Quaternary Ammonium Compound as Bactericide in Hide Brine Curing and Presoaking Processes

by

C. Veyselova[1], M. Birbir[2]* and D. Berber[2]

[1]*Anadolu University, Graduate School of Sciences*
26470 Tepebasi, Eskisehir, Turkey

[2]*Marmara University, Faculty of Arts and Sciences, Department of Biology,*
34722 Goztepe, Istanbul, Turkey

Abstract

Inactivation of bacterial activity on hides is necessary during brine curing and presoaking processes. Therefore, bactericidal effect of 0.02% quaternary ammonium compound containing didecyl dimethyl ammonium chloride and benzyl dimethyl ammonium chloride on the mixed culture of *Bacillus licheniformis*, *Bacillus pumilus*, *Enterococcus faecium*, *Staphylococcus intermedius*, *Pseudomonas luteola*, *Enterobacter cloacae* and *Vibrio fluvialis* was examined in the test media containing 26 % NaCl and organic substances by tube dilution method. In addition, the ability to grow of the mixed culture prepared from the test isolates was examined in the salt free Mueller Hinton Broth and in Mueller Hinton Broth containing 10 %, 15 % and 26 % NaCl during 72 days. Bacterial count of the mixed culture in Mueller Hinton Broth containing 26% NaCl was found higher than those in the the salt free Mueller Hinton Broth media after 2, 4 and 14 hours exposure times with the test agent. Our results showed that the test agent could be used in the brine solution where the salt slightly decreased effectiveness of the antibacterial agent. Additionally, it was observed that the isolates of the mixed culture were annihilated faster in the salt free Mueller Hinton Broth than the broth media containing different concentrations of NaCl. After the storage, bacterial counts in the Mueller Hinton Broth media containing different NaCl concentrations were not reduced to low levels. As a result, quaternary ammonium compound tested may be used in hide brine curing and presoaking processes to control Gram positive and Gram negative bacterial activities on hides. In addition, only NaCl application is not enough to control bacterial activity on hides.

Resumen

Es muy reconocido que la actividad bacterial en pieles debe ser desactivada durante su curado en salmueras y en el preremojo. Es así, cómo el efecto bactericida de 0,02% de un compuesto cuaternario de amonio que contiene didecil dimetil cloruro de amonio y bencil dimetil cloruro de amonio, ejercen sobre cultivos mixtos de *Bacillus lichenformis*, *Bacillus pumilos*, *Enterococcus faecium*, *Staphylococcus intermedius*, *Pseudomonas luteola*, *Enterobacter cloacae* y *Vibrio fluvialis* fueron examinados en un medio salino de prueba conteniendo 26% de ClNa y sustancias orgánicas, por el método de dilución por entubado. Adicionalmente, la capacidad de crecimiento del cultivo mixto preparado de los [microorganismos] aislados, fueron comprobados en un medio exento de sal, en un "caldo" Mueller-Hinton, así como también el mismo conteniendo 10%, 15% y 26% de ClNa respectivamente por 72 días. El conteo de colonias bacterianas del cultivo mixto en un medio Mueller-Hinton con 26% de sal se encontró mayor que los efectuados en el medio Mueller-Hinton exento de sal luego de 2,4 y14 horas de exposición con el agente [antibacteriano] siendo probado. Nuestros resultados demuestran que el agente en prueba puede utilizarse en la solución de salmuera donde la sal tan solo ligeramente merma la efectividad del agente antibacterial. Adicionalmente se observo que los microorganismos del cultivo mixto fueron aniquilados más rápidamente que los en el medio exento de sal Mueller Hinton, que en los medios conteniendo las diferentes concentraciones de ClNa. Luego del bodegaje de las pieles, conteos de las bacterias de las pruebas en los caldos conteniendo diferentes concentraciones de ClNa demostraron que las concentraciones de bacterias no fueron lo suficientemente reducidas. Cómo resultado, el compuesto de amonio cuaternario empleado puede usarse en preservar con en tratamientos basados en salmuera así como en procesos de preremojo para controlar actividad bacteriana tanto Gram positiva como Gram negativa en las pieles. Este trabajo indica que el empleo único de ClNa no sería suficiente para controlar la actividad bacterial en pieles.

*Corresponding author e-mail: mbirbir@marmara.edu.tr.
Manuscript received April 17, 2012, accepted for publication June 14, 2012

Introduction

Freshly slaughtered hides may contain normal skin microflora and the other bacteria coming from animal's feces or external sources. The bacteria secreting hydrolytic enzymes can cause hide deterioriation.[1] Therefore, brine and salt pack curing methods applied with boric acid or antimicrobials are commonly used in leather industry worldwide.[1,2] Furthermore, potassium chloride,[3] sodium sulfite and acetic acid,[1,4] different bactericides,[2,5] bacteriocin based preservative [6] and halocins[7,8] were suggested to control bacterial and archaeal populations on the hides by several researchers.

In addition to chemical treatments, gamma rays[9] electron beam irriditation[10] and electric current treatments[11-13] were recommended to inactivate microorganisms on hides. In spite of several suggestions for preservation of hides, salt curing method is still the most commonly used process in leather industry. It is thought that most of the bacteria on the salted hides may be controlled with salting process but our recent research results showed that salt pack cured hides contained bacterial population in high numbers. Berber and Birbir[14] isolated 10^4-10^8 c.f.u./g of bacteria from 36 salted hide samples. Most of these hides (83%) were imported from different countries such as Greece, England, U.S., Serbia, Bulgaria, Russia, Africa and Australia. It was found that 97% of the hide samples contained proteolytic (10^2-10^6 c.f.u./g) and lipolytic bacteria (10^3-10^7 c.f.u./g). Aslan and Birbir [15] found 10^4-10^8 c.f.u./g of bacteria from 10 salt pack cured hides. Proteolytic and lipolytic bacterial counts on these hides were found to be 10^4-10^7 c.f.u./g. A total of 396 Gram-positive bacteria comprising from 12 different genera and 47 bacterial species were isolated and identified from the hides. The total number of Gram positive proteolytic, lipolytic and both proteolytic and lipolytic isolates on the hides were found to be 278, 274 and 226, respectively. The most common Gram-positive genera on the salted hides were *Staphylococcus* (115 isolates), *Bacillus* (111 isolates) and *Enterococcus* (75 isolates).[16] A total of 256 Gram-negative bacterial isolates containing 21 different genera and 46 different bacterial species were isolated and identified from 10 hide samples. The total number of proteolytic, lipolytic and both proteolytic and lipolytic isolates on the hides were found to be175, 133 and 109, respectively.[17]

The most common Gram-negative genera on the salt-pack cured hides were *Enterobacter* (66), *Pseudomonas* (59) and *Vibrio* (32). These isolates showed both proteolytic and lipolytic activities in the highest number on the hides. Birbir and Ilgaz [18] have isolated *Bacillus cereus, Bacillus sphaericus, Bacillus firmus, Bacillus megaterium, Bacillus pumilus, Bacillus licheniformis, Kurthia variabilis* from 1 week stored salted hides and *Bacillus firmus, Bacillus brevis, Bacillus licheniformis, Bacillus licheniformis, Bacillus pumilus, Bacillus laterosporus, Bacillus sphaericus* and *Bacillus subtilis* from 2 months old stored hides. Aslan and Birbir[16,17] isolated and identified *Bacillus licheniformis*, *Bacillus pumilus*, *Enterobacter cloacae*, *Enterococcus faecium*, *Staphylococcus intermedius*, *Pseudomonas luteola*, *Vibrio fluvialis* from 10 salt-packed cured hides. In addition, *Bacillus pumilus, Bacillus licheniformis, Enterobacter cloacae, Pseudomonas luteola, Enterococcus faecium* and *Staphylococcus intermedius* were isolated from the main soak liquor treated with 0.02% of the test bactericide.[19]

If proteolytic and lipolytic bacteria on the salted hides can not be inactivated, these bacteria may continue damaging the hide during storage, presoaking and main soaking processes. Researchers stated that discoloration, serious grain peeling, hair slip, collagen fiber destruction, looseness, stickiness of the hide, inflammation of the epidermis, bad smell and voids in leather on the inadequately cured hides were important hide and leather defects caused by microorganisms.[2, 20-27] Therefore, it is necessary to inactivate the bacteria on the hides during storage before soaking. 0.02% of quaternary ammonium compound is used to inactivate bacteria producing hydrolytic enzymes in hide soaking process in Turkey.

In the previous study, 0.02% was found to be effective to reduce the number of bacteria to the safe level (<10^5 c.f.u./mL) in the main soak liquors[19] while its effectiveness in the presence of NaCl on the bacteria found on salted hides and soak liquor has not been investigated yet. Therefore, the aim of the study was to examine whether or not this agent can be used in the hide processes containing high amount of salt as in brine curing and presoaking. Moreover, this study also examined the ability to grow of the mixed culture of the isolates in the salt-free Mueller Hinton Broth and in the Mueller Hinton Broth media containing 10 %, 15 % and 26 % NaCl during 72 days. This test was performed to get an information on the ability to grow of bacteria on the salted hides during storage.

Experimental

The test bacteria used in this study were isolated and identified by the API® 20 E, API Staph, API 20 NE, API® 50CH and API 20 Strep test kits (BioMériux, Inc, France) in the previous study from the soaked hides treated with 0.02% of the quaternary ammonium compound containing didecyl dimethyl ammonium chloride and benzyl dimethyl ammonium chloride.[19] (the test solution was prepared by weighing 0.8 g of quaternary ammonium compound containing 12.5% didecyl dimethyl ammonium chloride and 12.5% benzyl dimethyl ammonium chloride in 1 L of final volume solution). Gram positive *Bacillus pumilus*, *Bacillus licheniformis*, *Staphylococcus intermedius* and *Enterococcus faecium*, *Gram negative Pseudomonas luteola*, *Vibrio fluvialis* and *Enterobacter cloacae* which found commonly both on the salted hides and in soaked liquors were selected as test isolates. The test isolates were resistant to 0.02% of the test agent used in the soak liquor.

Determination of Protease Activity

Proteolytic activity of the isolates was screened on tryptic soy agar medium containing 4% (w/v) gelatin. After incubation at 35 °C for 48 hours, the plates were flooded with saturated ammonium sulphate solution. Clear zones around the colonies were taken as evidence of protease activity.[28]

Determination of Lipase Activity

Lipolytic activity of the isolates was screened on agar medium containing 1% (w/v) Tween 80 and after incubation for 48 hours at 37 °C, opaque zones around the colonies were taken as evidence of lipase activity.[29]

Determination of Bactericidal Activity of the Test Agent on the Mixed Culture of the Test Bacteria in Salt-free MHB and MHB Containing 26% NaCl at 37°C and Different Contact Times.

One mL of the mixed culture of the test isolates adjusted to McFarland 0.5 (10^8 c.f.u./mL) were placed into the six sterilized test tubes and 9 mL of the salt free MHB (meat, infusion 2 g/L; casamino acids 17.5 g/L; starch 1.5 g/L) were added to test tube 1 to 3 and the other three test tubes, 4 to 6, 9 ml MHB containing 26% NaCl were added. Afterwards, the concentrations of 0.18%, 0.25% and 0.37% of the test agent were added into test tubes 1 to 3, respectively. Then, the same procedure was repeated for test tubes 4 to 6, containing media with 26 % NaCl. In addition, two tubes were used as control tubes (agent free). All of the test tubes were incubated at 37°C for 2, 4 and 14 hours. After the incubation, 1 mL of the bacterial suspensions were transferred into the test tubes containing 3% Tween 80, 0.3% lechitin and 0.5% sodium thiosulfate for neutralization of the agent. After neutralization, 100μL of this suspension were spread over the Mueller Hinton Agar (meat, infusion 2 g/L; casamino acids 17.5 g/L; starch 1.5 g/L and agar 20 g/L) and the plates were incubated at 37°C for 24 hours. At the end of incubation period, the colonies on the agar surface were counted and total number of colonies in 1 mL were calculated. The bacterial count before the experiment was 10^7c.f.u./mL.[30, 31]

Examining the Ability to Grow of the Mixed Culture of the Test Isolates at Different Salt Concentrations and Several Contact Times at 25°C.

MHB containing different concentrations of NaCl (10%, 15%, 26%) and salt-free MHB were used as test media. Firstly, 9 mL of MHB containing different salt concentrations were transferred into the four sterilized test tubes. One mL of the mixed culture of the test isolates adjusted to Mc Farland 0.5 were placed into the each test tube. The test tubes were incubated at room temperature (25°C) for different contact times (1 hour, 2 hour and 1, 3, 6, 7, 10, 15, 20, 30, 41, 52, 62 and 72 days). After the incubation periods, direct and serial dilutions of the bacterial suspension were spread over the surface of the agar plates containing MHA and incubated for 24 hours at 37°C. The bacterial number in the 1 mL and the reduction values were calculated.[11, 32]

All of the tests were done in triplicate in this study.

RESULTS AND DISCUSSION

In the present study, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas luteola* and *Enterobacter cloacae* showed both lipolytic and proteolytic activities while *Enterococcus faecium, Staphylococcus intermedius* and *Vibrio fluvialis* were found as protease positive and lipase negative (Table I).

TABLE I
Proteolytic and lipolytic activities of the test isolates.

Isolates	Proteolytic Activity	Lipolytic Activity
Bacillus licheniformis	+	+
Bacillus pumilus	+	+
Enterococcus faecium	+	-
Staphylococcus intermedius	+	-
Pseudomonas luteola	+	+
Enterobacter cloacae	+	+
Vibrio fluvialis	+	-

This study especially focused on inactivation of the pathogenic microorganisms, which could cause leather defects, with 0.02% of the test agent in brine curing and first soaking processes of hides. First soaking is a short process (2-4 hours) used in removing excessive salt , rehydrating and softening of the salted hides. First soaking liquor contains high amount of salt and organic substances originated from salted hides and organic substances in this process trigger the growth of microorganisms.

After 2, 4 and 14 hours, approximately 1 log increase of the mixed culture was seen in the test media containing both salt free MHB and MHB with NaCl 26% without the test agent (Table II).

TABLE II
The bacterial cell counts of the mixed culture in salt free MHB and MHB with 26% NaCl containing different concentrations of the test agent at 37°C

Bacterial cell numbers (c.f.u./mL)								
	Salt free MHB				MHB with 26% NaCl			
Time (h)	The test* agent free	0. 18%	0. 25%	0. 37%	The test agent free	0. 18%	0. 25%	0. 37%
2	$1.0x10^8$	$6.5x10^3$	$5.6x10^3$	$3.0x10^3$	$1.1x10^8$	$1.3x10^5$	$6.0x10^4$	$3.0x10^4$
4	$1.4x10^8$	$3.7x10^3$	$1.8x10^3$	$3.0x10^3$	$1.3x10^8$	$8.0x10^3$	$8.0x10^3$	$4.0x10^3$
14	$3.0x10^8$	$5.0x10^2$	$4.0x10^2$	$2.7x10^2$	$2.0x10^8$	$8.0x10^3$	$7.5x10^3$	$6.0x10^3$

*The test agent containing quaternary ammonium compound (didecyl dimethyl ammonium chloride and benzyl dimethyl ammonium chloride) was used at the two-flod increased concentration (0.02%) 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.

Bacterial count of the mixed culture in MHB containing 26% NaCl and different concentrations of the agent were found higher than those in the the salt free MHB after 2, 4 and 14 hours exposure times (Table II).

*The test agent containing quaternary ammonium compound (didecyl dimethyl ammonium chloride and benzyl dimethyl ammonium chloride) was used at the two-flod increased concentration (0.02%) 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.

When the test bacteria were exposed to concentrations of 0.18% of the test agent for short contact period (2 hours) such as first soaking process in the presence of 26% NaCl, >1 log reduction of the mix culture of bacteria were detected. On the other hand, >2 log reduction was recorded at the concentration of 0.25% and 0.37% of the agent. Additionally, >3 log of reduction of the test bacteria were found after 4 and 14 hours contact times with the test concentrations of the agent (Table II).

Our results were similar to Kallenberger's[33] study results. Kallenberger [34] has notified that chlorine, hypochlorite and quaternary amines are quite effective to control bacteria in hide processes. However, researcher emphasized that the effectiveness of antibacterial agents on bacteria might be reduced in the presence of high concentration of NaCl due to fatty emulsion structure of the agent.[33]

As it is known, salted hides can be stored for 2-6 months or much longer periods. Hence, the ability to grow of the mix culture of bacteria in the brine media containing 10%, 15% and 26% NaCl and organic substances were examined up to 72 days of storage at room temperature (25°C). Salt-free MHB was used to compare the ability to grow of the bacteria in MHB containing different NaCl concentrations. It was seen that the viable-cell counts in the salt-free MHB decreased faster than those in MHB containing different NaCl concentrations. Furthermore, it was found that viable-cell counts in 15% NaCl (10^5 c.f.u./mL) was found higher than those of 10% and 26% NaCl (10^4 c.f.u./mL) at the end of incubation period.

At the end of 1 hour, it was observed that the initial bacterial count of the mixed culture ($1.1x10^7$ c.f.u./mL) in the test medium containing 26% NaCl reduced to $5x10^6$ c.f.u./mL. The bacterial count gradually decreased to 10^4 c.f.u./mL at the end of 20th day but it did not changed significantly between 20th and 72nd days (Table IV).

At the end of 6 days, the count of bacteria was found to be $8x10^5$ c.f.u./mL in the MHB medium containing 15% NaCl, and this count was almost the same until 72nd day of storage (Table III).

One log reduction of bacterial count of the mixed culture was seen only in the media containing 15% NaCl after 3 days incubation at room temperature (Table III). Approximately, one log reduction of test bacterial count was seen in all of the test media after 6th day of incubation. The highest log reduction (4 log) of the count of test bacteria was recorded at the salt-free MHB after 62nd and 72nd days of incubation. At the end of incubation period, the viable-cell count in the salt-free MHB, the media containing 10%, 15% and 26% NaCl was reduced to 10^3, 10^4, 10^5 and 10^4 c.f.u./mL, respectively (Figure I).

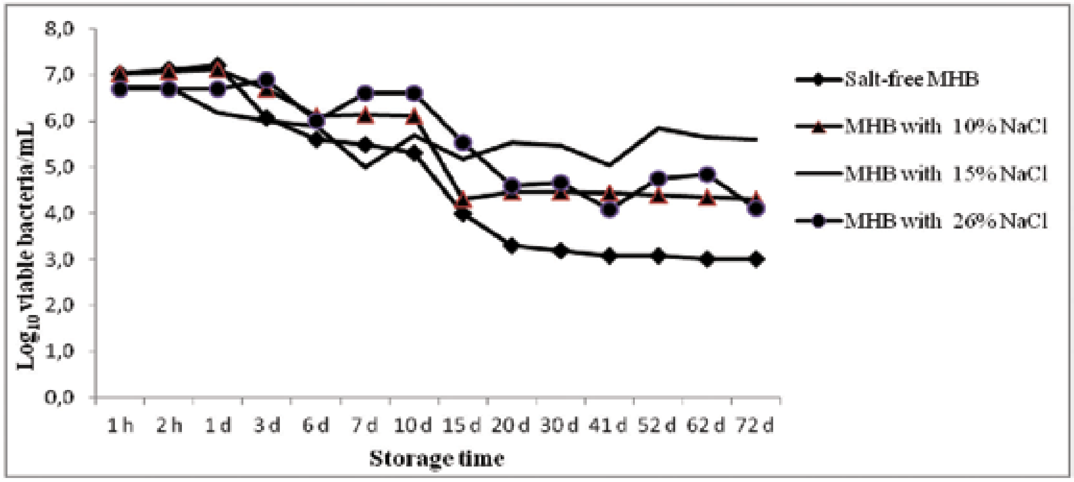

Figure 1. The bacterial count of the mixed culture in salt-free MHB and MHB containing different NaCl concentrations at the several contact times at 25°C.

Our results were similar to the results of previous studies.[14,15,18,22,35] Venkatesan and his colleagues[35] emphasized that the count of the salt-tolerant bacteria that grow on the media containing 10% and 20% NaCl raised significantly during storage of hides while, the salt-sensitive bacteria decreased with the period of storage. In another study, it was found that the count of salt-tolerant bacteria on the salted hides increased under prolonged storage conditions (2 months).[18] In addition, the count of halotolerant bacteria on 131 brine cured hides collected from 34 different hide curing facilities in US were explained between 10^3 and 10^4 c.f.u./g.[22] The population of bacteria on the salted hides collected from different countries were found between 10^4-10^8 c.f.u./g.[14] Aslan and Birbir [15] examined the population of bacteria on the salted hides collected from England, Australia and Turkiye. Researchers found that the bacterial population on the hides were between 10^5-10^8 c.f.u./g.

CONCLUSION

The results of this study showed that 0.02% quaternary ammonium compound may be used in the brine curing and presoaking processes of the hides. The bacterial count in the test media containing the test agent and NaCl has been decreased proportionally with the agent concentrations but high log reduction of the mixed culture has been seen at salt free test media. According to this result, we thought that salt slightly reduced the activity of the test agent. Furthermore, it has been shown that the cell count of Gram-positive and Gram-negative bacteria on the salted hides may not be reduced to the safe level with only NaCl treatment during storage at room temperature. After 72 days, bacterial numbers in the Mueller Hinton Broth media containing 10 %, 15 %, and 26 % NaCl were reduced to 10^4, 10^5 and 10^4 c.f.u./mL, respectively. This might be due to presence of salt tolerant bacteria. The salt may reduce the number of non-halo tolerant bacteria on the hides, however, the salt can not control the growth of salt-tolerant bacteria which may produce proteolytic and lipolytic enzymes. Therefore, it is certainly necessary to use effective bactericide in brine curing and presoaking processes of hides.

ACKNOWLEDGEMENTS

This work was supported by the Scientific Research Project Commission of Marmara University, Project No. FEN-C-YLP-060510-0143. We thank Scientific Research Project Commission of Marmara University and MCM Leather Chemical Industry. We also acknowledge Dr.Robert White and reviewers of JALCA for valuable suggestions and reviewing the English. If you don't mind I want to thank you and reviewers

REFERENCES

1. Bailey, D.G.; The Preservation of Hides and Skins. *JALCA* **98**, 308-319, 2003.
2. Mitchell, J.W.; Preventation of Bacterial Damage on Brine Cured and Fresh Cattle Hides. *JALCA* **82**, 372-382, 1987.
3. Bailey, D.G.and Gosselin, J.; The Preservation of Animal Hides with Potassium Chloride. *JALCA* **91**, 317-333,1996.
4. Hopkins, W.J.and Bailey, D.G.; Cattle Hide Preservation with Sodium Sulfite and Acetic Acid. *JALCA* **72**, 334-339, 1977.
5. Birbir, M. and Bailey, D.G.; Controlling the Growth of Extremely Halophilic Bacteria on Brine Cured Cattle Hides. *JSLTC* **84,** 201-204, 2000.
6. Hanlin, M.B.; Field, R.A.and Ray, B.; Characterization of Predominant Bacteria in Cattle Hides and Their Control by a Bacteriocin Based Preservative. *JALCA* **90**, 380, 1995.
7. Birbir, M.; Eryılmaz, S.and Ogan, A.; Prevention of Halophilic Bacterial Damage on Brine Cured Hide with Halocins. *JSLTC* **88**, 99-104 2004.

8. Birbir, M.and Eryılmaz, S.; Inhibiting of Lipolytic Haloarchaeal Damage on Brine Cured Hides with Halocin Producer Strains. *JSLTC* **91**, 69-72 2007.
9. Bailey, D.G.; Gamma Radiation Preservation of cattle Hides. A New Twist on an Old Story. *JALCA* **70**, 248-260, 1975.
10. Bailey, D.G.; DiMaio, G.L.; Gehring, A.G.and Ross, G.D.; Electron Beam Irradiation Preservation of Cattle Hides in a Commercial-Scale Demonstration. *JALCA* **96**, 382-392, 2001.
11. Birbir, Y. and Birbir, M.; Inactivation of Extremely Halophilic Hide-Damaging Bacteria via Low-level Direct Electric Current. *Journal of Electrostatics* 64, 791- 795, 2006.
12. Birbir M.; Özdoğru, B.; Birbir Y. and Ogan, A.; Extracellular Protease Activities of Extremely Halophilic Archaea and Their Control via Direct Electric Current. *JALCA* 92, **53-58,** 2008**.**
13. Birbir, Y.; Degirmenci, D. and Birbir, M.; Direct Electric Current Utilization in Destruction of Extremely Halophilic Bacteria in Salt That is Used in Brine Curing of Hides. *Journal of Electrostatics* 92, **53-58,** 2008.
14. Berber, D.and Birbir M.; Examination of Bacterial Populations in Salt, Salted Hides, Soaked Hides and Soak Liquors. *JALCA* 105, 320-326, 2010.
15. Aslan, E.and Birbir, M.; Examination of Efficiency and Sufficiency of Salt-Pack Curing Method. *JSLTC* **95**, 98-103, 2011.
16. Aslan, E. and Birbir, M.; Examination of Gram Positive Bacteria on Salt-Pack Cured Hides. *JALCA,* **106***,* 372-380, 2011.
17. Aslan, E. and Birbir, M.; Examination of Gram Negative Bacteria on Salt-Pack Cured Hides. *JALCA,* **107**, 106-115, 2012.
18. Birbir, M.and Ilgaz, A.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality. *JSLTC* **80**, 147, 1996.
19. Berber,D.;Birbir,M.andHacıoglu,H.;EfficacyAssessment of Bactericide Containing Didecyldimethylammonium Chloride on Bacteria Found in Soak Liquor at Different Exposure Times. *JALCA* **105**, 354, 2010.
20. Haines, M.B.; Quality Rawstock. *JALCA* **66**, 164-173, 1984.
21. Bailey, D.G. and Birbir, M.; The Impact of Halophilic Organisms on the Grain Quality of Brine Cured Hides. *JALCA* **91**, 47-51, 1996.
22. Bailey, D.G. and Birbir, M.; A Study of the Extremely Halophilic Microorganisms Found on Commercially Brine-Cured Cattle Hides. *JALCA* **88**, 285-293, 1993.
23. O'Flaherty, F.and Roddy, W.T.; The Chemistry and Technology of Leather. Volume IV-Evaluation of Leather, *Reinhold Publishing Corporation, The Science Pres Inc*. Ephrata, Pennsylvania, 1965.
24. Venkatesan,R.A.; Sugumar,M.; Nandy,S.C.and Santappa, M.; Survey of Incidence of Various Surface Defects in Goat and Sheep Skins in Madras, *Leather Science* **24**, 255-265, 1977.
25. Elliott, R.G.H.; Hides and Skins Improvement in Developing Countries. *FAO Agricultural Services,* Bulletin 67**,** 1988.
26. Roddy, W.T.; Relationship Between Hide Deterioration and Leather Defects. *Proceedings of the Fifteenth Research Conference,* pp.45-52, 1963.
27. Shede, P.N.; Kanekar, P.P.; Polkade, A.V.; Dhakephalkar, P.K.and Sarnaik, S.S.; Bacterial Succession On Raw Buffalo Hide and Their Degradative Activities During Ambient Storage. *International Biodeterioration & Biodegradation* **62**, 65-74, 2008.
28. Barnett, M.E.; Venghaus, J.D.; Microbiology Laboratory Exercises. *Wm.C. Brown Publishers Dubugue*, Iowa, 292, 1988.
29. Harley, J.P.; Prescott, L.M.; Laboratory Exercises in Microbiology. 5th Edition. *The McGraw-Hill Companies*, New York, 93, 2002.
30. Bilgehan, H.; Clinical Microbial Identification, Barış Yayınları, Ankara, 130, 2004.
31. Block, S.S.; Disinfection, Sterilization and Preservation, Fifth Edition, *Lippincott Williams and Wilkins*, U.S.A., 10, 2001.
32. Park, J-C.; Lee, M.S.; Lee, D. H.; Park, B. J.; Han, D-W.; Uzawa, M.; Takatori, K.: Inactivation of Bacteria in Seawater by Low-amperage Electric Current. *Applied and Environmental Microbiology* **69**, 2405, 2003.
33. Kallenberger, E.W.; Halophilic Bacteria in Hide Curing. Division of Graduate Studies and Research of the University of Cincinati. *Ph.D. Thesis*, Department of Basic Science Tanning Research of The Collage of Arts and Science, 1985.
34. Kallenberger, E.W.; Halophilic Bacteria in Brine Curing. *JALCA* **79**, 104, 1984.
35. Venkatesan, R.A.; Nandy, S.C.; Sen, S.N.; Effect of Storage and Pretanning Operation on the Bacterial Flora and Its Population on Goat Skin. *Leather Science* **17**, 395, 1970.