Preservation of Bovine Hide using Less Salt with Low Concentration of Antiseptic, Part I: Effectiveness of Developed Formulations

by

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Abstract

Bovine raw hides are commercially cured either with high salt concentration of about half the weight of actual hide or 95% saturated brine solution. This conventional technique is very popular due to the availability of common salt (sodium chloride) and its cost-effective procedure but it generates a huge pollution problem increasing salinity. As a result, an alternative method of using less or no salt for hide preservation needs to be developed. For the preservation of hide it is essential to arrest microbial attack on hide as the main constituent of raw hide is protein which is very susceptible for bacterial degradation. Such bacterial degradation leads to the putrefaction of raw hide before converting them into leather. Agricultural Research Services scientists at Wyndmoor, Pennsylvania, have developed antiseptic based formulations for hide curing where only 45% saturated brine solution is used. The newly developed formulations have been found more effective in limiting microbial growth for a longer time on cured skin than the regular brine process and thus preserve hides for more than 30 days. In-process analysis of cured hides during storage period reveals the compatibility of the alternative curing process in comparison to the traditional method. Therefore, this new development will not only preserve hide through better protection from microorganisms but also offers improved conservation of the environment.

Introduction

Raw hide and skin, the main byproducts of meat industry are the basic raw materials for the leather industry. The second major constituents of raw hide after moisture (60-70%) is protein (25-30%)[1] which includes globulin, albumin and fibrous proteins (collagen, 98%; elastin, 1%; and keratin, 1%).[2,3] Animal skin also contains a great variety of microorganisms, which are derived from air, water, soil, manure and extraneous filth.[4] In living animal, bacteria and microorganism on skins are held in control by the metabolic defenses of animal but the flayed skins become vulnerable for bacterial attack within 5-6 h of removal.[5,6] These microorganisms produce proteolytic and collagenolytic enzymes resulting in putrefaction of hide. The leather quality depends on the presence of necessary protein in raw hide. Therefore, it is extremely important to conserve protein from degradation in skin during the process of preservation.

For the preservation of hides, it is essential to arrest microbial attack as hides' protein is very susceptible to bacterial degradation. There are two ways of limiting or controlling microbial attacks, either by killing the microorganism which is called bactericidal or creating unfavorable conditions for the microorganisms to thrive, known as bacteriostatic. The bactericidal method employs chemicals that are usually harmful for humans or living species and costly. On the other hand, the bacteriostatic method utilizes salts (commonly sodium chloride) or other dehydrating agents which generate pollution problems in terms of total dissolve solid (TDS) increase or by increasing salinity of the soil which leads to a barren environment as far as plants are concerned.

The classic salt-based hide curing method employs 95% saturated brine solution or 40%-50% w/w sodium chloride on the raw weight of hide/skin.[7] Almost 75% of the salt used ends up in the effluent stream during soaking, which contributes to 40% of total solid content in the tannery effluent[8] creating major salt pollution in the environment. When the soils are irrigated with saline effluent discharge, the salts accumulate, unless they are leached out. Furthermore, saline irrigation water along with low-soil permeability, inadequate drainage, low rainfall and poor irrigation management, all cause salts to accumulate in soil, which has deleterious effects on crop production. Saline effluents

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from tanneries are manmade salinization which is causing destruction of the global bio-spheric mechanism. Degradation of soils by salinity and sodicity profoundly affects environmental quality.[9] The salts also affect release and solubilization of heavy metals in solution, with potential adverse effects on water quality and plant growth.[10] High concentration of sodium (Na^+) and chloride (Cl^-) affect plant directly by causing excessive uptake of these ions or indirectly by increasing soil pH. Germination of seeds is drastically inhibited by the high concentration of sodium chloride. Therefore, the development of an alternative hide preservation technique that addresses the ease of practice, cost effectivity, environmental benignity, leather quality and feasibility has become urgently necessary.

Salt free preservation methods such as controlled drying using a drying chamber[11] and radiation based curing either by using gamma rays (photon emission from radioactive materials) or electron beams[12] have been found expensive and difficult to adopt in a hide processing facility. Additionally, the possibilities of using potassium chloride,[13] soda ash,[14] preservatives such as benzalkonium chloride,[15] antibiotics like auromycin and terramycin,[16] neem oil[17] and boric acid[18] for preserving the hide have been explored. Higher cost, sub-optimal preservation efficiency, toxicity, poor quality of leather and adverse environmental impact are the main factors that explain why these methods have not been adopted commercially. Therefore, an alternative approach needs to be developed that eliminates/mitigates the problems associated with the existing hide preservation techniques.

This research has evolved a less salt containing curing system for salinity abatement employing low concentration of environmentally benign antiseptic which will pose no threat to the environment. The method reported in this paper provides better resistance to microbial growth on skin than the conventional method, preserving the hide for more than 30 days. The overall efficacy of the curing procedures was assessed by monitoring the parameters (i.e. water activity, moisture content, bacterial count, hair slip, odor, microscopic analysis and rehydration) of alternatively cured hide throughout the preservation period.

Experimental

Materials

Freshly flayed and de-fleshed bovine hides were acquired from a local meat packing facility, courtesy of JBS Packerland (Souderton, PA). The hide was split down the back into left and right segments. The sides were then cut into pieces that weighed approximately 800 – 1000 g each, with a dimension of 12 in x 12 in. All chemicals used for hide preservation listed in Table I were of commercial grade. Alkyltrimethylammonium bromide (ATMAB), Chlorhexidine di-gluconate (CDG), Lactic acid solution ≥ 85%, Peracetic acid solution, Hydrogen peroxide were purchased from Sigma Aldrich Chemical company (Milwaukee, WI). All other reagents used for the formulations were of the highest purity available from commercial suppliers. Brine solutions were prepared by dissolving specific amount of common salt (sodium chloride) in water and a salometer was used to measure their saturation level. The preparation of all formulations was carried out as detailed in Table I, where mixed or dissolved in tap water at room temperature (~21°C). All formulations were prepared ~12 h prior to the experiments.

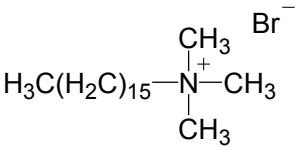
Protocol for the Alternative Hide Preservation

A 150% float (volume of sol/w of hide or v/w) was used for preservation treatment. Hide pieces were soaked individually in the 6-in-1 Dose drums (Dose Maschinenbau GmbH, Lichtenau, Germany) with in respective solutions for 18 h. During the treatment, the 6-in-1 Dose drums controls were set to 6 rpm for tumbling. A 95% saturated brine solution with 0.043% (v/v) bleach (NaOCl) was utilized for the control (F-A, Table I). This formulation is being used commercially for conventional hide preservation. For alternative methods (F-B to F-F, Table I), a 45% saturated brine solution was used along with other additives which cut the salt usages by more than 50%. After 18 hours of treatment, the hide samples were hung to dry, folded and stored at the ambient temperature of 38-40°C, and were monitored periodically for physical changes like smell and hair slip which are the indications of putrefaction.[19]

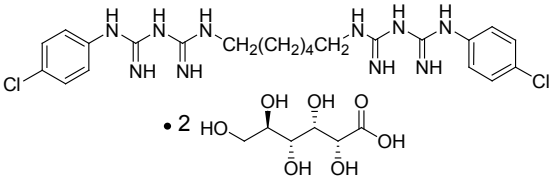
Table I
Composition of the developed curing formulations for hide preservation.

Formulations	Composition
F-A (control)	95% saturated brine soln. + 0.043% NaOCl (v/v)
F-B	45% saturated brine soln. + 0.6% ATMAB[ǂ] (wt./wt.) + 0.06% CDG[ǂ] (v/v)
F-C	45% saturated brine soln. + 0.6% ATMAB (wt./wt.) + 0.06% CDG (v/v) + 0.043% NaOCl (v/v)
F-D	45% saturated brine soln. + 0.6% ATMAB (wt./wt.) + 0.06% CDG (v/v) + H_2O_2 (135 ppm) + Peracetic Acid (80 ppm)
F-E	45% saturated brine soln. + 0.6% ATMAB (wt./wt.) + 0.06% CDG (v/v) + 0.043% NaOCl (v/v) + 2% Lactic Acid (v/v)
F-F	45% saturated brine soln. + 0.6% ATMAB (wt./wt.) + 0.06% CDG (v/v) + H_2O_2 (135 ppm) + Peracetic Acid (80 ppm) + 2% Lactic Acid (v/v)

The efficacy of the developed protocols was assessed by determining the water activity, moisture content, bacterial count, rehydration, microscopic analysis and physical properties of the treated hides throughout the preservation period.



ATMAB: Alkyltrimethylammonium bromide



CDG: Chlorhexidine di-gluconate

Efficiency Analysis of the Developed Formulations in Limiting Microbial Growth

To check the efficiency of the developed formulations, preliminary testing was carried out against naturally occurring aerobic bacteria on cattle hides. To conduct this, bacterial swab samples were collected from freshly procured cattle hides (JBS Packerland in Souderton, PA) using carcass sampling kits (Nasco Meat and Turkey Carcass Sampling Kit, Salida, California). Samples were diluted to make a 1:10 ml dilution in tryptic soy broth and incubated at 36°C for 24 hours. One milliliter of the bacterial culture was spread evenly throughout a tryptic soy agar (TSA) plate and allowed to dry. Uninoculated sterile commercially available antimicrobial disk were aseptically soaked in a test tube containing an individual formulation (F-A to F-F) for ~1 minute. Disk were evenly placed and lightly pressed on the TSA plates and incubated overnight at 36°C. After incubation, the bacterial growth surrounding each formulation infused disc was observed and recorded. If the natural bacteria were susceptible to a particular formulation, an area of "no growth" was observed around that individual disk. The area of no growth around a disk was referred to as the zone of inhibition, which was then measured in mm.

Determination of Water Activity and Moisture Content

Water in hides that is not bound to hide molecule can support growth of bacteria, yeast and molds. The term water activity (a_w) refers to this unbound water. During the preservation period the water activity of cured hides were checked periodically utilizing AquaLab Dew Point Water Activity Meter (Model # 4TEV).

Known weight (approximately 5 g) of hide pieces from control and experimental samples were collected after different periods of storage and their moisture content were determined using moisture analyzer, MF-50 (A&D Company Limited, Tokyo, Japan) operating at 50 to 200°C.

Determination of Bacterial Count

During storage, the specimens weighing ~ 5 g were cut from the samples treated with the different curing formulations and soaked separately in a 50 ml conical tube with 45 ml of sterile water. The soak solutions were prepared by shaking the tubes in an orbital shaker at 200 rpm for 30 min. After shaking, the soaking liquor was diluted to 1:10 ml in sterile water and vortexed for mixing. An aliquot of 0.1 ml of the mixed dilution was pipetted into sterile plates. Afterwards molten TSA agar was poured and shaken gently to get a uniform distribution of the diluted soaking liquor. The plates were incubated at 37°C for 24 h. The bacterial population was determined and expressed as log CFU per gram of hide. This analysis was carried out for all the cured samples after 7, 14, 21 and 28 days of storage.

Texture Analysis of the Cured Hides

A Brookfield CT3 texture analyzer, Middleboro, MA[20] was used to compare the relative hardness of the hides treated with the different curing formulations after 28 days of storage. A TA10 probe at 40g load was utilized to perform this test. The amount of total work on the cured hide under the predetermined load was calculated. The data points were collected from three locations on the hides to obtain a homogeneity in the results.

Scanning Electron Microscope Studies (SEM)

To assess the effect of the new formulations on fiber structure, bundle arrays, stains and shrinkage of the hide, SEM images of the preserved hide samples were taken after 28 days of storage and compared with the image of fresh hide sample. Samples were mounted on stubs and sputter gold coated for 1 min (EMS 150R ES, EM Sciences, Hatfield, PA). Samples were viewed with a FEI Quanta 200 F Scanning Electron Microscope, (Hillsboro, OR, USA) with an accelerating voltage of 10KV in high vacuum mode.

Rehydration of Cured Hides

Rehydration experiments for all the cured hides were carried out periodically. The data on the 28th day of storage are reported in this paper. Approximately, 5 g cured sample was put in 50 mL of water at room temperature (22±2°C) in a 100 mL beaker. The samples were removed from the beaker and gentle blotted on tissue paper to remove surface water and then weighed by an electronic balance with an accuracy of ±0.0001 g. The cured hides were evaluated for rehydration characteristics in respect of weight increase at every hour due to the absorption of water.

Hide Degradation Analysis

The cure hides were measured on the basis of color change, hair slip and odor on different time slots during storage period. These properties indicate the level of proteolysis of collagen and other sub protein constituent during the preservation period.

Results and Discussion

In this study, five novel formulations have been developed and tested for their effectiveness in hide preservation where, a 45% saturated brine solution in combination with bactericidal antiseptics is used. This new development reduces salt consumption by 50% from the conventional curing process, where 95% saturated brine solution is being used. This technology is adopted principally to address the pollution problem created from conventional curing methods either from soaking liquor used for hide preservation or/and tannery effluents. To develop the reported five formulations, a surfactant (ATMB) and an antimicrobial agents (CDG) have been used in common. ATMB is a quaternary ammonium compound which in addition to possess antibacterial properties.[21,22] ATMB is able to damage the cell membranes and destroy the cellular structure of various microorganisms including fungi, bacteria and other single cell organisms. ATMB is non-toxic to be applied directly to the skin. Chlorohexidine salts (CDG) dissociate in water and releases positively charged chlorhexidine cation which results bactericidal effect through the binding of this cationic molecule to negatively charged bacterial cell walls.[23] CDG is active against Gram-positive and Gram-negative organisms, facultative anaerobes, aerobes, and yeasts.[23] Among the additives, lactic acid (a alphahydroxy acid) is a well-known antimicrobial[24] and also acts as humectant[25] which attracts water and improve hydration of the stratum corneum of the skin. Alphahydroxy acid such as lactic acid also increases cohesion of the stratum corneum cells and thus reduces roughness and scaling. Also, two combinations of spore killing agents, hydrogen peroxide with peracetic acid[26] (F-D and F-F, Table 1) and sodium hypochlorite[27] (F-C and F-E, Table I) have been added to enhance the antimicrobial properties of the particular formulations.

Efficacy Tests for the Developed Formulations

Results revealed that disc infused with industry standard of 95% saturated brine solution (F-A) had no zone of inhibition when placed on treated TSA plates (Table II). However, the other formulations with 45% saturated brine solution and additional additives had zones of inhibition ranging from 1.8 –2.4 mm. Of the formulations, F-F showed greatest ability to inhibit the growth of the natural bacteria obtained from freshly obtained cattle hide. These data prove the effectiveness of the developed formulations on limiting the targeted microbial growth commonly found on bovine hides.

Table II
Measurement of the zone of inhibition by different formulations.

F-A (control)	F-B	F-C	F-D	F-E	F-F
0	1.9	2.2	1.8	2.0	2.4

Water Activity and Moisture Content

Water activity was found (Figure 1A) to be reduced throughout the storage times by all the formulations when compared to the initial reading of uncured fresh hide.

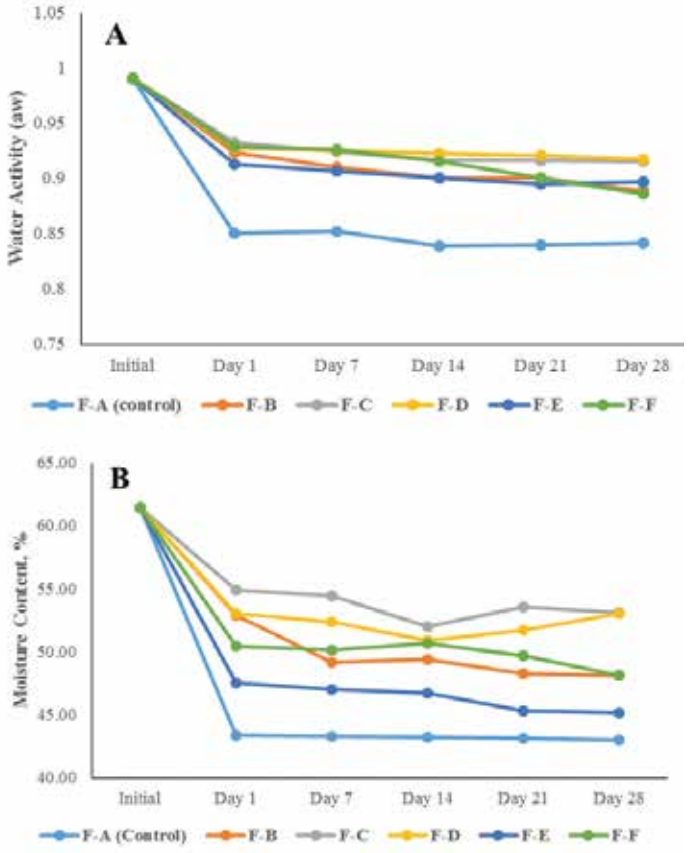


Figure 1. Water activity (A) and Moisture content (B) of cured hides during storage period.

Figure 1B shows the moisture content of the skins preserved with traditional and developed methods for 4 weeks of incubation. After 24 h alternatively treated hides were observed with a minimum of 6.52% (F-C) to a maximum of 13.92% (F-E) losses in moisture content where conventionally treated skin (F-A) showed expectedly lower moisture content (43.3%) due to high concentration of salt. At the end of the experimentation period of 28 days the reduction in moisture contents were recorded from 8.32% (F-C) to 16.29% (F-E) which is comparable to the result produced by the control procedure (F-A).

Bacterial Count in Cured Hides

The efficacy of a hide preserving formula mainly depends on the growth inhibitory activity of microorganisms on the skin. The level of hide putrefaction can be indirectly measured by the

amount of bacteria present on the preserved hide during the incubation period. Figure 2 shows the pictures of the bacterial population on TSA plates originated from the bovine hide cured by the different methods for 21 days. From comparative data in Figure 3, it is shown that alternatively cured hides pose better protection limiting microbial growth up to 21 days than the traditionally cured hide (F-A). The bacterial growth for F-A (control) treated hide became "Too Numerous To Count" (TNTC) within seven days of storage, where the formulations F-E and F-F were proven effective in limiting microbial growth on hides up to the end of the experiment.

Figure 2. Bacterial population on TSA plates from differently cured bovine hides for 21 days.

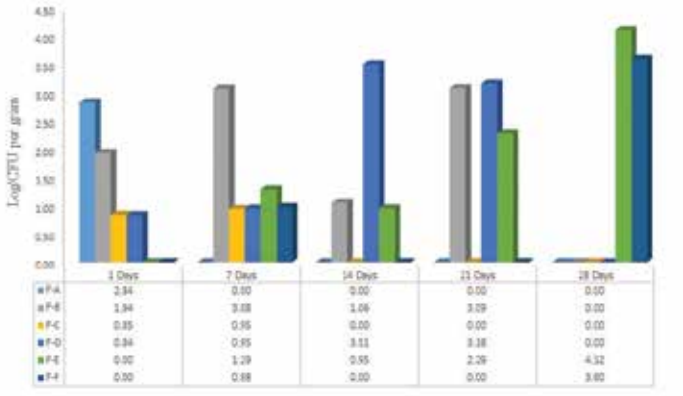


Figure 3. Bacterial growth on bovine hides over 28 days of preservation (Log CFU per gram).

Texture Analysis

The hardness of the samples was obtained by calculating the total work done by the same amount of predetermined load (force) on the surface of cured hides. The harder or tougher the sample is underneath the grain layer, the less work (in mJ) is accomplished with less amount of deformation under the same amount of force. From Figure 4, it was clear that, alternatively preserved hides were softer than the conventionally cured sample (F-A).

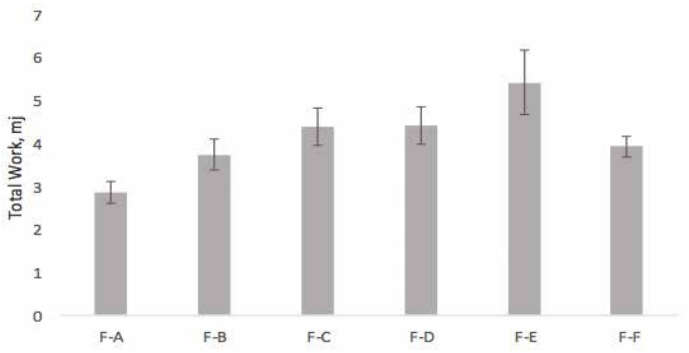


Figure 4. Relative hardness of differently cured hides: Less work on harder surface.

Microscopic Analysis of Cured Hides

SEM images for the cured samples (Figure 5, ii-vii) showed that the texture is aligned and non-overlapping with each other, which would provide well lustrous grain and texture after processing. No significant difference in the fiber structures and fiber orientation between the experimental (Fig 5, iii-vii) and control (Fig 5, ii) samples were seen. However, the SEM images at 1000x of the alternatively cured samples (Fig 5, iii-vii) showed relatively open structure, where the traditionally treated hide (Fig 5, ii) showed more compact structure as close as untreated raw hide (Fig 5, i).

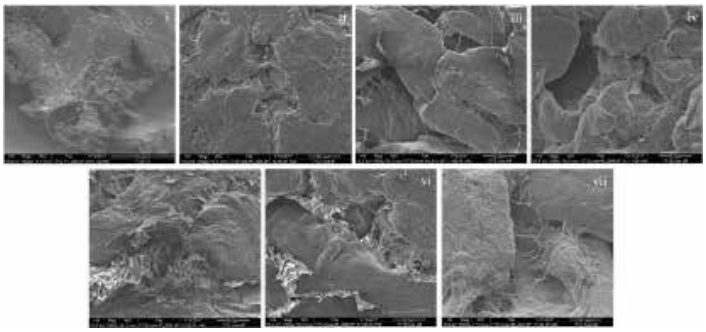


Figure 5. Scanning electron micrographs of cross-section of fresh (i) and preserved pelts after traditional, F-A (ii) and experimental curing F-B(iii), F-C (iv), F-D (v), F-E (vi), F-F (vii) for 28 days.

Rehydration Studies

Rehydration assay is an important analysis to assess the capability of the cured hides to absorb liquid. The faster the rate of rehydration resulting in significant advantages in tanning process by absorbing chemicals quickly.

From Figure 6, it is evident that all the alternatively cured hides absorb water at faster rates than the control sample (F-A) in the first hour. Samples, F-B, F-C and F-F show faster water absorbing capability than the control throughout the experiment and also the rehydration profiles of the other two samples are comparable to that of the traditionally cured hide.

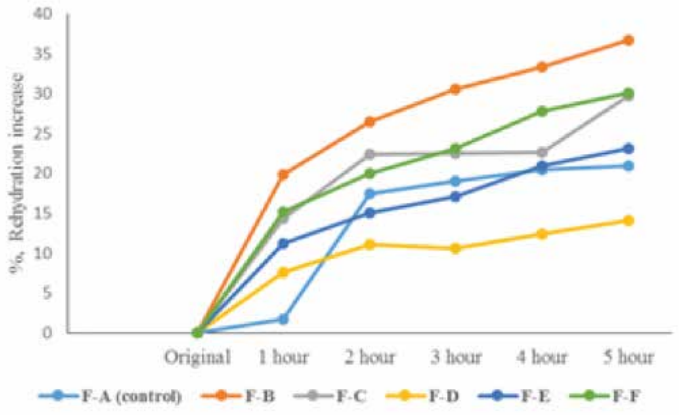


Figure 6. Rehydration profile of differently cured hides on 28th day of preservation.

Physical Changes of the Cured Hides

The changes in physical appearance of the differently cured hides are recorded during preservation period. Hair slip is one of the most common sign of hide putrefaction. The pungent odor and discoloration of hide are usually attributed to the waste products produced by bacterial activity. There was no color change or hair slip or bad odor observed from the control (F-A) and experimental samples (F-B to F-F) after being preserved for 28 days. However, discoloration, fully hair slip with pungent odor were detected from untreated raw hide within 3 days of storage, which was discarded and no attempt was made to process.

Conclusion

Controlling microbial growth on skin is the key to preserved hide for an extended period. From the diffusion test and bacterial colony count analysis at different stages it is evident that all of the developed formulations work better in limiting bacterial concentration than the traditional hide curing system. This effectiveness can be attributed to the combined strategies of bacteriostatic and bactericidal offered by these formulations. In these formulations, 45% brine solution helps to keep the moisture level of cured hides low creating an unhospitable environment for the bacteria to survive and the combination of ATMAB and CDG produces antiseptic properties to kill the bacteria at the same time. Additionally, these cured hides show overall better performance in absorbing water than the conventionally cured hide probably due to their open structures shown by the SEM images. The quick liquid absorbing capability of preserved hides results significant advantages in tanning process. The use of reduced amounts of salt and low concentration of antiseptics make this technology not only cost effective but also a viable option for combating the pollution problem of TDS arising from the traditional salt curing system.

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