Examination of bacterial population in main soak liquors in hide industry

Meral BİRBİR*, Didem YAZI, Derya DEĞİRMENCİ*****
and
Ayşen YUMURTACI****

**Department of Biology, Science and Arts Faculty, Marmara University, 34722 Göztepe, İstanbul, Turkey; Fax: 02163478783;E-mail: mbirbir@marmara.edu.tr.*
*** Institute for Graduate Studies in Pure and Applied Sciences, Marmara University; 34722, Göztepe, İstanbul, Turkey; E-mail: yazi47@hotmail.com.*
**** Institute for Graduate Studies in Pure and Applied Sciences, Marmara University; E-mail: dddegirmenci@yahoo.com .*
***** Institute for Graduate Studies in Pure and Applied Sciences, Marmara University; 34722, Göztepe, İstanbul, Turkey; E-mail: yumurtaciay@yahoo.com.*

SUMMARY

Cured hides are soaked in water for several hours to several days to remove salt, dirt, debris, blood and excess animal fats on the hides. Generally, soaking is the most important process in leather industry. Microbial populations in soak liquors can adversely effect the quality of leather. In this study, the presence of mesophilic bacteria was examined in 12 soak liquors representing twelve different hide curing facilities in Tuzla-İstanbul, Turkey. All of the examined soak liquors contained mesophilic, lipolytic and proteolytic bacteria. One, seven and four soak liquors contained 10^5, 10^6 and 10^7 colony-forming units (c.f.u.) of mesophilic bacteria/ml, respectively. Proteolytic and lipolytic bacterial numbers in the soak liquors were usually between 10^4 and 10^5 c.f.u./ml. Ten bacterial strains were isolated from the soak liquors. Although most of these strains showed positive catalase, oxidase, citrate utilization, Vogeus Proskauer and urease reactions, all of these strains showed negative methyl red and did not from indol from tryptophan. Nitrate was reduced to nitrite by all of strains tested. Due to presence of mesophilic, lipolytic and proteolytic bacteria in the soak liquors, effective bactericides or different treatment system should be used to prevent bacterial damage on hide.

INTRODUCTION

The extent of deterioration and the concentration of proteolytic enzymes present in a hide after post-mortem changes depend on a large number of factors, viz. time, temperature, number of bacteria present and intrinsic factors of the hide itself. To prevent putrefaction during transit and storage, hides must be cured. A fresh hide, when subjected to various operations prior to tanning, undergoes changes with respect to its bacterial population (Birbir and Ilgaz, 1996). Once the hides have been cured, they are then soaked in water for several hours to several days to remove salt, dirt, debris, manure, blood, excess animal fats and globular proteins which is found in fibrious structure of hide. Also soaking procedure restore the moisture content of the hides (Orlita, 2004; Rangarajan *et al*., 2003; Tancous, 1964). This

absorbed water rehydrates any dried inter-fibrillary protein loosing its cementing action on the fibres. The collagen fibres and keratin cells of the hair and epidermis also take up water to become more soft and flexible (Sharphouse, 1971).

Usually soaking is probably the most under-valued process in leather making. Many of the problems occuring in the final leather are a result of improper soaking. Also nitrogenous breakdown products, coagulated proteins and blood dissolved in soak liquors offer an ideal environment for microorganisms which come from hide or from external contaminants (Dahl, 1956).

Bacterial growth in soak liquor depends on temperature, time, pH and nutrients. Bacteria found in leather industry grow between 4.5^0C -35^0C which is commonly encountered in soak liquor. Most soaking process are done at above 15 ^{0}C. Soaking process without auxiliary agents takes 24 h for salted hides and 36-48 h for dried hides. There is a risk of damage to the leather making material when hides are soaked in long period compared to advised soaking duration. Rangarajan et al. (2003) mentioned that the typical main soak process in the U.S. ranged in duration from about 1.5 h to about 6 h. They also stated that this duration was 18-24 h in some European and South American tanneries. Evaluation of bacterial population in soak liquors give an idea about effectiveness of antimicrobial agents and contamination. Also alkaline medium and high organic content of hide in soak liquors support bacterial growth rapidly (Rangarajan *et al,* 2003). In our study the duration of soaking process of our samples ranged from 18 to 24 h for main soaking.

Under ideal conditions, proteolytic bacteria grow rapidly by attacking hide substance and cause an important reduction in the strength property on hides and holes on the leather (Tancous , 1964; Sharphouse, 1971). Before the hide is suitably flaccid it may become putrid and soak liquor damage the hide, the resultant leather may show tender or damaged grain, looseness and lack of solidity, particularly in the belly or thinner areas. Microbial activity on hide causes hair-slip, formation of putrefactive odors, loss of hide substance (Tancous , 1964; Sharphouse, 1971).

Mc-Laughlin and Highberger (1926) carried out investigations on goat skins undergoing soaking with respect to the bacterial count of the skin cured by different methods and indicated that a high percentage of the organisms were of the proteolytic type. Rangarajan et al. (2003) isolated a wide variety of bacteria from the soak liquor, including species of *Bacillus, Chromobacter, Pseudomonas, Clostridium, Lactobacillus* and *Serratia marcescens.*

Due to adverse effects of bacterial populations in soak liquors on hide quality, a microbiological study was conducted to determine the total numbers of mesophilic, proteolytic and lipolytic bacteria in soak liquors. Also metabolic activities of isolated strains from soak liquors were examined.

MATERIALS AND METHODS

Microbiological analysis

Twelve soak liquors were collected from different tanneries located in Tuzla-İstanbul, Turkey Leather Organized Tannery Region. pH and temperature values of the soak liquors were measured as soon as the samples were collected. Spread plate technique was used to determine the total numbers of mesophilic, proteolytic and lipolytic bacteria in soak liquors (Harley and Prescott, 1993). Twenty ml of soak liquor was placed in a flask containing 180 ml 0.85 % sterile physiological saline solution. The flask was placed in a shaking incubator (Edmund Bühler, Germany) for half an hour and 0.1 ml of direct soak liquor and serial dilutions of the soak liquor were spread onto the surface of the tryptic soy agar media, separately. Inoculated plates were incubated at 37 ^{0}C for two days. After the incubation period, the numbers of colonies were counted. All experiments were done duplicate. Colonies

with different morphology and colours were picked up and restreaked several times to obtain pure culture.

Morphological characteristics and biochemical tests

Isolated strains were examined for cell morphology. Cell morphology of exponentially growing liquid cultures were examined on freshly prepared wet mounts by light microscopy. Gram staining was performed as described before (Harley and Prescott, 1993). Oxidase, catalase, coagulase, indole formation from tryptophan, reduction of nitrate to nitrite, urease, methyl red, Voges- Proskauer, citrate utilization and lactose fermantation tests were done by using standart procedures (Harley and Prescott, 1993). Proteolytic and lipolytic activities of the test strains were performed as described before. Proteolytic and lipolytic activities were detected on media containing 1% skim milk and 1% Tween, respectively (Birbir and Kalli, 2000).

RESULTS and DISCUSSION

Mesophilic bacterial counts in soak liquors were usually between 10^6 and 10^7 c.f.u./ml while proteolytic and lipolytic strains in soak liquors were between 10^4 and10^5 c.f.u./ml. pH values of examined soak liquors were changed from 7 to 10 while temperatures of soak liquors were between 20 and 28 ^{0}C (Table 1).

Table 1. pH and temperature values and total bacterial numbers in soak liquors collected from different tanneries

Sample	pH	Temperature ^{0}C	Total mesophilic bacterial counts (c.f.u./ml)	Total lipolytic mesophilic bacterial counts (c.f.u./ml)	Total proteolytic mesophilic bacterial counts (c.f.u./ml)
1	9	28	$4.3X10^7$	$7.2X10^4$	$3.2X10^5$
2	10	25	$3X10^6$	$2.5X10^4$	$2X10^3$
3	9	27	$6X10^6$	$3.7X10^5$	$2.7X10^5$
4	10	26	$5X10^6$	$5\ X10^5$	$1,5\ X10^5$
5	9	20	$2.5X10^6$	$1.2X10^5$	$5X10^4$
6	7	25	$7.8X10^5$	$1X10^5$	$2X10^4$
7	10	28	$2X10^6$	$8.6X10^4$	$3.4X10^5$
8	10	26	$1X10^7$	$1.1X10^4$	$2X10^4$
9	10	27	$1X10^7$	$1X10^6$	$2\ X10^6$
10	9	26	$3X10^7$	$5X10^6$	$1,6\ X10^5$
11	9	27	$3X10^6$	$1,2X10^5$	$1X10^5$
12	10	28	$4X10^6$	$2\ X10^5$	$1X10^5$

Rangarajan et al. (2003) stated that bacterial population in soak liquors should not be higher than 10^5 c.f.u./ml but our results showed that microbial populations in the soak liquors except sample 6 contained more than 10^5 c.f.u./ml. (Table 1).

Birbir and Ilgaz (1996) explained that *Bacillus cereus, B. laterosporus, B.liquefaciens, B.megaterium, B. subtilis, Kurthia virabilis, Micrococcus rubens, Pseudomonas aureginosa, Staphylococcus aeurus* and *Staphylococcus epidermidis* have been isolated from 25 soaked hides.

Researchers reported that genera of *Bacillus, Clostridium, Proteus, Chromobacter, Lactobacillus, Micrococcus, Corynebacterium, Pseudomonas, Staphylococcus, Sarcina, Serratia* were isolated from soak liquors (Birbir et al., 1996; Pfleidere and Reiner, 1988; Yapıcı and Meriç Yapıcı, 2002).

Table 2. Biochemical test results of the strains isolated from different soak liquors

	Biochemical Tests							
Strains	Catalase	Oxidase	Citrate utilization	Voges Proskauer	Methyl red	Urease	Indole from trytophan	Nitrate reduction
A	+	+	+	+	-	+	-	+
B	+	-	+	+	-	+	-	+
C	+	-	+	+	-	+	-	+
D	+	+	+	+	-	+	-	+
E	+	+	+	+	-	+	-	+
F	+	-	+	+	-	+	-	+
K	-	+	+	+	-	+	-	+
L	+	-	+	+	-	+	-	+
M	+	-	+	+	-	+	-	+
N	-	+	+	+	-	+	-	+

Strains A, B, D and E showed gram-positive reactions, positive catalase and coagulase reactions (Table 2). Also these strains formed cell clusters. Because of these characteristic features these strains were thought to be *Staphylococcus aureus*.

C, F, L and M showed gram-negative reactions. These strains were coccobacil shaped. All of these strains showed positive catalase, Voges Proskauer, urease reactions, formed acid from lactose anaerobically and reduced nitrate to nitrite and utilized citrate as a carbon source. Strains C, F, L and M showed negative oxidase and methyl red reactions and did not form indole from tryptophan. Because of these characteristic features, these strains were thought to be belong to family *Enterobactericeae*.

Strains K and N formed chains were gram-positive and showed catalase negative reactions. Because of these characteristic features these strains were thought to be strains of genus *Streptococcus*.

These preliminary research result showed that the presence of different microorganism in the main soak liquor. More detailed study should be done to characterize microorganisms found in the main soak liquors. Because of high number of microbial population in soak liquors, effective bactericides or effective treatment systems should be used to reduce bacterial damage on hide during soaking process. It is known that some bactericides are not

effective because of high organic contents of soak liquors and some of bacterial species may develop resistance to bactericides on repeated usage. Hence, antibacterial activity of bactericides in soak liquors should be tested at different periods.

ACKNOWLEDGEMENTS

We Express our appreciation to Dr. Volkan Candar and Miss Yonca Eryasa for their kind permission to use Cognis library facility. We are grateful to the tanneries located in Tuzla Leather Organized Tannery Region in İstanbul, Turkey for supporting us soak liquors.

REFERENCES

[1] Birbir, M. and Ilgaz, A., Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality, *J. Soc. Leather Technol. and Chem.,* 80, 147-153, 1996.

[2] Birbir, M. and Kalli N**.,** Şereflikoçhisar Tuz Gölündeki Aşırı Halofilik Bakterilerin İzolasyonu ve İdentifikasyonu. Marmara Üniversitesi Araştırma Fonu, Proje No: 1999 Fen-6, 2000.

[3] Dahl, S., Prevention of Microbiological Deterioration of Leather. *Leather Chem. Assoc*., Vol L1, 3, 1956.

[4] Harley, J.P. and Prescott, L.M., Laboratory Exercises in Microbiology. 2th Edition, 76,78, 86, 99, 113, 1993. LACTOZA EKLEYELİM

[5] Mc Laughlin, G. and Highberger, J. H., On the Bacteriology of Goat Skin Soaking. *J. Amer. Leather Chem. Assoc*., 21, 280, 1926.

[6] Orlita, A., Microbial Biodeterioration of Leather and Its Control: A Review. *Int. Biodet. and Biodeg.*, 53(3) 157-163, 2004.

[7] Pfleiderer, E., and Reiner, R. Microorganisms in Processing of Leather in Biotechnology. (eds. By H.J. Rehm and G. Reed), VCH Weinheim, Germany, 66, 729–743, 1988.

[8] Rangarajan, R., Didato, D.T. and Bryant, S.D., Measurement of Bacterial Populations in Typical Tannery Soak liquors by Traditional and New Approaches. J *Amer. Leather Chem. Assoc*., 98:12, 473-510, 2003.

[9] Sharphouse I. H., *Leather Technician's handbook*, 11–12, 80–81, 132, 430–431, 456, 1971.

[10] Sykes, R.L., B. S.C. pH. D.Science for Students of Leather Technology, The British Leather Manufacturers, Research Association, Chapter 8 Eghan, Surrey

[11] Tancous, J.J., Microorganisms in the Skin and Hide Industry. American Institute of Biological Sciences. Washington, D.C. 1964.

[12] Venkatesan, R.A., Nandy, S.C. and Sen, S.N., Effect of Storage and Pretanning Operation on the Bacterial Flora and Its Population on Goat Skin. *Leather Sci.*, 17: 12, 395–404, 1970.

[13] Yapıcı, A.N. and Meriçli Yapıcı, B. Deri İşletmelerinde Karşılaşılan Mikrobiyal Olaylar ve Kullanılan Mikrobisidler, Teknik Bülten, Gemsan, Sayı 34 , 2002.