A Cleaner Process for Short-Term Preservation of Hides using Wheat Bran

by

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Abstract

Salting is the most common method to preserve hides and skins. However, this preservation system requires the generation of large amounts of contaminated salt, approximately three million tons per year. In recent years several researchers have suggested different methods for the short-term preservation of hides using plant-based formulations, which either minimize or even completely eliminate the use of salt in the process. In this work, the possibility of using wheat bran for this purpose was studied. Two methods of application (dry and aqueous solution) have been developed. They enable the preservation of hides for one month, reducing by half the salt used in the preservation stage without undermining the quality of the final leather. These two methods contribute to the improvement of the overall sustainability of the tanning process.

With dry application, the use of salt is avoided and preservation occurs because the hide is dried. The application in aqueous solution (10% wheat bran) requires its previous hydrolysis and a minimum amount of salt (10°Bé). The preservation occurs because the acidity of the hide is increased.

Introduction

Hides are the raw material used to manufacture leather. In turn, leather is used to create a wide range of diverse objects including shoes, garments, bags, and saddles. The process to transform hide into leather is called tanning. Basically, the process consists in chemically stabilizing the collagen protein, which is the main component of the hide. This stabilization is achieved by incorporating certain chemical products, such as basic chromium salts, which react with collagen forming complex structures into the hide. The stabilization transforms the hide (which is an easily degradable material due to bacterial attack) into leather, a stable and useful material to manufacture multiple objects. To prevent hides from putrefying in the time gap between the animal death and the tanning itself, it is necessary for them to undergo a preservation process.[1-3] The most widely used method is salt preservation,[4] which dehydrates the skin and prevents bacterial growth. An annual expenditure of at least 3 million tons of salt used to preserve hides has been estimated.[5] It must be pointed out that the salt being used throughout the whole process is not reusable, which raises serious environmental and economic concerns.

Salt-induced soil degradation is a serious threat to global agro-ecosystems.[6] Salt as a waste from different industrial processes cannot be indiscriminately discharged into the environment. The solid salt used in the preservation and also recovered in solid form contains bacteria, fat, dirt, among other elements. Its treatment is extremely expensive and therefore it is generally disposed of in a landfill. Another part of the salt already used is diluted in water, greatly complicating the subsequent purification processes of the residual floats. In fact, it is considered that more than 40% total dissolved solids (TDS) and 55% of chlorides in the resulting effluent of the total tanning process come from salt preservation.[7]

It is important to note that currently, in many cases, the hides are tanned a few days after the slaughter of the animal, which implies that very often a short-term preservation of approximately one month is enough. Another existing option is long-term preservation (salted or dried), which is absolutely required when the raw stock must remain stored for a long time before the start of the tanning operations.

For many years, a large number of researchers have tried to develop other preservation methods based on the total or partial replacement of salt by chemical products. In this regard research has been conducted on the hide preservation properties of chemicals such as boric acid combined with biocide[8] or naphthalene,[9] acetic acid combined with sodium sulfite[10] or with benzoic acid,[11] benzalkonium chloride,[12] formaldehyde,[13] sodium thiosulfate,[14] sodium metabisulfite,[15] sodium sulfate,[16] potassium chloride,[17] sodium carbonate,[18] oxide magnesium,[19] silicate,[20] silica-gel,[21] ozone,[22] synthetic polymers,[23] polysaccharides,[24] sodium hexafluorosilicate,[25] formic acid or formic acid combined with sulfuric acid,[26] chlorites and hypochlorites,[27] quaternary ammonium salts,[28] polyethylene glycol,[29] biocide,[30] and various antibiotics.[31-32]

Other lines of research include subjecting the hides to vacuum sealing,[33] cooling,[34] freezing,[35] electrical currents,[36] gamma irradiation,[37] irradiation with electron beams,[38] as well as the same irradiation combined with the use of bactericides[39] or with the use of gamma rays.[40]

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In recent years, several researchers have attempted to replace salt with plant-based formulations, thus valorizing the bioresources. Some of the plants tested were *Myrtus communis,*[41] *Origanum minutiflorum,*[42] *Azadirachta indica,*[43] *Sesuvium portulacastrum,*[44] *Wedelia chininsis*, *Cassia alata*, *Clerodendrum phlomidis*, *Solanum trilobatum* and *Calotropis procera,*[45] *Acalypha indica,*[46] *Terminalia chebula,*[47] *Lawsonia inermis,*[48] *Semecarpus anacardium,*[49] *Citrus sinensis,*[50] *Tamarindus indica,*[51] *Rumex abyssinicus,*[52] *Moringa oleifera*, *Averrhoa bilimbi*, *Muntingia calabura,* and *Leucaena leucocephala,*[53] The preservative action of these products is due to the fact that they contain antibacterial compounds.

The study presented in the paper follows this line of research studying the possibility of using wheat bran to replace part of the salt used in the short-time preservation of hides, thus contributing to improvements in the sustainability of the tanning process.

Wheat bran is one of three layers of the wheat kernel, specifically, the most external one. It is stripped away during the milling process and produces a very abundant and relatively inexpensive byproduct. It is estimated that 12 million tons protein from wheat bran are wasted annually worldwide.[54] Different alternatives to recycle such solid waste are being studied.[55,56] Wheat bran contains antibacterial and antioxidant compounds.[57] The most abundant one is ferulic acid,[58] but it also contains other phenolic acids (such as vanillic acid, syringic acid, coumaric acid), lignans[59] and alkylresorcinol.[60] For this reason, its potential use as a skin preservative has been studied.

Experimental

Materials

Bovine hide, wheat brand, and salt (NaCl) were used to carry out the tests.

Other equipment included a laboratory drum with temperature sensor (Inoxvic brand), a laboratory stove (Nahita brand), a laboratory scale (Cobos brand), and a pH meter (Crison brand).

Besides, p-iodonitrotetrazolium violet indicator (98%) was used to carry out the tests to determine hide putrefaction.

Methodology

The research was structured in five stages. The research is summarized in Table I and each stage is explained in detail below.

Test 1. Potential of wheat bran as a preservative of hides

The first stage consisted of a series of pre-tests to determine whether wheat bran may actually work as a hide-preserving agent.

In three of the tests wheat bran (25% on the weight of the hide) was applied solidly covering a piece of hide on both sides. In two of the tests 10% and 20% (on the weight of the hide) of salt (NaCl) were mixed with the wheat bran. The hides were then left to rest wrapped with a plastic at room temperature (approximately 20°C) for one month. Two clear symptoms of poor hide preservation are that the hair is easily pulled out and/or the smell of ammonia is detected. Each day the hides were unwrapped and these two tests were performed. Then the hides were wrapped again.

In another test, an amount of 40 g of wheat bran was mixed in 360 g of water and the amount of salt needed to reach a final density of 7.5°Bé (27.2 g). The mixture was stirred vigorously. The hide (40 g) to be preserved was introduced in the same container and allowed to marinate. When the float completely penetrated the hide reaching its center, the hide was removed from the float, wrapped in plastic and left to rest for a month at room temperature. The test was repeated carrying out the maceration at 40°C.

This last test was repeated changing the conditions of maceration. In this case the mixture of wheat bran, water and salt was allowed to stand in an oven at 40°C for three days before introducing the hide into the maceration vessel. The objective was to previously hydrolyze the bran so that the hide was macerated in an acidic solution at room temperature.

Table I

Summary of tests performed during the research

Test number	Test objective
1	Test to determine the potential preservative effect of wheat bran applied in different ways on the hide
2	Test to determine the preservative effect on the hide of wheat bran applied in liquid with different saline concentrations
3	Test to determine the preservative effect on the hide of wheat bran applied in liquid depending on the saline concentration and the maceration time
4	Test to compare qualitatively the presence of bacteria on the hide depending on the preservation method applied
5	Test to compare the physical properties between a hide preserved with salt and the hides preserved with the new methods tested with wheat bran

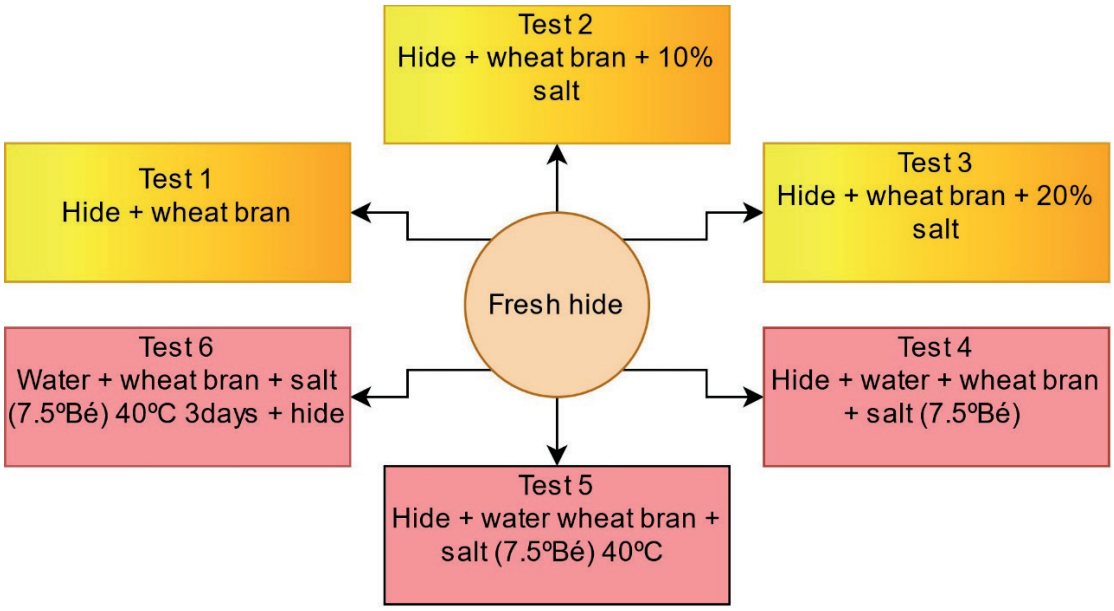

Figure 1. Tests on the hide preservative ability of wheat bran.

In these three tests the same controls were carried out as in the three previous tests. Figure 1 shows a diagram of the six tests carried out.

Test 2. Influence of the saline concentration in the preservation of hides

In the second stage the influence of the amount of salt on the preservation of a hide by maceration was studied by means of an aqueous mixture of wheat bran. Four identical solutions were prepared by mixing 40 g of wheat bran with 360 g of water. Increasing amounts of salt (41.5 g, 7.8 g, 100.8 g and 145.6 g) were added to each solution until saturation was reached. The final densities of each solution were 10, 15, 20 and 23.7°Bé. A piece of 40 g hide was immersed in each container and allowed to marinate. When the float completely penetrated the hide, reaching its center, the hide was removed from the float, wrapped in plastic and left to rest for a month at room temperature. The reference was an identical hide piece kept at 20°C without any preservation. Hair removal by pulling off, pH and the smell controls of the hides were performed periodically.

Test 3. Influence of maceration temperature and the moment in which the salt is added on the preservation of the hides

The third stage of the study examines how an increase in temperature during the maceration of wheat bran in water (with and without salt) may affect the preservation of the hide. Figure 2 shows a diagram of the eight tests carried out.

Two different types of maceration were performed. Eight identical solutions were prepared by mixing 40 g of wheat bran with 360 g of water. In four of the solutions, enough salt was added so that their final densities were 10, 15, 20 and 23.7°Bé. The maceration was carried out at 40°C until achieving pH stabilization. The solutions were allowed to cool to room temperature, filtered and then a piece of 40 g of hide was introduced in each of the containers. In the other four solutions a similar procedure was followed, although with a significant modification; the salt was added after maceration in the oven and just before introducing the hides into the already filtered solutions. Therefore, in some tests the maceration of the wheat bran, and therefore its acid hydrolysis, was carried out in saline medium and in the other tests the salt was added afterwards. When the float completely penetrated the hide, reaching its center, the hide was removed from the float, wrapped in plastic and left to rest for a month at room temperature. Hair removal by pulling off, pH and organoleptic controls of the hides were performed periodically.

Test 4. Comparison of the effectiveness of the preservation methods being tested

A comparative assessment of the degree of putrefaction of several of the hides preserved with wheat bran was carried out. The dry preservation method and the methods in which the preservation was carried out in liquid medium and had shown positive results were evaluated. In this

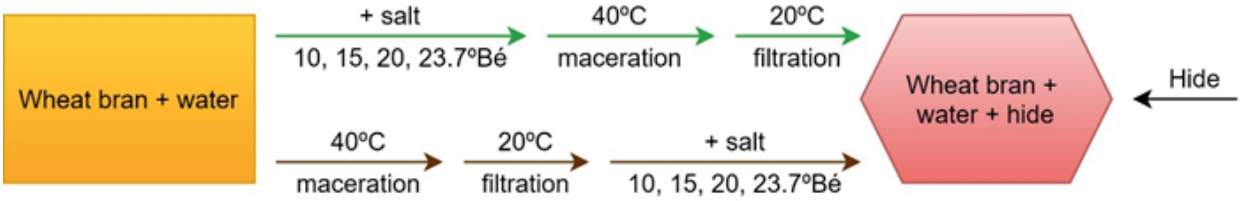

Figure 2. Different systems for preparing the preservative solution.

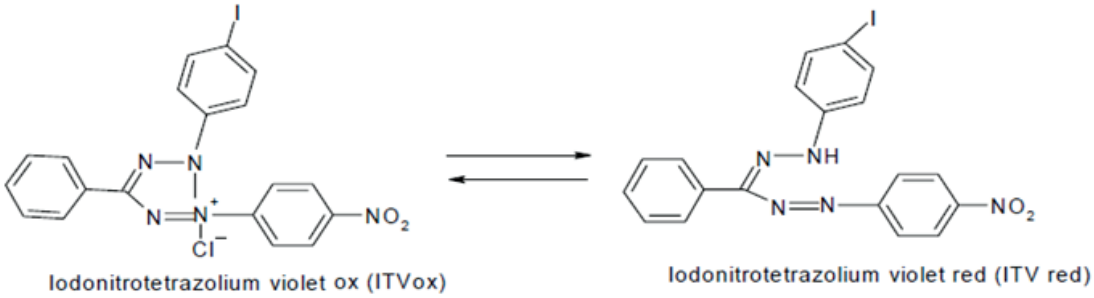

Figure 3. p-Iodonitrotetrazolium Violet reaction.

last case, only the three tests with minimal presence of salt (10 °Bé) were evaluated. The degree of putrefaction of the hide was measured using the p-iodonitrotetrazolium violet indicator. In the presence of bacteria, this indicator is reduced, forming a compound derived from formazan, which is violet colored and insoluble in water (Figure 3). This reaction can assess the degree of putrefaction of a hide.

Development of a comparative pattern to assess bacterial growth

In order to visually compare the effectiveness of each of the preservations a pattern was developed. A piece of salty hide was soaked for two days at room temperature so that bacterial growth would occur. Five test tubes were filled with different amounts of the resulting residual float, deionized water and p-iodonitrotetrazolium violet indicator, as shown in Table II.

The test tubes were left in an oven at 35 °C for 24 hours. Each tube modified its color depending on the number of bacteria it contained.

Comparison of bacterial growth in the various preservation methods tested

Six pieces of hide were subjected to the following preservation methods explained in the previous sections:

a) Wheat bran (dry).

b) 10% salt + wheat bran (dry).

c) 20% salt + wheat bran (dry).

d) Aqueous solution of macerated wheat bran + salt (10°Bé) at room temperature + hide.

e) Aqueous solution of macerated wheat bran + salt (10°Bé) at 40°C (3 days) + hide.

f) Aqueous solution of macerated wheat bran at 40°C (3 days) + hide + salt (10°Bé).

Table II

Comparative pattern. Content of each tube

Test tube	0	1	2	3	4	5
Indicator	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Deionized water	5 mL	4 mL	3 mL	2 mL	1 mL	0 mL
Residual float	0 mL	1 mL	2 mL	3 mL	4 mL	5 mL

In the case of methods d, e and f, the tests were carried out with the minimum amount of salt (10°Bé) that had allowed obtaining good preservation results in previous tests.

The pieces were left in an oven at 35°C for 48 hours to accelerate possible bacterial growth. Subsequently they were soaked for two days at room temperature. Results showed that the final pH was the same for all floats. In six test tubes, 5 mL of each residual float was mixed with 1 mL of p-iodonitrotetrazolium violet indicator and left for 12 hours in an oven at 35°C. Finally, the coloration of each tube was compared with that of the tubes of the standard to establish a relative order of effectiveness of the preservation systems being tested.

Effect of salt on bacterial growth

An additional test was carried out to assess the preservation effect of the salt added to the aqueous solution of macerated wheat bran kept at 40°C for three days before adding the salt and the hide to be preserved. The process was similar to the one followed in the previous test except for the modification of doubling the amount of indicator to intensify the resulting color and thus facilitate the interpretation of the results. Five tests were carried out with densities of 0, 10, 15, 20 and 23.7 °Bé and the results were visually evaluated.

Test 5. Effect of the type of preservation on the physical properties of the final leather

Four pieces of hide were preserved for one month according to the following methods:

Wheat bran (dry).

Aqueous solution of macerated wheat bran at 40°C (3 days) + hide + salt (10°Bé).

Aqueous solution of macerated wheat bran + salt (10°Bé) at 40°C (3 days) + hide.

Salting (traditional).

In other words, from each of the different methods tested throughout the research, those with the highest salt savings that allowed to preserve the hide correctly were chosen.

The pieces of hide were chrome tanned following a standard formulation.

The Official IUP (Physical Test Methods) and IUC (Chemical Test Methods) methods specified below were followed to analyze the leather: *IUP 6 (ISO 3376:2011) Determination of tensile strength and percentage extension;*[61] *IUP 8 (ISO 3377-2:2002) Determination of tear load - Part 2: Double edge tear;*[62] *IUP 9 (ISO 3379:2015) Determination of distension and strength of surface (Ball burst method);*[63] *IUP 16 (ISO 3380:2015) Determination of shrinkage temperature up to 100°C.*[64]

Three experts compared the following organoleptic properties of the leathers obtained: fullness, softness, grain tightness, and grain smoothness.

Results and discussion

Test 1. Potential of wheat bran as a hide preservation agent
In the three tests in which the wheat bran was applied in solid form, the hides suffered significant dehydration. The test in which no salt was added after two days was practically dry (water content decreased from 70% to 15%) and maintained a constant pH of 6. When salt was added to the tests, less dehydration occurred, keeping the hides somewhat moist, and the pH value decreased slightly until 5.5. After one month none of the three tests showed any sign of putrefaction.

In the three tests in which the hide was macerated in aqueous solution, it took five days for the liquid to penetrate the hide. In the test carried out at room temperature, the pH dropped to 5. In the two tests with working conditions at 40°C, the final pH was 4.5. In all three cases, the hairs could be easily removed by pulling them off by hand after a few days since the beginning the test and the pieces of hide gave off a slight ammonia smell. That is, clear signs of putrefaction were observed.

In the first case, the preservative effect was due to dehydration, since bacterial growth was halted in the absence of water.

In the second case it was observed that the presence of salt was not enough to stop bacterial growth. It was also concluded that higher temperatures accelerate the acid hydrolysis of wheat bran, yet the compounds released were not capable of slowing bacterial growth on their own. Therefore, under the tested conditions, the preservation of the hides was not possible.

In summary, under the conditions tested, preservation of the hide with solid wheat bran is possible and preservation with wheat bran macerated in water is not possible.

Test 2. Influence of saline concentration on the preservation of hides
In the four tests the macerating solution crossed the hides in five days whereas the pH value remained between 5.5 and 6.

The blank test hide (no preservation) showed signs of putrefaction after 5 days. The hairs were easily removed by hand and the piece of hide gave off ammonia smell.

The four tests in which the maceration solution contained different amounts of salt (10, 15, 20 and 23.7°Bé) did not show differences in behavior. After one month none of the four tests showed any sign of putrefaction and the hairs were difficult to remove by simply pulling them off by hand. The hides were flexible, which means that they retained a significant degree of moisture.

Taking also into account the result of the previous test, in which the same procedure was followed although with a different amount of salt (7.5°Bé), it was concluded that the presence of a certain amount of salt (10°Bé) is necessary for the preservation method to succeed. The salt greatly contributes to stop bacterial growth and enables the preservation of the hides with no signs of putrefaction for at least one month.

Test 3. Influence of maceration temperature and the moment in which the salt is added on the preservation of the hides
After two days in the oven at 40°C, the pH of the macerating solutions without salt was 4.5 and this value remained constant. On the other hand, in the macerated solutions with salt the final stabilized pH was 5. This value was reached after three days in the oven at 40°C, except in the most concentrated saline solution when it was achieved in 4 days. In both cases, when the pieces of hide were introduced in filtered maceration solutions at room temperature, it took four days for the macerating solution to penetrate the hides.

After one month none of the tests showed any sign of putrefaction and the hair was extremely difficult to pull off by hand.

The results of the test show that the higher the temperature, the faster the hydrolysis of wheat bran, thus facilitating the appearance of acidic compounds that possibly influence the preservation of hides. The presence of salt slows acid hydrolysis and prevents the release of some acidic compounds, as indicated by the final pH difference between the two types of maceration solutions.

Test 4. Comparison of the effectiveness of the preservation methods

Development of a comparative pattern of bacterial growth
The colorations of the tubes that served as a comparative pattern can be seen in Figure 4.

The liquid contained in tube 0, which does not contain residual float from the soaking of hides, is transparent, and the coloration becomes violet as the amount of the residual float increases. Therefore, the

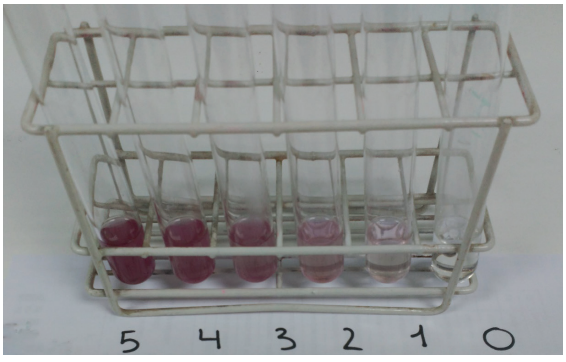

Figure 4. Comparative pattern of bacterial growth. More color intensity means more bacteria.

more intense the violet color in the tube, the more bacteria will be contained in the liquid.

Comparison of the bacterial growth in the different preservation methods being tested

In the comparative test, the final pH of all the residual soaking floats was 6. The colorations obtained at the end of the test can be observed in Figure 5 (tests a, b and c) and Figure 6 (tests d, e and f). The comparison with the pattern tubes may be found in Table III.

Results indicate that bacterial growth is more pronounced in the case of hides preserved by applying dry wheat bran. The addition of salt stops bacterial growth, but the other preservation systems tested slow it down to a much greater degree.

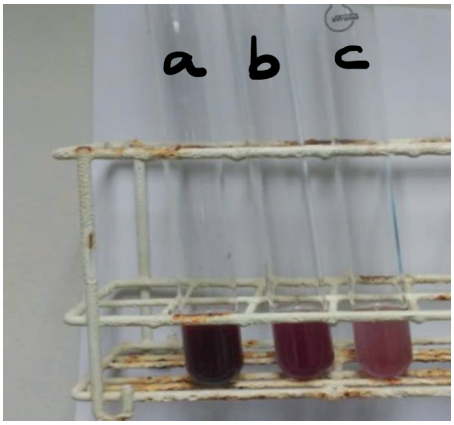

Figure 5. Bacterial growth versus dry preservation methods: **a.** Wheat bran; **b.** Wheat bran + 10% salt; **c.** Wheat bran + 20% salt.

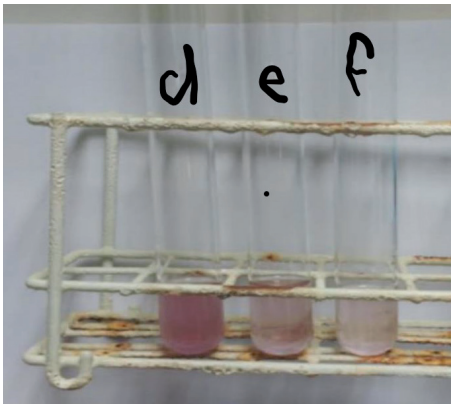

Figure 6. Bacterial growth versus aqueous solution preservation: **a.** Wheat bran + salt (10°Bé) at room temperature + hide; **b.** Wheat bran + salt (10°Bé) at 40ºC + hide; **c.** Wheat bran at 40°C + hide + salt (10°Bé).

The reason is that in the absence of salt, bacterial growth does not stop until the piece of hide is dry. On the other hand, when the piece of hide is introduced into a saline solution, as the dissolved salt penetrates it, bacterial growth slows down. The penetration of the saline solution is faster than the drying, and as a consequence the bacterial growth stops sooner. However, as seen in previous tests, bacterial growth on preservation by drying stops quickly enough to allow the hide to remain in good condition for a month.

It is also observed that the heat treatment of the macerated aqueous wheat bran contributes to slowing down the bacterial growth in the hide. This is probably because the acid hydrolysis of wheat bran is encouraged by releasing compounds with a bactericidal effect. When performing the heat treatment without salt, the hydrolysis is even more salient, the final pH is lower and the bactericidal effect is clearly increased. The results suggest the need to carry out future investigations with longer preservation periods to check whether the effectiveness of the methods being tested is only short term or whether its effectiveness may persist in medium or long-term periods.

Effect of salt in bacterial growth

This test was carried out to assess the influence of the salt adding procedure for the hide preservation methods described in the previous test with lower bacterial growth.

The colorations obtained can be seen in Figure 7.

Figure 7. Effect of salt on bacterial growth. **A:** 0°Bé; **B:** 10°Bé; **C:** 15°Bé; **D:** 20°Bé and **E:** 23.7°Bé.

Table III

Comparison of the bacterial growth using the pattern tubes

Test	Coloring versus pattern tubes
Wheat bran (dry)	Much more intense than tube 5
10% salt + wheat bran (dry)	More intense than tube 5
20% salt + wheat bran (dry)	Similar to tube 5
Aqueous maceration of wheat bran + salt (10°Bé) at room temperature + hide	Between tube 3 and tube 4
Aqueous maceration of wheat bran + salt (10°Bé) at 40°C (3 days) + hide	Similar to tube 2
Aqueous maceration of wheat bran at 40°C (3 days) + hide + salt (10°Bé)	Similar to tube 1

Table IV

Results of physical tests

Type of preservation	Shrinkage Temperature (°C)	Tensile strength (N/mm2)	Extension (%)	Tear load (N/mm)	Distension (mm)
Salted (Traditional)	107.5	24.12	44.6	126.65	17.34
Wheat bran (dry)	106.5	29.91	31.1	109.09	17.80
Aqueous maceration of wheat bran at 40°C (3 days) + hide + salt (10°Bé)	108	23.32	40.5	103.26	16.28
Aqueous maceration of wheat bran + salt (10°Bé) at 40°C (3 days) + hide	107	29.68	49.0	162.70	16.17

It must be noted that in order to get a final color similar to that of the standard tube 0 (Figure 4, blank with no contamination) a density of 15°Bé in the preservation float was required. Likewise, it was observed that the higher the solution density, the lower the bacterial growth. Therefore, the results suggest that a solution with a density of 10°Bé enables a good preservation of the hide, at least in the short term.

Test 5. Effect of the type of preservation on the physical properties of the final chromium-tanned leather

The results can be seen in Table IV.

The differences in the results obtained are not significant and can be attributed to the own anisotropy of the hides. Therefore, from the viewpoint of physical properties, the four methods tested for the preservation of hides are equally acceptable.

All leathers showed good organoleptic properties except for the leather resulting from hides preserved in the aqueous maceration at 40°C. These showed some signs of grain cracking, which is indeed a serious inconvenience and therefore made us dismiss this method.

Environmental benefits

All the methods tested show significant savings in salt. Also, in most of the methods essayed wheat bran can be recovered for other uses by following the appropriate treatment. The majority of these uses are related to food, biochemistry or their use as fuel.

There are two salt preservation methods. The first one is with salt grain, in which case, at least 45-50% salt is used on the hide weight (Wu, 2017). Depending on the type of hide, this amount can reach up to 70-100%. In the second method, hides are tumbled in a paddle with saturated brine (36% w/v at 15°C), which is equivalent to a density of 26°Bé approximately, with a ratio kg hide/L float of 1/5 (Covington, 2011).

These methods are long-term preservation (2-3 years). Thus, in the three methods of dry application that were tested, salt savings range from 25% to 45% on the hide weight, when compared with the minimum quantities used in traditional salting.

In the two methods in which the wheat bran is macerated, the final solution contains 10.5% salt, while in a traditional preservation with brine it contains approximately 26% salt. These values are approximate since they vary slightly depending on the temperature. In any case, results show savings of 15.5% of salt per liter of brine. In both cases, the salt used is minimized by 50%. Whatever the chosen method, and taking into account that it is estimated that the annual salt expense to preserve skins is about 3 million tons, the proposed alternatives involve very significant savings in salt and, therefore, remarkable environmental benefits.

On the other hand, wheat bran can be recovered and reused. In the case of dry application, once the hide is preserved, the wheat bran can be recovered by shaking the hide. The washed and dried wheat bran can be reused (i.e. as a biofuel). In the case of application in a liquid medium, once the hide is preserved, the wheat bran can be recovered and reused after filtration, washing and subsequent drying.

Conclusions

Results show that the use of wheat bran in the preservation of hides avoids damage by bacterial attacks for at least one month. The systems successfully tested enable the hides to be preserved either by sprinkling the wheat bran on the hides or by macerating the wheat bran in water and subsequently impregnating the hides with the resulting solution. The quality of the final leathers obtained from hides preserved by the tested systems is comparable to the quality of leathers obtained from hides preserved by traditional methods. These new methods allow a very important reduction from the salt used in the traditional methods of hide preservation by salting, which leads to much better environmental outcomes, as has been explained above. The systems we have tested have proven to be valid for short-term preservation. Future research will be necessary to confirm or rule out whether the new systems are valid in medium or long-term periods.

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