Examination of Bacterial Populations in Salt, Salted Hides, Soaked Hides and Soak Liquors

by

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Abstract

This study was performed to evaluate the efficiency of salt curing and soaking methods containing antibacterial agents. The bacteria adversely affecting hide quality because of hide preservation method and soaking process were examined in salt (n=40), salted hide (n=36), soaked hide (n=34) and soak liquor (n=19) samples obtained from different tanneries in Leather Organized Tannery Region, Tuzla-İstanbul, Turkiye. Most of the hides examined in this study (83%) were imported from abroad. The pH, moisture content %, ash content % and salt saturation % of the salted hides were determined. In the salted hides, the pH values of all samples (pH 6–9) and moisture content (49–66%) in 28% of the samples were found to be appropriate for bacterial growth. It was determined that ash content in 25% (10–14%) of the salted hides and salt saturation in 22% (65–84%) of the salted hides were suitable for the growth of mesophilic bacteria. Despite the salt-curing of hides, proteolytic and lipolytic mesophilic bacteria were isolated from the hides in high numbers. Proteolytic and lipolytic extremely halophilic bacteria were observed in the most samples of salt and salted hides. These bacterial counts were 10^2–10^4 c.f.u./g in the salt samples whereas they were 10^2–10^6 c.f.u./g in the salted hides. Although proteolytic and lipolytic mesophilic bacteria were 10^5–10^8 c.f.u./g in 97% of the soaked hide samples, they were 10^5–10^6 c.f.u./ml only in 42% of the soak liquors. In conclusion, it was determined that this hide preservation method was not adequate to inactivate bacterial activity. Hence, bacterial activity was high in the salted and soaked hides and also the concentration of antibacterial agents used in the soaking process was not sufficient. It is believed that eliminating of these problems will provide to increase hide quality.

Resumen

Este estudio se efectuó para evaluar la eficiencia de la preservación por salado y los métodos de remojo conteniendo agentes antibacteriológicos. La bacteria que adversamente afectan la calidad de la piel debido al método de preservación y proceso de remojo fue examinada en muestras de la sal (n=40), piel salada (n=36), piel remojada (n=34) y baño de remojo (n=19), obtenidas de diferentes tenerías en La Región Designada para Curtiembres Normalizadas, Tuzla-Estambul, Turquía. La mayoría de las pieles revisadas en este estudio (83%) fueron importadas del exterior. El Ph, el porcentaje de contenido de humedad, el contenido porcentual de cenizasy el porcentaje de saturación de la sal fueron determinados. En los cueros salados, el pH de todas las muestras (pH 6–9) y el contenido de humedad (49–66%) en un 28% de las muestras se encontró como apropiado para crecimiento bacterial. Se determinó que el contenido de ceniza (10–14%) en un 25% de las pieles y la saturación salina (65–84%) en un 22% de las pieles saladas, fueron favorables para la reproducción de bacteria mesofílica. Aun con el tratamiento por sal de las pieles, bacteria proteolítica y lipolítica-mesofílica, fueron aisladas en altas poblaciones. Bacteria proteolítica y lipolítica extremadamente halofílica fueron observadas en la mayoría de las muestras de sal y pieles saladas. Los conteos de bacteria fueron de 10^2-10^4 ufc/g en las muestras de sal mientras que fueron de 10^2-10^6 ufc/g en las pieles saladas. Aunque la bacteria proteolítica y lipolítica-mesofílica fueron de 10^5–10^8 ufc/g en un 97% de las pieles remojadas, fueron solo de 10^5–10^6 ufc/ml en un 42% en los baños de remojo. En conclusión, se determinó que este método de preservación de la piel no es adecuado para inactivar la actividad bacteriana. La actividad bacterial, entonces, fue alta en las pieles saladas y remojadas, así como también la concentración de los agentes bactericidas utilizados en el remojo no fue suficiente. Se cree que la eliminación de estos problemas conduciría a un aumento en la calidad de la piel.

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INTRODUCTION

Since the animal body may be exposed to a variety of microorganisms originated from environmental sources such as air, water, soil, feces, manure and slaughterhouse, many species of bacteria easily colonize on the hide surface. Hair follicles on skin or hide are fairly appropriate structures for microorganisms to easily colonize. Moreover, the secretions of skin glands are rich in microbial nutrients such as urea, amino acids, lactic acid and lipids. Even though normal microbial flora of hides containing mesophilic bacteria in healthy animals do not cause any damage on hide, it begins to vary and destroy the hide immediately after the animal is slaughtered and flayed. Hide containing water, protein and fats offers an ideal environment for microbial growth. If there is a sufficiently high population of bacteria on hide, the coordinated expression of the bacterial enzymes may cause irreversible damages on hide.[1-5] The goal of hide preservation with salt and bactericides is to prevent the bacterial damage on hides during their storage and transportation periods.[1] Salt pack curing and brine curing methods are commonly used in the hide preservation in Turkiye. The most important point in salt pack curing method is to apply salt containing bactericides on the hide homogenously. In case this method is applied inadequately, mesophilic and halophilic bacteria can grow on hide. Mesophilic bacteria are commonly found in nature and warm-blooded animals.[5] Salt lakes and saline environments include halophilic and extremely halophilic bacteria. Salt obtained from salt lakes are used in the preservation of hide.[6-8] Hence, salted hides may contain mesophilic, halophilic and extremely halophilic bacteria in high numbers. Proteolytic and lipolytic mesophilic, halophilic and extremely halophilic bacteria on salted hides may cause discoloration of the flesh side of hide, hair slips, pin pricks, degradation of entire follicle, holes in grain surface, partially removal of grain layer, distruption of collagen fibers, loose grain, grain peeling and uneven dyeing in leathers.[9-13]

Salted hides are soaked in water for rehydration in the soaking process and this process helps to remove salt, blood, manure, fats, globular proteins found in fibrous structure of hide. The duration of main soaking processes may change according to tanneries in different countries and ranges from about 1.5 hours to 24 hours. Soak liquors containing high organic substances serve as an ideal environment for bacterial growth.[14] The proteolytic and lipolytic mesophilic bacteria on salted hides can reach considerably high numbers and may cause serious damages on soaked hides during the soaking process by affecting the hide quality adversely.

To prevent bacterial attacks on hides during the soaking process, a variety of bactericides are added into soak liquors. The efficiency of bactericides on proteolytic and lipolytic bacteria in soak liquors may be insufficient due to high organic content of soak liquors. Furthermore, some of the bacteria may be resistant to bactericides applied to the cured hide and soak liquors and these resistant strains may grow in the presence of the bactericides on the cured hides and in the soak liquors. These bacterial strains contain resistance plasmid and may transfer this resistance plasmid to other bacterial strains by conjugation.[5, 7, 14, 15]

The most important factors which affect hide quality adversely are curing and the soaking process of hides in hide industry. The damage occured from these processes cannot be prevented with the other tanning processes. Hence, it is certainly necessary to control proteolytic and lipolytic mesophilic and halophilic bacterial numbers in salt, on salted and soaked hides and in soak liquors periodically to have an idea about inactivation of bacteria during the curing and soaking processes. In addition to this, by reason of adverse effects of bacterial population on salted hides, soaked hides and in soak liquors, it is necessary to control proteolytic and lipolytic bacteria in the cured and soaked hide samples collected from different tanneries. To understand the efficiency and sufficiency of salt pack curing method used in different countries including Turkiye, total halophilic, mesophilic, proteolytic and lipolytic bacterial populations in salt, on salted (imported from different countries and collected from Turkiye) and in soaked hides and soak liquors were examined. Furthermore, moisture content, ash analysis and salt saturation of salted hides were determined for the evaluation of salt curing process.

EXPERIMENTAL

In the present study, salt used in curing of hide (n=40), salted hide (n=36), soaked hide (n=34) and soak liquor (n=19) samples were collected from different tanneries in Leather Organized Tannery Region, Tuzla-İstanbul, Turkiye. Most of the salted hides examined were imported (83%) from different countries such as Greece, England, U.S., Serbia, Bulgaria, Russia, Africa and Australia. The rest of the salted hides were (17%) salt pack cured hides in Turkiye. Then, these samples were immediately placed into sterile sample bags and containers and they were carried on ice during the transportation. Before the experiments, all of the salted hide samples were cleaned thoroughly of hair, fat and dirt. Then, the hide samples were cut into small pieces.

Measurement of pH in the salted hides

Five grams of the salted hides were put into the flasks containing 100 ml of sterile distilled water at 20°C and then they were placed in a shaking incubator (Edmund Bühler, Germany) for 1 hours. All pH measurements of the salted hides were done by using a pH meter (Sartorius Professional Meter PT- 10P, Goettingen, Germany).[10]

Moisture, ash and salt saturation of the salted hides

The salted hide samples were weighed before drying in an

oven at 102˚C for 5 h. The drying procedure was repeated until first weight is equal to second weight.[10] Then, the dry samples were placed in ceramic crucibles and ashed in a muffle furnace at 600˚C for 8 hours. After cooling, the samples were weighed to determine ash content.[10,16] Salt saturations of the examined hide samples were calculated according to standard methods.[10,17]

Examination of extremely halophilic bacteria in salt and salted hide samples

Spread plate technique was used to determine the total numbers of extremely halophilic, proteolytic and lipolytic halophilic bacteria in salt samples and on the salted hides.[18] 20gr of each salt and salted hide sample was placed into flask containing 180 ml 25% NaCl solution. The flasks were placed in a shaking incubator (Edmund Bühler, Germany) for 3 hours at 25˚C in 100 rpm and direct and serial dilutions of the bacterial suspension were spread onto the surface of the agar plates containing Brown medium (2g $MgSO_4.7H_2O$, 2g KCl, 3g sodium tri-citrate, 5g yeast extract, 20g agar and 250g NaCl in 1000 ml of distilled water; pH 7). After the incubation at 39˚C for one month, the numbers of colonies were counted. The bacteria in the salt and salted hide samples were tested for degradation of gelatin and Tween 80 by standard methods using 2% (w/v) gelatin and 1% (w/v) Tween 80, respectively. All experiments were done in duplicate.

Examination of mesophilic bacteria in the salted hides, soaked hides and soak liquors

Spread plate technique was used to determine the total numbers of mesophilic, proteolytic and lipolytic bacteria in the salted hides, soaked hides and soak liquors.[18] 20ml of each soak liquor and 20 gr of each salted hide and soaked hide were separately placed in a flask containing 180ml 0.85 % sterile physiological saline solution. The flasks were placed in a shaking incubator (Edmund Bühler, Germany) for half an hour at 25˚C in 100 rpm and direct and serial dilutions of the bacterial suspension were spread onto the agar plates containing Nutrient agar.

After the incubation at 37˚C for two days, the numbers of colonies were counted. Proteolytic and lipolytic activities of bacterial strains in the salted hides, soaked hides and soak liquors were determined according to previously described test methods.[18,19] All experiments were done in duplicate.

RESULTS AND DISCUSSION

Microbiological analysis of the salt samples

Total extremely halophilic (10^2–10^5 c.f.u./g), proteolytic (10^2–10^4 c.f.u./g) and lipolytic (10^2–10^4 c.f.u./g) halophilic bacteria were isolated from all of the salt samples examined. The total extremely halophilic bacterial numbers were 10^4–10^5 c.f.u./g (89%) in 36 and 10^2–10^3 c.f.u./g (11%) in 4 of the salt samples (Table I). These results support previous studies with regard to the total extremely halophilic bacterial numbers in salt obtained from Tuz Lake (10^4–10^6 c.f.u./g), Kaldırım and Kayacık salterns (10^5–10^7 c.f.u./g) and Tuzköy salt mine (10^5–10^6 c.f.u./g) in Turkiye.[20,21]

pH, moisture, ash content and salt saturation analysis of the salted hide samples

The efficacy of curing method was checked by determining the pH, moisture, ash content, salt saturations and total bacterial counts on the salted hides. These parameters affect the growth of microorganisms on salted hides. At the optimum pH values on which microorganisms grow, it is clear that hide damage will be more. In our study, pH values were measured as 6 (5%), 7–7.5 (53%) and 8–9 (42%) in the salted hides (Table II).

Schmitt and Deasy[22] determined that pH values in hides were decreasing continuously in the course of time during the preservation process and they suggested that this reduction in pH value was occurred due to postmortem changes. Polkade[4] stated that fresh raw hides had an appropriate pH, high moisture and nutrient content for the microbial growth. In the other study, it was reported that bacteria hydrolyze the hide at pH 7.5.[10,23] Taking into consideration that pH range is between 4 and 9 for the most microorganisms living in natural

TABLE I

Mean, median, mode and range values of total extremely halophilic, proteolytic and lipolytic halophilic bacterial numbers in the 40 salt samples.

	Mean	Median	Mode	Range
Total Extremely Halophilic Bacteria (c.f.u./g)	8.2×10^4	3.5×10^4	1×10^4	5.9×10^5
Total Proteolytic Extremely Halophilic Bacteria (c.f.u./g)	2.7×10^3	2.5×10^2	1×10^2	3.9×10^4
Total Lipolytic Extremely Halophilic Bacteria (c.f.u./g)	8.5×10^3	1×10^3	2×10^3	7.9×10^4

environments, pH values of the examined hide samples in our study were reasonably appropriate (6–9) for the bacterial growth. Moisture contents were found as very low (26–39%) in 4, normal (41–48%) in 22 and high (49–66%) in 10 of the 36 hide samples (Table II). Bailey[1] reported the standards of moisture content as 40–48%, ash content as 14–48% and salt saturation as ≥ 85% for salted hides. It was emphasized that moisture content lower than 40% leads to poor rehydration and higher moisture content than 48% supports bacterial growth on hides. Moreover, Bailey[1] mentioned that these minimum standards are necessary for the long term hide preservation under the good conditions. On the basis of these standards, moisture contents of the examined hides were found as normal at the most of the hides examined in the present study. According to our results, 10 samples were under high risk for the bacterial damage. Low moisture content is not preferred in leather making processes. If moisture content of hide is insufficient, cracking on grain surface of salted hides may occur. Ash contents were evaluated as low (10–14%) in 9, normal (15–23%) in 26 and high (49%) in 1 of the 36 hide samples. It was demonstrated that salt saturations in 28 of the 36 salted hides were ≥ 85-100% (86–100%) and in 8 of the 36 salted hides were lower than 85% (65–84%). Ash contents and salt saturations in most (75–78%) hide samples examined supported the growth of extremely halophilic bacteria (Table II).

TABLE II

Mean, median, mode and range values of pH, moisture content %, ash content % and salt saturation % in the 36 salted raw hides.

	Mean	Median	Mode	Range
pH	7.63	7.50	7.50	3.00
Moisture content %	45.87	46.56	41.03	39.94
Ash content %	17.33	15.74	16.66	39.00
Salt saturation %	92.20	95.15	100.00	34.63

TABLE III

Mean, median, mode and range values of total mesophilic, proteolytic and lipolytic mesophilic, extremely halophilic, proteolytic and lipolytic halophilic bacterial numbers in the 36 salted hides.

	Mean	Median	Mode	Range
Total Mesophilic Bacteria (c.f.u./g)	5.1×10^7	1.1×10^7	1×10^8	3.2×10^8
Total Proteolytic Mesophilic Bacteria (c.f.u./g)	4.3×10^5	1×10^5	1×10^5	2.5×10^6
Total Lipolytic Mesophilic Bacteria (c.f.u./g)	2.9×10^6	1.6×10^5	5×10^4	5×10^7
Total Extremely Halophilic Bacteria (c.f.u./g)	6.3×10^6	2.1×10^5	1×10^5	9.9×10^7
Total Proteolytic Extremely Halophilic Bacteria (c.f.u./g)	5.3×10^5	1.3×10^4	1×10^2	6.5×10^6
Total Lipolytic Extremely Halophilic Bacteria (c.f.u./g)	2.4×10^5	4.2×10^3	0	6.2×10^6

Microbiological analysis of the salted hides

Due to inappropriate salt curing, total mesophilic bacteria (10^4–10^8 c.f.u./g) and extremely halophilic bacteria (10^3–10^8 c.f.u./g) were observed in high numbers on the salted hide samples in our study. It was found that 97% of the examined samples had proteolytic (10^2–10^6 c.f.u./g) and lipolytic mesophilic bacteria (10^3-10^7 c.f.u./g). It was determined that 94% and 81% of the samples had proteolytic extremely halophilic bacteria (10^2–10^6 c.f.u./g) and lipolytic extremely halophilic bacteria (10^2–10^6 c.f.u./g), respectively (Table III). The percentage of proteolytic halophilic bacteria on the salted hides was found to be higher than previous studies.[11,13,24] Also, the total extremely halophilic, total proteolytic and lipolytic extremely halophilic bacterial numbers in the salted hides were higher than that of salt samples. These findings showed that the salted hides stored in the tanneries provided appropriate conditions for growth of extremely halophilic bacteria (10^3–10^8 c.f.u./g) in high numbers. Therefore, it is necessary that the salt used in hide preservation should not contain proteolytic and lipolytic extremely halophilic bacteria or these bacteria in salt should be inactivated.

Solar salt is produced by natural evaporation of seawater or brine and solar salt is not exposed to any treatment before it is applied to hides. Previous studies results showed that unprocessed solar salt contained high numbers of proteolytic and lipolytic extremely halophilic bacteria.[21,25] Since the hides were cured with this unprocessed solar salt, extremely halophilic bacterial counts were high in the salted hides examined. It was found that extremely halophilic bacterial counts were fairly high in 72% of the hides (10^5–10^7 c.f.u./g). These findings were consistent with the previous studies reporting the extremely halophilic bacterial numbers (10^5–10^8 c.f.u./g) in the brine cured hides.[9,24] Researchers demonstrated that proteolytic bacteria which originated from unprocessed solar salt digested the grain surface of hide and caused a complete disruption of collagen fibers.[11] Furthermore, it was explained that these bacteria produced sponge-like vesicles within hide and light stains on the suede surface of finished double-face leathers.[12,13]

It is recommended that halocins, bile salts and antimicrobial agents may be used in inactivation of halophilic microorganism in salt to prevent halobacterial damage on salted hides.[6,20,26-28] Antimicrobial activity tests against the bacteria which were isolated from salted hides should be done continuously to control bacterial resistance against antimicrobial agents used in commonly in hide industry. However, it is necessary to use antimicrobial agents, which is not effective on bacterial ribosomes because mitochondria and chloroplasts contain ribosomes (70S) similar to ribosomes of bacteria (70S). Antimicrobial agents which inhibit protein synthesis of bacteria may also affect protein synthesis of mitochondria and chloroplasts.[5]

TABLE IV

Mean, median, mode and range values of total mesophilic, proteolytic and lipolytic mesophilic bacterial numbers in the 34 soaked hides.

	Mean	Median	Mode	Range
Total Mesophilic Bacteria (c.f.u./g)	1.4×10^8	1.1×10^8	2×10^8	8.9×10^8
Total Proteolytic Mesophilic Bacteria (c.f.u./g)	1.86×10^7	2.4×10^7	2×10^6	1.49×10^8
Total Lipolytic Mesophilic Bacteria (c.f.u./g)	1.7×10^7	2.8×10^6	2.8×10^6	1.2×10^8

TABLE V

Mean, median, mode and range values of total mesophilic, proteolytic and lipolytic mesophilic bacterial numbers in the 19 soak liquors.

	Mean	Median	Mode	Range
Total Mesophilic Bacteria (c.f.u./ml)	8.3×10^6	4×10^6	1×10^7	4.2×10^7
Total Proteolytic Mesophilic Bacteria (c.f.u./ml)	4.1×10^5	7.2×10^4	1.2×10^5	5×10^6
Total Lipolytic Mesophilic Bacteria (c.f.u./ml)	1.8×10^5	2×10^4	0	2×10^6

Bailey *et al*.[29] emphasized that electron beam irridation may be used to prevent bacterial growth on raw hides until 3 weeks. Furthermore, Bailey (1) suggested that KCl, which has important roles in opening of stomata, osmotic and ionic balance and activation of enzymes in plant cells[30] may be used in preservation of raw hides instead of NaCl.

Also, Birbir *et al*.[7] showed that a 0.5 A direct electric current was very effective method to kill proteolytic and lipolytic extremely halophilic bacterial populations in salt used in hide preservation.

Microbiological analysis of the soaked hide and soak liquor samples

Total mesophilic bacterial numbers were 10^7–10^8 c.f.u./g in 29 and 10^5–10^6 c.f.u./g in 5 of the 34 soaked hides. Total proteolytic mesophilic bacterial numbers were 10^6–10^8 c.f.u./g in 28 and 10^4–10^5 c.f.u./g in the 6 soaked hides. Total lipolytic mesophilic bacterial numbers were 10^6–10^8 c.f.u./g in 31 and 10^4–10^5 c.f.u./g in the 3 soaked hides (Table IV).

It was determined that total mesophilic bacterial numbers in the soak liquor samples were 10^5–10^7 c.f.u./ml. In these samples, total proteolytic mesophilic bacterial numbers were 10^5–10^6 c.f.u./ml in 8 and 10^2–10^4 c.f.u./ml in 9. Total lipolytic mesophilic bacterial numbers were 10^5–10^6 c.f.u./ml in 8 and 10^3–10^4 c.f.u./ml in 8 and 10^1–10^2 c.f.u./ml in 2 samples (Table V).

Although most tanneries add antibacterial agents to the soak liquors, high mesophilic bacterial numbers were obtained from the soaked hides (10^5–10^8 c.f.u./g) and soak liquors (10^5–10^7 c.f.u./g) examined. While proteolytic (10^4–10^8 c.f.u./g) and lipolytic (10^5–10^8 c.f.u./g) mesophilic bacteria were isolated from all of the soaked hides, proteolytic (10^2–10^6 c.f.u./g) and lipolytic (10^1–10^6 c.f.u./g) mesophilic bacteria were isolated from 89% and 95% of the soak liquors, respectively.

Total mesophilic, proteolytic and lipolytic mesophilic bacterial numbers in the soaked hides were higher than that of bacterial numbers in the salted hides. The numbers of these bacteria were found to be low in the soaked liquors. The high bacterial numbers on the soaked hides were thought to be related with bacterial population on the salted hides. When the salted hides containing bacteria in high numbers are soaked, these bacteria will grow rapidly because of water and high organic content on the soaked hides. On the other hand, the decrease in bacterial numbers in the soak liquors may be related with usage of antimicrobial agents. These results suggested that the bacterial damage on hides may occur during the soaking process.

These experimental results proved that the mesophilic bacteria on the salted hides may contaminate the soak liquors easily and these bacteria may grow rapidly both on the soaked hides and in soak liquors due to inadequate antibacterial agents used in the soaking process.

Conclusion

Our results showed that the salt and antibacterial agents used in hide preservation and the soaking process were insufficient to prevent growth of the mesophilic and halophilic bacteria on the salted hides (obtained from abroad and Turkiye) and soaked hides examined. Unless the growth of bacteria on hides is prevented, some of the bacteria on the salted hide may diffuse into collagen fibers and may cause loss of elasticity of leather. It is not easy to kill the bacteria penetrated into the collagen fibers. Therefore, it is necessary to use more effective bactericidal applications on these bacteria during the hide curing and soaking processes. The effect of bactericide which will be used should be tested on the bacteria isolated from tanneries before application. If the proteolytic and lipolytic bacteria cannot be inactivated during hide curing process, these bacteria may cause quality reduction of leather during other processes.

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