Efficacy Assessment of Bactericide Containing Didecyldimethylammonium Chloride on Bacteria Found in Soak Liquor at Different Exposure Times

by

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Abstract

In this study, the efficacy of the bactericide containing didecyldimethylammonium chloride (quaternary ammonium compound) on the bacteria found in the main soak liquor at tannery was examined at different exposure times of 10 min., 30 min., 60 min., 120 min., 180 min., 240 min., 300 min., 360 min., 420 min. and 480 min. The presence of proteolytic and lipolytic mesophilic bacterial populations in high numbers in the main soak liquors showed that the recommended concentration of the bactericide (0.4 g/l) was not effective to control bacterial populations. Based on these results, the recommended concentration of the bactericide in the main soaking process was doubled (0.8 g/l) and it was observed that this concentration was considerably effective in controlling the bacterial growth. In addition, the bacterial flora in the main soak liquor treated with this bactericide was examined. *Enterobacter gergoviae, Enterobacter sakazakii, Enterobacter amnigenus biogrup I, Enterobacter cloaceae, Pseudomonas fluorescens, Pseudomonas luteola, Pseudomonas aeruginosa, Pseudomonas putida, Enterococcus avium, Enterococcus faecium, Lactococcus lactis ssp. lactis, Aerococcus viridans, Vibrio parahaemolyticus, Kocuria varians, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus sciuri, Staphylococcus xylosus, Staphylococcus cohnii ssp. urealyticus, Staphylococcus epidermidis, Staphylococcus intermedius, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus cohnii ssp. cohnii, Staphylococcus warneri* and *Micrococcus spp.* were isolated and identified from the main soak liquor after doubling the bactericide concentration. As a conclusion, the recommended dose of commonly used bactericides should be tested periodically in main soaking process at tanneries to inactivate various bacterial populations found in soak liquors.

Resumen

En este estudio, la eficacia de los bactericidas que contienen cloruro de didecyldimetilamonio (compuesto de amonio cuaternario) sobre la bacteria que se encuentra en el licor del remojo principal en la curtiembre fue examinado a diferentes tiempos de exposición de 10min, 30min, 60min, 120min, 180 min., 240 min., 300 min., 360 min., 420 min. y 480 min. La presencia de poblaciones de bacterias proteolíticas y lipolíticas mesófilas en gran número en el licor del remojo principal mostraron que la concentración recomendada del bactericida (0,4 g / l) no fue efectiva para controlar las poblaciones bacterianas. Con base en estos resultados, la concentración recomendada del bactericida en el proceso de remojo principal se duplicó (0,8 g / l) y se observó que esta concentración era considerablemente efectiva para controlar el crecimiento bacteriano. Además, la flora bacteriana en el licor del remojo principal tratados con este bactericida fue examinado. *Enterobacter gergoviae, Enterobacter sakazakii, Enterobacter amnigenus biogrup I, Enterobacter cloaceae, Pseudomonas fluorescens, Pseudomonas luteola, Pseudomonas aeruginosa, Pseudomonas putida, Enterococcus avium, Enterococcus faecium, Lactococcus lactis ssp. lactis, Aerococcus viridans, Vibrio parahaemolyticus, Kocuria varians, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus sciuri, Staphylococcus xylosus, Staphylococcus cohnii ssp. urealyticus, Staphylococcus epidermidis, Staphylococcus intermedius, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus cohnii ssp. cohnii, Staphylococcus warneri and Micrococcus spp.* fueron aislados e identificados a partir del licor del remojo principal después de doblar la concentración del bactericida. Como conclusión, la dosis recomendada de bactericidas de uso general debe ser probado periódicamente en el proceso de remojo principal en las curtiembres para inactivar diversas poblaciones de bacterias en el licor de remojo.

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Introduction

Hide curing which controls bacterial activities on hide is temporary preservation method until a hide is processed into a final product while soaking process which is the first tannery operation removes excess salt, blood, soluble proteins, dirt and manure of hides. Furthermore, this process softens up and rehydrates the partially dehydrated cured hide to bring its original condition. Inspite of bacteriostatic effect of sodium chloride used in hide curing, the bacteria on salted hides cannot be completely inactivated and these bacteria find an ideal medium to grow at an enourmous rate in the soak liquors and on the soaked hides. This phenomenon was confirmed by several researchers and our previous study.[1-3] Birbir and Yazi collected a total of 36 salted hides, 34 soaked hides and 19 soak liquors to examine the proteolytic and lipolytic mesophilic bacteria in these samples. It was determined that 97% of the examined samples contained proteolytic (10^2-10^6 c.f.u./g) and lipolytic mesophilic bacteria (10^3-10^7 c.f.u./g).[1] Proteolytic and lipolytic mesophilic bacterial numbers in the soaked hides were between 10^4 and 10^8 c.f.u./g while these bacterial numbers in the soak liquors were 10^2-10^6 c.f.u./g and 10^1-10^6 c.f.u./g, respectively. Under conditions in which a high local population density of cells is attained in cell's local environment, the bacterial numbers increase on the soaked hides, cell to cell chemical communication, or quorum sensing, occurs among the bacteria. Then, the bacterial species in high numbers employ invasiveness and other virulence factors to enhance pathogenicity.[4] Previous study results showed that when the hide curing process was not applied adequately, proteolytic and lipolytic halophilic and mesophilic bacteria on the hide will continue to damage the hide during storage.[1,5-7] In addition, if the bacterial populations which were originated from inadequately cured hides in soak liquors cannot be prevented with effective concentration of bactericides, the bacteria accumulated both on the flesh and epidermis sides may penetrate into dermis layer and damage collagen fibers of hide affecting leather quality adversely.[5,8]

Putrid odor, lustreless or blind sections in the grain, hair-slip, loose grain, reduced firmness, pitting holes, putrefaction marks on the grain may occur on soaked hides due to bacterial attack.[9,10] McEvoy and his colleagues stated that the grain layer was very important for the attractive appearance of leather and damage or removal of this layer would cause undesirable sueding on the surface of finished leather.[11] Orlita compared the salt packed Indian goat skins and native cow hides in terms of bacterial growth depending on soaking process. The researcher observed that the goat skins became slimy on the flesh side with putrid odour after 40–48 h and perforated in the grain side after 3 days. In addition, the author observed the perforations in cow hides within 5–7 days.[12]

It is certainly necessary to add effective bactericides to the hide at curing and soaking processes to prevent bacterial damage. It is known that tanneries use different bactericides to control bacterial activity in soak liquor but our previous studies on the soak liquors treated with different bactericides showed that every soak liquor contained bacteria in high numbers.[1,13]

Although the presence of the bacterial species in soak liquors have been documented in the previous studies,[6,12,14] there is no detailed report evaluating the efficacy of bactericides during soaking process at different periods. Hence, the goal of this study was to evaluate the effectiveness of the commonly used bactericide at the recommended dose by the manufacturer at different exposure times of 10 min., 120 min., 240 min., 360 min. and 480 min. during soaking process at the tannery and determine an effective concentration of the bactericide on the bacteria found in the soak liquors. Morever, a detailed study during the main soaking process was conducted to follow changes in content and numbers of bacteria at different exposure times at two-fold concentration of the bactericide (0.8 g/l).

Experimental

An evaluation of effectiveness of the bactericide at manufacturers' recommended dose

The test bactericide containing didecyldimethylammonium chloride (12.5% w/v) and benzyl dimethyl ammonium chloride (12.5% w/v) is a representative quaternary ammonium compound and has an antibacterial activity by disrupting of bacterial cell membranes.[4*]

In this study, the bactericide at the recommended dose (0.4 g/l) was added to the main soak liquor for evaluation of its effectiveness on the bacteria found in the liquor. The soaking procedure had been performed at 18–20°C in the paddle (3.500 kg hide /13.000 liter water) at the tannery. Spread plate technique was used to determine the total numbers of mesophilic, proteolytic and lipolytic mesophilic bacteria in the liquor.[15] A 100 μl quantity of each soak liquor collected before and after the application of bactericide (10 min., 120 min., 240 min., 360 min. and 480 min.) was separately placed in a test tube containing 9.9 ml 0.85% sterile physiological saline solution (10^{-2} dilution). Dilution was also repeated twice (10^{-6} dilution). Direct and serial dilutions (10^{-2}, 10^{-4} and 10^{-6}) of

1. The test bactericide containing quarternary ammonium compounds (didecyldimethylammonium chloride and benzyl dimethyl ammonium chloride) was used at the recommended dose (0.4 g/l) and two-fold increased concentration (0.8 g/l) of a proprietary bactericide developed by *MCM Leather Chemical Industry Trade Limited Company, Turkiye* and indicates that this product contains 25% (w/v) of proprietary active ingredients.

the bacterial suspension were plated onto Nutrient agar and the agar plates containing 2% (w/v) gelatine and 1% (w/v) Tween 80 under the aseptic conditions for the determination of total mesophilic, proteolytic mesophilic and lipolytic mesophilic bacteria, respectively.[15, 16] After the incubation at 37°C for 48 h, the numbers of colonies grown on the agar plates were counted. Clear zones around the colonies were taken as an evidence of protease and lipase activities. All experiments were done in duplicate. Since the total mesophilic bacterial numbers were determined above 10^5 c.f.u./ml, the recommended concentration of bactericide was doubled (0.8 g/l) to increase the efficacy of bactericide on bacteria in the soak liquor.

Determination of effective concentration of the bactericide

In the application of two-fold increased concentration of the bactericide, the samples of soaking liquors were collected before and after the application of bactericide (10 min., 30 min., 60 min., 120 min., 180 min., 240 min., 300 min., 360 min., 420 min. and 480 min.). The total numbers of mesophilic, proteolytic and lipolytic mesophilic bacteria were determined as described above. Furthermore, total bacterial numbers belonging to family of *Enterobacteriaceae*, genera of *Staphylococcus*, *Pseudomonas* and *Enterococcus,* which were encountered in high frequencies on the salted hides in the previous study [2], were determined on the selective media after 24-48 h incubation at 37°C. Eosine methylene blue agar (Merck), Baird Parker RPF (Biomèrieux) agar, Cetrimide agar (Merck) and D-coccosel agar (Biomèrieux) were used to isolate the strains belonging to family *Enterobacteriaceae*, genera of *Staphylococcus*, *Pseudomonas* and *Enterococcus*. Characteristic colonies of the bacteria belonging to family *Enterobacteriaceae*, genera of *Staphylococcus*, *Pseudomonas* and *Enterococcus* were picked up and restreaked several times to obtain pure culture. The strains isolated from the main soak liquors were identified by a variety of tests: Gram reaction, cell morphology, catalase and oxidase activities and the ability to use different compounds. Gram staining was performed using earlier described procedures.[15] Catalase activity was determined by adding a 3% (w/v) H_2O_2 solution to colonies on the agar plates. Isolated strains was rubbed using a platinum inoculating loop on filter paper moistened with the oxidase test reagent (tetramethyl-p-phenylene-diamine dihydrochloride) for the presence of cytochrome oxidase. The presence of dark purple coloration on the filter paper was taken as an evidence of oxidase activity. The API 20 E, API Staph, API 20 NE and API Strep test kits (Biomèrieux) were used to determine the isolates belonging to family *Enterobacteriaceae* and genera of *Staphylococcus*, *Pseudomonas* and *Enterococcus*. The isolates were grown on selective media at 37°C for 24 h and suspended in sterile saline solution (0.85% NaCl) to adjust the density of the bacterial cultures to McFarland No. 0.5 or 4 as described in the manufacturers' instructions. The culture dilutions were then loaded to the test strips. These test strips were incubated at 37°C for 18–24 h. The results of all biochemical tests were read and evaluated after incubation period.

Results And Discussion

Table I presents total numbers of mesophilic, proteolytic and lipolytic bacteria in the main soak liquors treated with the manufacturers' recommended dose of the bactericide (0.4 g/l) before and after the application of the test bactericide (10 min., 120 min., 240 min., 360 min. and 480 min).

One $\log_{10}$ reduction was detected in the number of total mesophilic, proteolytic and lipolytic mesophilic bacteria at exposure time of 10 min. to the bactericide in the main soak liquor. Organic substance in soak liquor may affect bacteriostatic or bactericidal effects of bactericide on bacteria.[17] It was thought that organic substances and bacteria released from hide may not be transferred into the soak liquor within the first 10 min. of the soak process. Thus, we found that the recommended dose of the bactericide was effective to reduce the bacterial numbers at 10 min. exposure time but after 120 min. exposure time, the proteolytic and lipolytic mesophilic bacterial numbers again started to increase due to high contents of organic substances and bacteria in the main soak liquor. Therefore, the more organic substance and bacteria was transferred into the main soak liquor, the more bacterial numbers were detected in the soak liquors treated with the manufacturers' recommended dose of bactericide at following exposure times (360 min. and 480 min.). (Table I)

The duration of main soaking process may change on the type and condition of the cured stock, physical conditions of the raw stock and fluctuations in the beamhouse operations.[18] This duration may range from about 1.5 hours to 24 hours in different countries.[19] Most tanneries usually prefer 24-hour soaking process for adequate rehydration of hides. The duration of soaking process in Turkiye is usually between 12–18 hours. Longer soaks may cause risk of bacterial damage on hides. Orlita[12] mentioned that quality of leather is influenced adversely by the number and species of proteolytic and lipolytic bacterial strains in soak liquors. Also**,** the author stated that bacterial growth is promoted by higher temperatures and extended soaking process. Rangarajan *et al*.[19] emphasized that bacterial numbers in soak liquors should be less than 10^5 c.f.u./ml. According to our results, it was determined that manufacturers' recommended dose of the bactericide tested was not effective to reduce bacterial number to a reasonable level that the bacterial damage would be controlled on hide. Hence, concentration of the bactericide was increased two-fold (0.8 g/l) to control bacterial numbers in main soak liquor.

The total numbers of mesophilic, proteolytic and lipolytic mesophilic bacteria, total bacterial numbers belonging to

family *Enterobacteriaceae* and genera *Staphylococcus*, *Pseudomonas* and *Enterococcus* were 1×10^7, 2×10^3, 2×10^3, 8.5×10^3, 3×10^5, 3.2×10^2 and 2×10^1 c.f.u./ml, respectively before treatment with the bactericide in two-fold concentration. Only total numbers of mesophilic bacteria (10^7 c.f.u./ml) and total bacterial numbers belonging to genus *Staphylococcus* (10^5 c.f.u./ml) in the main soak liquor were found to be high (Table II).

Two $\log_{10}$ reduction (from 10^7 to 10^5 c.f.u./ml) was detected in the number of total mesophilic bacteria in the main soak liquors after 10 min. exposure with the bactericide and then the number of total mesophilic bacteria did not change during soaking process (480 min. treatment). One $\log_{10}$ reduction (from 10^5 to 10^4 c.f.u./ml) was detected at the total bacterial number belonging to genus *Staphylococcus* after exposure time of 10 min. with the bactericide and also another one $\log_{10}$ reduction (from 10^4 to 10^3 c.f.u./ml) was recorded after exposure time of 180 min. After this exposure time, total number of bacteria belonging to genus *Staphylococcus* did not change until the end of soaking process (480 min). The numbers of total proteolytic mesophilic (10^3 c.f.u./ml) and total number of bacteria belonging to genus *Enterococcus* (10 c.f.u./ml) were found to be the same during soaking process (480 min). We observed that total number of bacteria belonging to family *Enterobacteriaceae* remained constant (10^3 c.f.u./ml) until 360 min. exposure time. Then, the bacterial number increased to 10^4 c.f.u./ml at 420 min. exposure time and remained the same (10^4 c.f.u./ml) until the end of soaking process. The total number of bacteria belonging to genus *Pseudomonas* remained unchanged (10^2 c.f.u./ml) until 240 min. exposure time. After this exposure period, the total number of bacteria belonging to genus *Pseudomonas* were detected as 10^3 c.f.u./ml until the end of soaking process (Table II).

Consequently, the two-fold increase in the manufacturers' recommended dose of bactericide for the main soak process reduced all the bacterial numbers at the reasonable level ($\leq 10^5$ c.f.u./ml) that will not give damage to hide. As mentioned before,[19] bacterial population above 10^5 c.f.u./ml in soaking process may cause bacterial defects on hide. In this study, we demonstrated that bacterial numbers in soak liquor can be reduced by doubling the bactericide concentration applied in manufacturers' recommended dose. The total bacterial numbers of mesophilic, proteolytic and lipolytic mesophilic bacteria and total numbers of bacteria belonging to family *Enterobacteriaceae* and genera *Staphylococcus*, *Pseudomonas* and *Enterococcus* were decreased to the required levels by the two-fold increase (0.8 g/l) in the bactericide concentration.

To prevent bacterial damage on hide, quaternary ammonium compunds (QACs), isothiazoles, halogenated organic compounds e.g. bronopol (2-bromo-2nitro-propane-1.3-diol), thiocarbamates and others, e.g. sulphur containing heterocycles like derivates of benzothiazole (for example thiocyanomethylthiobenzothiazole (TCMTB)), and glutaraldehyde are commonly used bactericides in the leather industry especially in the soaking process.[20] Doxycycline HCl, which is an antibiotic, has been used in fresh hides to control bacterial growth.[21]

Bilgi *et al*.[3] examined the effect of commercial bactericide containing quarternary compounds (% 0.4) on bacteria in the main soaking float under the conditions with different NaCl concentrations. The researchers observed low bacterial numbers in the presence of bactericide. They found that the numbers of total aerobic mesophilic, proteolytic and lipolytic bacteria were 10^4 c.f.u./ml in the main soak liquors containing bactericide whereas these bacterial numbers were 10^7–10^8 c.f.u./ml in control samples. Orlita[12] isolated six perforation-

TABLE I

Total numbers of mesophilic, proteolytic and lipolytic mesophilic bacteria in the main soak liquors treated with the bactericide at the manufacturers' recommended dose (0.4 g/l).

Exposure times of main soak liquor to the test bactericide*	Total numbers of mesophilic bacteria (c.f.u./ml)	Total numbers of proteolytic mesophilic bacteria (c.f.u./ml)	Total numbers of lipolytic mesophilic bacteria (c.f.u./ml)
Before treatment with the bactericide	10^5	10^4	10^3
After 10 min.	10^4	10^3	10^2
After 120 min.	10^5	10^5	10^3
After 240 min.	10^5	10^5	10^3
After 360 min.	10^5	10^5	10^4
After 480 min.	10^6	10^6	10^5

* The concentration of active ingredient of the test bactericide is 0.10 g/l.

causing strains of bacteria which are *Bacillus subtilis*, *B. megaterium*, *B. anthracoides*, *B.pumilus* and *Pseudomonas aeruginosa* from the soak liquors and emphasized that the addition of bactericides to the soak liquors is required especially in prolonged soaking process or in summer.

Also, we focused on the determination of pathogenic bacteria in the main soak liquor after doubling the bactericide concentration to 0.8 g/l. We isolated and identified *Enterobacter gergoviae, Enterobacter sakazakii, Enterobacter amnigenus biogrup I, Enterobacter cloaceae, Pseudomonas fluorescens, Pseudomonas luteola, Pseudomonas aeruginosa, Pseudomonas putida, Enterococcus avium, Enterococcus faecium, Lactococcus lactis ssp. lactis, Aerococcus viridans, Vibrio parahaemolyticus, Kocuria varians, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus sciuri, Staphylococcus xylosus, Staphylococcus cohnii ssp. urealyticus, Staphylococcus epidermidis, Staphylococcus intermedius, Staphylococcus haemolyticus, Staphylococcus lugdunensis, Staphylococcus cohnii ssp. cohnii, Staphylococcus warneri* and *Micrococcus spp.* in this study. The presence of the bacteria belonging to genera *Proteus*, *Corynebacterium*, *Sarcina*, *Bacillus, Micrococcus, Chromobacter*, *Clostridium*, *Serratia, Lactobacillus, Pseudomonas* and *Staphlycoccus* in soaking process was also found in the previous studies.[6, 12, 14, 19, 22]

To the best of our knowledge, this is the first study which were isolated and identified microorganisms such as *Enterobacter gergoviae*, *Enterobacter sakazakii*, *Enterobacter amnigenus biogrup I*, *Enterobacter cloaceae*, *Pseudomonas fluorescens*, *Pseudomonas luteola*, *Pseudomonas putida*, *Enterococcus avium, Enterococcus faecium*, *Lactococcus lactis ssp. lactis*, *Aerococcus viridans*, *Vibrio parahaemolyticus*, *Kocuria varians*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus sciuri*, *Staphylococcus xylosus*, *Staphylococcus cohnii ssp. urealyticus*, *Staphylococcus*

TABLE II

Total numbers of bacteria in the main soak liquors treated with the two-fold increased concentration of the test bactericide

Exposure times of main soak liquor to the test bactericide	Total numbers of mesophilic bacteria (c.f.u./ml)	Total numbers of proteolytic mesophilic bacteria (c.f.u./ml)	Total numbers of lipolytic mesophilic bacteria (c.f.u./ml)	Total numbers of bacteria belonging to family *Enterobacteriaceae* (c.f.u./ml)	Total numbers of bacteria belong to genus *Staphylococcus* (c.f.u./ml)	Total numbers of bacteria belong to genus *Pseudomonas* (c.f.u./ml)	Total numbers of bacteria belong to genus *Enterococcus* (c.f.u./ml)
Before treatment with the bactericide	1×10^7	2×10^3	2×10^3	8.5×10^3	3×10^5	3.2×10^2	2×10^1
After 10 min.	1.5×10^5	1.2×10^3	1×10^3	6×10^3	1×10^4	1.6×10^2	2×10^1
After 30 min.	2×10^5	3.5×10^3	1×10^3	1.5×10^3	2.5×10^4	5.4×10^2	1×10^1
After 60 min.	8.4×10^5	4×10^3	8×10^4	9×10^3	1×10^4	5.4×10^2	2×10^1
After 120 min.	3.3×10^5	1×10^3	1.5×10^4	5.5×10^3	1×10^4	2.85×10^2	1×10^1
After 180 min.	6.4×10^5	1×10^3	2.3×10^4	6×10^3	2×10^3	4.8×10^2	1×10^1
After 240 min.	1.4×10^5	3×10^3	4.5×10^4	1.9×10^3	3×10^3	9.9×10^2	1×10^1
After 300 min.	1.3×10^5	2×10^3	1×10^3	6.5×10^3	2×10^3	1.1×10^3	1×10^1
After 360 min.	1×10^5	5×10^3	1.5×10^3	8×10^3	1×10^3	1×10^3	1×10^1
After 420 min.	1×10^5	5×10^3	2.5×10^3	1.3×10^4	1×10^3	1×10^3	1.5×10^1
After 480 min.	3×10^5	2×10^3	1×10^3	1.1×10^4	2×10^3	1×10^3	—

epidermidis, *Staphylococcus intermedius*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus cohnii ssp. cohnii* and *Staphylococcus warneri* from the main soak liquor.

Conclusion

In conclusion, isolation of a variety of microorganisms in high numbers from the main soak liquors showed the inadequacy of preservation of the salted hides. Bacterial damages can be prevented by effective bactericides applied to salt curing and soaking processes. We suggest that examination of efficacy of bactericides on the bacteria isolated from soak liquors should be frequently tested in tannery conditions.

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References

1. Birbir, M. and Yazi, D.; Examination of Bacterial Population in Salt, Salted and Soaked Raw Hides. Scientific Research Project No. FEN–DKR–290506–0113, Marmara University, 2009.

2. Birbir, M. and Aslan, E.; Isolation and Characterization of Microorganisms Found on Salted Hides. Scientific Research Project No. FEN–C–YLP–030408–0080, Marmara University, 2009.

3. Bilgi, S.T., Yapici, B.M and Yapici, A.N.; Determination of Bacterial and Fungal Numbers in Floats of Pre-tanning Operations. *African Journal of Biotechnology* **8**, 1602–1607, 2009.

4. Madigan, M.T., Martinko, J.M., Dunlop, P.V. and Clarck, D.P.; Brock Biology of Microorganisms, 12th ed., Pearson Benjamin Cummings, pp. 329–417, 2009.

5. Bailey, D.G. and Birbir, M.; The Impact of Halophilic Organisms on the Grain Quality of Brine Cured Hides. *JALCA* **91**, 47–51, 1996.

6. Birbir, M. and Ilgaz, A.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality. *JALCA* **80,** 147–153, 1996.

7. Bitlisli, B.O., Karavana, H. A., Basaran, B., Sarı, O., Yasa, I. and Birbir, M.; The Effect of Conservation Defects on the Suede Quality of Double-Face. *JALCA* **99**, 494–501, 2004.

8. Haines, M.B.; Quality Rawstock. *JALCA***66**, 164–173, 1984.

9. John, G.; Possible Defects in Leather Production. Definitions, Causes, Consequences, Remedies and Types of Leather. Druck Partner Rübelmann GmbH, Carl-Benz-Strasse 11, D-69495 Hemsbach, pp 33–35, 1997.

10. Yapici, A.N.; The Effect of Using a Fungicide Along with Bactericide in the Main Soaking Float on Microbial Load. *African Journal of Biotechnology* **7** , 3922–3926, 2008.

11. McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Bailey, D.G., Blair, I.S. and McDowell, D.A.; The Effects of Treating Bovine Hide with Steam at Subatmospheric Pressure on Bacterial Numbers and Leather Quality. *Letters in Applied Microbiology* **37**, 344–348, 2003.

12. Orlita, A.; Microbial Biodeterioration of Leather and Its Control: A Review. *International Biodeterioration & Biodegradation* **53**, 157–163, 2004.

13. Birbir, M., Yazi, D., Değirmenci, D. and Yumurtacı, A; Investigation of Bacterial Populations Present in the Soak Liquors. *Journal of Leather Science*, **2** ,11–15, 2008.

14. Yapici, B.M., Yapici, A.N., Karaboz, İ. and Tozan, M.; Deri Sektöründe Kullanılan Bazı Bakterisitlerin Etkinliğinin Tespiti Üzerine Bir Araştırma. I. Ulusal Deri Sempozyumu, İzmir, 7–8 Ekim, 2004.

15. Harley, J.P. and Prescott, L.M.; Laboratory Exercises in Microbiology, 5th ed. The McGraw-Hill Companies, New York, NY, pp. 93–95, 117-120, 143–144, 161-163, 291–295, 2002.

16. Atlas, R.M.; Handbook of Microbiological Media for the Examination of Food. 3rd ed., CRC Press, USA, pp. 330, 2004.

17. Oie, S., Kamiya, A., Tomita, M., Katayama, A., Iwasaki, A. and Miyamura, S.; Efficacy of Disinfectants and Heat against *Escherichia coli* O157:H7. *Microbios* **98**, 7–14, 1999.

18. Anderson, R.; Wet Processing, Beamhouse Processing. *Leather*, 18–24, 1992.

19. Rangarajan, R.; Didato, T.D. and Bryant, S.; Measurement of Bacterial Populations in Typical Tannery Soak Solutions by Traditional and New Approaches. *JALCA* **98**, 477–485, 2003.

20. Confederation of National Associations of Tanners and Dressers of the European Community. The European Tanning Industry Sustainability Review- A contribution to the world summit on sustainable development, http://www.euroleather.com, 2002.

21. Stockman, G., Didato, D. and Hurlow, E.; Antibiotics in Hide Preservation and Bacteria Control. *JALCA* **102** ,62–67, 2007.

22. Pfleiderer, E. and Reiner, R.; Microorganisms in Processing of Leather in Biotechnology. Rehm, H.J.; Reed, G.Eds; VCH Weinheim, Germany, 66, 729–743, 1988.