, it may be several hours to several days before they are salted. Bacterial growth can be well advanced in this length of time, and much damage may result. A treatment that would allow an operator to hold hides and skins for about three days without damage would be very useful. Furthermore, hides exported to Japan and Europe have been reported to have arrived in an unsatisfactory condition. Improved curing methods would help to alleviate this problem.

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In some studies on the sterilization of collagen, Giffie (1) found that benzalkonium chloride (BAC), a mixture of alkyldimethylbenzyl ammonium chlorides, was an effective disinfectant. In addition, it appeared to be actively bound to the collagen, since it could not be removed by washing with water. The absorption of this material on a wide variety of substances is well-known. Grassmann and Hausam (2,3) and Hausam (4) investigated a product called Rohzephirol (now called Germex) which contained an alkyldimethylbenzyl ammonium chloride and found the treated skins to be in very good condition, although many microorganisms survived. The leather quality was improved, but there appeared to be a small loss of yield. Weidemann (5) tested four quaternary ammonium compounds, including benzalkonium chlorides, for sterilizing hides. His objective was to prevent contamination of the meat by bacteria from the hides. In laboratory tests such materials reduced the microflora by over 99% but were considered to be too costly for practical purposes at the concentrations tested.

The present investigations were undertaken to determine whether BAC would be effective for short-time holding of hides and skins, whether it would improve salt-curing, and whether it would interfere in processing or be detrimental to leather quality.

MATERIALS AND METHODS

Laboratory tests.—Freshly flayed calfskin was obtained at a local slaughterhouse and either used at once or frozen and thawed as needed. Agitation during treatment was accomplished by tumbling in jars. During the tests skin pieces were incubated in closed dishes over water at 28°C.

At the conclusion of the incubation period the skin pieces were placed in 500 ml. of water in Fernbach flasks and shaken on a reciprocating shaker for 5 minutes. This water was then filtered through glass wool, and the skin pieces were washed with an additional 500 ml. of water, which was also filtered through the glass wool. The combined washings were made up to one liter, and aliquots of these solutions were used for the determination of the soluble nitrogen and a bacterial count.

Numbers of bacteria were determined by plating appropriate dilutions using a nutrient medium designed for materials containing quaternary ammonium salts (6). Soluble nitrogen was determined by the official ALCA Kjeldahl method (7).

Tannery test.—Fresh calfskins were obtained from a local slaughterhouse, treated as described in the experimental section, and placed in a pack. The temperature of the room in which these skins were held ranged between 40°F. and 65°F. Pickup of BAC was estimated by a procedure supplied by the manufacturer. Tablets are placed in the solution, and the color that develops indicates the approximate concentration of the BAC. The tablets

contain methyl orange and bromophenol blue in an appropriate weight ratio along with an anionic detergent. When this material is added to an aqueous quaternary ammonium solution, the anionic detergent reacts stoichiometrically with the quaternary ammonium compound to form a nonionized salt. The dyes impart a distinctive and characteristic color to the solution, which is indicative of whether there is an excess of the quaternary ammonium compound or the anionic detergent in the solution (8). At the completion of the test, the skins were taken to a tannery and processed with the regular production.

Tensile strength, elongation, grain crack, and slit tear determinations were run on specimens of the leather after conditioning them at 73°F. and 50% R.H. Chrome and fat determinations were made by the ALCA-recommended procedures (7). Shrinkage temperatures were also determined.

EXPERIMENTAL RESULTS

Fifty-gram samples of fresh calfskin were treated for one hour as described in the previous section with solutions containing 0.1, 0.2, 0.3, and 0.4% BAC. The ratio of skin to solution was 1 to 6. Duplicate sets of samples were treated, and determinations of bacteria and soluble nitrogen were made after 3 days and 7 days exposure over water at 28°C.

Table I shows the results of this test. Bacteria on the pieces treated with BAC were about one-half as numerous as on the untreated control at the 3-day incubation period. More significant, however, was the difference in types of organisms present with and without the BAC treatment. The control had a typical putrid odor and contained the usual putrefactive type of heavy, slimy growth. The flora from the treated skins contained many organisms of the pseudomonas type which are notoriously resistant to disinfectants. The odor of these pieces was not objectionable, and there was no evidence of damage to the skin. The BAC inhibited the development of degradative bacteria, resulting in a significantly lower amount of soluble nitrogen at all levels of treatment.

After 7 days incubation the number of viable bacteria on the treated samples was no higher than at 3 days, whereas on the control they had increased almost ten times. Soluble nitrogen did increase somewhat on the treated samples but was less than half that of the control. The treated skin pieces were still in very good condition, and putrid odors had not developed.

There was very little difference in the numbers of bacteria, amount of soluble nitrogen, and the condition of the skin pieces at the different levels of BAC. This is in agreement with the results of Weidemann (5).

BAC was tested as an adjunct with salt for curing calfskin pieces by greensalting and by brining. In order to make the test more drastic and thus obtain results more quickly, skin pieces were inoculated with a heavy sus-

TABLE I
SHORT-TIME PRESERVATION OF FRESH CALFSKIN WITH BAC

No.	Treating Solution*	3-day Incubation			7-day Incubation		
		Soluble N† % on skin wt.	Bacteria† per g. skin	Condition	Soluble N % on skin wt.	Bacteria per g. skin	Condition
1	Control, water only	0.21	6×10^9	Slimy growth, putrid odor	0.39	2×10^{10}	Very putrid odor, all hair loose
2	0.1% BAC	0.13	2.7×10^9	Small amounts of yellowish growth, slight odor, not objectionable	0.14	7.2×10^8	Ammoniacal odor, hair very loose
3	0.2% BAC	0.09	2.4×10^9	"	0.19	3.6×10^9	Ammoniacal odor, hair fairly loose
4	0.3% BAC	0.05	1.8×10^9	"	0.14	2.8×10^9	"
5	0.4% BAC	0.07	2.4×10^8	"	0.16	3.8×10^8	"

*Skin pieces tumbled 1 hour in treating solution. 1-6 float.

†Determinations made immediately after treatment on skin pieces treated with 0.1% BAC showed 0.01% soluble nitrogen and 1×10^4 bacteria per g.

pension of bacteria. This suspension was prepared in 20% saline from salt-cured hide that had been allowed to decompose partially. There were many red-colored organisms present. Brine treatment was carried out for 24 hours, with intermittent agitation for the first 4 hours.

The results of the brining tests are given in Table II. In the absence of BAC, putrefaction was evident by the 26th day from the putrid odor and the presence of pink discoloration. Pieces treated with 0.2% BAC in the brine did not show decomposition until after about 3 months storage over water. Even then, they were not putrid, but ammonia was present, and the hair was loose. There was no significant difference whether the skin pieces were treated before or after inoculation.

TABLE II
THE INFLUENCE OF BAC ON THE ACCELERATED STORAGE OF BRINE-CURED AND INOCULATED CALFSKIN*

Treatment	State of Preservation and Appearance after Storage over Water at 28°C. for:			
	12 days	26 days	50 days	89 days
Saturated brine alone†	very good	pink, putrid	red, strong NH ₃ , putrid	red, putrid, hair loose
Saturated brine containing 0.2% BAC†	very good	very good	very good, flesh dull	dull, NH ₃ , hair loose

*100-gram lots.

†Results were the same whether the treatment preceded or followed inoculation.

In the green-salting tests pieces of fresh calfskin were treated with 0.2% BAC solution for 1 hour with mild agitation at a ratio of 6 parts of solution to 1 part skin. They were then drained and inoculated by immersion in the bacterial suspension described above. Controls were run without the BAC treatment and without inoculation. The pieces of skin were covered with 50% of their weight of fine salt and stored over water at 28°C. Results are recorded in Table III. Inoculated pieces without BAC had started to putrefy after 12 days and had turned red and had a putrid odor after 26 days. Pieces with the BAC treatment did not show any signs of decomposition until after 50 days, when some pink spots appeared on the flesh. A putrid odor and hair looseness was found upon examination after the 89th day. The flesh of these pieces had a yellowish tint after 12 days and then turned a dull grayish color. This may have been due to the growth of Pseudomonads which subsequently died. Without inoculation the pieces remained in good condition for 50 days

whether they had been treated with BAC or not. After about 3 months the untreated pieces were pink and had a putrid odor and loose hair, whereas the treated pieces were still in good condition.

TABLE III
THE INFLUENCE OF BAC ON THE CURING OF
GREEN-SALTED CALFSKIN PIECES

No.	Treatment		State of Preservation and Appearance after Storage over Water at 28°C. for:			
	BAC Solution %	Inoculated	12 days	26 days	50 days	89 days
1	none	yes	flesh pink	red, putrid	red, NH ₃ , putrid	red, NH ₃ , putrid, hair loose
2	0.2	yes	very good, flesh yellowish	very good, flesh dull	pink spots on flesh	grey, NH ₃ , putrid, hair loose
3	none	no	very good	very good	very good	pink, putrid hair loose
4	0.1	no	very good	very good	very good	very good
5	0.15	no	very good	very good	very good	pink spots, hair tight

Plant-scale test.—Three lots of ten freshly flayed, 12–17 lb. calfskins were obtained from a local slaughterhouse. The control lot, which was designated No. 3, was treated first. These skins were made into a pack in an unused root cellar by covering the flesh side of each skin with about one-third of its weight of fine salt as it was placed on the pile. The pack was covered with a plastic sheet.

The skins for Treatment No. 1 were placed in a wooden drum in four times their weight of 0.1% (1,000 p.p.m.) BAC solution. The drum was turned slowly for 1 hour, after which the skins were removed and horsed up and drained for 1½ hours. These skins were then placed in the pack by salting as with the controls. A plastic sheet separated the two lots. The skins of Treatment No. 2 were given the same BAC treatment as those for Treatment No. 1 but were placed in the pack without salting. The treatments and conditions are summarized in Table IV. After the storage period the skins were taken to a tannery for processing. The technical director of the tannery and the foreman of the hide cellar examined the skins and found all of them to be in excellent condition. No difficulty was experienced in processing any of the skins into leather.

TABLE IV
TREATMENT, STORAGE CONDITIONS, AND STATE OF CURED
CALFSKINS IN PLANT-SCALE TEST

Treatment No.	Treatment	Pickup of BAC %	Days in Pack	Cellar Temp. °C.	State of skins after Storage*
1	0.1% BAC for 1 hr., then salted	40-50	34	40-65	excellent
2	0.1% BAC for 1 hr., no salt	40-50	4	50-54	excellent
3	Salt only		40	40-65	excellent

*Tanners' evaluation.

When examined in the crust, the leather from Treatment No. 1 and Treatment No. 3 was comparable to the regular tannery production; whereas that from No. 2, which was not salted, was not uniform as to temper and appearance. This indicates the importance of sodium chloride in curing. Four skins from each treatment were processed into finished leather before the chemical and physical tests were run.

Chemical analyses.—The results of the chrome and fat analyses and the shrinkage temperatures are given in Table V. The chrome contents are somewhat higher in all samples than would be expected, but there were no significant differences in the leathers of the three treatments.

TABLE V
ANALYSIS OF CALFSKIN LEATHER

Treatment	Stage	Chrome (Cr ₂ O ₃) %	Fat %	Ts °F
0.1% BAC for 1 hr., then salted	finished	6.6	2.5	98-100
	crust	6.5	2.8	5' boil
0.1% BAC for 1 hr., no salt	finished	6.4	2.7	97-98
	crust	6.5	2.6	5' boil
Salt only	finished	6.2	2.4	92-94
	crust	6.3	2.6	5' boil

The values for fat are lower than normal for this type of leather, but again there was no difference between treatments. The finished leather from all three treatments had a lower shrinkage temperature than the crust leather. This is unusual but was not caused by the experimental treatments.

Physical tests—Tests were run on samples of leather from each skin. The skins were sampled according to the provisional method of the ALCA (7), 5'' in from the base of the tail and 2'' down from the backbone. Measurements were made of percent elongation, load to break, and strength in the tensile determination; load to tear in the slit tear test; and percent extension and load to crack in the grain crack procedure (8).

Averages of the ten determinations for each characteristic in each treatment are given in Table VI. Statistical analysis of variance was used on each set of data, as shown in Table VII. The results indicate that there were significant differences between the treatments in the slit tear strength and the resistance to grain cracking. Leather made from skins treated with BAC and salt (Treatment No. 1) showed a significantly improved slit tear resistance over leather produced from skins cured by the other treatments.

TABLE VI
PHYSICAL PROPERTIES OF CALFSKIN LEATHER

	Treatment No.		
	1 Average	2 Average	3 Average
<i>Tensile</i>			
Elongation, %	52.6	51.5	50.1
Load to break, lb.	81.5	83.2	84.3
Strength, p.s.i.	2809.0	2964.0	2813.0
<i>Slit tear</i>			
Load to tear, lb.	24.8	21.7	22.6
<i>Grain crack</i>			
Extension to cracking, %	37.6	50.4	35.5
Load to crack, lb.	447.0	537.5	394.5

As to grain crack, there were significant differences between treatments both in extension to cracking and load to crack. Both properties were significantly higher in Treatment No. 2 (BAC alone) than in the other two treatments.

TABLE VII
ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F	F _{0.05}
<i>Tensile Strength, p.s.i.</i>					
Total	29	2,818,480			
Treatments	2	156,140	78,070	0.72	3.55
Skins	9	719,546	79,950	0.74	2.46
Error	18	1,942,794	107,933		
<i>Tensile-Elongation, %</i>					
Total	29	1,801.2			
Treatments	2	31.4	15.7	0.28	3.55
Skins	9	753.9	83.8	1.48	2.46
Error	18	1,015.9	56.4		
<i>Tensile-Load to Break, lb.</i>					
Total	29	1,814.0			
Treatments	2	39.8	19.9	0.30	3.55
Skins	9	569.3	63.3	0.95	2.46
Error	18	1,204.9	66.9		
<i>Slit Tear-Load to Tear, lb.</i>					
Total	29	149.0			
Treatments	2	50.9	25.45	10.8†	3.55
Skins	8	53.9	6.74	2.85*	2.51
Error	18	42.4	2.36		
<i>Grain Crack-Extension to Cracking, %</i>					
Total	29	4,984.2			
Treatments	2	1,300.9	650.45	4.65*	3.55
Skins	9	1,164.9	129.43	0.92	2.46
Error	18	2,518.4	139.91		
<i>Grain Crack-Load to Crack, lb.</i>					
Total	29	445,496.7			
Treatments	2	104,651.7	52,325.88	4.01*	3.55
Skins	9	106,096.7	11,788.52	0.90	2.46
Error	18	234,748.3	13,041.57		

*Significant at 5% level.

†Significant at 1% level.

DISCUSSION AND RESULTS

Although alkyldimethylbenzyl ammonium chloride compounds are used extensively in many sanitizing applications, they have received very little attention for the preservation of hides and skins. Our results indicate that

fresh skins treated with BAC can be held for several days without becoming damaged by microorganisms. If no salt treatment was included in the cure, the leather was not as uniform as would be desired, but it should be borne in mind that much acceptable leather has been produced from unsalted hides during war times.

No difficulty was experienced in processing hides treated with BAC, and the physical properties were at least equal to those of the untreated controls. It appears that material of this type could be used to good advantage by small butchers for the preservation of hides and skins until they are picked up and salted. Results also indicate that BAC would be useful as an adjunct in salt-curing hides and skins, particularly in cases where they are to be shipped under humid conditions.

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