

The Effect of Conservation Defects on The Suede Quality of Double-Face

Kürk-Süet Kalitesine Konservasyon Hatalarının Etkisi

* Behzat Oral Bitlisli, * Hüseyin Ata Karavana, * Bahri Başaran, * Özcan Sarı, **İhsan Yaşa, *** Meral Birbir

SUMMARY

In this research, the effects of microorganisms on suede quality of double-face leather were examined. The bacterial numbers on salted sheepskin samples were found as 1.8×10^6 - 4.3×10^7 cfu/g on solid medium and 1.9×10^6 - 4.7×10^7 cfu/g on halophile medium containing 10-30% NaCl. However it was found that salted sheepskins contained 4.4×10^1 - 2.0×10^4 cfu/g of fungi. While 53-74% of halophilic bacteria and 39-58% of fungi showed proteolytic activity, 47-62% of halophilic bacteria and 35-54% of fungi showed lipolytic activity. It was observed that defective light stained areas on suede surface of finished double-face leathers were caused by microorganisms on salted sheepskins and that these defects correspond to 35% of the total double-face leathers amount. Consequently, it was decided that the proteolytic and lipolytic activities of microorganisms on the flesh side of the sheepskins decreased the suede quality and caused economical losses.

Keywords: Double face, Skin conservation, Proteolytic and halophilic activities, Suede quality

ÖZET

Bu araştırmada kürk-süetlerin süet kalitesi üzerine mikroorganizmaların etkisi incelenmiştir. Tuzla konserveleşmiş koyun derisi örneklerinin bakteri sayısı katı ortamda 1.8×10^6 - 4.3×10^7 cfu/g olarak ve %10-30 NaCl içeren halofil ortamda 1.9×10^6 - 4.7×10^7 cfu/g olarak bulunmuştur. Tuzlu koyun derilerinin 4.4×10^1 - 2.0×10^4 cfu/g sayıda fungus içerdiği belirlenmiştir. Halofilik bakterilerin % 53-74' ü ve fungusların %39-58' i proteolitik aktivite gösterirken, halofilik bakterilerin %47-62' si ve fungusların %35-54' ü lipolitik aktivite göstermiştir. Kürk-süetlerin süet tarafındaki açık renkli kusurlu kısımların mikroorganizmalardan kaynaklandığı ve toplam kürk-süetlerin % 35' inde bu kusurların bulunduğu gözlemlenmiştir. Sonuç olarak; koyun derilerinin et kısımlarındaki mikroorganizmaların proteolitik ve lipolitik aktivitelerinin süet kalitesini düşürdüğü ve bunun ekonomik kayıplara neden olduğu ortaya konulmuştur

Anahtar Kelimeler: Kürk-süet, Deri konservasyonu, Proteolitik ve halofilik aktiviteler, Süet kalitesi

* Ege University, Faculty of Engineering, Department of Leather Engineering, 35100 Bornova-İZMİR

** Ege University, Science Faculty, Microbiology Department, 35100 Bornova-İZMİR

*** Marmara University, Science and Art Faculty, Biology Department, 34722 Göztepe-İSTANBUL
e-mail: oral.bitlisli@ege.edu.tr, huseyin.ata.karavana@ege.edu.tr, bahri.basaran@ege.edu.tr

* JALCA Vol. 99 2004' te yayımlanmıştır.

Geliş Tarihi: 12.10.10

Basım Kabul Tarihi: 14.01.11

Introduction

The term double-face is used to describe all skins where the fur or wool is used for garments or footwear, the flesh side being presented either as a suede nap or finished ¹. In double-face production, the flesh side quality of raw skins, besides the wool side, has great importance. Therefore, the selection of skins for double-face production is extremely significant ¹. The flesh side of the skin should not have any defects to obtain a perfect suede effect. Conservation defects on skins decrease the quality of the suede and may cause serious economical losses. Conservation defects were generally considered as hair slips by tanners but the microorganisms on the skins might have caused damages on the flesh side, although hair slip is not observed.

Skin providers use auxiliary chemical substances such as boric acid besides salt in conservation process ². Biocide utilization is sometimes not preferred due to its high price. Skins used in international trade may have to be stored for a long time under unsuitable conditions during transportation or in tannery stores. Damages dependent on microorganism activities may occur on hides and skins that are exposed to unsuitable conditions. These damages on hides and skins may cause uneven dyeing in leathers ^{3, 4}.

Highly saline conditions in a local habitat exert a strong selective pressure on the biota, favoring the development of halotolerant and halophilic microorganisms ^{5, 6}. These microorganisms can also survive in a brine solution and even in a properly "cured" hide ^{6, 7}. When halophilic bacteria grow on hide, they excrete hydrolytic enzymes onto the surface of the hide. Then, their extracellular proteolytic enzymes are capable of attacking and degrading the proteinous material of hides and skins ⁷. The majority of halophilic bacteria and molds isolated from salted hides and skins have lipolytic and proteolytic enzymes ^{7, 8}.

Fungi are also important microorganisms in leather industry. Fungi reproduce by forming vegetative and generative spores under suitable environmental conditions in the tannery. Spores and vegetative mycelia are easily transferred by air and physical contact during the processing of leather. Fungi proliferate especially on pickled, vegetable-tanned, chrome-tanned hides and skins and finished leathers. However, raw hides and skins are also a natural substrate for many species of fungi as well as bacteria ⁸. Mold growth may cause discolorations on the surface of pickled, vegetable-tanned, chrome-tanned hides and skins and finished leathers. Some

mold discolorations can be removed by washing, but others cause undesirable changes in the physical and chemical properties, rendering the leather unfit for commercial purposes. Previous studies showed that fungal growth on leather and leather products greatly devalues their quality ⁹.

Reports on fungi from extremely hypersaline environments are very few. However, lack of representation in literature may not reflect the inability of fungi to colonize these extreme environments, but rather the little effort that has been invested to find them ¹⁰. In addition to this, the important role of fungi in the degradation of organic materials in hypersaline environments as salted skins, hides, salt lake and the marine ecosystem have been paid little attention ¹¹.

In the present study, some chemical properties of salted sheepskins imported for double-face production were analyzed, and the number of halotolerant bacteria, halophilic bacteria, fungi and the percentage of lipolytic and proteolytic microorganisms on the skins were enumerated. Furthermore, the effects of microorganisms on the suede quality of finished double-face leathers were examined.

Material and Methods

Material

Raw Sheepskins and Double-Face Leathers

In this study, 5300 pieces of drum salted sheepskins imported for double-face production by a tannery located in Denizli, Aegean Region of Turkey, and 3000 pieces of double-face leathers obtained by processing these skins were examined. The skins were processed in three different parties of 1000 pieces each and they were all dyed in brown. Sample amounts that would represent the parties were calculated according to sampling formula (Eqn.1) ¹².

$$n = 0.2 \sqrt{x} \quad (1)$$

where n = sample amount,
 x = greatness of the party

Isolation Media

Halophile medium and modified nutrient agar containing 0%, 10%, 17%, 25% and 30% (w/v) were used to isolate halotolerant and extremely halophilic bacteria from the salted sheepskins. Halophile medium contained 5 g KCl, 5 g

MgCl₂.6H₂O, 5 g NH₄Cl, 5 g MgSO₄.7H₂O, 10 ml of 1% ferric-citrate solution, 50 ml of trace element solution, 30 ml of yeast extract solution (150g/l), 30 ml of peptone solution (150g/l), 10 g agar and 925 ml distilled water¹³. Trace element solution (per liter of distilled water) consisted of 1 mg CuSO₄.5H₂O, 220 mg ZnSO₄.7H₂O, 10 mg CaCl₂.6H₂O, 180 mg MnCl₂.4H₂O, 6.3 mg Na₂MoO₄. Modified nutrient agar contained 23 g nutrient agar, 5 g nutrient gelatin, 5 g beef extract and 1000 ml distilled water^{14, 15}. Modified malt extract agar [30 g malt extract, 5 g peptone, 15 g agar, 10 g glucose, 1.0 g yeast extract, streptomycin (100 µg/ml) and 1000 ml distilled water with the same concentrations of NaCl described above] was used to isolate fungi from the salted sheepskins¹⁶. The pH of halophile medium, modified nutrient agar and modified malt extract agar were adjusted to 7.0, 7.7 and 5.6 with 1 N KOH and 1 N HCl, respectively.

Halophile medium and modified malt extract agar containing % 1 skim milk or % 1 tributyrin were used to determine proteolytic and lipolytic microorganisms on the salted sheepskins¹⁷.

Methods

Chemical Analysis of Raw Sheepskins

In order to evaluate the quality of salted sheepskins, analysis of salt saturation,¹⁸ pH determination,¹⁹ moisture content,¹⁸ ash determination,¹⁹ salt determination,²⁰ and boric acid content²¹ were examined.

Microbiological Analysis of Raw Sheepskins

Isolation of microorganisms

Spread plate technique was used to determine the numbers of halotolerant, extremely halophilic bacteria and fungi on the salted sheepskins²². Twenty five grams of salted sheepskin samples cut into half inch squares were placed in a flask containing 225 ml of 25% NaCl (w/v) 7. The flasks were placed in a shaking incubator (New Brunswick G-625 Environmental Inc. Shaker) for two hours and 0.1 ml of serial dilution of the sample was spread onto the surface of the three different media. Inoculated plates were incubated at 37 °C for one week for halotolerant and extremely halophilic bacterial growth and at 27 °C for three weeks for fungal growth. After the incubation periods, the numbers of colonies were counted. All experiments were done in duplicate. Fungal isolates were identified according

to the taxonomic identification proposed by Raper et al.,^{23,24} Pitt,²⁵ Barnett,²⁶ Domsch et al.²⁷.

Determination of Defective Double-Faces and Color Evaluation Test

In order to determine the number and percentage of defective double-face leathers, 3000 pieces of finished double-faces were examined subjectively using day light source in Datacolor International (model Tru-View 2) color matching booth. Thus, the defective pieces among the 3000 finished double-faces were determined, which provided us with the precise percentage of the defective double-faces within the party.

Following this procedure, Minolta Spectrophotometer (model CM-508d) connected to a computer was used to detect numerically the color difference between the even dyed areas and the stained areas on the suede surface of the defective double-faces. Colorimetric measurements were carried out on samples selected from defective double-faces according to sampling formula (Eqn.1). L* (lightness), a* (red-green color coordinate) and b* (yellow-blue color coordinate) color parameters were directly read using D65 illuminator and 10° standard observer²⁸. h° (hue), defined as (Eqn.2), describes the color tone and C* (chroma), defined as (Eqn.3), describes the vividness²⁹.

$$h^{\circ} = \arctan b^{*} / a^{*} \quad (2)$$

$$C^{*} = [(a^{*})^2 + (b^{*})^2]^{1/2} \quad (3)$$

The device was calibrated using a standard white plate before the measuring process. By means of a standard reading area with a diameter of 8 mm, 5 measurements for each were taken for the area that was dyed in a light color directly on the suede surface and the areas with even dyeing. The averages of these measurements were also taken.

Results

Chemical Properties of Raw Sheepskins

Preservation of hides and skins is one of the main subjects of leather industry. Raw skin can easily be spoiled because of its biological structure. Hence, the preservation of the fresh skins that have been obtained by flaying the animals, under the appropriate conditions, is necessary before taking the skins into processing.

Since the sheepskins have a compact and long wool structure, the curing process is not as easy as it is in hide. Especially, in sheepskins that will be processed to become fur and suede, both wool and flesh sides should be preserved perfectly. However, the skin suppliers apply the standard curing operation since they don't know which product type the raw skin they sell will turn into. On the other hand, tanners check whether the wool is easily removed from the skin as a control criterion of conservation of sheepskins. But some defects that are not taken into consideration can cause the quality of the leather to decrease.

The general method for preservation of sheepskins is by wet salting the skins in stack or drum cure. In the conservation process of sheepskins, 100% salt is used in stack curing and 45% salt is used in drum curing based on skin weight ². If skins are to be held longer than three weeks to one month, they will need to contain sufficient salt to be preserved over this period.

It is known that one of the most important requirements for growth of halophilic bacteria and molds is the proper moisture content. A well preserved skin should have a moisture content of between 40 and 48% of the skin weight and sufficient salt to provide at least 80-85% saturation, in that moisture ^{2, 18, 30}. This corresponds to 12.5% of salt on skin weight ². Knowledge of the conditions under which brine cured hides and skins can be safely stored and transported, particularly on long sea voyages, is a matter of particular importance ⁸. Nancy and Venkatesan ³¹ showed that deterioration, as exhibited by loss in tensile strength, could be attributed to high temperature and high relative humidity, which brought hydrolytic attack on collagen.

Chemical analysis results of the salted sheepskins are given in *Table I*. Generally in the curing process of sheepskins, approximately 0.5% boric acid is used taking the salt weight as a base to inhibit the halophilic bacteria by reducing the pH degree below ^{6, 2}. Similarly, it was detected that in the conservation process of the skins examined in this research boric acid has been used and the pH degree was found to be below ⁶.

It was found that the moisture content of the salted sheepskins was slightly high while boric acid content on the salted sheepskins was low to control the growth of extremely halophilic bacteria. Also low pH and slightly high moisture content supported the growth of fungi on the samples. Salt concentration of the salted sheepskins was sufficient to

Table 1. Chemical Analysis of Raw Sheepskins

Moisture (%)	48.25 ± 0.89
Ash (%)	25.11 ± 0.83
Salt (%)	23.71 ± 0.85
Salt Saturation (%)	146.2 ± 7.52
Boric Acid (%)	0.39 ± 0.01
pH	5.81 ± 0.17

prevent non-halophilic bacterial growth, but this salt amount supported the growth of extremely halophilic bacteria on the salted sheepskins.

Consequently, deterioration of the salted sheepskins during storage was thought to be associated with exposure to high humidity. The deleterious influence of high humidity on the salted sheepskins was also increased by acidic pH.

Microbiological Process of Raw Sheepskins

One of the most important factors affecting leather quality is microbial spoilage ^{8, 32}. Salt is widely used to protect raw hides and skins from microbial spoilage. However, halophilic and halotolerant microorganisms found in salt and the environment can be introduced to the raw hides and skins by salting process. Under unsuitable transportation and storage conditions, these microorganisms can flourish on hides and skins and subsequently begin a degenerative process ^{15, 33}. The isolation and identification of the halophilic bacteria on brine cured hides and the proteolytic activity of these isolates have been studied in detail ^{7, 14, 32, 34}.

As seen in *Table II*, the extremely halophilic bacterial numbers on the salted sheepskin samples were found as 1.8x10⁶-4.3x10⁷ cfu/g on modified nutrient agar containing 10-30% NaCl (w/v) and 1.9x10⁶-4.7x10⁷ cfu/g on halophile media containing 10-30% NaCl (w/v). In the study of Birbir et al., ¹⁵ the total halophilic bacterial number on brine cured hides was found as 1x10⁷-7x10⁹ cfu/g. A significant difference between bacterial numbers on two different media used for isolation was not found in our study. Colonies on both media were circular, creamy, light pink, opaque and 1-5 mm in diameter after appropriate incubation time.

In this study, NaCl content of salted sheepskins was found 23.7% (*Table I*). Bacterial growth was not observed on modified nutrient agar and halophile medium without salt.

Table 2. Total Number of Halophilic Bacteria (cfu/g) at 37 °C for One Week on Modified Nutrient Agar and Halophile Media and Total Number of Fungi (cfu/g) at 27 °C for Three Weeks on Modified Malt Extract Agar with Various Salt Concentrations.

MEDIA	Salt Concentration %				
	0	10	17	25	...30
MNA ^a	0	1.8x10 ⁶	4.3x10 ⁷	2.8x10 ⁷	2.4x10 ⁶
HM ^b	0	1.9x10 ⁶	4.7x10 ⁷	3.1x10 ⁷	2.1x10 ⁶
mMEA ^c	3.1x10 ³	2.0x10 ³	4.1x10 ⁴	1.4x10 ⁴	1.1x10 ¹

a; Modified Nutrient Agar

b; Halophile Media

c; Modified Malt Extract Agar

The results showed that 23.7% NaCl of the sheepskins inhibited non halophilic bacteria. These results agreed with the previous research ¹⁴.

The genera of mold that are most commonly found on leather are *Aspergillus*, *Penicillium* and *Paecilomyces*. Mold growth may cause various discolorations on the skins, hides and leathers. The mold growth itself may be brownish tan, black, yellow, red or green. Also the by products of molds turn the color of the chrome to dark green ⁹.

In this study, fungal growth was clearly seen on flesh side of salted sheepskins (*Figure 1*). All of the salted sheepskins showed abundant mold growth causing white, yellow and brownish tan discoloration and all gave off an unpleasant musty odor. The total fungus number was found

as 1.1x10¹-4.1x10⁴ cfu/g on modified malt extract agar containing 0-30% NaCl (*Table II*). Seven species of filamentous fungi were isolated from salted sheepskins and identified as *Aspergillus terreus*, *A. niger*, *A. fumigatus*, *Penicillium restrictum*, *P. citrinum*, *Alternaria* sp. and *Cladosporium* sp. The results showed that a remarkably diverse fungal population may be found on salted sheepskins. These filamentous fungi can also be recovered from natural environments such as soil and hypersaline lakes ^{35, 36}. Tolerance to high salt concentration is a common feature of fungi species¹⁶. Some species of *Aspergillus* and *Penicillium* can survive at above 25% NaCl ³⁷.

Total number of halophilic bacteria and fungi, total number of proteolytic and lipolytic microorganisms and their percentages are presented in *Table III*. While 53-74% of total

Table 3. Total Number of Halophilic Bacteria and Fungi on Media with 1% Skim Milk and 1%Trybutyrin and Percentage of Proteolytic and Lipolytic Microorganisms.

MEDIA	Salt Concentration %				
	0	10	17	25	30
HM+1% Skim Milk	0	1.4x10 ⁶	3.1x10 ⁷	1.9x10 ⁷	1.1x10 ⁶
Proteolytic Bacteria(%)	0	74	66	61	53
HM+1% Trybutyrin	0	1.2x10 ⁶	2.8x10 ⁷	1.7x10 ⁷	9.6x10 ⁵
Lipolytic Bacteria(%)	0	62	60	54	47
Mmea+1%Skim Milk	1.0x10 ⁴	9.6x10 ³	1.7x10 ³	5.4x10 ²	3.0x10
Proteolytic Fungi(%)	58	48	42	39	27
MMEA+1%Trybutyrin	9.7x10 ³	9.4x10 ³	1.6x10 ³	4.9x10 ²	2.0x10
Lipolytic Fungi(%)	54	47	41	35	18

halophilic bacteria and 27-58% of total fungi were found to be proteolytic, 47-62% of total halophilic bacteria and 18-54% of total fungi were lipolytic. It was also determined that the higher the salt concentration was in isolation media, the lower the total numbers and percentages of proteolytic and lipolytic microorganisms were.

The screening of the halophilic bacteria excreting hydrolytic enzymes as proteases and lipases was carried out on the agar media supplemented with 1% skim milk for proteases and 1% Tween 80 for lipases^{17, 38}. Routinely, casein, azocasein, Hammerstein casein, milk, skim milk and oligopeptides are used to determine for qualitative and quantitative protease activities^{17, 38, 39, 40}. Similarly, there are many substrates to detect for lipase activity such as trybutyrin, Tween 80, olive oil, p-nitrophenil palmitate⁴¹.

Birbir et al.⁴² examined 18 selected extremely halophilic strains isolated from Sereflikoçhisar Salt Lake (Turkey) for their proteolytic and lipolytic activities. They found that approximately 94.4% and 72% of all strains hydrolyzed gelatin and Tween 80. They mentioned that when the salt obtained from Sereflikoçhisar was used directly in brine curing, the halophilic bacteria in the salt would digest the collagen, thus lowering the value of leather and representing a huge annual loss to the Turkish tanning industry.

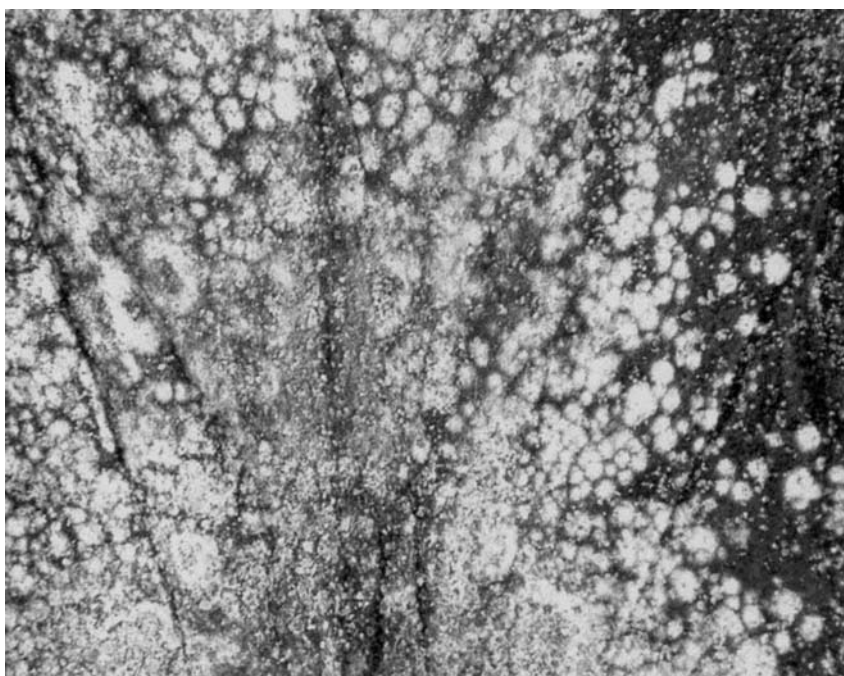


Figure 1. - Fungi on the flesh side of salted sheepskin

Sánchez-Porro et al.¹⁷ found 201 protease and 207 lipase producers from a total of 9848 bacteria colonies which were isolated from different hypersaline environments located in South Spain. Their study in hypersaline environment revealed that enzymes of halophiles were expected to show optimal activities in extreme hypersaline conditions, thus halophiles producing extremozymes would be of invaluable help for biotechnological applications.

Defective Double-Face Ratio and Color Evaluation

One of the most critical aspects of the marketability of a product is its color⁴³. The control and measurement of color in leather processes has become increasingly important in today's economies⁴⁴. The quality control of leather is the assessment of these shade differences to the standard⁴³. Spectrophotometric techniques such as colorimetric color measurements are used to analytically evaluate color uniformity or stability of materials^{43,45,46,47,48}. Color quality is determined by the requirements of the end user thus, poor quality leather due to color defects is not acceptable⁴⁴.

Color quality control is conducted subjectively in a color matching booth in most tanneries. Similarly, in our study a subjective examination of 3000 pieces of double-face leathers in the color matching booth revealed that 1050 pieces of these leathers had light uneven dyeing on their suede surfaces (*Figure 2*). This defect, which appears as light stains on the suede surface, was observed to correspond to 35% of the total double-face amount.

Conservation defects on hides and skins affect the fixing properties of dyestuff to leather substance and accordingly, even dyeing and homogeneity of the parties in a negative way. Microorganism activities on hides and skins especially during conservation, storage and transportation might cause undyed areas or light stains on the leather grain^{3, 4}. Due to the damages caused by proteolytic and lipolytic microorganisms on the flesh side of skins, less dyestuffs may be fixed during dyeing process and this state may be observed as light stains on the suede surface. As a matter of fact, L*(lightness)

value was found to be higher due to stained areas dyed in lighter color when the color coordinates obtained at the end of spectrophotometric measurements employed on the suede surfaces of defective double-faces were examined. These areas, which appear in light color because of less dye fixing, has given a higher C* (chroma) value (Table IV).

On the other hand, depending on the changes in a* and b* values, it is possible to determine the color differences on a leather surface. Low values of a* and b* define a dark color, whereas higher values of a* and b* indicate lighter color. As seen in Table IV, a* and b* values in the areas with light stains were higher than even dyed areas. Lower values of h° indicate a lighter color and lower values were obtained from stained areas.

Conclusions

The most interesting conclusion of our study is that fungi, as well as extremely halophilic bacteria, can grow on salted skins under the appropriate conditions. Proteolytic and lipolytic activities of extremely halophilic bacteria and fungi on skins may affect the quality of the final product in different extents depending on the type of the finished leather. The defects are seen more markedly especially in leathers like

suede and nubuck, for which the natural appearance is important. This research results showed that the light stained appearance on the suede surfaces of defective double-faces could be covered only by finishing process. However, the finishing process removes the suede appearance and lowers the sale price of the finished product.

Serious economical losses in leather industry may occur due to the defects on hides, skins and finished leathers. This situation creates a great problem for the countries that

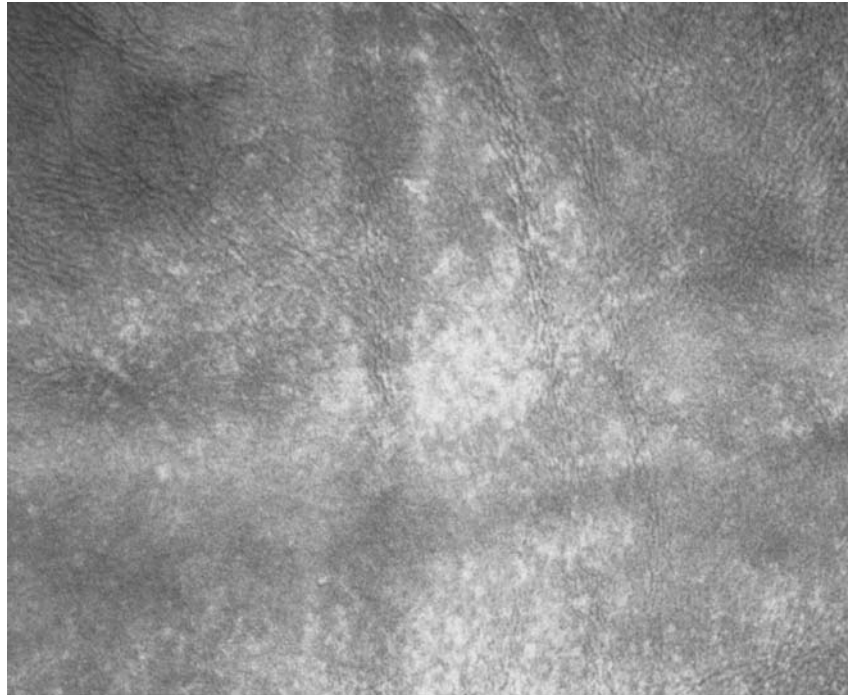


Figure 2. - Light stains on the suede surface of the double-face

Table 4. Average Values Belonging to the Color Coordinates of Even and Stained Areas on the Suede Surface of Defective Double-faces

Party No	Areas on Suede Surface	L*	a*	b*	C*	h°
1	Even Dyed Area	24.94 + 1.40 ^b	15.51 + 0.44	17.13 + 0.43 ^b	23.11 + 0.51 ^b	42.16 + 0.75 ^a
	Stained Area	32.65 + 0.99 ^a	16.20 + 0.31	20.89 + 0.30 ^a	26.44 + 0.36 ^a	37.78 + 0.53 ^b
2	Even Dyed Area	26.01 + 0.92 ^b	16.16 + 0.27	18.51 + 0.72 ^b	24.57 + 0.70 ^b	41.12 + 0.62 ^a
	Stained Area	34.68 + 0.65 ^a	16.66 + 0.19	22.11 + 0.51 ^a	27.69 + 0.50 ^a	37.03 + 0.44 ^b
3	Even Dyed Area	28.79 + 0.64 ^b	15.93 + 0.20	18.94 + 0.42 ^b	24.74 + 0.45 ^b	40.07 + 0.25 ^a
	Stained Area	34.29 + 0.56 ^a	16.09 + 0.17	20.98 + 0.36 ^a	26.44 + 0.39 ^a	37.50 + 0.22 ^b

have a high production capacity and need to import hides and skins. Therefore, we suggest that precautions such as salt choice, utilization of sufficient amount of salt, selection of effective biocide, suitable transportation and storage conditions, and detailed application of these precautions in trade agreements between skin providers and tanners should be taken into consideration, which will eventually help to reduce the losses in quality.

References

1. N. Streater, and S. Ward, *The production of double-face sheepskins including: Focus on woolskin dyeing techniques.* *World Leather* 12(1), p. 45-51, 1999.
2. K. T. W. Alexander, D. R. Corning, A. D. Covington, B. M. Haines, N. C. J. Lamb, P. D. Kemp, M. P. Walker and R. M. Webster,; *A Course For Fellmongers, British Leather Confederation, England, 91p., 1988.*
3. K. Eitel,; *Das färben von leder. Bibliothek des Leders Band 5, Umschau Verlag, Frankfurt am Main Germany, 336 p., 1987.*
4. M. F. Alam, *The development of hide curing salt.* *Leather International* 204(5), 42-44, 2002.
5. N Gunde-Cimerman, P. Zalar, de Hoog, G. S. and Plemenitas, A.; *Hypersaline waters in salterns-natural ecological niches for halophilic black yeasts.* *FEMS Microbiology Ecology* 32, 235-240, 2000.
6. Oren, A.; *Diversity of halophilic microorganisms: Environments, phylogeny, physiology and applications.* *Journal of Industrial Microbiology & Biotechnology* 28, 56-63, 2002.
7. D. G. Bailey and M. Birbir,; *A study of the extremely halophilic microorganisms found on commercially brinecured cattle hides.* *JALCA* 88, 291-299, 1993.
8. D. Didato, J. Bowen, and E. Hurlow,; *Microorganism control during leather manufacture.* *Leather Technologists Pocket Book Chapter 20, (M. K. Leafe ed.), The Society of Leather Technologists and Chemists, East Yorkshire, England, p.339-352, 1999.*
9. M. Birbir, O. Ozyaral, C. Johansson, and A. Ilgaz,; *Mold strains isolated from unfinished and finished leather goods and shoes.* *JALCA* 89, 14-19, 1994.
10. B. Javor,; *Hypersaline environments-microbiology and biogeochemistry, Springer, Berlin, 1989.*
11. Buchalo, A. S., Nevo, E., Wasser, S. P., Oren, A. And Molitoris, H. P.; *Fungal life in the extremely hypersaline water of the Dead Sea. First records. Proceedings of Royal Society of London B* 265, 1461-1465, 1998.
12. Lange, J.; *Qualitätsbeurteilung von leder, lederfehler, lederlagerung und lederpflege. Bibliothek des Leders Band10, Umschau Verlag, Frankfurt am Main Germany, 339p., 1982.*
13. <http://www.dsmz.de/media/med652.htm> (accessed 28.10.2003)
14. Birbir, M., Kallenberger, W., Ilgaz, A. and Bailey, D. G.; *Halophilic bacteria isolated from brine cured cattle hides.* *JSLTC* 80, 87-90, 1996.
15. Birbir, M. and Ilgaz, A.; *Isolation and identification of bacteria adversely affecting hide and leather quality.* *JSLTC* 80, 147-153, 1996.
16. Kis-papo, T., Grishkan, I., Oren, A., Wasser, S. P. and Nevo, E.; *Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea.* *Mycol. Res.* 105(6), 749-756, 2001.
17. Sánchez-Porro, C., Martin, S., Mellado, E. and Ventosa, A.; *Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes.* *Journal of Applied Microbiology* 94(2), 295-300, 2003.
18. Bailey, D. G.; *Preservation of hides and skins. Leather Technologists Pocket Book Chapter 1, (M. K. Leafe ed.), The Society of Leather Technologists and Chemists, East Yorkshire, England, p.5-21, 1999.*
19. Anonymous; *Official Methods of Analysis, Society of Leather Technologists and Chemists, Northampton, England, 1996.*
20. Hakimoglu, I.; *Chemical Analysis Methods for Leather Industry. Leather Research Institute-Istanbul, Turkey, Publication No.4, 1989, (in Turkish).*
21. Boron, H.; *Vereinfachte Bestimmung des Bars. In phanzen mit 1:1 Diantrimid, Fresenius zf Andyclem* 143, 339-349, 1954.
22. Litchfield, C. D. and Gillevet, P. M.; *Microbial diversity and complexity in hypersaline environments: A preliminary assessment.* *Journal of Industrial Microbiology & Biotechnology* 28, 48-55, 2002.
23. Raper, K. B. and Thom, C.; *A Manual of Penicillia, The Williams and Wilkins Comp., Baltimore, 1949.*
24. Raper, K. B. and Fennell, P. I.; *The Genus Aspergillus, The Williams and Wilkins Comp., Baltimore, 1965.*
25. Pitt, J. I.; *The Genus Penicillium and Its Telemorphic States Eupenicillium and Talaromyces, Academic Press, London, 1979.*
26. Barnett, H. L.; *Illustrated Genera of Imperfect Fungi, Burgess Publication Comp., 1960.*
27. Domsch, K. H., Gams, W. and Anderson, T. H.; *Compendium of Soil Fungi, Academic Press, London, 1980.*
28. CIE; *Colorimetry 2nd Ed., CIE Publication No.15.2., Commission Internationale de l'Eclairage, Vienna, 1986.*
29. McLaren, K.; *Colour space, colour scales and colour difference.*

-
- Colour Physics for Industry Chapter 4, (ed. Roderick McDonald), the Society of Dyers and Colourists, H Charlesworth & Co Ltd, Huddersfield, p.97-115, 1987.
30. Harmancioglu, M. and Dikmelik, Y.; Raw Skin. Özen Press, İzmir, Turkey, 1993, (in Turkish).
 31. Nancy, J. C. and Venkatesan, R. A.; Defects in hides, skins and leather due to animal diseases. *Leather Science* 21, 219-230, 1974.
 32. Bailey, D. G. and Birbir, M.; The impact of halophilic organisms on the grain quality of brine cured hides. *JALCA* 91, 47-51, 1996.
 33. Kallenberger, W. E. and Lollar, R. M.; Halophilic bacteria thrive in seasonal cycles. *JALCA* 81, 248-265, 1986.
 34. Birbir, M.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality in Hide Industry, PhD. Thesis, Marmara University, Atatürk Education Faculty, Istanbul, Turkey, 112p., 1991.
 35. Abdel-Hafez, S. I. I.; Survey of the mycoflora of desert soils in Saudi Arabia. *Mycopathologia* 80, 3-8, 1982.
 36. El-Gindi, A. A. and Saad, R. R.; Fungi of virgin and cultivated soil of Salhiah Desert, Egypt. *Zentralblatt Für Mikrobiologie* 145, 547-551, 1990.
 37. Tresner, H. D. and Hayes, J. A.; Sodium chloride tolerance of terrestrial fungi. *Applied Microbiology* 22, 210-213, 1971.
 38. Sánchez-Porro, C., Mellado, E., Bertoldo, C., Antranikian, G., and Ventosa, A.; Screening and characterization of the protease CP1 produced by the moderately halophilic bacterium *pseudoalteromonas* sp. Strain CP76. *Extremophiles* 7, 221-228, 2003.
 39. Kumar, C. G. and Takagi, H.; Microbial alkaline proteases: From a bioindustrial viewpoint. *Biotechnology Advances* 17, 561-594, 1999.
 40. Giménez, M. I., Studdert, C. A., J. Sánchez, J. J. and De Costa, R. E.; Extracellular protease of *natrialba magadii*: Purification and biochemical characterization. *Extremophiles* 4, 181-188, 2000.
 41. Gupta, R., Rathi, P., Gupta, N. and Bradoo, S.; Lipase assays for conventional and molecular screening: An overview. *Biotech. App. Biochem* 37, 63-71, 2003.
 42. Birbir, M., Kalli, N. and Johansson, S.; Examination of salt quality of Sereflikochisar lake used in the Turkish leather industry. *JSLTC* 86, 112-117, 2002.
 43. Sidney, L. Jay, Jr.; Color control for the leather industry. *JALCA* 86, 353-363, 1991.
 44. Randall, D. L.; Color measurement and control in leather. *JALCA* 89, 309-319, 1994.
 45. Abril, M., Campo, M. M., Önenç, A., Sanudo, C., Alberti, P. and Negueruela, A. I.; Beef colour evolution as a function of ultimate pH. *Meat Science* 58, 69-78, 2001.
 46. Baloglu, E., Hizarcioglu, S. Y. and Karavana, H. A.; An alternative evaluation method for swelling studies of bioadhesive tablet formulations. *Pharmaceutical Development and Technology* 9, 1-5, 2004.
 47. Marjoniemi, M. and Mäntysalo, E.; Studies on multicomponent spectroscopic analysis of dye solutions. *JALCA* 87, 249-258, 1992.
 48. Kim, W. J., Heo, J. S., Shin, S. B. and Ko, J. Y.; Measurement of colour difference in the leather dyeing process with natural dyes. *JSLTC* 87, 25-29, 2003.