THE Journal OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

May 2014 Vol. CIX, No. 5 *JALCA* 109(5), 138-175, 2014

110th Annual Convention

to be held at the
Gideon Putnam Resort
Saratoga Springs, New York
June 18 - 20, 2014

For more information go to
www.leatherchemists.org/
annual_convention.asp

CONTENTS

COMMUNICATIONS FOR JOURNAL PUBLICATION

Manuscripts, Technical Notes and Trade News Releases should contact
Mr. Robert F. White, *Journal Editor*, 1314 50th Street, Suite 103, Lubbock, TX 79412, USA.
E-mail: jalcaeditor@prodigy.net Mobile phone: (616) 540-2469

Contributors should consult the Journal Publication Policy at
http://www.leatherchemists.org/journal_publication_policy.asp

ISSN: 0002-9726

JOURNAL OF THE
AMERICAN LEATHER CHEMISTS ASSOCIATION

Proceedings, Reports, Notices, and News
of the
AMERICAN LEATHER CHEMISTS ASSOCIATION

OFFICERS

Steve Lange, *President*
Leather Research Laboratory,
University of Cincinnati
5997 Center Hill Avenue, Bldg. C
Cincinnati, OH 45224

Sarah Drayna, *Vice-President*
LANXESS Corporation
6150 S. Conservancy Dr.
New Berlin, WI 53151

David Peters, *Vice-President Elect*
Twin City Hide
105 Hardman Ct., Suite 100
So. Saint Paul, MN 55075

COUNCIL

Goetz Hagen
Tannin Corporation
4331 Chesapeake Dr.
Charlotte, NC 28216

Chris Koelblinger
Horween Leather Co.
2015 N. Elston Ave.
Chicago, IL 60614

Gary Rennerfeldt
Tyson Foods, Inc.
800 Stevens Port Drive
Dakota Dunes, SD 57049

Andreas W. Rhein
Tyson Foods, Inc.
800 Stevens Port Drive
Dakota Dunes, SD 57049

John Rodden
Union Specialties, Inc.
3 Malcolm Hoyt Drive
Newberyport, MA 01950

Susan Steele
Buckman USA
1256 N. McLean Blvd.
Memphis, TN 38108

EDITORIAL BOARD

Dr. Meral Birbir
Department of Biology
Marmara University
Istanbul, Turkey

Chris Black
Consultant
St. Joseph, Missouri

Dr. Eleanor M. Brown
Eastern Regional Research Center
U.S. Department of Agriculture
Wyndmoor, Pennsylvania

Dr. Anton Ela'mma
Retired
Perkiomenville, Pennsylvania

Elton Hurlow
Buckman International
Memphis, Tennessee

Prasad V. Inaganti
Wickett and Craig of America
Curwensville, Pennsylvania

Dr. Cheng-Kung Liu
Eastern Regional Research Center
U.S. Department of Agriculture
Wyndmoor, Pennsylvania

John Moore
Retired
Petaluma, California

Dr. Edwin H. Nungesser
Dow Chemical Company
Spring House, Pennsylvania

Dr. Roger A. Pinto
GST AutoLeather, Inc.
Livonia, Michigan

Dr. Mila Aldema Ramos
Eastern Regional Research Center
U.S. Department of Agriculture
Wyndmoor, Pennsylvania

Dr. J. Raghava Rao
Central Leather Research Institute
Chennai, India

Andreas W. Rhein
Tyson Foods, Inc.
Dakota Dunes, South Dakota

Dr. Bi Shi
National Key Laboratories
Sichuan University
Chengdu, China

George Stockman
Buckman USA
Memphis,Tennessee

Maryann M. Taylor
Eastern Regional Research Center
U.S. Department of Agriculture
Wyndmoor, Pennsylvania

Brandon Yoemans
S. B. Foot Tanning Co.
Red Wing, Minnesota

PAST PRESIDENTS

G. A. Kerr, W. H. Teas, H. C. Reed, J. H. Yocum, F. H. Small, H. T. Wilson, J. H. Russell, F. P. Veitch, W. K. Alsop, L. E. Levi, C. R. Oberfell, R. W. Griffith, C. C. Smoot, III, J. S. Rogers, Lloyd Balderson, J. A. Wilson, R. W. Frey, G. D. McLaughlin, Fred O'Flaherty, A. C. Orthmann, H. B. Merrill, V. J. Mlejnek, J. H. Highberger, Dean Williams, T. F. Oberlander, A. H. Winheim, R. M. Koppenhoefer, H. G. Turley, E. S. Flinn, E. B. Thorstensen, M. Maeser, R. G. Henrich, R. Stubbings, D. Meo, Jr., R. M. Lollar, B. A. Grota, M. H. Battles, J. Naghski, T. C. Thorstensen, J. J. Tancous, W. E. Dooley, J. M. Constantin, L. K. Barber, J. J. Tancous, W. C. Prentiss, S. H. Feairheller, M. Siegler, F. H. Rutland, D.G. Bailey, R. A. Launder, B. D. Miller, G. W. Hanson, D. G. Morrison, R. F. White, E. L. Hurlow, M. M. Taylor, J. F. Levy, D. T. Didato, R. Hammond, D. G. Morrison, W. N. Mullinix, D. C. Shelly, W. N. Marmer, S. S. Yanek, D. LeBlanc, C.G. Keyser, A.W. Rhein, S. Gilberg

THE JOURNAL OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION (USPS #019-334) is published monthly by The American Leather Chemists Association, 1314 50th Street, Suite 103, Lubbock, Texas 79412. Telephone (806)744-1798 Fax (806)744-1785. Single copy price: $8.50 members, $17.00 non-member. Subscriptions: $175 for hard copy plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers; $175 for ezine only; and $195 for hard copy and ezine plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers.

Periodical Postage paid at Lubbock, Texas and additional mailing offices. Postmaster send change of addresses to The American Leather Chemists Association, 1314 50th Street, Suite 103, Lubbock, Texas 79412.

Website: www.leatherchemists.org E-mail: ALCA@leatherchemists.org

COPYRIGHT 2014. THE AMERICAN LEATHER CHEMISTS ASSOCIATION

DIFFUSION AND REACTION BEHAVIOR OF PROTEASES IN CATTLE HIDE MATRIX VIA FITC LABELED PROTEASES

by

JIANZHONG MA,*[1,2] XUEYAN HOU,[1] DANGGE GAO[1] AND JING ZHANG[3]
[1]*College of Resources & Environment,*
Weiyang Campus, Shaanxi University of Science & Technology,
XI'AN 710021, SHAANXI PROVINCE, PR CHINA

[2]*Key Laboratory of Auxiliary Chemistry and Technology for Chemical Industry,*
Ministry of Education, Shaanxi University of Science & Technology
XI'AN 710021, SHAANXI PROVINCE, PR CHINA

[3]*College of Foreign Languages and Communications,*
Shaanxi University of Science & Technology,
XI'AN 710021, SHAANXI PROVINCE, PR CHINA

ABSTRACT

Enzymes play an important role in the biological treatment of animal skin collagen in the leather making process. In this work, three proteases (2709, LimeG and SoakL) with different molecular weight were used in the treatment of cattle hide. Protein and hydroxyproline absorbance was measured to evaluate the treatment effectively. Proteases labeled by Fluorescein Isothiocyanate (FITC) were used to treat the cattle hide to observe the diffusion and reaction behavior of proteases in cattle hide matrix. The results indicate that when the cattle hide was treated with smaller molecular weight protease, the degradation degree of the protein and collagen was more than that of the cattle hide treated with larger molecular weight protease. The fluorescence microscopy images demonstrate that during the early stages, proteases chiefly diffused into the cattle hide matrix through pores and hair follicles, and then diffused into the inner layer via hair follicles to hydrolyze inter-fibrillary proteins for opening up collagen fibers. In the present investigation, a visible assessment for the diffusion and reaction behavior of proteases in the enzymatic treatment of cattle hide matrix was reported.

INTRODUCTION

As a kind of biomaterials, animal skin may often have been discarded after humans ate the meat. However, animal skin collagen is the raw material for leather manufacture. Cattle hide is one of the most important leather-making raw materials. The constituents of cattle hide are very complex, including collagen fibers and many other inter-fibrillary proteins, such as albumin, globulin, mucin, mucinoid, glycosidoprotein. Among those, collagen fibers are the main constituent parts needing to be protected in the leather making process. On the contrary, other components have to be removed by chemical or physical methods. The common chemicals are surfactants, acids, alkalis and salts, but most of these chemicals lead to increased pollution of the environment.[1] As an environmentally friendly material, enzymes have been widely applied in cosmetics, medicines, food, the leather industry[2-5] and others fields[6-8] because of their excellent properties. In the leather industry, enzymes are usually used to treat animal skins to remove the non-collagen components.[9-12] Up to the present, however, essential theoretical research on enzymatic treatment of animal skins is still lacking. For example, the action mechanism of enzymes in the animal skin matrix was not clear. Cattle hide matrix, an insoluble substrate, is different from soluble substrates. For example, for some soluble substrates in enzymatic reactions, enzymes dissolve into the solution together with soluble substrates and then react regardless of the enzyme size. However it is necessary to protect cattle hide matrix in leather making process. When cattle hide is treated by enzymes to remove the non-collagen proteins, enzymes have to permeate into the cattle hide matrix and contact with the proteins inside the cattle hide matrix, and then enzymes will hydrolyze the inter-fibrillary proteins.

---

*Correspondence author e-mail: majz@sust.edu.cn, Tel.: +86 29 8616 8010.
Manuscript received October 3, 2013, accepted for publication February 6, 2014.

Therefore, the molecular weight of enzymes has an effect on the enzymatic treatment of cattle hide matrix. It may be difficult for larger molecular weight enzymes to enter cattle hide matrix, but the larger molecular weight enzymes hydrolyze the collagen on the cattle hide surface unduly instead. Maybe it is better to choose appropriate molecular weight enzymes in different leather making processes according to the specific collagen fiber structure. It has been reported that the lower molecular weight proteases were easier to diffuse into the wool, leading to extended fiber damage, which was the major obstacles for the use of proteases for wool finishing applications, but the larger molecular weight protease controlled enzymatic hydrolysis of wool surface.[13]

In order to determine the distribution of enzymes in animal skin matrix, researchers have already done some work. Proteases in different sections of the pelt unhaired by enzymes were transferred to casein, and the degradation reaction of enzyme and casein would produce white spots which could be used to determine the enzyme penetration rate and quantity.[14] However, the limitation was that if enzymes penetrated into the skin matrix, which had already lost activity, enzymes could not be determined. No better and easier method for detecting the presence of enzymes and their diffusion behavior in the animal skin matrix has been reported so far.

FITC is a well-known probe for labeling biologically relevant proteins.[15-16] Strong fluorescence of FITC can be observed as a yellow-green substance at excitation wavelength of 490-495nm on an epifluorescence microscope or under UV illumination.[15] FITC can react with the amino-group of proteins. Therefore, FITC labeled proteins have been used in much research to achieve visualization investigation.[13, 17-19] In this study, FITC labeled proteases were used to detect the position and penetration of proteases in the cattle hide matrix and provide a visible method to keep track of the diffusion or penetration behavior of proteases when the cattle hide matrix was treated with protease. By this means, the behaviors of several proteases with different molecular weight were compared in the diffusion and hydrolytic attack to the cattle hide. At the same time, the diffusion and reaction mechanisms of proteases in the enzymatic treatment process were proposed.

MATERIALS AND METHODS

Materials

Fluorescein Isothiocyanate (Sigma) and dialysis bags were purchased from Hefei Bomei Biotechnology Co., Ltd, China. Foline-Phenol (Sigma F-9252) was purchased from Shanghai Just Scientific Co., Ltd. Proteases (Genencor LimeG and Genencor SoakL) were provided by Genencor Bio-Products Co., Ltd. Alkaline protease 2709 was purchased from Beijing Donghua Qiangsheng Biotechnology Co., Ltd. Casein, a biochemical reagent, was purchased from Beijing AOBOX Biotechnology Co., Ltd. Antiseptic KL, degreasing agent DN and auxiliaries WT-H were of commercial grade, provided by Sichuan Desaier Chemical Industry Co., Ltd. Other reagents were of analytical grade. Wet salted cattle hide was provided by a tannery.

Instrumentation

A TU-1900 Double Beam UV-vis Spectrophotometer (Beijing Persee General Instrument Co., Ltd.) was used to determine protein and hydroxyproline in the enzymatic bath. Enzymatic treatment of cattle hide matrix was performed on a SHZ-A Immersion Oscillator (Shanghai Pudong Physical Optical Instrument). A TFM-300D Trinocular Upright Fluorescence Microscope (Shanghai Tuanjie Instrument) was used to detect the FITC labeled proteases in the cattle hide matrix. The molecular weight of proteases was measured on a DIONEX ultimate 3000 Liquid Chromatograph equipped with PLaquagel-OH Mixed Column.

Determination of the Protease Activity

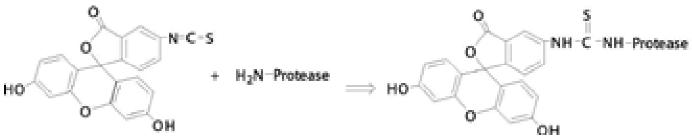
The activity of protease was determined by UV Spectrophotometry according to the national standard.[20] The 0.5% (w/v) protease solutions and 1% (w/v) casein solution were prepared using 0.1M Glycine-NaOH buffer (pH9.0 for SoakL and 2709, pH10.0 for LimeG). Then 1mL protease solution and 2mL casein solution were missed and incubated at 40 for 10min. The reaction was stopped by adding 4mL 0.4mol/L trichloroacetic acid (TCA). The mixture was settled at room temperature for 15min and filtered. The absorbance of the filtrate was measured at 275nm by UV Spectrophotometer. Then the concentration of tyrosine was calculated. Blanks were also prepared in the same way except that TCA was added prior to casein solution.

Enzymatic Treatment of Cattle Hide Matrix

Enzymatic treatment was carried out in a flask with a bath ratio of 1:200 (the mass of cattle hide to water) in an Immersion Oscillator. The dosages of chemicals were calculated on the basis of cattle hide weight. The hide in this study was fleshed pre-soaking hide. All samples were from a single hide. They were cut into 10cm×10cm pieces and soaking by using 0.1% (w/w) Antiseptic KL, 0.1% (w/w)degreasing agent DN (non-ionic surfactant), 0.3% (w/w) auxiliaries 0.4% (w/w) WT-H and Na_2CO_3 , shaken at 25°C for 1h, then 20 units of protease for per gram cattle hide were added to the bath. At intervals, the enzyme bath withdrawn was instantly kept in boiled water for 10min to inactivate proteases[21] and then the bath samples withdrawn were analyzed to determine the released protein and hydroxyproline.

FITC Labeled Protease

Proteins, including proteases, contain a large number of primary amino groups. The FITC molecule contains active isothiocyanate group (–N=C=S), which can easily react with primary amine group of the protease molecule at alkaline pH to form thiourea bond.[22] The labeled reaction is shown as follows.



To investigate the diffusion of proteases with different molecular weights, proteases were fluorescently labeled with FITC. The cattle hide matrix was treated with FITC labeled protease solutions, and then the longitudinal sections of cattle hide were observed on a TFM-300D Trinocular Upright Fluorescence Microscope.

The FITC labeled protease reaction was carried out under alkaline and dark conditions to avoid the fluorescein loss of fluorescence. Phosphate buffer of pH 8.0 was used for the preparation of FITC solution and proteases solution. The 0.2mL 1mg/mL FITC solution was slowly drop-wise added to 10mL 2mg/mL protease solution, stirred in dark condition at 4°C for 8h. After the labeled reaction, free FITC was removed through dialyzing for 24h, and the dialysate was changed every 2h over night until the absorbance of dialysate at 495 nm was approximately zero because the maximum absorption wavelength of FITC was 495 nm. Then the FITC labeled protease solutions were diluted to 45mL and 10cm×10cm pre-soaking and fleshed cattle hide was treated in each of the FITC labeled protease solutions at 25°C, shaken for 24h. Then the treated cattle hide was washed with distilled water and then frozen and sliced. The longitudinal sections of cattle hide were analyzed on a TFM-300D Trinocular Upright Fluorescence Microscope. Figure 1 is a schematic that illustrates the idea of this research.

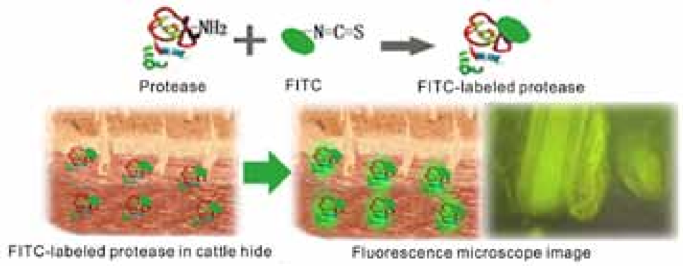


Figure 1. The schematic of FITC labeled protease to detect the behavior of protease

Analysis

Determination of protein in the enzymatic bath

The proteins hydrolyzed by proteases in the bath were determined according to Folin-Lowry's method. In this method, proteins reacted with Foline-phenol reagent to form a blue substance, which could be measured at 640 nm on a TU-1900 Double Beam UV-vis Spectrophotometer. The protein absorbance in this study was the absorbance of complexes of the protein and chemicals at 640nm. The protein absorbance was the average of two replications.

Determination of Hydroxyproline in the Enzymatic Bath

The determination of hydroxyproline was carried out according to the chloramine-T oxidation method. The sample was hydrolyzed in hydrochloric acid and release hydroxyproline, which was then oxidized by the chloramine-T to produce oxides containing pyrrole ring. Perchloric acid destroyed the excess chloramine-T. The hydroxyproline oxide reacted with dimethylaminobenzaldehyde to form red compounds, which could be determined at 558nm on a TU-1900 Double Beam UV-vis Spectrophotometer. The hydroxyproline absorbance in this study was the absorbance of complexes of the hydroxyproline and chemicals at 558nm. The hydroxyproline absorbance was the average of two replications.

Determination of Protease Molecular Weight

The molecular weight of protease was recorded on a DIONEX ultimate 3000 Liquid Chromatograph equipped with PLaquagel-OH Mixed Column, 300*7.5mm. The standard molecular weight samples were polyethylene glycol (PEG) and polyethylene oxide (PEO) in the range of 106-442800Da. The mobile phase was 0.02% $NaNO_3$ aqueous solution, and the column temperature was 40°C with flow rate of 1.0mL/min.

RESULTS AND DISCUSSION

Enzymatic Treatment of Cattle Hide Matrix

Enzymatic hydrolysis was very complicated which might be affected by many factors, including the type, specificity[11] and optimal reaction conditions of enzymes. Even the enzyme molecular weight plays an important role in the enzymatic treatment of some special insoluble substrates, such as cattle hide matrix and wool[13]. In this experiment, three proteases (LimeG, SoakL and 2709) with different molecular weights were used in the cattle hide enzymatic treatment. The molecular weight of proteases was determined by a DIONEX 3000 Liquid Chromatograph. The weight average Mws of Lime G, Soak L and 2709 are listed in Table I.

TABLE I
Molecular weights of 2709, Lime G and Soak L.

Proteases	2709	Lime G	Soak L
Weight Average Mw(kDa)	9,000	141,000	198,000

In the treatment process, proteases hydrolyzed protein in the cattle hide matrix and released protein and hydroxyproline from the hide matrix. The protein and hydroxyproline contents in the enzymatic bath were represented as absorbance of the complexes of the protein or hydroxyproline with the chemicals at 640nm and 558nm respectively. The released protein and hydroxyproline absorbance treated with different proteases are shown in Figure 2 and Figure 3, which indicate that both protein and hydroxyproline absorbance was time-dependent whether in presence of proteases or not.

The protein absorbances in the enzymatic bath of cattle hide treated with LimeG, SoakL, 2709 and the control (without protease) were very different with the increase of treating time. For the control, the least protein was released with a slower releasing rate. The protein-releasing behavior of cattle hide treated with LimeG was very similar to that of the control. When the cattle hide was treated with 2709, more protein was released and the released rate was faster than that of the cattle hide treated with LimeG and SoakL, but the protein absorbance of cattle hide treated with SoakL was between that of the cattle hide treated with LimeG and 2709. Overall, for the four treating processes with different proteases and the control, proteins increased fast in the first 2h because most of the soluble proteins on the surface of cattle hide dissolved in water or some proteins on the surface were hydrolyzed by proteases and the proteins released in the bath rapidly. After 2h, proteases began to diffuse into the cattle hide matrix and hydrolyze the protein slowly and led to the slower increasing rate of protein. As the treatment time prolonged, the proteases gradually lost activity and had less hydrolysis action on the protein. After 15h, the released protein decreased correspondingly because there was also less soluble protein remaining after 15 hours.

As is known to all, collagen is a kind of protein and hydroxyproline is the collagen-specific amino acid. Proteases inevitably attacked collagen in the enzymatic treatment of cattle hide matrix. The higher the concentration of hydroxyproline in the enzymatic treatment bath was, the more serious the impairment in the cattle hide collagen was[21]. In this experiment, the damage to collagen was determined in terms of the concentration of hydroxyproline in the enzymatic treatment bath. Similar to the protein absorbance, the hydroxyproline absorbance in Figure 3 indicates that hydroxyproline increased rapidly in the first 4h. Subsequently, the increasing rate became slower. This may be explained by the fact that at the initial stage, the proteases had higher activity and hydrolyzed collagen. After 4h, the proteases began to lose activity and lead to a weaker hydrolysis of the collagen. The hydroxyproline concentration in the bath treating with the proteases and the control was in descending order: 2709≈LimeG>SoakL>control. The molecular weights in Table 1 indicate that the molecular weights of the three proteases were in descending order: SoakL, LimeG, and 2709. Because almost 95%-98% collagen was located at the grain layer and reticular layer of cattle hide[23] the released hydroxyproline concentration was related to the protease molecular weight to some degree. The smaller the molecular weight of protease was, the higher the concentration of hydroxyproline was. However, high molecular weight proteases could hardly penetrate into the grain layer and the reticular layer in a short time. Therefore, larger molecular weight proteases just stayed in the upper levels of the cattle hide matrix that contained less collagen, while smaller molecular weight proteases could enter the inner layer among collagen fibers to damage collagen and released hydroxyproline eventually.

The Effect of FITC on the Activity of FITC Labeled Protease
The activities of the proteases labeled by FITC and the free proteases have been determined by Folin method in order to investigate the effect of FITC on the proteases activities. Figure 4 indicates that the activities of FITC-LimeG, FITC-SoakL and 2709 were close to those of the free LimeG, SoakL and 2709. This result was consistent with the literature reported.[24] Therefore; the FITC labeled proteases would have

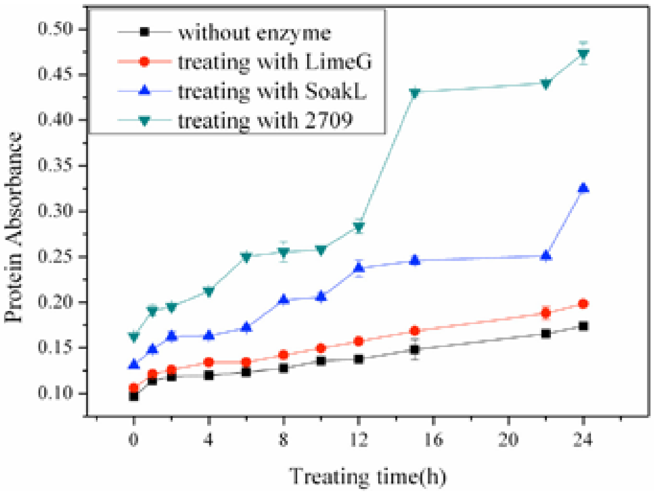


Figure 2. Protein absorbance in the bath treated with different proteases

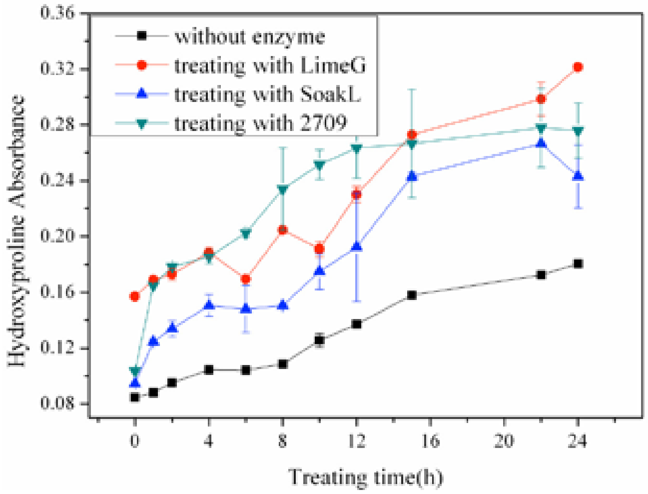


Figure 3. Hydroxyproline absorbance in the bath treated with different proteases

equivalent hydrolysis capability in the cattle hide enzymatic treatment process. On the other hand, with a low molecular weight, FITC would not affect the molecular weight of the proteases. The penetration and hydrolysis of FITC labeled proteases could reflect those of the free proteases in the cattle hide matrix equally.

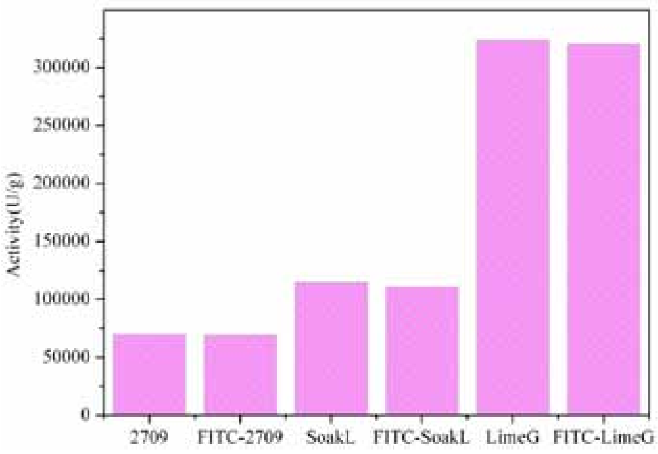


Figure 4. Activity of the free proteases and FITC labeled proteases.

Effect of Molecular Weights of Proteases on the Diffusion

In the fluorescence microscopy images (Figure 5), the green parts were FITC labeled proteases. The number A(A0,A1), B(B0,B1) and C(C0,C1) represent FITC-2709, FITC-LimeG and FITC-SoakL distributed in the different levels of the cattle hide matrix when the treating time was 12h and 24h. It is obvious that most of the proteases distributed widely in the cattle hide matrix after being treated for 24h, but the proteases only existed around the hair follicles when being treated for 12h. This indicates that during the diffusion process, most of the proteases diffused from pores into hair follicles but not through the epidermis at the initial stage because the epidermis is mainly composed of keratin, which has strong resistance to enzymes, as does collagen. General protease hardly hydrolyzes the natural keratin due to the close keratin alpha helix and a large number of peptide chains disulfide bonds on the natural keratin[23]. In the cattle hide enzymatic process, the proteases first diffused into the hair follicles might hydrolyze the protein around the hair follicles and promote the penetration of water to dissolve the soluble protein. Then the channel was opened and more proteases enter the cattle hide matrix. With the treating time prolonged, proteases diffused in the cattle hide matrix and degraded the inter-fibrillary proteins to disperse the collagen fibers.

Comparing A0, B0 and C0, we found that before the cattle hide matrix was treated with FITC-2709 for 12h, FITC-2709 had already diffused to the bottom of the hair follicles. When the cattle hide matrix was treated with FITC-LimeG for 12h, FITC-LimeG existed around the hair follicles. When the cattle hide matrix was treated with FITC-SoakL for 12h, most of FITC- SoakL existed around the upper half of the hair follicles and only a small part penetrated into the middle and lower parts of the hair follicles. This result indicates that proteases with smaller molecular weight were more easily to penetrate into the inner cattle hide matrix. On the contrary, larger molecular weight proteases might diffuse in the upper layer of the cattle hide matrix.[25] Figure 6 reveals the diffusion schematic diagram of larger and smaller molecular weight proteases in the cattle hide matrix. Figure 6a is the diffusion

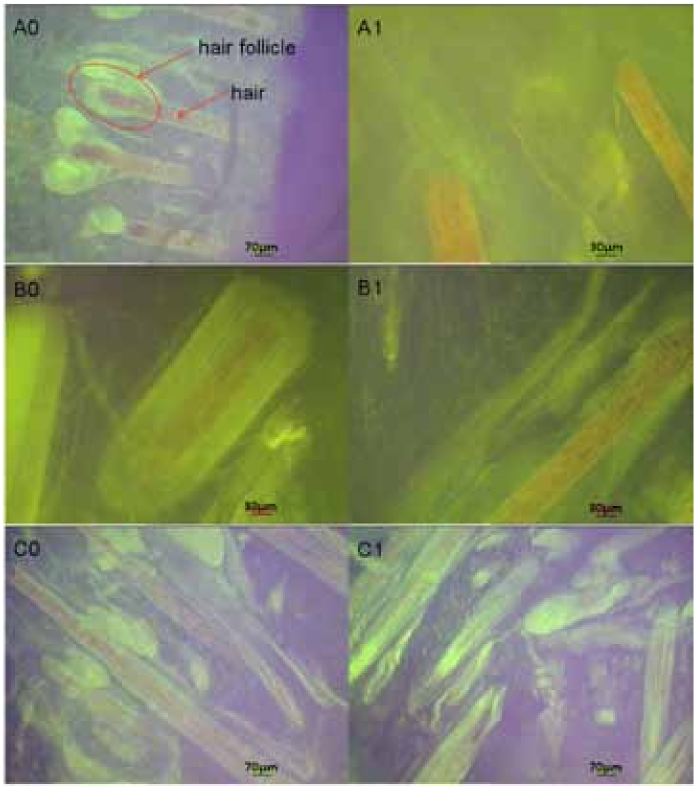


Figure 5. Fluorescence microscopy images of longitudinal sections of cattle hide treated with FITC labeled protease: (A0) FITC labeled 2709 treated cattle hide for 12h; (A1) FITC labeled 2709 treated cattle hide for 24h; (B0) FITC labeled LimeG treated cattle hide for 12h; (B1) FITC labeled LimeG treated cattle hide for 24h; (C0) FITC labeled SoakL treated cattle hide for 12h; (C1) FITC labeled SoakL treated cattle hide for 24h.

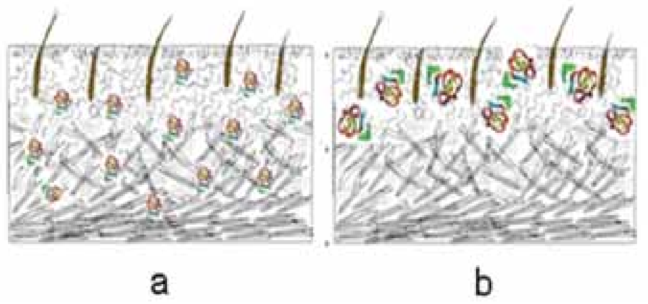


Figure 6. Schematic diagram: effect of protease molecular weight on the penetration of cattle hide matrix. a) smaller molecular weight protease diffusing into inner layer of cattle hide matrix; b) larger molecular weight protease diffusing into upper layer of cattle hide matrix.

schematic diagram of smaller molecular weight protease in the inner layer of cattle hide matrix, while figure 6b is the diffusion schematic diagram of larger molecular weight protease in the upper layer of cattle hide matrix. This may explain the preliminary result (Figure 2 and Figure 3) that the hydrolysis degree of different molecular weight proteases to the protein and collagen in the cattle hide was different.

Diffusion and Reaction Mechanism of Proteases in Cattle Hide Matrix

The cattle hide matrix is usually divided into four layers from upper epidermis to the flesh side, including epidermis, grain layer, reticular layer and subcutaneous tissues. The composition and collagen fiber weave of each layer are quite different.[26-28]Epidermis is the outmost layer of a skin. The main composition of epidermis is keratin, which has to be removed in the leather making process. Grain layer contains fibers, muscles, arteries, sweat glands, sebaceous glands, hair, et al and most of these have to be removed in different operations. In grain layer, the fibers are thin and loosely woven. Reticular layer is the most important part for leather because it contains most of collagen fibers tightly woven with the angle of weave approximately 45° that are beneficial to the strength of the leather. Subcutaneous tissues is mainly fatty adipose tissue, which must be mechanically removed at the initial stage.

According to the protein and hydroxyproline releasing behavior (Figure 2 and Digure 3) and the fluorescence microscopy images (Figure 5), the mechanism model of proteases diffusion and reaction was proposed in Figure 7. Because of the special structure of cattle hide matrix, the larger interstices between hair and hair follicles or pores made proteases first get into the matrix chiefly through hair pores or hair follicles and diffuse into the inside layer slowly. Simultaneously the penetrated proteases hydrolyzed protein around the hair follicles in stage b. As the increase of hydrolysis, some proteases entered the grain layer through hair follicles immediately but not through epidermis. In grain layer, proteases hydrolyzed more inter-fibrillary proteins, promoting the penetration of more proteases. Then from stage b to c, some soluble protein dissolved in water and the insoluble protein was hydrolyzed by the penetrated proteases. The space between the fibers became larger and larger, which was good for the later penetration of the proteases. At the same time, some proteases approached the flesh side and acted on the subcutis, but the proteases diffusion rate from flesh side was much slower than that from the grain layer because of the dense tissue structure and insoluble subcutis. From stage c to d, the fibers opened up gradually and became looser. At this time, the non-keratinised components were destroyed[29] and the epidermis was damaged to some degree because of alkaline condition for long time, and the majority of proteases dispersed in the cattle hide matrix through both epidermis and the flesh side.

CONCLUSION

The present study reported a visible method via FITC labeled proteases to investigate the diffusion and reaction behavior of proteases in the cattle hide matrix. The way proteases got into the cattle hide matrix was that the vast majority of proteases first entered hair follicles and then diffused through hair follicles into the grain layer and reticular layer, hydrolyzing the inter-fibrillary proteins. Moreover, the diffusion of proteases into the cattle hide matrix was dependent on molecular weight of proteases. Smaller molecular weight proteases were easier to diffuse into cattle hide matrix and degrade the inter-fibrillary proteins to disperse the collagen fibers.

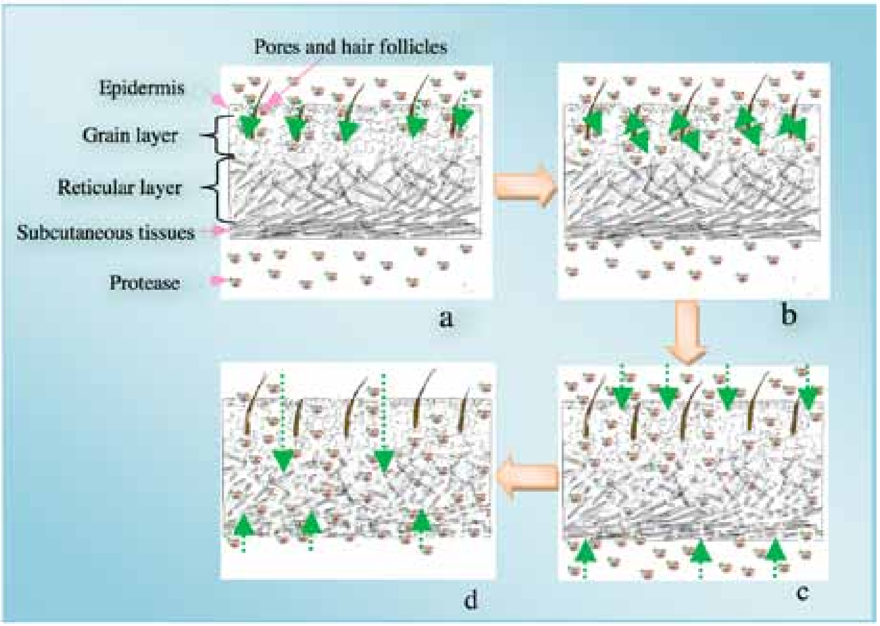


Figure 7. Mechanism model of protease penetration and reaction in cattle hide matrix. The green dashed arrows indicate the direction of protease diffusion.

ACKNOWLEDGEMENTS

This project was supported by Youth National Science Foundation of China (21104042), Doctor Science Research Foundation of the Education Ministry of China (20106125120003) and Graduate Innovation Fund of Shaanxi University of Science and Technology. We thank Shanghai Tuanjie Instruments Co., Ltd for providing TFM-300D Trinocular Upright Fluorescence Microscope, Northwest University for helping us with molecular weight measurement of proteases.

REFERENCES

1. Thanikaivelan, P., Rao, J.R., Nair U.B., Ramasami, T.; Progress and recent trends in biotechnological methods for leather processing. *Trends in Biotechnol.* **22**, 181–188, 2004.
2. Dettmer, A., Anjos, P. and Gutterres, M.; Special review paper: Enzyme in the leather industry. *JALCA* **108**, 146-158, 2013.
3. Swarnalatha, B., Sairam, G. Sekaran; Production of alkaline protease by Pseudomonas aeruginosa using proteinaceous solid waste generated from leather manufacturing industries. *Bioresour. Technol.* **99**, 1939–1944, 2008.
4. Ma, J.Z., Yang, X.Y., Gao, D.G., Lv, B., Yu, Y.; Study of JPK-1 Compound Enzyme, *Fine Chemicals* **25**, 894–899, 2008.
5. Liu, L., Liu, Q., Li, J., Du, G., Chen, l.; Characterization of Gelatin and Casein Film Modified by Microbial Transglutaminase and the Application as Coating Agents in Leather Finishing. *JALCA* **107**, 13-20, 2012.
6. Kumar, A, General, T., Bhaskar, N., Suresh,V., Sakhare, Z., Halami, M., Gowda,R., Mahendrakar, N.; Utilization of tannery fleshings: Optimization of conditions for fermenting delimed tannery fleshings using Enterococcus faecium HAB01 by response surface methodology. *Bioresour. Technol.* **101**, 1885–1891, 2010.
7. Brougher, D.S., Oleas, T.B., Kohn, R.A.; Interactions of α-Amylase and calcium chelator during neutral detergent fiber analysis. *J. Agric. Food Chem.* **53**, 5716–5718, 2005.
8. Eker, B., Zagorevski, D., Zhu, G.Y., Linhardt, R.J., Dordick, J.S.; Enzymatic polymerization of phenols in room-temperature ionic liquids. *Mol. Catal. B: Enzym.* **59**, 177–184, 2009.
9. Kanth, S.V., Venba, R., Madhan, B., Chandrababu, N.K., Sadulla, S.; Cleaner tanning practices for tannery pollution abatement: Role of enzymes in eco-friendly vegetable tanning. *J. Cleaner Prod.* **17**, 507-515, 2009.
10. Palop, R., Marsal. A., Cot, J.; Optimization of aqueous degreasing process with enzymes and its influence on reducing the contaminant load. *The Society of Leather Technol. Chem.* **84**, 170–176, 2000.
11. Puvanakrishnan, R., Manohar, B.M., Rajaram, A., Puvanakrishnan, R.; Ecofriendly lime and Sulfide free enzymatic dehairing of cattle and hides using a bacterial alkaline protease. *Chemosphere* **70,** 1015–24, 2008.
12. Foroughi, F., Keshavarz, T., Evans, C.S.; Specificities of proteases for use in leather manufacture. J. *Chem. Technol. Biot.* **81,** 257–261, 2006.
13. Araujo, R., Silva, C., Machado, R., Casal, M., Cunha, A.M., Rodriguez-Cabello, J.C., Cavaco-Paulo, A.; Proteolytic enzyme engineering: A tool for wool. *Biomacromolecules* **10**, 1655–1661, 2009.
14. Yang, Y.Z.; Test of enzyme in dehaired pelt. *China Leather* **31**, 20–22, 2002.
15. Hoffmann, C., Dudal, J.L., Patel, S., Gallet, O., Pauthe, E.; Fluorescein isothiocyanate labeled human plasma fibronectin in extracellular matrix remodeling. *Anal. Biochem.* **372**, 62–71, 2008.
16. Thiele, C, Ganzle, M.G. and Vogel, R.F.; Fluorescence labeling of wheat proteins for determination of gluten hydrolysis and depolymerization during dough processing and sourdough fermentation. *J. Agric. Food Chem.* **51**, 2745–2752, 2003.
17. Homer, K. A. and Beighton, D.; Fluorometric determination of bacterial protease activity using fluorescein isothiocyanate labeled proteins as substrates. *Anal. Biochem.* **191**, 133–137, 1990.
18. Law, B. and Tung, C.H.; Structural Modification of Protease Inducible preprogrammed nanofiber precursor. *Biomacromolecules* **9,** 421–425, 2008.
19. Chen, J., Liu, T.W., Lo, P.C., Wilson, B.C., Zheng, G.; "Zipper" molecular beacons: A generalized strategy to optimize the performance of activatable protease probes. *Bioconjugate Chem.* **20,** 1836–1842, 2009.
20. QBT 1803-1993, General methods of determination for industrial enzymes.
21. Song, J., Tao, W.Y., Chen, W.Y.; Kinetics of enzymatic unhairing by protease in leather industry. J. Cleaner Prod. 19, 325–331, 2011.
22. Miyata, S. and Morita, S.; A new method for visualization of endothelial cells and extravascular leakage in adult mouse brain using fluorescein isothiocyanate. J. Neuro. Sci. Meth. 202, 9–16, 2011.
23. Liao, L.L.; Tanning chemistry and technology. Beijing: Science Press, 2005.
24. Zhang, C., Song, X.J., Wang, J., Wen, J.; Stability and fluorescence spectra of lipase labeled with FITC, *Chinese Journal of Applied Chemi*stry **25**, 1381–1384, 2008;
25. Carla J.S.M., Silvaa, M., Prabaharana, Georg Gübitzb, Artur Cavaco-Paulo; Treatment of wool fibers with subtilisin and subtilisin-PEG. *Enzyme Microbial Technol.* **36**, 917–922, 2005.

26. Basil-Jones, M.M., Edmonds, R.L., Norris, G.E. and Haverkamp, R.G.; Collagen Fibril Alignment and Deformation during Tensile Strain of Leather: A Small-Angle X-ray Scattering Study. *J. Agric. Food. Chem.* **60**, 1201–1208, 2012.
27. Basil-Jones, M.M., Edmonds, R.L., Cooper, S.M. and Haverkamp, R.G.; Collagen Fibril Orientation in Ovine and Bovine Leather Affects Strength: A Small Angle X-ray Scattering (SAXS) Study. *J. Agric. Food Chem.* **59**, 9972–9979, 2011.
28. Wang, H.R.; English for Leather Technology. Beijing: China Light Industry Press, 2005.
29. He, X.X., Cheng, H.M., Chen, M., Wu, Lian and Li, Z.Q.; Epidermis morhology: Investigation of cattle hide during unhairing by transmission electron microscope. *JSLTC* **96**, 119–122, 2012.

SKIN CHARACTERISTICS OF *CERVUS ELAPHUS* L. FROM REPUBLIC OF TUVA IN RUSSIA

by

URANA DANDAR,[1] SELIME MENTEŞ ÇOLAK,[1] REMZIYE DEVECI,[2] ARIFE CANDAŞ ADIGÜZEL ZENGIN[1] AND GÖKHAN ZENGIN*[1]
[1]*Department of Leather Engineering, Engineering Faculty, Ege University*
35100 IZMIR, TURKEY

[2]*Department of Molecular Biology, Faculty of Science, Ege University*
35100 IZMIR, TURKEY

ABSTRACT

Characteristics of an animal raw skin are closely related to the physical properties of finished leather obtained from the skin. Although numerous studies have been performed on determination of physical properties of tanned deerskin, characteristics of raw deerskin have not yet been described in literature. In the present study, data on morphological, histochemical and chemical characteristics of wild red deer (*Cervus elaphus* L.) skins obtained from Republic of Tuva within the Russia Federation are presented and the relevant differences between skins of other animal species described. Table Top Scanning Electron Microscopy (TSEM) and Scanning Electron Microscopy (SEM) were used to determine the morphological characteristics of raw deerskins. For histochemical studies the cross section of skins were observed by Research Microscopy (RM). Hydroxyproline content, Total Kjeldahl Nitrogen (TKN), volatile matter, fat content, water-soluble-matter, sulphated total ash and fatty acid contents of raw deerskins were analyzed to further characterize the chemical composition of deerskin. Deerskin has comparatively higher TKN values, lower fat content with higher unsaturated fatty acid ratio, when compared to sheepskin. TSEM and SEM analysis showed that fur fibers and guard hairs of red deer skin were characterized by large cortex and medulla respectively that provides good isolation properties. In addition, high hydroxyproline content, low fat content, and tightly packed collagen fibers revealed by histochemical observations consolidates the long lasting property of deer skin. Assessment of skin characteristics enabled gathering data on physical and chemical properties of red deer skin, which is significant in choosing appropriate potential raw material that will produce the most suitable leather for a specific application.

INTRODUCTION

The red deer (*Cervus elaphus*) is one of the largest deer species and has a large global distribution extending from Europe and North Africa through central Asia, the Far East, North America and Siberia.[1-3] Tuva Republic is located in South Siberia within the Russian Federation, on the Mongolian border. The region is characterized by mountain steppe and thin coniferous forest called taiga. The climate is continental, with annual temperature variation from -40°C in winter to +40°C in summer. Herding and hunting are the main livelihood practices, which are important economic activities in the region. According to official estimates there are presently about 12 thousand red deer in the Republic of Tuva. Red deer where its meat is widely used as a food source is of great importance and plays a key role in Tuvan people's subsistence.[4-6]

Deer skin, which is a byproduct of meat production, is heavier and owns a larger surface area when compared to ovine leather.[7-8] It could be considered as an isotropic material that is similar to woven textiles, providing a more uniform area for pattern lay out and cutting due to their less variable physical strength throughout the skin. They are mainly used in clothing industry due to their desirable properties such as soft touch and special grain that offers a suitable material for long lasting garments.[6] It can also be utilized as a raw material for production of high cost, fashion outerwear materials, upholstery, automotive, glove, bag, shoe upper and chamois leathers.[9]

---

*Corresponding author e-mail: zenginhan@gmail.com
Manuscript received October 30, 2013, accepted for publication February 12, 2014.

Earlier studies provided information about assortment, optimal sampling positions, physical, chemical, physico-mechanical properties and possible usage areas of tanned deer skin[6-8, 10-12] and less study has been performed about the characteristic properties of raw deer skins. Elastic fibers surrounding the hair follicles of deer[13] and the hair follicle characteristics of forest deer and mountain reindeer[6] were identified by several researchers. Hartl et al. 1990 investigated the genetic variability and differentiation in Central Europe red deer (*Cervus elaphus* L.)[14] and Fukunaga et al. 2009 reported seasonal variations of morphological and biochemical characteristics of Yeso sika deer (*Cervus nippon yesoensis*).[15] This study is important because it will provide information about raw skin characteristics of *Cervus elaphus* L. which hasn't been reported in literature until now.

In the present work, wild red deer skins (*Cervus elaphus* L.) of Republic of Tuva in Russia are characterized by determination of morphological, histochemical, and chemical properties. Identification of deer skin characteristics is of primary importance to enable a better understanding of fiber type variability that is responsible for the skin's elasticity and mechanical resistance; to exploit raw material for production of long-lasting leather outerwear materials and to provide some basic information about the quality of deer skins.

EXPERIMENTAL

Materials

Two skins of wild male *Cervus elaphus* L. of Artiodactyla class of Cervides family known as Red Deer[16] were obtained from local hunters in autumn season from Republic of Tuva in Russia.

Methods

Samples for histochemical analysis were taken from the croupon region of wild deerskin right after slaughtering and were kept in ice for the first 18 hours and then immediately fixed in formalin (10%; Merck, Darmstadt, Germany). Following the sampling, skins were flayed and cured with salt for further morphological and chemical analyses.

Morphological and Histochemical Investigations of Deer Skins

Morphological Analysis

Tabletop Scanning Electron Microscopy (TSEM) and Scanning Electron Microscopy (SEM) investigations were performed for morphological study in accordance with the previously published procedures; Cadirci et al. 2010[17] and Zengin and Afsar 2011[18] respectively. Magnifications up to 1mm were performed with TSEM and for better visualization of cross-section and higher magnifications SEM was used.

Histochemical Analysis

Deer skin specimens were fixed in formalin solution (10%) and embedded in paraffin. 3 µm thick vertical sections were stained with Van Gieson's staining technique[19] for histochemical study. The images of cross section of deer skin were taken using a research microscope (Leica DM 4000B) equipped with a digital camera (Olympus DP7).

Chemical Characterization of Deer Skins

Preservation salt was removed from flesh side of raw deerskins prior to chemical analysis. The samples were taken from sampling area as described in TS EN ISO 2418[20] and separated into five groups depending on their sampling area such as croupon, neck, shoulder, belly and foot. Test samples were dried at 45°C for 48h in a circulating air-drying oven, milled and prepared in accordance with TS EN ISO 4044.[21]

Determination of Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen values of deerskins were determined in accordance with Kjeldahl nitrogen method.[22, 23]

Determination of Hydroxyproline Content

Hydroxyproline content of deerskins was determined following the previously published method Çolak et al. 2008.[24] The measurement of hydroxyproline was performed spectrophotometrically using chloramin T by a modified version of Taugard method and the results were given as μg/mg.

Determination of Volatile Matter and Fat Content

The volatile matter and fat content of deer skin samples were determined according to the standards of TS EN ISO 4684[25] and TS EN ISO 4048[26] respectively.

Determination of Water-soluble Matter, Water-soluble Inorganic Matter and Water-soluble Organic Matter

The procedures recommended in TS EN ISO 4098[27] were followed for the determination of water-soluble matter, water-soluble inorganic matter and water-soluble organic matter.

Determination of Sulphated Total Ash and Sulphated Water-insoluble Ash

Sulphated total ash and sulphated water-insoluble ash of the samples were determined by following the standard procedure described in TS 4125 EN ISO 4047.[28] Prior to determination of sulphated total ash, samples were washed in 250 ml distilled water and dried at 45°C for 48h in a circulating air-drying oven to prevent the interference of preservation salt.

Analysis of Fatty Acid Methyl Esters by Gas Chromatography (GC)

Analysis of fatty acid methyl esters was performed using a gas chromatography (Agilent 7890) with hexane (GC grade; Merck) as solvent, and data were acquired by solution software. Natural fat of deer skin obtained by TS EN ISO 4048[26] was trans-esterified according to TS EN ISO 12966-2[29] and analyzed following standard TS 4664 EN ISO 5508.[30]

Statistical Analysis

The effects of skin type, region and interaction of these two factors were analyzed. The statistical analysis of data was performed using Minitab for Windows (ver. 14.0, Minitab Inc., State College, PA). Analysis of variance was performed on each attribute ($p < 0.01$). Duncan's post hoc test was used for multiple comparisons when significant interactions were observed.

RESULTS AND DISCUSSION

Morphological and Histochemical Investigations of Deer Skins

Morphological Characteristics

The guard hair, fur fiber, cross sections of fibers, epidermis and dermis of wild deer skin are illustrated in Figure 1 and 2. They were composed of epithelial and connective tissues. The medulla proportions of guard hairs (primary hair follicle) were high and the medulla cells had polygonal and/or honeycomb shape. The group of seven or eight fur fibers (secondary hair follicle) was located around one guard hair.

Two types of fibers, fur fiber and guard hair were observed on wild red deer skin surface. In literature, guard hairs of wild animals were described as straight, resilient, and coarse and fur fibers were described as fine, soft and often having a silky hair like form.[31, 33] The SEM images of deer skin samples revealed that the guard hair (A), which was long and had a large diameter and strong hair structure, was located vertically in deep layers of papillary.

Approximately 80-85% of deer guard hair was consisted of medulla, resulting in hollow core. Fur fibers (B), described as the second type of fiber were very fine and closely located to the grain surface (Figure 3). The ratio of medulla in fur fibers was lower in comparison to guard hair. Some parts of cuticle cells were not clearly visible. Accordingly, the fur fibers mainly consisted of cortex, with an approximate ratio of 2/3 to total thickness, which provides flexibility and resistance to fur fibers. In terms of location of hairs through papillar and reticular layer, deer skin shows similarity to sheep skin. However, deer hair is fewer in number and more straight compared to sheep skins.

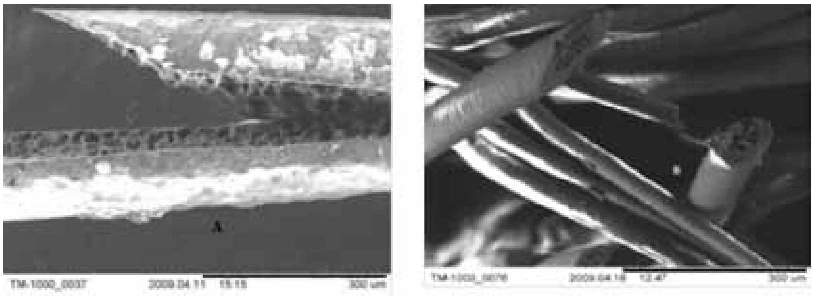


Figure 1: TSEM micrograph of the cross section of the guard hair (A) [31] and fur fibers (B) (*300).[32]

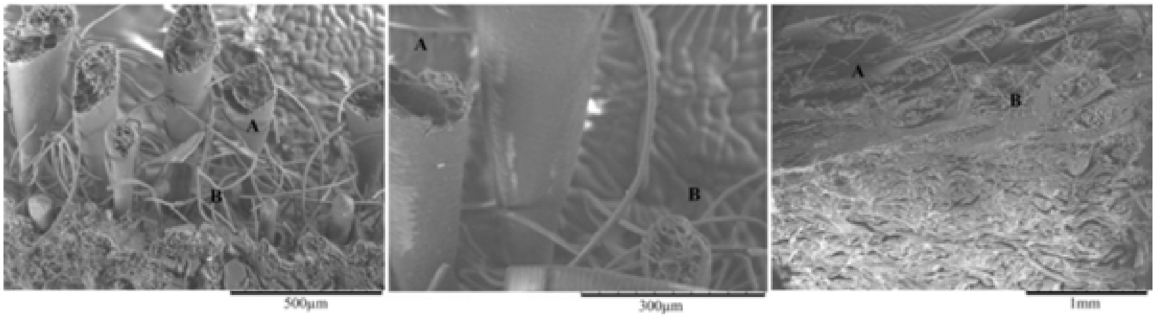


Figure 2: TSEM micrographs of guard hairs (A), fur fibers (B) and the cross section of wild deer skin with magnification factor 200, 300 and 60, respectively.[32]

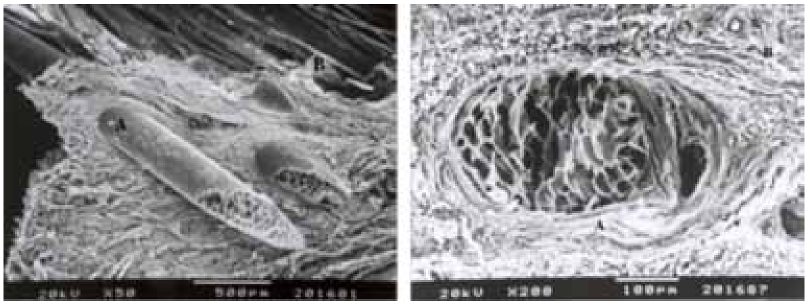


Figure 3: SEM micrographs of the guard hairs (A) and fur fibers (B) of deer skin.[32]

Fukunaga et al. 2009 reported that calf skin and deer skin are similar in size and biochemical composition however collagen fiber and fiber bundle structure of deer skin has unique characteristics.[15]

Histochemical Characteristics

The collagen fibers stained pink-red by Van Gieson's, are loosely packed in the form of fine bundles in stratum papillare layer and oriented parallel to epidermal structures (superficial epidermis and epidermis surrounding the hair roots). In stratum reticulare layer, which is located under the stratum papillare, collagen fibers are arranged densely in coarse bundles and oriented sequentially in longitudinal and transverse directions (Figure 4).

Considerable high extension of deer leather by comparison with both woven textiles and ovine leathers[8] is mainly associated with coarse and rather loosely interwoven corium fiber bundles of deer skin that provide elasticity and well suited to the production of clothing leather.[34]

Chemical Analysis

Samples for chemical analysis were taken from five different regions of wild deer skins that are presented in Table 1. Significant interactions ($p < 0.01$) between deer skins and regions were observed for all chemical analyses, except soluble matter in dichloromethane ($p > 0.01$).

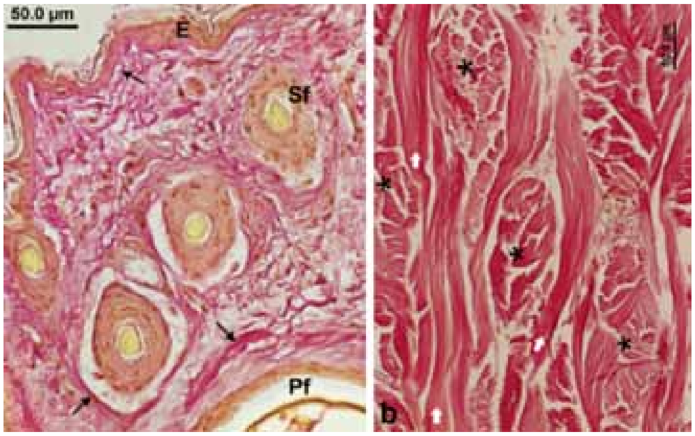


Figure 4: Dispersion of collagen fibers (□) in stratum papillare (a) and longitudinally (●) and transverse (▲) localization of collagen fibers in stratum reticulare (b). E, epithelium; Pf, primary hair follicle; Sf, secondary hair follicle; (Paraffin section, van Gieson's stain).

Determination of Total Kjeldahl Nitrogen (TKN)

There were significant differences ($p < 0.01$) among two skins and their different regions in addition to their significant interaction effect. The first deerskin had lower TKN values than second deerskin (Table I). Croupon region had the highest TKN value among other skin regions, while the lowest TKN values were obtained from shoulder and belly of first and second deer skin respectively. Neck and foot regions had the

TABLE I
The chemical properties of wild deerskins depending on five different regions

Results	Deer Skin	Regions of deer skins				
		Croupon	Shoulder	Neck	Belly	Foot
Total Kjeldahl Nitrogen (%)	1	74.62±0.74^{A}	65.93±0.34^{D}	71.26±0.19^{B}	62.25±0.45^{E}	69.25±0.47^{C}
	2	73.78±0.21^{A}	61.41±0.50^{C}	73.16±0.13^{A}	69.08±0.62^{B}	70.42±0.15^{B}
Hydroxyproline content (µg/mg)[24]	1	134.7±1.56^{B}	132.1±0.57^{B}	139.4±0.50^{A}	106.1±0.64^{C}	92.6±1.20^{D}
	2	118.8±1.27^{B}	147.7±0.28^{A}	118.5±2.12^{B}	88.7±0.35^{D}	102.0±0.85^{C}
Volatile Matter (%)	1	5.71 ± 0.02^{A}	4.43±0.07^{B}	4.12±0.04^{BC}	3.82±0.16^{C}	6.17±0.13^{A}
	2	7.02 ± 0.01^{A}	6.12±0.55^{B}	6.92±0.05^{A}	6.56±0.22^{AB}	6.16±0.12^{B}
Water soluble matter (%)	1	4.68±0.24^{A}	5.11±0.20^{A}	5.27±0.13^{A}	3.55±0.10^{B}	0.93±0.06^{C}
	2	4.05±0.09^{A}	3.74±0.18^{A}	0.10±0.21^{C}	3.41±0.07^{A}	2.19±0.10^{B}
Water soluble organic matter (%)	1	1.29±0.22^{A}	1.44±0.22^{A}	1.44±0.41^{A}	0.56±0.15^{B}	0.47±0.07^{B}
	2	2.70±0.42^{A}	1.28±0.47^{B}	0.01±0.03^{D}	1.25±0.05^{B}	0.61±0.07^{C}
Water soluble inorganic matter (%)	1	3.39±0.46^{AB}	3.67±0.02^{A}	3.83±0.15^{A}	2.99±0.14^{B}	0.46±0.02^{C}
	2	1.35±0.39^{B}	2.46±0.42^{A}	0.09±0.01^{C}	2.16±0.03^{A}	1.58±0.02^{B}
Sulphated total ash (%)	1	0.40±0.00^{B}	0.34±0.00^{B}	5.09±0.26^{A}	0.23±0.01^{C}	0.31±0.09^{BC}
	2	0.25±0.02^{BC}	0.33±0.05^{B}	1.08±0.01^{A}	0.19±0.08^{C}	1.11±0.05^{A}

$^{A\text{-}D}$ Means in the same row followed by different uppercase letters represent significant differences ($p<0.01$)

next highest TKN values after croupon region for both deer skins. The TKN values obtained from deer skins were higher than Black Anatolian (62.78%), East Anatolian Red (56.05%), and Holstein (63.23%) bovine hides.[35] Similarly to deer skins, variation of TKN values for different hide regions was reported, and croupon provided the highest TKN values 63.94% and 62.58% for Black Anatolian and East Anatolian Red bovine hides respectively.[36]

Determination of Hydroxyproline Content

The hydroxyproline content is one of the important parameter that gives specific information on the quality of leathers, hides and skins. There were significant ($p < 0.01$) differences in hydroxyproline content of skins and regions, their interaction was also found significant (Table I). The first deerskin had statistically higher hydroxyproline content. Among different regions, shoulder had the highest hydroxyproline content whereas neck and croupon had the next highest value for all deerskins. In previously published literature, shrinkage temperature increase of deer skins were reported to be directly proportional to the hydroxyproline content of deer skins and highest results were obtained from shoulder (66.4°C), neck (64.5°C) and croupon (65°C) respectively.[24]

In raw materials hydroxyproline content provide long-lasting and durable leathers in terms of mechanical and physical properties due to the increased stability of collagen triple helix.[37] For this reason, higher hydroxyproline content of raw deer skins produces high quality finished deer leathers with respect to long lasting, resistant and durable properties. In literature, different percentages of hydroxyproline in the total amino acid content were reported for cow (92%), calf (94%), pig (95.5%), shark (79%) and fish (53%) skins.[24]

Determination of Volatile Matter and Fat Content

There were significant differences in volatile matter content of two deerskin samples (Table 1). The second deerskin had significantly higher average volatile matter 6.56% than the first deerskin 4.86%, excluding the effect of regions. This is probably due to different structure of deer skins depending on life habits, season, age, sex, environment and breeding of the animal.[38] The amount of salt used in preservation, type of preservation and relative humidity of air could also affect these results.[39] For volatile matter results significant differences ($p < 0.01$) among five different regions of deer skins were observed. The croupon and foot regions had the highest values, and the lowest values ($p < 0.01$) were determined from belly and neck regions of the first deer skin sample. On the other hand, lowest volatile matter was obtained from foot and shoulder region of the second deer skin, while the highest values were obtained from croupon and neck regions ($p < 0.01$). These regional variations should always be considered as the consequence of inconsistent characteristic of leather material[38] that depends on raw material and processes applied in leather production.[40]

Although various fat contents of different type of raw materials such as sheepskin (5-30%), goat skin (3-10%) and cattle hide (2-4%) have been previously reported,[39, 41] there isn't any published data related to fat content of deer skin. In this study, the first deerskin (1.39%) had lower natural fat than second skin sample (1.64%) when the regional differences were not taken into account. No significant interaction ($p > 0.01$) but differences ($p < 0.01$) between two skins and skin regions was found. The natural fat content was determined as 1.80^{A}, 1.74^{A}, 1.62^{A}, 1.25^{B} and 1.16^{B}% for the shoulder, belly, neck, croupon and foot regions respectively without considering the skin differences. High fat cell content in sheep skins produces tough but spongy leather, because after the removal of fat in degreasing process, the empty fat cells play no part in attaching the corium to the grain layer but only interrupt the weave, resulting in loose skin and reduced mechanical strength.[42] Therefore it can be deduced that low fat content of deer skin provides better structure and higher strength properties.[9] Moreover low natural fat content of deer skins reduces the need for a degreasing process that provides more environmentally friendly leather manufacture resulted in high quality leather products.

Determination of Water-soluble Matter, Water-soluble Inorganic Matter and Water-soluble Organic Matter

The average water-soluble matter of the first deerskin in the form of chloride, sulphate, carbonate, phosphate and silicate[39] was found significantly higher than second deerskin, 3.91% and 2.70% respectively (Table I). The highest results regarding water-soluble matter, water-soluble inorganic matter and water-soluble organic matter were obtained from croupon, shoulder and neck regions while the lowest values were determined from the foot region of the first skin sample ($p < 0.01$). The highest significant value for water-soluble matter and water-soluble organic matter was obtained from croupon region of the second deerskin. The shoulder and belly region had the highest amount of water-soluble inorganic matter. Finally, the lowest significant value was determined from the neck region of the second sample considering all these three analyses ($p < 0.01$).

Determination of Sulphated Total Ash and Sulphated Water-insoluble Ash

Ash content of hides and skins vary from species to species[35] and in several studies, sulphated total ash results of different cattle hides were reported as in a range of 1.81, 2.22, 2.27 and 3.95%.[35, 36, 43] However, the sulphated total ash results of deer skins were found lower than the mentioned values. The difference between the ash content values could be related to leather type and environmental conditions. There was a significant difference in sulphated total ash and sulphated water-insoluble ash results among two skins and skin regions. Besides, interaction of skins with region was significant ($p < 0.01$). The total ash content of the first deerskin was found significantly higher than the second deerskin. The neck region had the highest total ash content for both skin types, while the lowest value was obtained from belly region ($p < 0.01$). This difference could be derived from skin type and salt used in preservation, which is responsible for the nearly entire ash content.[44]

Determination of Fatty Acid Methyl Esters by GC

The major saturated acids; palmitic (C16:0), stearic (C18:0), tridecanoic (C13:0), myristic (C14:0) and unsaturated acids; palmitoleic (C16:1), oleic (C18:1*cis)*, C18:3 (*trans*), C20:3, oleic (C18:1n9c), α-linolenic (C18:3n3) and long chained nervonic acid (C24:1) determined for deerskins were presented in descending order of abundance in Table II.

TABLE II
Fatty acid composition of different skins

Fatty Acid		Sheep[18]	Pig skin[45]	Deer[32]
C11:0	Undecylic acid	-	-	0.53
C12:0	Lauric	-	-	0.17
C13:0	Tridecylic acid	-	-	6.56
C14:0	Myristic	2.45	-	4.63
C14:1	Myristoleic	0.18	-	0.47
C15:0	Pentadecylic acid	-	-	1.5
C16:0	Palmitic	27.09	1.08	31.77
C16:1	Palmitoleic	3.32	44.91	4.43
C17:0	Margaric	0.18	-	0.80
C18:0	Stearic	42.35	1.53	13.23
C18:1	Oleic	16.55	49.36	8.98
C18:2	linoleic	-	-	1.59
C18:3	Linolenic	-	-	2.80
C20:3n6	Eicosadienoic acid	-	-	1.5
C20:5n3	Eicosapentaenoic acid	-	-	2.03
C22:0	Behenic acid	-	-	1.21
$C_{22:6n3}$	Docosahexaenoic acid	-	-	2.1
C24:0	Lignoceric acid	-	-	1.68
C24:1	Nervonic acid	-	-	2.88

Palmitic, stearic and oleic acid had the highest concentration in fatty acid composition of deerskin samples, which is similar to sheepskins. Deer skin contains less oleic acid and much higher palmitic acid than both sheep and pig skin.[18] Unsaturated fatty acid ratio of deer samples was found nearly 25% of total fatty acids, which primarily affects the melting point of fat, which is lower than sheep skin.

CONCLUSION

In the present study, the characteristics of wild red deer (*Cervus elaphus* L.) skins obtained from Republic of Tuva within the Russia Federation were investigated by morphological, histochemical and chemical methods to exploit this as a raw material for the production of long lasting and high quality leather outerwear. Morphological results revealed that red deer skins have two different hair types; guard hair (primary hair follicle) and fur fiber (secondary hair follicle). The guard hair of deer skin provides a protection against environmental conditions due to their high medulla proportion. The special location of fur fibers around guard hair and their high cortex layer supports this protection and gives flexibility and resistance to fur fibers. The collagen fiber orientation in deerskin was categorized as longitudinally and transverse and the fibers were tightly packed in these two directions. This structural characteristic of collagen resulted in high strength properties and elasticity for resultant leather products. The hydroxyproline and TKN content of deerskins were found higher than the other reported values, which is a good indication of long-lasting properties. In addition, deer skin characteristics showed similarities to calfskin, in terms of low fat and sulphated total ash content. The essential fatty acid methyl esters of deerskin were comparable to sheepskins; however they differ in their amount.

Consequently, the results of this study demonstrated that raw material of red deer skins could be utilized as an ideal raw material for the production of long lasting outerwear materials providing high strength properties. Unique wool characteristic of red deer skins could perfectly promote the utilization of this raw material in other usage areas where also the thermal insulation property is needed.

ACKNOWLEDGEMENT

The authors would like to thank to Turkey Prime Ministry State Planning Organization for the supply of equipment (Project no: 2007 DPT 001) and Ege University Research Project Department Directorate for financial support (Project no: 04 MUH 007).

REFERENCES

1. Corbet, G. B.; The Mammals of the Palaearctic Region: A Taxonomic Review. BritishMuseum (Nat. Hist.), London, 38-63, 1978.
2. Koubek, P and Zima, J.; Cervus elaphus. The Atlas of European Mammals., eds A.J. Mitchell-Jones, G. Amori, W. Bogdanowicz, B. Kryßtufek, P.J.H. Reijnders, F. Spitzenberger, M. Stubbe, J.B.M. Thissen, V. Vohralik & J. Zima, Academic Press, London, UK, 388–389, 1999.
3. Wilson, D and Ruff, S.; The Smithsonian Book of North American Mammals, Washington and London, Smithsonian Institution Press, 1999.
4. Lomanova, N.V., Borisov, B.P., Volodina, O.A., Gubar, J.P., Ljapina, M.G., Komissarov, M.A., Mosheva, T.S., Naumov, A.A.,Sidorov, S.V., Tsarev, S.A., Judina, T.V., Fokin, S.J., Blohin, J.J., Zverev, P.A., Kozlova, M.V., Mezhenev, A.P., Romanov and J.M.; Wild Animals of Russian Federation (Biology, Safety, Usage and Resources) 2008-2010, Chapter 9, p.10-15, 2010.
5. Eldom, S and Wilder, T.; Buckskin: The Ancient Art of Braintanning, A Practical Guide to Home Tanning by the Wet-Scrape Method, Boonville, California, USA, Paleotechnics, 2001.
6. Korpiharju, T.; Investigations into Properties and Use of Reindeer Skin and Leather and Influence of Defects on Leather Properties. *JALCA* **89**, 357-363, 1994.
7. Bruce, C. M., Laing, R. M., Niven, B. E and Carr, D.J.; Laminated Cervine Leather for Apparel. *JSLTC* **89**, 1-7, 2005.
8. Lange, S., Laing, R. M., Niven, B. E., Scobie, D. R., Young, S. R and Gore, S. E.; Deer Leather as a Material for Outwear. *JSLTC* **86**, 157-162, 2002.
9. Herfeld, H.; Die Tierische Haut, Bibliothek des Leders, Umschau Verlag Breidenstein GmbH, Frankfurt am Main, 1st Edition, Part II, 75, 1990.
10. Clark, J. E and Webster R. M.; Leather Production from New Zealand Deerskins. *The Royal Society of New Zealand Bulletin* **22**, 303-306, 1985.
11. Gore, S. E., Laing, R. M., Lange, S., Scobie, D. R and Young, S.R.; Changes to Surface Flaws on Deer Hides During Processing to Garment Leather. *JSLTC* **86**, 183-187, 2002.
12. Gore, S. E., Laing, R. M., Carr, D. J and Niven, B. E.; Extension and Recovery of Cervine Garment Leather. *JSLTC* **88**, 56-62, 2004.
13. Starcher, B., Aycock, R. L and Hill C. H.; Multiple Roles for Elastic Fibers in the Skin. *Journal of Histochemistry & Cytochemistry* **53**, 431-443, 2005.
14. Hartl, G. B., Willing, R., Lang, G., Klein, F and Köller, J.; Genetic Variability and Differentiation in Red Deer (*Cervus elaphus* L) of Central Europe. *Genet. Sel Evol.* **22**, 289-306, 1990.
15. Fukunaga, S., Yoshie, A., Yamakawa, I and Nakamura, F.; Seasonal Variations of Yeso Sika Deer Skin and Its Vegetable Tanned Leathers, XXX. IULTCS Congress, Beijing China, October 11-14, 1-4, 2009.
16. Dandar, U.; A Study on the Characteristics of Hairy Shoe Upper Leather Made of Deer Skin. Master Science Thesis, (in Turkish), Ege Univ. Graduate School of Natural and Applied Science, Bornova, Izmir, 2005.
17. Cadirci, B. H., Ozgunay, H., Vural, C and Yilmaz, O.; A New Defect on Leather: Microbial Bio-Film. *JALCA* **105**,129-134, 2010.
18. Zengin, G and Afsar, A.; Use of Natural Fat Emulsions in Fatliquoring Process and Investigation of Fatty Spue Formation. *JALCA* **106**, 83-91, 2011.
19. Drury, R. A. B., Wallington, E. A and Cameron, S. R.; Carleton's Histological Technique, 4th Edition, Oxford University Press, Ely House, London, 1967.
20. TS EN ISO 2418; Leather - Chemical, Physical and Mechanical and Fastness Tests - Sampling Location, *Turkish Standard (TSE)*, Ankara, Turkey, 2006.
21. TS EN ISO 4044; Leather – Chemical Tests- Preparation of Chemical Test Samples, *Turkish Standard (TSE)*, Ankara, Turkey, 2009.
22. Bradstreet R. B.; Kjeldahl method for organic nitrogen. *Anal Chem* **26**, 185–187, 1954.
23. TS 4134; Leather - Determination of Nitrogen Content and Hide Substance. Titrimetric method, *Turkish Standard (TSE)*, Ankara, Turkey, 1985.
24. Çolak, S. M., Dandar, U and Çavdar, Z.; A Study on Research Some Structural Properties of Deer Hides. *Journal of Animal Production* **49**, 38-46, 2008.
25. TS EN ISO 4684; Leather - Chemical Tests - Determination of Volatile Matter, *Turkish Standard (TSE)*, Ankara, Turkey, 2009.
26. TS EN ISO 4048; Leather - Chemical Tests - Determination of Matter Soluble in Dichloromethane and Free Fatty Acid Content, *Turkish Standard (TSE)*, Ankara, Turkey, 2009.
27. TS EN ISO 4098; Leather — Chemical tests — Determination of water-soluble - matter, water-soluble inorganic matter and water-soluble organic matter, *Turkish Standard (TSE)*, Ankara, Turkey, 2009.
28. TS 4125 EN ISO 4047; Leather- Determination of Sulphated Total Ash and Sulphated Water- Insoluble Ash, *Turkish Standard (TSE)*, Ankara, Turkey, 2000.
29. TS EN ISO 12966-2; Animal and Vegetable Fats and Oils - Gas Chromatography of Fatty Acid Methyl Esters - Part 2: Preparation of Methyl Esters of Fatty Acids, *Turkish Standard (TSE)*, Ankara, Turkey, 2011.
30. TS 4664 EN ISO 5508; Animal and Vegetable Fats and Oils-Analysis by Gas Chromatography of Methyl Esters of Fatty Acids, Turkish Standard (TSE), Ankara, Turkey, 1996.

31. Zengin, G., Dandar, U., Çolak, S.M and Deveci, R.; Structural Characteristics of Skins and Wools of Deers Living in Republic of Tuva in Russia, I. International Leather Engineers Symposium, April 29 – May 1, Izmir, Turkey, 547-554, 2009.
32. Zengin, G., Dandar, U., Çolak, S.M and Deveci, R.; Characterization of Red Deer Skins, XXXI. IULTCS Congress, 27 – 30 September, Valencia, Spain, A19, 2011.
33. Bachrach, M.; Microscopical Study of Guard Hair and Fur Fibers (Chapter 36), Fur a Practical Treatise, 3rd Edition, 556, 1953.
34. Haines, B.M.; The Fiber Structure of Leather, Conservation of Leather and Related Materials, Ed. Marion Kite and Roy Thomson, Elsevier Ltd, Printed in China, 1st Edition, Part 3, 15, 2006.
35. Armutak, A and Dağlıoğlu, S.; Examination of the Skin Structure of Holstein Cattles which are Farmed around Istanbul with Histological and Chemical Methods. *Journal of Faculty of Veterinary Medicine* **21**, 170-196, 1995.
36. Fırat, U. B and Artan, M. E.; Examination of the Skins Structure obtained from Anatolian Black and East Anatolian Red Breeds in Turkey by Histological and Chemical Methods: III. The Comparative Quantitative Examination of the Chemical Compounds of Skins. *Journal of Faculty of Veterinary Medicine* **21**, 395-402, 1995.
37. Reich, G and Taeger, T.; What is Leather? The Structure and Reactivity of Collagen, From Collagen to Leather – The Theoretical Background, 1st Edition, BASF Service Center, Published in Ludwigshafen, Germany, 9, 2007.
38. Thorstensen T. C.; Practical Leather Technology, 4th Edition, Krieger Publishing Company, Malabar, Florida, 1993.
39. Lange, J.; Qualitaetsbeurteilung von Leder, Lederfehler, Lederlagerung und Lederpflege, Bibliothek des Leders, Umschau Verlag Breidenstein GmbH, Frankfurt am Main, 1st Edition, Part IV, 210, 1982.
40. Thomson, R.; Testing Leathers and Related Materials, Ed. Marion Kite and Roy Thomson, Elsevier Ltd, Printed in China, 1st Edition, Part 6, 58, 2006.
41. Harmancıoğlu, M and Dikmelik, Y.; Raw Skin: Structure, Composition, Properties, Özen Ofset Publisher, Chapter 3, 85-124, 1993.
42. Leather Clothing Its Manufacture and Maintenance, 1st ed., British Leather Confederation Fabric Care Research Association, p. 11, 1989.
43. Yakalı, T and Dikmelik, Y.; An Investigation on Some Physical and Chemical Properties of Young Bull Raw Skin and Finished Leather. *Doğa Bilim Derg. Vet. Hay. Tar. Orm* **6**, 67-72, 1982.
44. Bailey D. G.; Preservation of Hides and Skins, Leather Technologists Pocket Book, Ed. Malcolm K. Leafe, 4th Edition, The Society Of Leather Technologist and Chemists, Published in UK, 14, 1999.
45. Shalbuev, D., Slavgorodskaya, M., Zengin, G. and Dandar, U.; Comparative analysis of the fats which has different chemical contents by chromatography. VI. International Science Practical Conference, Leather and Fur in XXI Century: Technology, Quality, Environmental, Management, Education, Ulan Ude, Russia, 261-264, 2011.

NUTRIENT BALANCE IN AEROBIC BIOLOGICAL TREATMENT OF TANNERY WASTEWATER

by

JIANFEI ZHOU,[1] YA-NAN WANG,[2]* WENHUA ZHANG[2] AND BI SHI[1,2]
[1]*Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University,*
CHENGDU 610065, P. R. CHINA

[2]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan* UNIVERSITY,
CHENGDU 610065, P. R. CHINA

ABSTRACT

The effect of nutrient composition of tannery wastewater on aerobic biological treatment, particularly on the removal of ammonia nitrogen (NH_3-N) and total nitrogen (TN) was investigated based on the theory of nutrient balance. During the biological treatment of conventional tannery wastewater, total organic carbon (TOC) and total phosphorus (TP) were almost exhausted. However, the removal of TN was unsatisfactory, suggesting that tannery wastewater contains excessive N source and insufficient C and P sources. When extra C and P sources were added together into the wastewater, the removals of NH_3-N and TN increased significantly. Under the optimal ratio of TOC:TN:TP (10:1:0.2), the removals of NH_3-N and TN were 100% and 80%, respectively, and the added C and P sources were almost consumed by activated sludge. This means that a good nutrient balance was achieved in this condition. Accordingly, when ammonium salts are replaced by organic acids and phosphates in the deliming and bating processes, it is reasonable to assume that the change of wastewater composition would favor the aerobic biological treatment of wastewater.

INTRODUCTION

Tannery wastewater commonly contains a large amount of ammonia nitrogen (NH_3-N) and total nitrogen (TN) due to the addition of ammonium salts in deliming and bating and the removal of non-collagenous proteins from raw hide/skin in beamhouse processes.[1] The end-of-pipe treatment is regarded as an effective approach to solving the serious impact of nitrogen. However, conventional secondary treatment (biological treatment) could not achieve satisfactory N removal efficiency. Therefore, a special N removal treatment, usually nitrification-denitrification, should be further performed.[2-4] On the other hand, the strategy of reducing N pollution at the origin, especially the use of non-ammonia deliming and bating technologies, has been recommended as a better solution. In our previous research, sodium hexametaphosphate, citric acid and sodium citrate have been successfully used in non-ammonia deliming and bating processes as substitutes for ammonium salts.[5, 6] With the use of these ammonia free chemicals, redundant P and C will be introduced into the wastewater although N is eliminated. It is known that the content of P in conventional tannery wastewater is extremely low due to the fact that nearly no P-containing chemicals are used. Therefore, we are deeply concerned with the introduction of P as it is also a restrictive nutrient in most freshwater systems that would cause eutrophication as the same as N.[7] In addition, citric acid and citrate contribute a great deal of total organic carbon (TOC) in wastewater, though they are reported to be easily biodegraded.[8] Anyway, the change of nutrient composition in wastewater will necessarily influence the aerobic biological treatment process.

---

*Corresponding author e-mail address: wangyanan@scu.edu.cn
Manuscript received January 2, 2014, accepted for publication February 13, 2014.

Aerobic biological treatment is a process that uses biodegradable organic matter in wastewater and oxygen to promote the growth of microorganisms, and hence removes the organic pollutants. Among a multitude of biological treatment processes, activated sludge process is regarded as the most classical method. This process involves activated sludge (a suspended biological floc composed of microorganisms) and oxygen in the adsorbing, oxidizing and removing organic pollutants in wastewater.[7, 9] In order to maintain the growth and reproduction of activated sludge, energy and C sources as well as several nutrients (mainly N and P sources) must be available in adequate amounts for microbial metabolism.[7] It has been reported that the nutrient balance is controlled by the mass ratio of C:N:P, generally around 100:5:1 for aerobic biological treatment, where N and P mean the contents of TN and total phosphorus (TP) in wastewater, respectively, and C commonly means COD or BOD in different literature.[10-14] It is worth noting that COD or BOD can be converted into TOC to represent the content of C[15-17] as a linear relationship can be established between TOC content and COD or BOD content for a given wastewater.[7] Furthermore, it is convenient to determine TOC and TN contents simultaneously by an instrument within 20 min. In this study, the effect of addition of C and P sources on aerobic biological treatment of tannery wastewater, especially the removal of NH_3-N and TN, was investigated. The optimal ratio of C:N:P in tannery wastewater, where the amount of C was characterized by TOC, was suggested. The results are also valuable for evaluating the effect of input change of nutrients caused by reformation of leather processing on biological treatability of tannery wastewater.

EXPERIMENTAL

Materials

Tannery wastewater and activated sludge were collected from Ruixing Leather Co., Ltd. (Haining, China). The wastewater was sampled from the primary clarifier, which was ready for biological treatment. The concentrations of TOC, NH_3-N, TN, TP as well as the pH of the raw wastewater were determined. The activated sludge was sampled from the aeration tank. The chemicals used for biological wastewater treatment and analysis of wastewater were of analytical grade.

Procedures for Aerobic Biological Wastewater Treatment

The wastewater and the activated sludge were mixed in a 2000 mL measuring glass, where the ratio of wastewater and sludge was 2:1 (v/v). An aeration device was used for the biological wastewater treatment (Figure 1). The ambient temperature (25°C) was controlled by an air-conditioner. After aerobic biological treatment, the mixture of wastewater and sludge was sampled for mixed liquor suspended solids (MLSS) and sludge volume (SV) determinations. At the same time, the mixture was centrifuged at 5000 rpm for 10 min. Then the supernatant was used for the measurements of TOC, NH_3-N, TN and TP.

Figure 1. Aeration device for biological wastewater treatment

Analytical methods

TOC and TN were measured by using TOC/TN analyzer (LiquiTOC, Elementar, Germany). NH_3-N was determined by distillation and titration method.[18] TP was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Optima 8000, PerkinElmer, USA) with acid digestion pretreatment.[18] MLSS and SV were measured according to the standard methods for the examination of wastewater.[18]

Aerobic Biological Treatment with the Addition of C and P Sources

Four groups of aerobic biological treatment trials were performed according to the procedures above. Group 1 was control without addition of C and P sources. Glucose (900 mg/L, based on the content of C, similarly hereinafter) was added in group 2 as C source. Trisodium phosphate (24 mg/L, based on the content of P, similarly hereinafter) was added in group 3 as P source. C and P sources (900 mg/L of glucose and 24 mg/L of trisodium phosphate) were added in group 4. Samples were collected after 0, 12, 24, 48, 72 and 96 h of biological treatment, and analyses were carried out immediately.

Aerobic Biological Treatment with Different C/N Ratios

Six groups of aerobic biological treatment trials were performed according to the procedures above. Group 1 was control without addition of C and P sources. Different amounts of glucose were added in the other five groups as C source to ensure that the ratios of TOC to TN (C/N) in the wastewaters were approximately 5:1, 8:1, 10:1, 13:1 and 16:1 (w/w, similarly hereinafter), respectively. Simultaneously, an equal amount of trisodium phosphate (24 mg/L) was added in each of the five groups as P source so that the ratio of TN to TP (N/P) was 1:0.2. Samples were collected before and after biological treatment for 48 h, and analyses were performed as described above.

Aerobic Biological Treatment with Different N/P Ratios

Five groups of aerobic biological treatment trials were performed according to the procedures above. Group 1 was control without addition of C and P sources. An equal amount of glucose (900 mg/L) was added in each of the other four groups as C source so that the C/N ratio was 10:1. At the same time, different amounts of trisodium phosphate were added in the four groups as P source to ensure that the N/P ratios in the wastewaters were approximately 1:0.05, 1:0.1, 1:0.2 and 1:0.5, respectively. Samples were collected and analysed before and after biological treatment for 48 h.

Aerobic Biological Treatment using Different P Sources

Three groups of aerobic biological treatment trials were performed according to the procedures above. Glucose was used as the C source for each group. Trisodium phosphate (TSP, $Na_3PO_4 \cdot 12H_2O$), sodium dihydrogen phosphate (MSP, $NaH_2PO_4 \cdot 2H_2O$) and sodium hexametaphosphate (SHMP, $(NaPO_3)_6$) were used as the P source, respectively. The ratio of TOC:TN:TP (C:N:P) was set to be approx. 10:1:0.2. Samples were collected and analysed before and after biological treatment for 48 h.

RESULTS AND DISCUSSION

Characteristics of Raw Tannery Wastewater and Activated Sludge

Table I shows the characteristics of the tannery wastewater after primary treatment. The pH of the wastewater was 7.95, which was suitable for aerobic biological treatment. The content of NH_3-N in wastewater (61.5 mg/L) accounted for about 50% of TN (124.2 mg/L), and the other half should be mainly organic nitrogen. As chemicals containing P were seldom used in leather processing except a little phosphated fatliquor, the TP concentration in wastewater was only 1.2 mg/L. As a result, the original ratio of C:N:P in the wastewater was approx. 3:1:0.01. The activated sludge used for the experiment was present in the form of tawny floc with good settling properties. The pH of the sludge was 8.40.

Effect of Addition of C and P Sources on Aerobic Biological Treatment

In order to investigate the effect of addition of C and P sources on aerobic biological treatment, four groups of treatment trials were conducted. The results are shown in Figure 2. For the group with the addition of both C and P sources, the rates of NH_3-N and TN removal increased significantly compared with the other groups (Figure 2(a) and 2(b)). NH_3-N was exhausted after treatment for 24 h. TN concentration dropped to the minimum with the removal reaching more than 80% after treatment for 48 h. At the same time, most of the C source added was consumed (Figure 2(c)). The residual carbon should be non-biodegradable matter. In addition, Figure 2(d) shows that the concentration of TP in the C and P added wastewater decreased to 2 mg/L after treatment for 12 h and then remained stable. It should be noted that the determined initial concentration of TP in the wastewater was approx. 7 mg/L, although the offer of P was 24 mg/L. This may be due to the adsorption of phosphate on activated sludge. Actually this part of phosphate was also available for microorganisms. All the results above indicated that assimilation of microorganisms to was predominant in the initial 48 h, and C, N and P sources were used as nutrients for the microbial cell synthesis and growth. This could also be demonstrated by the increases of MLSS and SV, as shown in Figure 3. By contrast, the groups with the addition of single C source or P source as well as control resulted in poorer removals of NH_3-N and TN. During the treatment, the concentration of TN in wastewater decreased from 120 mg/L to 90 mg/L in the initial 48 h, and then slightly increased probably due to the autolysis and endogenous respiration of activated sludge. Moreover, TOC and TP in control group were almost exhausted at the end of biological treatment. These facts demonstrated that lack of C source and/or P source limited the consumption of N source by the activated sludge. Conventional tannery wastewater was rich in N source and lack of C and P sources in terms of the nutrient balance of activated sludge (see Table I and control group in Figure 2). Therefore, the strategies that can reduce N impact and supplement appropriate amounts of C and P in leather processing could be suggested in order to meet the nutrient requirement of aerobic biological wastewater treatment.

Effect of C/N Ratio on Aerobic Biological Treatment

Based on the results of the previous section, the duration of aerobic biological treatment was set to be 48 h for the following experiments. Six groups of aerobic biological treatment trials were carried out using different C/N ratios and equal N/P ratio (1:0.2). The effect of C/N ratio on wastewater treatment is shown in Figure 4 and Table II. The removal of NH_3-N reached 100% for all the groups with the addition of C and P sources, and the removal of TN in these groups was much higher than control (Figure 4). When the C/N ratio was

TABLE I
Characteristics of the tannery wastewater after primary treatment.

pH	TOC (mg/L)	NH_3-N (mg/L)	TN (mg/L)	TP (mg/L)
7.95	413.7	61.5	124.2	1.20

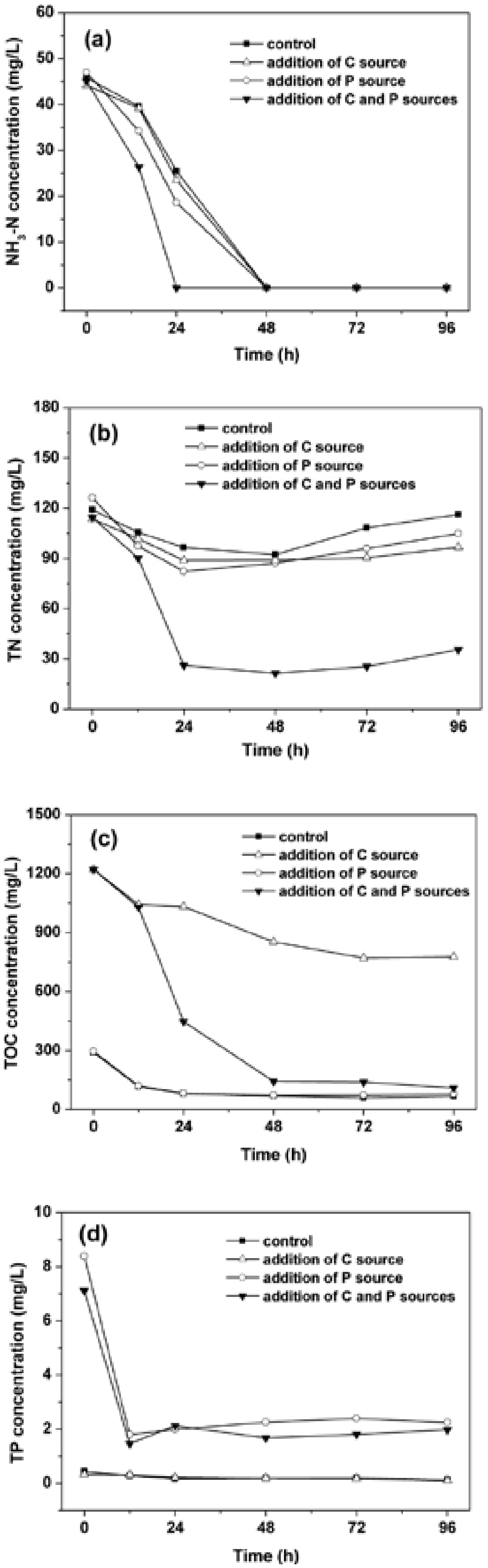


Figure 2. Effect of addition of C and P sources on aerobic biological wastewater treatment: (a) NH_3-N; (b) TN; (c) TOC; (d) TP.

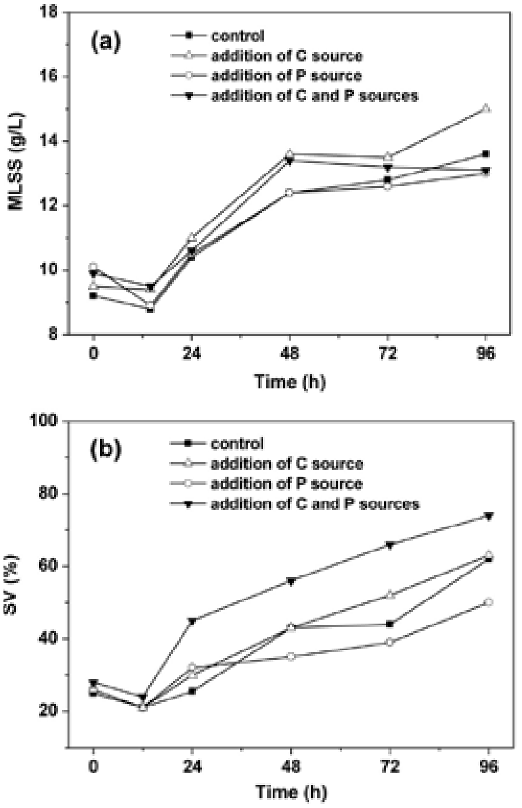


Figure 3. Effect of addition of C and P sources on activated sludge properties: (a) MLSS; (b) SV.

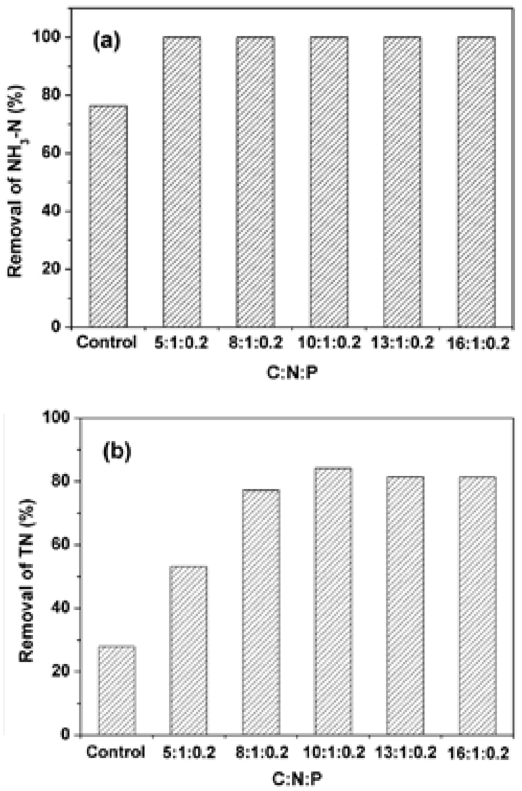


Figure 4. Effect of C/N ratio on aerobic biological wastewater treatment: (a) NH_3-N; (b) TN.

TABLE II
Wastewater parameters before and after treatment using different C/N ratios.

C:N:P	TOC (mg/L)		TN (mg/L)		TP (mg/L)	
	0 h	48 h	0 h	48 h	0 h	48 h
Control	271.6	66.4	120.6	87.0	0.21	0.02
5:1:0.2	660.1	71.2	119.3	55.9	10.21	1.63
8:1:0.2	994.7	82.2	119.8	27.3	9.65	1.56
10:1:0.2	1198.5	102.8	118.0	18.7	9.88	1.24
13:1:0.2	1651.5	259.7	118.3	21.9	7.78	1.59
16:1:0.2	1979.2	645.5	112.3	20.8	8.29	1.73

5:1, it was found that the removal of TN was only 53% (Figure 4(b)), and the residual TOC in wastewater was nearly the same as control (Table II). This means that C source was insufficient using this C/N ratio, resulting in a large surplus of N source. The removal of TN increased to the peak of 84% in the C/N ratio of 10:1, and then showed no more enhancement with further increase in C/N ratio. Meanwhile, the residual TOC in wastewater kept rising (Table II), suggesting that the C source added was excessive in the C/N ratios of 13:1 and 16:1. In general, the optimal C/N ratio for aerobic biological treatment was 10:1.

Effect of N/P Ratio on Aerobic Biological Treatment

The investigation on the effect of N/P ratio on aerobic biological treatment was further performed based on the optimal C/N ratio of 10:1. Figure 5(a) shows that NH_3-N was exhausted after treatment for 48 h in all the experimental groups where both C and P sources were added, which was similar to the results shown in Figure 4(a). The removal of TN as well as the consumption of TOC was positively correlated with the offer of P (Figure 5(b) and Table III). When the N/P ratio was 1:0.2 or 1:0.5, the removal of TN achieved 80%, and the residual TOC was around 100 mg/L. However, the TP concentration in wastewater was as high as 5.74 mg/L in the N/P ratio of 1:0.5, indicating that P source was excessive in this condition. On the contrary, P source was inadequate for nutrient need of aerobic biological treatment in the N/P ratio of 1:0.1 or 1:0.05, which led to relatively low removals of TN and TOC. In consideration of nutrient balance of activated sludge, the optimal ratio of C:N:P was 10:1:0.2.

Effect of Different P Sources on Aerobic Biological Treatment

In order to investigate the effect of different P sources on aerobic biological treatment, three types of phosphates, i.e. TSP ($Na_3PO_4 \cdot 12H_2O$), MSP ($NaH_2PO_4 \cdot 2H_2O$) and SHMP ($(NaPO_3)_6$) were used as the P source for the treatment, respectively. The ratio of C:N:P was adjusted to 10:1:0.2 for all the groups. Table IV shows that the removals of TOC, TN and TP in the group using TSP as P source were higher than those in the other two groups. This may be due to the fact that TSP was dissolved in the wastewater as orthophosphate, which could be directly available to the activated sludge.[19] SHMP, as a type of polyphosphate, should be firstly converted into orthophosphate and then utilized by activated sludge, which probably resulted in slower removal of TOC and TN. [19] But in fact, the addition of SHMP as P source is still effective to remove the pollutants in the tannery wastewater.

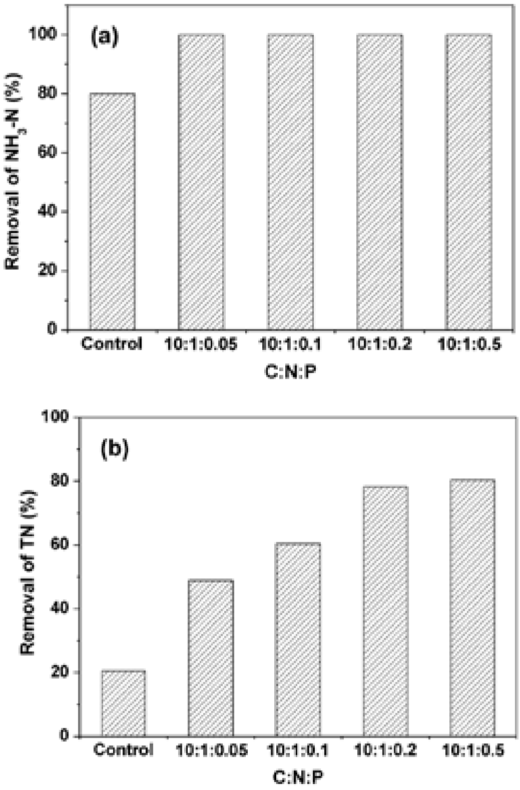


Figure 5. Effect of N/P ratio on aerobic biological wastewater treatment: (a) NH3-N; (b) TN.

TABLE III
Wastewater parameters before and after treatment using different N/P ratios.

C:N:P	TOC (mg/L)		TN (mg/L)		TP (mg/L)	
	0 h	48 h	0 h	48 h	0 h	48 h
Control	234.2	64.3	116.6	92.8	0.15	0.06
10:1:0.05	1126.3	428.4	113.3	57.9	1.33	0.40
10:1:0.1	1195.6	239.9	110.6	43.8	3.42	2.41
10:1:0.2	1150.1	116.7	116.6	25.4	5.85	1.42
10:1:0.5	1150.1	100.8	117.1	23.1	15.19	5.74

TABLE IV
Wastewater parameters before and after treatment using different P sources.

P source	TOC (mg/L)		NH_3-N (mg/L)		TN (mg/L)		TP (mg/L)	
	0 h	48 h	0 h	48 h	0 h	48 h	0 h	48 h
$Na_3PO_4·12H_2O$	1099.6	89.2	43.4	0	116.1	27.5	10.30	1.46
$NaH_2PO_4·2H_2O$	1145.7	195.7	43.4	0	115.8	55.0	14.97	3.88
$(NaPO_3)_6$	1151.7	187.7	43.8	0	118.7	54.4	10.80	4.54

Discussion on the Nutrient Balance in Aerobic Biological Treatment

In the view of nutrient balance in aerobic biological treatment, C and P sources in conventional tannery wastewater are insufficient to some extent, whereas N source is excessive due to the use of ammonium salts in deliming and bating as well as the removal of non-collagenous proteins from the raw hide/skin. In our previous research, SHMP, citric acid and sodium citrate have been successfully applied in non-ammonia deliming and bating processes as substitutes for ammonium salts.[5,6] The reformation of technologies is inevitable to change the nutrient composition of tannery wastewater. The contents of C and P sources in wastewater would increase, while the content of N source would decrease significantly, which makes the modified ratio of C:N:P closer to the requirement of nutrient balance in comparison with the original ratio. Therefore, it can be inferred that aerobic biological treatment of the wastewater produced from the non-ammonia process should be easier than that of conventional tannery wastewater, and the difficulty of nitrogen removal will be alleviated greatly.

In addition, although the wastewater and the activated sludge used in this study were collected from a typical tannery, the optimized ratio of C:N:P (10:1:0.2) for the aerobic biological treatment may not be perfectly suitable for other tanneries in consideration of the differences in leather processing, wastewater composition and activated sludge. However, the exploration of the biological wastewater treatment in terms of nutrient balance is still instructive. Besides the reformation of leather processing mentioned above, a comprehensive treatment of tannery wastewater together with other industrial wastewaters rich in C and P (such as pulp and paper mill wastewater, organic chemical industry wastewater) may be an effective solution where the complementary nutrients supplied by these wastewaters could meet the requirement of nutrient balance, and thus favor the aerobic biological treatment. As industrial parks have begun to spring up in China, the comprehensive treatment of wastewaters from different industries becomes more possible.

Conclusions

Conventional tannery wastewater is commonly rich in N, but lacks C and P in terms of nutrient balance, which limits the aerobic biological treatment of the wastewater by activated sludge. The addition of C and P sources in wastewater, especially in the C:N:P ratio of 10:1:0.2, was shown to remarkably improve the removal of NH_3-N and TN. These results infer that the strategies of reducing N input and appropriately introducing C and P in leather processing would be favorable for the construction of nutrient balance in wastewater and the increase in efficiency of biological wastewater treatment.

ACKNOWLEDGEMENT

This project is financially supported by the National Key Technologies R&D Program (2011BAC06B11), the National High Technology R&D Program (2013AA06A306) and the Fundamental Research Funds for the Central Universities (2014SCU11027).

REFERENCES

1. Wang, Y. N., Zeng, Y. H., Chai, X. W., *et al*.; Ammonia nitrogen in tannery wastewater: distribution, origin and prevention. *JALCA* **107**, 40-50, 2012.
2. Saeed, T., Afrin, R., Al Muyeed, A., *et al*; Treatment of tannery wastewater in a pilot-scale hybrid constructed wetland system in Bangladesh. *Chemosphere* **88**, 1065-1073, 2012.
3. Insel, H. G., Gorgun, E., Artan, N., *et al*; Model based optimization of nitrogen removal in a full-scale activated sludge plant. *Environ. Eng. Sci*. **26**, 471-480, 2009.
4. Ganesh, R., Balaji, G., Ramanujam, R. A.; Biodegradation of tannery wastewater using sequencing batch reactor—Respirometric assessment. *Bioresour. Technol.* **97**, 1815-1821, 2006.
5. Zeng, Y. H., Lu, J. H., Liao, X. P., *et al*.; Non-ammonia deliming using sodium hexametaphosphate and boric acid. *JALCA* **106**, 257-263, 2011.
6. Wang, Y. N., Zeng, Y. H., Liao, X. P., *et al*.; Removal of calcium from pelt during bating process: an effective approach for non-ammonia bating. *JALCA* **108**, 120-127, 2013.
7. Metcalf & Eddy Inc.; Wastewater engineering: treatment and reuse (Fourth edition), Tsinghua University Press, Beijing, China, 2003.
8. Koh, K.; Citric Acid in Detergent Industry. *Detergent & Cosmetics* **23**, 130-132, 2000.
9. Grady Jr., C. P. L., Daigger, G. T., Lim, H. C., *et al*.; Biological wastewater treatment, Chemical Industry Press, Beijing, China, 2003.
10. Azbara, N., Yonar, T.; Comparative evaluation of a laboratory and full-scale treatment alternatives for the vegetable oil refining industry wastewater (VORW). *Process Biochem*. **39**, 869-875, 2004.
11. Sapari, N.; Treatment and reuse of textile wastewater by overland flow. *Desalination* **106**, 179-182, 1996.
12. Kajitvichyanukul, P., Suntronvipart, N.; Evaluation of biodegradability and oxidation degree of hospital wastewater using photo-Fenton process as the pretreatment method. *J. Hazard. Mater.* **138**, 384-391, 2006.
13. Ammary, B. Y.; Nutrients requirements in biological industrial wastewater treatment. *African Journal of Biotechnology* **3**, 236-238, 2004.
14. Sayadi, S., Allouche, N., Jaoua, M., *et al*.; Detrimental effects of high molecular-mass polyphenols on olive mill wastewater biotreatment. *Process Biochem*. **35**, 725-735, 2000.
15. Liu, Y., Zhang, T., Fang H. H. P.; Microbial community analysis and performance of a phosphate-removing activated sludge. *Bioresour. Technol*. **96**, 1205-1214, 2005.
16. Vainberg, S., Togna, A. P., Sutton, P. M., *et al*.; Treatment of MTBE-contaminated water in fluidized bed bioreactor. *J. Environ. Eng.-ASCE* **128**, 842-851, 2002.
17. Kida, K., Ikbal, Sonoda, Y., *et al*.; Influence of mineral nutrients on high performance during anaerobic treatment of wastewater from a beer brewery. *Journal of Fermentation and Bioengineering* **72**, 54-57, 1991.
18. Clescerl, L. S., Greenberg, A. E., Eaton, A. D.; Standard methods for the examination of water and wastewater 20th edition, American Public Health Association, Washington DC, 1998.
19. Zhang, S. H., Water Treatment Microbiology, Chemical Industry Press, Beijing, China, 2005.

STUDIES OF ETHIOPIAN SHEEPSKINS AS AN OPPORTUNITY FOR VALUE ADDITION, PART II: OPTIMIZATION AND CHARACTERIZATION OF WANKE UPPER AND GARMENT LEATHERS

by

H. Mohammed,[1] G. Aysanew,[1] R. Aravindhan,[2] J. Raghava Rao[2]* and N.K. Chandra Babu[2]
[1]*Leather Industry Development Institute,*
Addis Ababa, Ethiopia

[2]*Central Leather Research Institute, Council of Scientific & Industrial Research*
Adyar, Chennai, India

ABSTRACT

Ethiopian tanners face a shortage of raw material input for the production of leather. The government strategically planned for importing raw skins from neighboring countries and also for effective utilization of available raw material resources in the country. The meat of Wanke sheep is in high demand in international markets, but the skin commands low price not only due to low availability but also less demand by tanners due to natural problems associated with the skin. Hence, tanners treat the skin as a reject. The problems of Wanke skin include high natural fat deposition, thin substance and low strength. Usually, leathers made out of Wanke skins have low selections compared to Ethiopian sheep skins and are mainly utilized for making lining leather. In this work, efforts have been made to develop a process technology for making high value leathers with improved properties from Wanke sheepskin.

INTRODUCTION

A major problem with the Ethiopian leather sector is from the supply of raw hides and skins. In addition there is a competition for the best origin (highland) sheepskin, which drives the price abnormally to a high level. Tanneries are not interested in purchasing sheepskins coming from lowland areas due to the poor quality of the raw material. Wanke or Black head sheepskin is one such variety where Ethiopian tanners could not produce high value leather.

Ethiopian tanners have faced a shortage of raw material as an input for the production of leather. Moreover the government has planned more effective utilization of available raw material resources in the country. Among the available resources, Wanke sheepskins take priority position as an untapped resource and found significantly in the lowland regions of the country. Wanke sheepskin is also important because the meat of Wanke Sheep is highly in demand in the international market.[1] Their size is good; almost 80-90% are either large or extra large. In addition the price of the raw material is very low. Even though Wanke sheepskins have many economical advantages because of absence of appropriate technologies for solving the problems of Wanke sheepskin, it is treated as a reject for tanneries to process it to leather. Thus the main objective is to develop a process technology for making high value leather from Wanke sheepskins.

---

*Corresponding author e-mail: clrichem@mailcity.com; Tel: + 91 44 2441 1630; Fax: + 91 442491 1589.

Manuscript received October 4, 2013, accepted for publication February 25, 2014.

MATERIALS AND METHODS

Dry salted Wanke sheepskins were used for the study. Leather processing chemicals starting from soaking to crust preparation used were of commercial grade from different chemical companies.

Process Design and Standardization

The problem associated with the Wanke sheep skins have been understood after thorough study of the histology, SEM and chemical characterization (Part I).[2] Based on the observations made, process methodologies were devised to address the problem, and a number of trials were conducted to standardize the process for making high quality leather from Wanke sheep skins. The strategy was mainly aimed at solving the following issues viz;

- Proper degreasing
- Improving the thickness of Wanke sheep skin
- Improving the fullness of the leather
- Improving the softness of the leather
- Improving the strength properties of the leather

Physical Characterization of the Skin

Strength Characteristics

Wanke sheepskins were converted to crust leathers following the process strategy designed. The resulting crust leathers were tested for physical strength properties. Sampling and conditioning were done according to the standards ISO 2418:2005 and ISO2419:2005.[3,4] Mechanical properties such as tensile strength and elongation at break,[5] tear strength and tear load,[6] softness (with rim size of the ring at 25mm) were measured.[7] These properties of the leather were used to compare the leathers produced from Wanke sheep skin and those from Abyssinia highland sheep skin.

Organoleptic Properties

The organoleptic properties such as softness, fullness, roundness, smoothness of grain, uniformity of color and overall appearance of the crust leather were evaluated. The values range from 1-10; higher value represent better is the property. The result of the organoleptic property of the leather was used as the basis in planning further improvement in the overall property of the leather made from Wanke sheepskin.

Pilot Scale Trials for Making Wanke Garment and Ladies Upper Leathers

Optimized post tanning processes were employed to develop garment and ladies upper leather from Wanke sheep skins. 25 Wanke sheepskins were used for each pilot scale trial. The leather products were analyzed for physical and organoleptic characteristics.

RESULT AND DISCUSSION

Processing Strategy

The recipes for processing the skins were designed based on the available information on problems associated with Wanke sheepskin and also on the histological and chemical characteristics determined.[2] Particularly the high fat content, poor substance and rib marks were taken into consideration. Owing to the high fat content, thorough degreasing was planned. However, in order to make sure that Wanke sheep skin doesn't have double layer and that the layers should not separate into two when the fat between the layers is removed during processing (taking the case of New Zealand Merino sheep skin), pretanning was carried out before degreasing. This strategy would help in protecting the matrix from collapsing due to removal of excessive fat in Wanke sheepskin. The Wanke sheepskin was pretanned with a phenolic replacement syntan. After stabilizing the matrix with pre-tanning syntan, the skins were thoroughly degreased, repickled followed by chrome tanning and basification. After degreasing, the skins were subjected to beaming on wooden beams to mechanically degrease the skin. It was observed that without pretanning also there was no problem of double layer effect on Wanke sheepskins. Thus pretanning was not carried out for subsequent trials.

The next strategy was planned to improve the thickness of the Wanke sheepskins. In order to do so, the skins were subjected to longer liming. The duration of liming was close to 50 hours, which resulted in better opening up of the fibers and gave softer leather. The next strategy focused on improving the fullness of the leather, for which Wanke sheepskins were tanned using vegetable tanning materials. Subsequently, the chrome tanned and vegetable tanned Wanke leathers were retanned using proper choice of syntans and fatliquors to produce garment and upper leathers, respectively. The process recipes for making full chrome garment and semi chrome upper leathers are shown in Table I and II, respectively. The physical properties of the crust leathers processed using the optimized process recipe is presented in Table 3. From the table, it could be observed that the leather products satisfy the standard requirement of sheep garment and light use sheep upper. From the % elongation and the softness values it could be inferred that the full chrome leathers are much softer than the semi chrome leathers, and the former would be much suitable for garment or softy upper leathers and the latter would be suitable for making upper leathers. Scanning Electron Microscopic images of crust leathers produced from full chrome garment and semi chrome upper leathers are shown in Fig. 1 a and b, respectively. It could be observed from Fig.1a that the fibers are uniformly arranged, whereas the fibers of the semi chrome upper leathers are more compact and coated as observed in Fig. 1b._

TABLE I
Optimized process recipe for full chrome garment leather from dry salted Wanke Sheep skins.

Process	Percent	Chemicals	Time	Remark
Soaking	200	Water		
	0.1	Soda Ash		
	0.25	Preservative		
	0.1	Wetting Agent		Leave overnight
Painting	10	Water		
	2	Sodium sulphide		
	5	Lime		
Apply the paste uniformly on to the flesh side and pile it over night. Next day Unhairing				
Reliming	100	Water		
	10	Lime		
Dip the unhaired skins in this lime liquor for 2 days. Then do fleshing				
Deliming	100	Water		
	1	Ammonium Chloride	45’	pH< 8.0
Bating	0.25	Bating Agent	60’	Temp 36-38^{o}C
				Drain/wash
Pickling	100	Water		
	10	Salt	10’	^{o}Be 6-7
	0.2	Formic Acid		
	1	Sulphuric Acid	3x15’+40’	pH 2.0-2.5
				Two day ageing
Depickling	100	Water		
	8	Salt	20’	
	0.6	Sodium bicarbonate	20’	
	0.6	Sodium bicarbonate	20’	
	0.6	Sodium bicarbonate	20’	pH 4.5-5.0
Degreasing	4	Degreasing agent	2 hours	
Washed with 4% saline water 4 times and then Beamed				
	4	Degreasing agent	2 hours	
Washed with 4% saline water 4 times and then Beamed				
Repickling	100	Water		
	10	Salt	10’	
	0.2	Formic Acid		
	1	Sulphuric Acid	3x 10’	pH 2.6-2.8;

Table I continues on next page.

Table I continued.

Process	Percent	Chemicals	Time	Remark
				drain 50% liquor
Tanning	50	Water		
	7	Basic Chromium Sulphate	30’	
	100	Water	30’	
Basification	0.5	Sodium formate		
	0.5	Sodium bicarbonate	3x15’+45’	pH 3.6-3.8
	2	Phenolic replacement syntan	30’	
After piling for 48 hours, sam set and shave the leathers to a desired thickness (0.6 mm). Subsequently, post tanning was carried out.				
Add	200%	Water, 45°C		
	0.5	Formic Acid	30’	pH=3.0-3.3
				Drain/Wash/Drain
Re-chroming	100	Water, 40°C		
	3	Aliphatic Aldehyde	15’	
	5	Basic Chromium Sulphate	60’	
	1	Sulfited fish oil	20’	
	2	Sodium Formate	x5’+20’=4.0	
Neutralization	100	Water, 40°C		
	1	Sodium Formate		
	2.5	Neutralizing syntan	45’	pH 6.0/6.2
	0.3	Sodium Bicarbonate	45’	Drain/Wash/Drain
	100	Water, 45°C		
	3	Acrylic resin		
	2	Aromatic Sulphonic acid syntan	30’	
Dyeing	4	Red	60’	
Fatliquoring	50	Water, 60°C		
	5	Lecithin based fatliquor		
	4	Alkyl phosphates and neutral oils		
	6	Synthetic fatliquor	60’	
Fixation	2	Formic Acid (1:10 cold)	20’	
	1.5	Formic Acid (1:10 cold)	20’	pH 3.4/3.6
				Drain/Wash/Drain
		Pile and L/O/N Next day sam set and Hang over dry		

TABLE II
Optimized process recipe for semi-chrome upper from dry salted Wanke sheepskins. (Process from soaking to degreasing are similar to earlier Table I).

Process	Percent	Chemicals	Parameter	Time
Tanning	50	Water		
	5	Salt		10’
	12	Wattle tannin powder		60’
	12	Wattle tannin powder	Check Ø	60’
	50	Water		60’
	0.5	Formic Acid	pH=3.5	30’
		Pile O/N		
		Next day		
	50	Water		
	15	Myroblan		60-120
	0.5	Formic Acid	pH=3.0-3.5	30’
		Drain, Wash, pile O/N		
Stripping	100	Water		
	1	Sodium Sulphite		30’
		Drain, Wash, Drain		
	100	Water		
	1	Oxalic acid		30’
		Drain, Wash, Drain		
	50	Water		
	0.25	Formic Acid	pH=3.0	20’
Rechroming	6	Basic Chromium sulphate		
	4	Chrome syntan		
	1	Chrome stable synthetic fatliquor		
	1	Ester based fatliquor		
	1	Sulphited fish oil		60’
	4	Protein filler		60’

Table II continues on next page.

Table II continued.

Process	Percent	Chemicals	Parameter	Time
Basification	1	Sodium formate		
	1	Sodium bicarbonate	pH=4.0	3x10’+ 30’
		Drain/Wash/Pile		
Neutralization	100	Water		
	4	Neutralization syntan	pH=5.5	45’
	50	Water		
	0.25	Bio polymeric syntan		20’
Retanning	2	Acrylic resin syntan		20’
	2	Synthetic fatliquor		20’
	2	Phenolic replacement syntan		
	4	Melamine resin		
	4	Wattle tannin powder		
	2	Dispersing Agent		60’
Fatliqoring	50	Water		15’
	3	Sulfited fish oil		
	2	Synthetic fatliquor		
	8	Synthetic fatliquor		
	2	Lanloline based fatliquor		
	5	Lecithin based fatliquor		
	3	Semi synthetic fatliquor		
	0.1	Preservative		60’
	3	Wattle tannin powder		30’
	4	Formic Acid		3x15’+30’

TABLE III
Physical Characteristics of Crust Leathers resulted from optimized process.

Physical tests	Direction	Full Chrome		Semi Chrome	
		Abyssinia	Wanke	Abyssinia	Wanke
Tensile Strength (Mpa)	Parallel	20±0.50	20±1.00	19±2.00	23±1.00
	Perpendicular	12±0.50	16±0.50	16±2.00	18±1.00
Elongation (%)	Parallel	38±0.50	44±0.50	35±1.00	42±0.50
	Perpendicular	64±0.50	43±1.00	43±2.00	40±0.50
Tear Strength (N)	Parallel	27±0.50	24±1.00	31±1.00	27±1.00
	Perpendicular	36±1.00	25±1.00	26±0.50	21±1.00
Softness		4.57±0.13	5.77±0.33	4.9±0.20	4.93±0.46

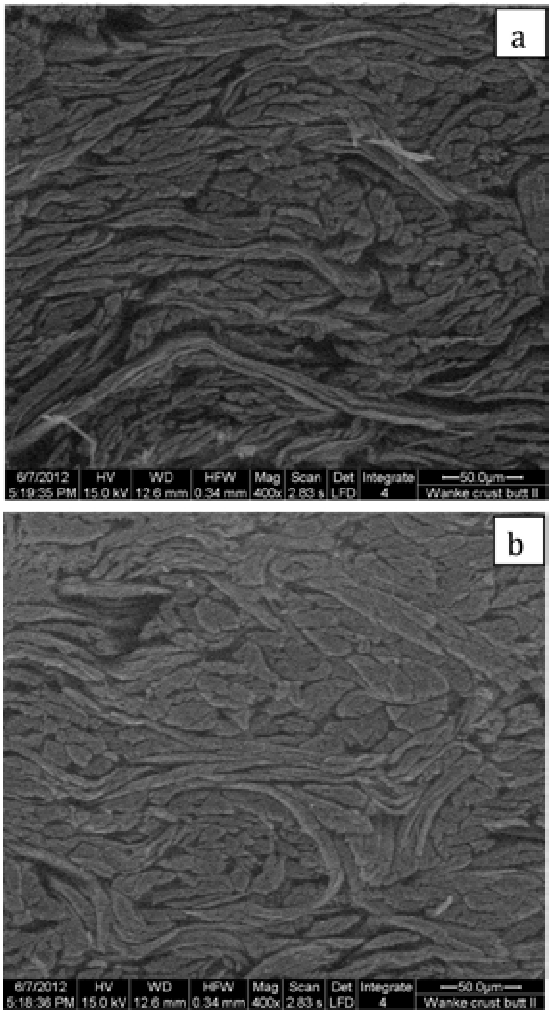


Figure 1. SEM image of cross section of Wanke leathers at crust stage (a) full chrome garment leather and (b) semi-chrome upper leather.

Organoleptic Properties

Leathers produced after thorough degreasing followed by chrome tanning and improved post tanning gave better fullness and softness suitable for garment leather. On the other hand, the leathers produced by semi chrome process and improved post tanning showed best results in terms of fullness and softness, which are suitable for upper leathers. Evaluation of the properties such as softness, fullness, roundness, smoothness of the grain and general appearance of crust leathers for full chrome and semi chrome leather were made and the results of the evaluation are presented in Table 4. It is observed from the organoleptic properties that both the type of leathers (Full chrome and semi chrome) produced from Wanke sheepskins exhibited better bulk properties.

Pilot Scale Trials for Making Wanke Garment and Ladies Upper Leathers

The optimized recipe as provided in Table 1 and 2 were employed for processing 25 samples of Wanke sheepskins to garment leathers and ladies upper leathers, respectively. Developed leathers showed better bulk properties and strength characteristic on par with the results obtained in lab scale trials. In order to establish the efficient utility of these leathers, it was proposed to develop garments and ladies shoes using the developed leathers.

The physical characteristics carried out on full chrome and semi chrome leathers made from Wanke sheepskins are tabulated in Table 5 and 6, respectively. From the table it is observed that full chrome and semi chrome Wanke crusts leathers showed improved strength properties on par with the standard range for upper and garment leathers. Slight improvement in the thickness of the semi chrome leather was also observed, which could be attributed to the vegetable tanning of the leathers. Table 7 shows the organoleptic properties of garment and upper leathers processed in pilot scale trails. It could be observed that the properties of leathers were comparable to that of the results obtained in experimental trials.

TABLE IV
Organoleptic properties of Crust Leathers resulted from optimized process.

Parameters	Full chrome		Semi chrome	
	Wanke	Abyssinia	Wanke	Abyssinia
Softness	9	9	9	9
Fullness	9	8	9	9
Roundness	8	8	8	8
Smoothness of grain	8	9	8	7
Uniformity of color	8	8	8	8
Overall appearance	9	9	9	9

TABLE V
Physical Test Results of full chrome Wanke sheep garment leather (Pilot scale trials).

Physical Tests	Test Result
Tensile strength (N/mm^2)	29±1.00
Percent Elongation	94±2.00
Double Edge tear load-parallel (N)	30.40±1.05
Double Edge tear load-perpendicular (N)	32.10±1.20
Average double Edge tear (N)	31.30±1.80
Double edge tear load (N/mm)	46.60±1.10
Single Edge tear load-parallel (N)	12.60±1.60
Single Edge tear load-perpendicular (N)	18±2.00
Average single edge tear load (N)	14.90±0.80
Single edge tear load (N/mm)	20.60±0.90

TABLE VI
Physical Test Results of semi-chrome Wanke sheep upper leathers (Pilot scale trials).

Physical Tests	Test Results
Tensile Strength (MPa) Parallel	23±1.00
Tensile Strength (MPa) perpendicular	18±1.00
Elongation (%) Parallel	42±0.50
Elongation (%) Perpendicular	40±0.50
Thickness (mm)	0.75
Tear Strength (N) Parallel	27±10
Tear Strength (N) Perpendicular	21±10
Softness	4.93±0.50

TABLE VII
Organoleptic property of Wanke leathers.

Parameters	Full Chrome (for Garment)	Semi Chrome (for Upper)
Softness	9	9
Fullness	9	9
Roundness	8	8
Smoothness of grain	8	8
Uniformity of color	8	8
Overall appearance	9	9

Figure 2. Leather and products developed from Wanke sheepskins.

Ladies garment and a pair of ladies shoes were designed and developed in the design studio of Leather Industry Development Institute, Addis Ababa, Ethiopia. Developed products are shown in Fig. 2. The designers did not face any problem in handling the leathers during the preparation of garment or footwear.

CONCLUSIONS

Leather making from Wanke sheepskin was limited to low value leathers. The problem of this skin to make value added leather, such as shoe upper and garment leather, existed for many years; but scientific approach to deal with the problem was not followed to solve the problem. This research report has tried to understand the problem and the possible solution scientifically. Characterization of Wanke sheepskin through various scientific tools led to understanding of the problem associated with the skin and development of suitable process strategy to make value added leather. Leathers made from Wanke skins through full chrome process exhibit good softness, fullness, fine grain and improved strength properties suitable for softy upper and garment leather. On the other hand, the semi-chrome processing of Wanke skins resulted in leathers with uniform fullness, roundness and fine grain characteristics suitable for upper leather.

ACKNOWLEDGEMENTS

The work has been carried out as a part of Twinning Project between CSIR-Central Leather Research Institute, Chennai, India and Leather Industry Development Institute, Addis Ababa, The Federal Democratic Republic of Ethiopia. (CSIR-CLRI communication no.:1037)

REFERENCES

1. Berhanu, G., Hoekstra, D., Samson, J.; Heading towards commercialization? The case of live animal marketing in Ethiopia. Improving Productivity and Market Success (IPMS) of Ethiopian Farmers Project Working Paper 5. ILRI (International Livestock Research Institute), Nairobi, Kenya. 2007.
2. Mohammed, H., Aysanew, G., Aravindhan, R., Gnanamani, A., Raghava Rao, J. Chandra Babu, N.K.; Studies on Ethiopian sheep skins as an opportunity for value addition Part I: Histological, microscopic and chemical characterization of Abyssinian and Wanke sheep skins, *JALCA* **109**, 76-81, 2014.
3. ISO 3376:2002, Standard test method for determination of Tensile strength of leather.
4. ISO 3377:2002, Standard test method for determination of Tear strength and Tear Load.
5. IUP 36, Measurement of leather softness.
6. SLC 9 (IUC 9), Substances extractable with dichrolomethane, Official methods of analysis. The Society of Leather Technologist and Chemists, Northampton, UK, 1996.
7. IUC 10, Determination of nitrogen and hide substance.

LIFE LINES

Jianzhong Ma, professor, received his Doctor of Polymer Chemistry and Physics Degree from Zhejiang University, China, in 1998; and then did his postdoctoral research at Eastern Regional Research Center of United States Department of Agriculture from 1999 to 2000. His main research interests are synthesis and preparation mechanism of polymer additives (leather chemicals), preparation and application of inorganic-organic composite materials.

Xueyan Hou studied at the Shaanxi University of Science & Technology since 2006, majoring in leather chemistry and engineering. In 2010, she joined the group of Prof. Jianzhong Ma. In 2012, she began her doctoral research focusing on the stabilization of enzymes and their application in the leather industry.

Dangge Gao, associate professor, received her doctorate in 2009 in Shaanxi University of Science & Technology, Xi'an, China. After she finished her doctoral study, she worked in Shaanxi University of Science & Technology and focused on inorganic-organic composite materials in leather and textile.

Jing Zhang, senior lecturer at Shaanxi University of Science & Technology, has a MA in English linguistics from South-Central University for Nationalities. Her areas of research and teaching include Applied Linguistics, Comprehensive English (for English Majors), English for Non-English Major Postgraduate with particular interest in TEFL and TESOL.

Urana Dandar, born in Russia Federation Republic of Tuva, graduated from Ege University Leather Technology Department, Turkey, in 2002. She received a MSc degree in 2005, and presently is a PhD student in the Leather Engineering Department. She is working on shoe upper leather, fur production, leather sludge, utilization of leather wastes, mobility of chromium and elimination of harmful substances in leather production. She has been working as a specialist at Leather Engineering Department since 2013.

Selime Menteş Çolak is an Associate Professor in the Department of Leather Engineering at Ege University, Turkey. She has been working in the same department since 1988. Her research areas are utilization of leather waste, biodiesel production, cleaner production for leather, rawhide and leather processing technologies.

Remziye Deveci is an Assoc. Prof. Dr. in the Department of Biology, Section of Molecular Biology at Ege University, Turkey. Her research areas are cell biology, developmental biology and glycobiology. She has been using histology and histochemistry techniques since 1983, transmission electron microscopy techniques since 1991, antibody/lectin labeling techniques in light, floresans and transmission electron microscopy since 1995 and immunoblotting and lectin-blotting techniques since 2007.

Arife Candas Adıgüzel Zengin rceivd a MSc in Leather Engineering from Ege University, Turkey, in 2004 and a PhD from the same university in 2010. She started working as a research assistant in Leather Engineering Department in 2001, and was at the Aalborg University, Denmark, to perform her PhD studies between 2006 and 2007. Her field of interests includes enzyme production and applications in leather engineering, biotechnology, cleaner processing methods for leather, and leather processing technologies.

Gökhan Zengin earned his MSc and PhD degree from Ege University, Turkey. He was research assistant, 1999-2013, assistant professor since 2013 and obtained his Associate Professor degree at January 2014 at Leather Engineering Department of Ege University. He's research areas are the characterization of animal skins, environmental cleaner technologies, by-products of collagenic materials, evaluation and optimizations of the leather processes, elimination of the leather wastes and historical leathers.

Jianfei Zhou received his M.Sc. degree in applied chemistry at Shaanxi University of Science & Technology in 2006. After graduating, he worked as a faculty member at Wenzhou University. Now he is undertaking his PhD research at Sichuan University. His research focuses on treatment of leather waste.

Yanan Wang received his Ph.D. degree in Leather Chemistry and Engineering at Sichuan University, China, in 2013. After graduating, he joined Sichuan Univerisity as a faculty member. His research interests include tanning chemistry and clean technology of leather manufacture.

Wenhua Zhang, see *JALCA* **100**, 447, 2005

Bi Shi, see *JALCA* **99**, 220, 2004

Mohammed Hussien Seid is working as a Senior Leather Technology Researcher at the Leather Industry Development Institute (LIDI), Addis Ababa, Federal Democratic Republic of Ethiopia. Presently, he is pursuing his MSc (Leather Technology) at CSIR-Central Leather Research Institute (CLRI), India, under the Twining program between CSIR-CLRI and LIDI.

Aysanew Gorems Melesse is working as Leather Technology Researcher at Leather Industry Development Institute (LIDI), Addis Ababa, Federal Democratic Republic of Ethiopia. Presently, he is pursuing his PhD at CSIR-Central Leather Research Institute (CLRI), India, under the Twining program between CSIR-CLRI and LIDI.

R. Aravindhan, see *JALCA* **106**, 208, 2011

J. Raghava Rao, see *JALCA* **93**, 156, 1998.

N. K. Chandrababu, see *JALCA* **103**, 401, 2008.

ALCA 110TH ANNUAL CONVENTION

JUNE 18-20, 2014

GIDEON PUTNAM RESORT, SARATOGA SPRINGS, NY

Welcome to an exciting venue of events that will be unfolding for us from June 18 to June 20, 2014. We will host the 110th Annual Convention at the beautiful Gideon Putnam Resort at Saratoga Spring, New York. It is a first class facility that you can learn more about by logging on to their website at **http://www.gideonputnam.com**.

Again this year will be an email around mid March containing all details about the convention. Also voting for our Slate of Candidates will be done via email in late April. The online convention registration form has been updated and is ready for use or you can print out a form and send it to us. Visit **www.leatherchemists.org/annual_convention.asp** to download the registration form. Continue checking this website for new information and announcements about the 110th Annual Convention.

The schedule this year is entirely new. Prior to the official opening of the convention, the annual golf tournament will be held Wednesday, June 18, at the Saratoga Spa Gold Course beginning at noon. Pre-registration for golfers is a must to facilitate the start of the tournament. Further information on the golf outing will be found under the golf tab under this section.

The official opening of the convention will begin with Registration on Wednesday, June 18, from 5 to 7 pm in the Lobby followed by a Cocktail Reception and Dinner on the Gideon Patio. It will be a great time to have conservations with old and new associates in the leather industry.

ALCA President Steve Lange will open the Technical Program at 8 am on Thursday, June 19. This year's technical program is being organized by Vice-President Sarah Drayna and will offer a wide array of leather technologies covering tanning to finishing to environmental issues can be viewed in the coming months under this section of our website under Technical Program. The John Arthur Wilson Lecture will feature Jakov Buljan of Croatia. The title of his presentation is "Some Considerations about International Technical Cooperation in the Tanning Sector." The technical sessions will end at 4:45 pm followed by the Annual Fun Run at 5:30 pm. Thursday's activities will be capped off by a cocktail party at Portico from 7:15 to 8 pm followed by the Awards Banquet from 8 to 10 pm at the Arches. This will be an exciting evening that you won't want to miss.

Technical papers will resume Friday morning at 8 am with the Annual Business Meeting ending the morning sessions. At noon everyone is invited to attend the Sports Activities Awards Luncheon at the Arches from 12:15 to 1:15 pm. Prizes will be awarded for the Fun Run and golf outing as well as door prizes. The convention will close after the Sports Activities Awards Luncheon.

Please make plans now to join us for a wonderful time at the Gideon Putnam Resort.

Doug Morrison
Convention Chair

JOB POSTING

INTERNATIONAL MARKETING MANAGER AT TEXPAC HIDE & SKIN LTD. FORT WORTH, TX

TASKS: Develop international marketing plans; oversee research & analysis of global trade data & sales to Asian tanneries; manage inventory control (production/distrib. analysis & distrib. schedule) & cost/benefit analysis (determine industry FOB prices); manage QA. REQUIRED: MS in Management OR BS in Management + 5 yrs' exp. in project and staff management; Fluency in Mandarin Chinese; Travel to China 2-3 times per yr.

Send resume to **jzitnik@texpacftw.com.**

ALCA 110th ANNUAL CONVENTION

Tentative Technical Program Starting Thursday, June 19, 2014

Partial Listing of Oral Presentations
(Subject to Change — check this section of our website for updates on the technical program)

John Arthur Wilson Memorial Lecture
"Some Considerations about International Technical Cooperation in the Tanning Sector"
by Jakov Buljan, Leather Consultant, Ex-Chief of UNIDO Leather and Leather Products Unit, Zagreb, Croatia

Eagle Ottawa LLC Sponsored Speaker
"The Significance of Getting the Science Correct When Fighting for the Leather Industry"
by Mike Redwood, LeatherNaturally!, UK

Discussion Panel
with Stephen Sothmann /USHSLA, Dietrich Tegtmeyer/Vice President of the IULTCS
and John Wittenborn/Leather Industries of America, Washington, D.C.
Topics to Include Current Industry Positions on Carbon Footprint

End Users Panel
with Kerry Brozyna/Wolverine and Other Major Companies

Discussion Panel of Machine Suppliers
including Emiliano Valle/Vallero International and Alessandro Burlando/Alpe/bService

Other Papers to be Presented on Thursday and Friday:

Chemistry of Syntans and Their Influence on Leather Quality by Jochen Ammen, BASF, Ludwigshafen, Germany

Powdered Hide Model for Vegetable Tanning. II Hydrolysable Tannin by Eleanor M. Brown, United States Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA

Chrome Update by Eric Dick, Elementis LTP Inc., Milwaukee, WI

Understanding the Enzyme-Assisted Beamhouse Process by Marcelo Fraga de Sousa, Buckman Laboratorios Ltda., Brazil

Prospects and Development of Innovative Technologies for Leather Production in the Republic of Uzbekistan by T.J. Kodirov, Tashkent Institute of Textile and Light Industry, Uzbekistan

Preparation of Nonwoven and Green Composites from Tannery Solid Wastes by Cheng-Kung Liu, United States Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA

Overview of Brazilian Cattle Chain, the Demands for Animal Protein, and the Forecast for the Future Years by Guilherme Motta, JBS Brazil, Brazil

Development of an Alternative Low Salt Bovine Hide Preservation Using PEG and Crude Glycerol by Mila Aldema-Ramos, United States Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA

Utilization of Agricultural By-Products to Supplement Gelatin in Preparation of Products for Leather by Maryann M. Taylor, United States Department of Agriculture, Eastern Regional Research Center, Wyndmoor, PA

Multifunctional Effects of Collagen and Keratin Based Biopolymer Colloid Systems and Their Cosmetic Applications by Jan Sedliacik, Technical University in Zvolen, Slovakia

Leather in the 21st Century by Simon Yarwood, World Trades Publishing/World Leather Magazine, UK

THE 55TH JOHN ARTHUR WILSON MEMORIAL LECTURE

by

JAKOV BULJAN

Jakov Buljan, leather consultant and ex-Chief of UNIDO Leather and Leather Products Unit, will present the 55th John Arthur Wilson Memorial Lecture at our Annual Meeting at 8 am on Thursday, June 19, 2014. The title of his presentation is:

Some Considerations About International Technical Cooperation in the Tanning Sector

ABSTRACT

Growth rates of the past and projections for the future point towards substantial livestock to support a strong tanning sector as well as towards an increasing gap between the raw hides and skins available and the assumed growing needs of the human population. The amount of pollution generated by the tanning industry is also significant. Taking into account the salt for curing, chemicals for processing and wastewater treatment, the ratio of chemicals used to fresh hide/skin weight is approx. 1:1. Furthermore, the major part of the chemicals added is actually not retained in leather; based on the present global input of approx. 10 million tons w.s. hide and skins weight the amount of process chemicals discharged into is of the order of some 7.0 million tons/year. Some 360 million $m^3$of wastewater discharged/year contain about 1.8 million tons of COD, 1.2 million tons of Total Kjeldahl Nitrogen (TKN), 0.5 million tons of Cr, and, worst of all, more than 4.5 million tons of Total Dissolved Solids (TDS).

Relocation of the substantial part of the tanning and downstream industry from North to South (*the big shift*) in recent decades also meant relocation of pollution for which developing countries were quite unprepared. Many of them have turned to United Nations Industrial Development Organization (UNIDO) for help. UNIDO is a specialized agency from the UN family, with headquarters in Vienna, Austria. Its mandate is to promote sustainable industrial development as an important driver of economic growth and thus contribute towards eradication of poverty. Sustainable development also incorporates the social and industry-related environmental and energy challenges. As of 1 January 2014, the Organization had 171 Member States and employed close to 700 staff at Headquarters and other established offices. UNIDO's regular budget, covering staff and operating costs, is funded by contributions from its Member States assessed along the principles of the UN system, the largest contributor being Japan. Programs and projects are mainly funded through voluntary contributions from donor countries and institutions (the largest contributor is the EU), as well as from multilateral funds. External inputs are always are complemented by substantial local financing. Experience has shown that due to its inherent features multilateral cooperation is often more efficient and preferred over bilateral.

Some typical projects developed and implemented by UNIDO Leather & Leather Products Unit tackling pollution prevention, wastewater treatment, finishing, occupational safety and health, gender equality, ecolabelling etc. In the last 30 years, UNIDO was to some extent involved in tackling up to 20% of all tannery effluents generated in developing countries; its particular experience is in dealing with Common Effluent Treatment Plants (CETPs) servicing old or new tannery clusters. It is recognized that coagulation and flocculation as well as sludge conditioning before dewatering, crucial steps in wastewater treatment as performed today, would be impossible without the pioneering work on colloids by J.A. Wilson.

Training tailored to the specific needs of recipients is an essential part of technology transfer. Modern means of communications offer fundamentally new possibilities of learning, especially for the young, "click & slide" generations. Five modules of the *Animated Visual Training Tool (AVTT),* supplementing the booklet *Introduction to the treatment of tannery effluents* have been made available for wider public use.

A difficulty encountered in many projects was the (mis)perception that advanced cleaner technologies can limit end-of-pipe treatment to some simple and cheap procedures.

A very special feature of UNIDO activities is its Leather and Leather Products Industry Panel, a global forum comprising some 20 reputable specialists from all walks of trade. The Panel's primary function is to review and suggest the relevant topics and priority issues to be addressed by UNIDO TA. The latest paper produced for the Panel was the Carbon Footprint study for the leather industry, Shanghai 2012. UNIDO is traditionally a significant contributor to IULTCS/IUE documents and reference source for EU Best Available Technology (BREF) norms.

Nowadays, UNIDO assistance to the tanning industry remains that of a globally present catalyst focused on environmental issues but increasingly acting in the e-World.

The most pressing challenges confronted by the industry and the future of leather as material (e.g. durability vs. fashion changes/ *short-termism/consumerism*) is also briefly discussed.

Relocation of capacities in different directions in a globally free trade is a continuous process requiring high flexibility; the transfer North to South in both tanning and leather products is already being replaced by South-to-South movement. In any case, pollution prevention and treatment of waste remain the key ingredients of international technology transfer; eventually, it also helps leveling the competition ground. Ultimately, cooperation is much more than solidarity: development with all its spin off benefits (employment, education, better living standards, more balanced population growth etc.) is the *conditio sine qua non* for ensuring global stability.

OBITUARY
HAROLD D. DIEPHOUSE

Former Grand Haven resident Harold Diephouse, aged 65, passed away in his home in Waterloo, Iowa, on March 14, following a brief battle with cancer.

Harold was born on October 30, 1948. He graduated from Fruitport High School in 1966.

Harold began his career in the leather industry in 1968 with Eagle Ottawa Leather Company, Grand Haven, Michigan, as a production worker. He joined the technical team in 1971 obtaining his hands on experience and training from Arthur Hirsch and Hans Gassman. He was soon promoted from Lead Leather Technician to Wet End Technical Manager and further to Wet End Technical Manager World Wide, where he was responsible for all of Eagle Ottawa's Wet End processing. In 1992 he joined Horween Leather Company in Chicago, Illinois as Technical Manager. Harold returned to Eagle Ottawa in 1995 as Technical Manager for Eagle Ottawa Milwaukee and was transferred to Eagle Ottawa Waterloo, Iowa. In 2005 Harold joined GST AutoLeather as Supplier Quality Engineer and was promoted to Corporate Supplier Quality Manager having responsibilities for incoming materials to all GST locations. In 2011 he went to work for Twin City Tanning having retiring from there in 2012.

Harold was very active in local youth sports as a coach. From 1973 to 1078, he coached peewee football for the Tri-Cities Football League. During that same time period he also coached little league baseball for the Tri-Cities Kids League.

In 1981, Harold began coaching track and field. As the head coach of the Lakeshore Track Club, Harold led his team to consecutive Athletic Congress National Championship meets in Merced, California in 1981 and Omaha, Nebraska in 1982.

In the fall of 1982, Harold returned to the sidelines as the head coach of the Grand Haven High School's freshman football team.

In 1984 Harold became the assistant coach of the Spring Lake High School Girls Cross Country Team, and became their head coach for the 1985 and 1986 seasons. His love of track and field culminated in the opening of The Athlete's Shop in Grand Haven, the first local sporting goods store to carry a then unknown brand of running shoes called Reebok.

Harold also enjoyed restoring classic cars and was a frequent exhibitor at car shows all over the mid-west.

Harold joined the ALCA in 1989, having been a member for over 23 years before retiring. He was a member of the Education Committee since 2002 and served on the O'Flaherty Award Committee in 2008. His education includes 2 years at Muskegon Business College in Business Management, 1 year at Hamilton College in Business Management, and a technical certificate from the ALCA.

Harold is survived by his wife Rita Ann (Waterloo, IA), his sons Timothy Paul (Connie) of Nunica, Jeremy Garth (Grand Rapids), and Adam John (Waterloo, IA), four grandchildren; one sister, Donna Middlebrook (Porters Corners, NY) and two brothers, Ralph (Sarasota FL) and Bobby (Cincinnati, OH).

INTERNATIONAL UNION OF LEATHER TECHNOLOGISTS AND CHEMISTS SOCIETIES

NEWS RELEASE FROM THE IULTCS

14 MARCH 2014

SCIENTIFIC FACTS ABOUT CHROMIUM: NOW AVAILABLE IN 6 LANGUAGES

The presentation, "***Chromium and Leather Research: A balanced view of scientific facts and figures***" has generated much interest in the leather industry and received wide acclaim. The IULTCS Executive Committee has received reports from around the world where this material has been used successfully to calm fears and ensure meaningful debate with various people and organizations with concerns about use of chromium tanning compounds. Chromium III, which is used exclusively by the leather industry, can be used safely and the formation of unwanted chromium VI can be managed. To ensure this happens is the responsibility of all in the leather industry.

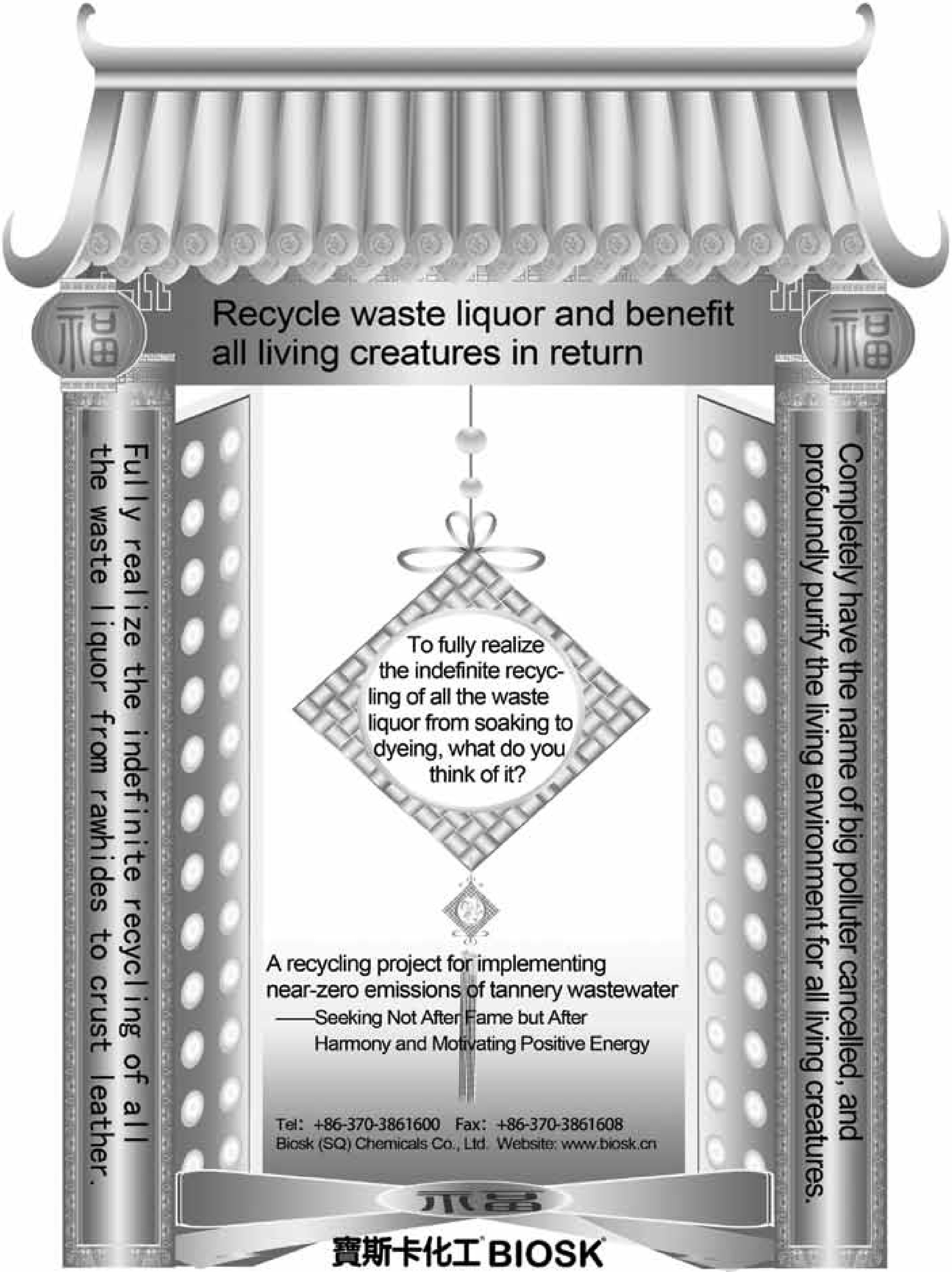
To ensure better understanding of chromium chemistry, the abovementioned presentation has now been made accessible to a wider audience. The IULTCS has translated the material into 6 languages. Versions in English, Chinese, Spanish, Turkish, Japanese, and Portuguese are now available on the IULTCS website under the section "IULTCS Information Releases" (see **www.iultcs.org**).

The next meeting of the IULTCS Executive takes place on March 31st during the APLF in Hong Kong. One issue to be discussed — and one that is of much importance for the industry — is the development by the International Union Chemical Commission of a standard for "*Critical Chemical Substances in leather*" along with appropriate leather test methods. Increased testing and Restricted Substances Lists (RSL's) that have little meaning or relevance for leather is an increasing burden for the industry. During the IULTCS meeting, the executive will also review updates and progress from its other Commissions involved in leather testing, environment, training, and research. The Executive Committee will also approve final plans for the 10th "*Asian International Conference on Leather Science and Technology*" which will be held in Okayama, Japan in November of this year and review plans for the next IULTCS World Congress which is scheduled for Brazil in 2015.

End News Release

For editorial Information: Elton Hurlow
Email: **elhurlow@buckman.com**
Tel: **+1 901 272 6689**

PRESIDENT: Dr. Patricia Casey, Lanús Este, AR-1824 Buenos Aires, Argentina. • E-mail: **pcasey@fonseca.com.ar**
EXECUTIVE SECRETARY / TREASURER: Dr. Campbell Page / IULTCS Secretariat, c/o VESLIC, Postfach 505, CH 4016 Basel, Switzerland • Website: **www.iultcs.org** • E-mail: **office@iultcs.org**



Few people realize that Leather Making is the world's oldest manufacturing process, thus the world's oldest industry. Tanning—the process of converting hides and skins into leather—is also the world's first science. Also, because of the pure craftsmanship involved, tanning may well be the world's first art form.

Anyone who doubts that a sheepskin has up to 30,000 fibers per square inch has only to count them.

NOTHING TAKES THE PLACE OF LEATHER

INDEX TO ADVERTISERS

Biosk	IV
Buckman Laboratories	Inside Front Cover
Chemtan	III
Chemtan	Back Cover
Chemtan	Inside Back Cover
Elementis	V
Stahl	II
Union Specialities, Inc.	VI