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Valorization of Pomegranate Peel Waste as Retanning Agent in Leather Industry and Investigation of Its Effect on Prevention of Cr(VI) Formation

by

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Abstract

Although Cr(VI) is not used in any step of the manufacturing process, the entity of Cr(VI) in leather and leather products has become a concern in the leather industry. Formation of free radicals may bring about conditions for the oxidation of free Cr(III) (if exists in leather) to Cr(VI) and these oxidations are usually catalyzed by heat and UV radiation. Antioxidants have an important role in order to inhibit oxidant compound activity by donating an electron to compounds. There are many studies on the prevention of Cr(VI) formation in leather production. The inhibiting properties of Cr(VI) formation were usually investigated by using commercial vegetable tannins (polyphenols) together with other retanning agents in retanning process. However, no study has been conducted on the possible use of pomegranate peel tannins as an alternative retanning agent, which is known as an important source of antioxidants, in leather making and their ability to prevent Cr(VI) formation. In the present study, pomegranate peels were extracted in different solvents and total phenolic content and antioxidant power of the extracts were determined by Folin-Ciocalteu, FRAP (The Ferric Reducing Ability of Plasma) and TEAC/ABTS (Trolox-Equivalent Antioxidant Capacity/2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) methods. Furthermore, different amounts of pomegranate peel extracts were used in retanning processes and the leathers were investigated in terms of increases in thickness, color changes as well as effect on prevention of Cr(VI) formation. The results revealed that besides noticeable retanning performance, pomegranate peel extract has a spectacular effect on prevention of Cr(VI) formation as compatible with its antioxidant power.

Introduction

Chrome tanning is the most favored technology in the leather industry worldwide due to its ability to produce leather with attributes desirable for high-quality leather such as excellent hydrothermal stability, better dyeing characteristics and softness. So much so that today approximately 80-90% of leather tanned is chromium based. However, the technology has come under censure globally for its severe environmental impacts and adverse effects on human health and other organisms.[1] Cr(III) salts, mainly chromium (III) hydroxide sulphate ($Cr(OH)SO_4$), are ordinarily used in the chrome tanning process. Although Cr(VI) compounds are not used in tanning process, the chemicals used in production and other factors such as a chemical reaction facilitated by heat, UV radiation, changes in pH and oxidizing agents etc., may cause oxidation of Cr(III) to Cr(VI) upon ageing of leather products. These deformations generally emerge due to the oxidant group free radicals, which are dependent on these factors.[2] Antioxidants interact with free radicals, terminating the adverse chain reactions and converting them to harmless products.[3] Plant-derived antioxidants (vegetable tannins) are active substances obtained from natural plants through extraction and sometimes purification techniques.[4-6] They mainly include polyphenols, flavonoids, terpenoids and aromatic compounds and their composition can vary widely depending on the characteristics of the plants and the extraction procedures.[5,6]

Vegetable tannins (plant-based polyphenols) are used in tanning and retanning processes in leather making due to the fact that they are water soluble and easily interact with collagen predominantly via multiple hydrogen bonds. Mimosa, quebracho, sumac, tara, valonea and chestnut are the most common vegetable tannins used commercially for this purpose. In recent years, researchers have attempted to explore the use of new plant materials for tanning/retanning processes.[7-11] However, there is no report regarding the use of pomegranate peel extract as a retanning agent in leather making for prevention of Cr(VI) formation.

Pomegranate (Punica granatum L.) belongs to Punicaceae family and it is an important fruit crop adaptable to a wide range of agro-climatic conditions. Pomegranate fruit is mainly grown in Iran, Tunisia, Turkey, Spain, Egypt, Morocco, USA, China, India, Argentina, Israel and South Africa.[12,13] Pomegranate peel (PoP) includes approximately 30–40% portion of the pomegranate fruit and remains as a byproduct after juicing the fruit.[13,14] Large parts of fruits are wasted in processing industries (which can amount to fifty percent in case of pomegranate) causing environmental pollution. PoP is considered a waste, however it is composed of notable polyphenolic compounds such as phenolic acids, anthocyanins and tannins (ellagitannins, punicalagin, punicalin and gallotannins).[15] PoP color is primarily due to the presence of anthocyanins (one class

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of flavonoids).[13,16] Owing to this, it possesses a higher antioxidant activity than the edible portion (arils).[13,17,18] Indeed, PoP holds significant free radical scavenging, anti-microbial, antiatherogenic and antimutagenic properties.[19-23] Therefore, PoP should be considered as a co-product with juice extraction and not a waste.[15,24] In recent years, there has been an increase in interest for beneficial phytochemicals present in fruit peels and utilization of them in food, pharmaceutical and cosmetic industries.[13]

The aim of this study is to investigate the possible utilization of pomegranate peels as a valuable new source of tannin for leather industry.

Materials and Methods

Materials

The pomegranate peels used in the trials were supplied from a food company operating in Torbali/Izmir. The peels were rinsed with distilled water so as to remove dirt (dust, mud etc.). Then they were dried in ambient conditions for one day, and then in an oven at 40°C for two days. The dried peels were broken into pieces of about 0.5-1 cm and kept dry, in the dark, and at ambient temperature.

Wet-blue sheep leathers (breed of Métis) were used in retanning experiments.

Methods

Determination of moisture content of pomegranate peels: The moisture content of pomegranate peels was determined according to SLC 113[25] test method with three replications and mean data was given as result.

Preparation of extracts: 20 g dehumidified pomegranate peels were pre-soaked overnight in 400 mL distilled water before the extraction process. In order to determine the effect of temperature on extraction efficiency and tannin content, pomegranate peels were subjected to Koch extraction separately for 8 h at 70°C and 90°C. Water was used as solvent in the extractions.

Determination of maximum extractable materials (MEM) from pomegranate peels: Maximum extractable material from pomegranate peels was determined according to the method described by Sivakumar et al.[26] with slight changes. Twenty grams of pomegranate peels were soaked in 400 mL of distilled water (Float-I) in a clean flask and magnetically stirred at constant temperatures of 70°C and 90°C for 8 h, separately. The flask was covered with aluminum foil to prevent water evaporation.

Extract solution was filtered after 8 h, then the total solids of Float-I was determined. Remaining pomegranate peels were re-extracted similarly for following two days, and samples were taken every day after 8 h of extraction and total solids were also determined for each filtered extract solution (Float II and Float III). The total maximum soluble material per gram of the pomegranate peels was calculated as MEM.

Determination of extraction yields and tannin contents of extract solutions: Extraction yields and tannin contents of solutions obtained from both Koch extractions and MEM experiments (total solutions collected in 3 days) were determined.

Extraction yields were calculated according to the Formula 1. The extraction trials were repeated three times and mean data were given.

$$(\%) \text{ Extraction Yield} = (\text{extract obtained (g)} / \text{pomegranate peels used (g)}) \times 100 \qquad \text{(Formula 1)}$$

The pomegranate peel extracts were analyzed to determine the amounts of total solids (SLC 114[27]), total solubles (SLC 115[28]), non-tannin content (SLC 116[29]), and tannin matter absorbable by hide powder (SLC 117[30]). Analyses were performed in triplicate and mean data were given.

Total phenols and antioxidant powers of pomegranate peels: Before the analyses, pomegranate peels were ground using a tilting grinder machine (Super Mixer SM 108 Model). A 0.1 g sample of pomegranate peel powder was extracted overnight in 100 mL of solvent in dark ambient before the total phenol and antioxidant analyses. In order to determine the possible effects of different solvents on extractable total phenols and correspondingly antioxidant power of pomegranate peels, water (100 mL) and water:ethanol (50:50 mL), water:methanol (50:50 mL), water:acetone (50:50 mL) mixtures were used as solvent. All analyses were carried out with two replications.

The total phenolic content of pomegranate peels was determined by Folin-Ciocalteu method, which was adapted from Swain and Hillis[31], at 725 nm with a spectrophotometer (Shimadzu UV-1601 Spectrophotometer) and quantification was done on basis of the standard curve of gallic acid concentration ranging between 15 and 400 μmol/L (r^2=0.9929). The amount of the total phenolic content is expressed as mg of gallic acid equivalent (GAE) in g extract.

As is known, there is no single universal method to reliably investigate antioxidant powers of plant materials. Therefore FRAP (Iron (III) Reducing Antioxidant Power)[2,32,33] and TEAC/ABTS (Trolox Equivalent Antioxidant Capacity)[2,34] methods were used in order to obtain comparable and reliable results for determining antioxidant and antiradical activities of pomegranate peel extracts.

In the method of Trolox Equivalent Antioxidant Capacity (TEAC/ABTS), the antioxidant activity of pomegranate peel extracts was determined at 734 nm with a spectrophotometer and the quantification was performed according to the standard curve of Trolox concentration ranging between 100 and 1500 µmol/L (r^2=0.9943). FRAP method was also carried out in the same way, standard curved was obtained with Trolox ranging from 100-1200 µmol/L (r^2=0.9970), UV measurements were done at 593 nm. In both methods, the antioxidant power is expressed as mg of Trolox equivalent (TE) in g extract.

Usage of extract solutions in retanning process: From the comparison of yields and tannin contents of extract solutions obtained from Koch extractions performed at 70°C and 90°C, it was seen that there was no remarkable difference. Therefore, it was decided to use the solutions obtained at 70°C in retanning processes.

The croupon areas of wet-blue leathers were divided into 30x30 cm pieces as experimental samples. Pomegranate peel extracts were used at 1%, 2.5%, and 5% dry matter on the wet-blue leather weight and two leather pieces were used for each process. The retanning process was prescribed in Table I. The extract solution was not used in control samples.

Post-Retanning Tests

Determination of filling coefficient: In order to determine changes in the thickness of retanned leathers depending on different ratios of extract solution usage, the thicknesses of the leathers from 10 different areas were measured in wet form before retanning (T_1) (after neutralization) and after retanning (T_2) according to ISO 2589[35]. Filling coefficients were calculated according to Formula 2.

$$(\%)\ \text{Filling coefficient} = ((T_2 - T_1) / T_1) \times 100 \qquad \text{(Formula 2)}$$

Determination of color changes: The color differences of leathers retanned with different ratios of pomegranate peel extracts were determined by using Minolta CM-2600d spherical spectrophotometer with CIE 100 standard observer angle and CIE standard D65 daylight source. The colors of all retanned leather samples and their color differences against the control sample were evaluated according to the CIE Lab color coordinate system.

Artificial ageing of leather samples and Cr (VI) analysis: Pomegranate peel extracts' possible effects on prevention of Cr(VI) formation were investigated. First of all, the initial Cr(VI) contents of control and pomegranate peel extract retanned leather samples were determined. Then, two ageing methods were used in order to

Table I
Retanning recipe

Process	Amount (%)	Product	Temperature (°C)	Duration (min.)	pH
Washing	200	Water	35		
	0.2	HCOOH		30	
Draining					
Neutralization	150	Water	35		
	0.5	Neutralizing syntan	35	15	
	0.3	$NaHCO_3$		45	5.5-6.0
Draining					
Washing	200	Water	35	30	
Draining					
Retanning	150	Water	40		
	x	Extract solution		20	
Fatliquoring	3	Sulphited fish fatliquor	45	45	
	3	Sulphited natural fatliquor			
	2	Synthetic fatliquor			
	1	Synthetic paraffin			
Fixation	1	HCOOH		30	
	0.2	HCOOH		30	3.5
Washing	200	Water	35	20	

determine Cr(VI) formation under thermal pre-ageing and drastic conditions. In the first method, the leather samples were subjected to the thermal pre-ageing (80°C for 24 h) procedure according to the ISO 10195[36] standard. In the second method[2], leather samples were exposed to UV light (360 nm) and kept at 80°C for 7 days. Cr(VI) analysis was performed immediately on the leathers which were cooled after the ageing processes. Cr(VI) contents in leathers were determined according to the ISO 17075-1[37] test method.

Results and Discussion

Moisture Content, Extraction Yields and Tannin Contents of Pomegranate Peels: Moisture content of pomegranate peels was found to be 5.5(±0.2)%.

As a result of the trials on the MEM of pomegranate peels; it was determined that the increase in temperature caused a significant increase in extractable total solids, while the share of tannins in the solids decreased noticeably (Table II). As a matter of fact, this situation showed that the increasing temperature in MEM trials caused a significant increase in the quantity of non-tannins transferred to the solution rather than the quantity of tannins.

However, from the Koch extraction of pomegranate peels at different temperatures, a slight increase in the amount of total solids and tannins extracted (Table II) was determined by increasing temperature. But, since this increase was not very significant and considering the possible negative effects of high temperatures in extraction on the chemical structures of tannins, it was decided to use parameters of 70°C and 8 h in the extraction of pomegranate peel extracts that were used in the retanning experiments.

Total phenolic contents and antioxidant powers of pomegranate peels: Considering the total phenolic contents of pomegranate peel extracts; the highest amount of phenolics was obtained by using water:methanol mixture as solvent, followed by water:acetone, water:ethanol and water (Table III). Similarly, Orak et al.[38] determined the total phenolic contents of pomegranate peels, which extracted in water, methanol, and ethanol, between 126.11-212.48 µg GAE/mg in extract, to be the lowest value in water and the highest value in methanol extraction.

The antioxidant powers of the pomegranate peels, extracted in different solvents, according to ABTS and FRAB methods were shown in Table III. It is clearly seen that the highest antioxidant power was obtained from water:acetone extraction in both methods. Singh et al.[13] remarked that there seems to be close relationship between antioxidant power and the levels of phenolic compounds extracted by using different solvents. Abid et al.[39] reported that acetone extraction of pomegranate peels exhibited

Table II

The effect on extraction yields and tannin contents of different extraction temperatures

	Koch Extractions		**MEM Extractions**	
Extraction Temperature (°C)	Extraction Yield (Total Solids) (%)	Tannin Content (%)	Extraction Yield (Total Solids) (%)	Tannin Content (%)
70	43.4(±0.3)	53.8(±0.2)	55.3(±0.4)	53.7(±0.4)
90	45.5(±0.4)	54.5(±0.2)	67.1(±0.5)	40.8(±0.3)

Table III

Total phenolic contents and antioxidant powers of pomegranate peels extracted in different solvents

Type of Solvent	**Total phenolic content (mg GAE/g)**	**Antioxidant power according to ABTS (mg TE/g)**	**Antioxidant power according to FRAP (mg TE/g)**
Water	116.3(±1.2)	554.1(±13.3)	511.8(±16.5)
Water:Ethanol	136.0(±3.5)	645.3(±6.7)	547.1(±5.3)
Water:Methanol	208.8(±4.0)	840.6(±3.7)	573.7(±1.9)
Water:Acetone	195.1(±2.4)	885.5(±7.2)	577.3(±1.9)

the highest antioxidant activity (97.82% according to the DDPH method) as compared to water and ethanol extract. On the other hand, Elfalleh et al.[40] determined that methanolic pomegranate peel (7.5 TEAC mmol/100 g DW) exhibited higher ABTS activities than aqueous one (3.80 TEAC mmol/100 g DW) and indicated that these differences were due to the content and quality of the phenolic compounds extracted in different solvents.

Filling coefficient and color changes of retanned leathers: Filling coefficient test results of the leathers retanned with 1, 2.5, and 5 % pomegranate peel extract were given in Table IV. From the evaluation of the results, it was seen that the thicknesses of the leathers increased for all samples in conjunction with increasing amount used in retanning. The maximum increment in filling coefficient was determined to be 10.6% with 5% pomegranate peel ratio which can be considered as a remarkable solo performance for retanning.

Table IV

Filling coefficient values of retanned leathers

Pomegranate peel amount used in retanning	Filling coefficient (%)
Control Sample	0.6(±0.3)
1 %	5.0(±0.7)
2.5 %	8.0(±1.1)
5 %	10.6(±0.9)

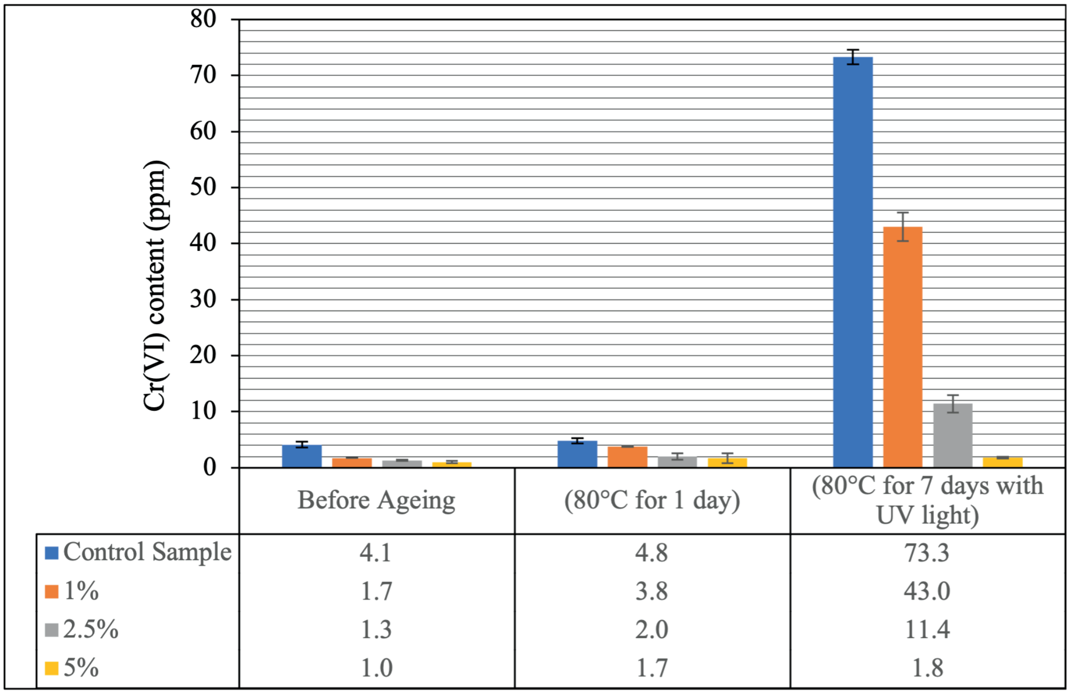
Depending on their chemical structures, colors and percentages of use, retanning agents cause changes in leather physical properties (fullness, firmness, color, handle etc.). Together with other properties, the color imparted by retanning agents to the leather is important, as it has a direct effect on dyeing and the final color. That's why the color of the leathers retanned with pomegranate peel extracts was measured. The data of the color measurements of the leathers retanned with pomegranate peel extracts and comparisons with control sample were given in Table V. Color measurement of leathers was performed before and after ageing. Unretanned and unaged leather was used as control sample. From the examination of the data, regardless of the amount of pomegranate peel used in retanning, it was observed that the color of the leathers significantly shifted towards yellow and slightly shifted towards green compared to the control sample. There was

Table V

Color measurement values of leathers retanned with pomegranate peel extracts at different ratios before and after ageing

Sample	L	a	b	dL	da	db	dE
Before Ageing							
Control Sample	81.56	-4.22	0.47	------	------	------	------
1 %	77.19(±1.0)	-5.90(±0.5)	20.05(±0.5)	-4.37(±1.0)	-1.68(±0.5)	19.58(±0.5)	20.16(±0.6)
2.5 %	77.44(±0.9)	-5.02(±0.2)	19.31(±0.7)	-4.11(±0.9)	-0.80(±0.2)	18.83(±0.7)	19.31(±0.8)
5 %	74.55(±1.0)	-4.42(±0.1)	19.77(±0.5)	-7.01(±1.0)	-0.20(±0.1)	19.30(±0.5)	20.55(±0.7)
After Ageing (80 °C for 7 days with UV light)							
Control Sample	83.56(±1.1)	-3.72(±0.3)	0.87(±0.4)	2.01(±1.1)	0.50(±0.3)	0.40(±0.4)	2.17(±1.1)
1 %	77.86(±0.7)	-5.09(±0.2)	20.62(±0.7)	-3.70(±0.7)	-0.87(±0.2)	20.15(±0.7)	20.51(±0.8)
2.5 %	76.90(±0.9)	-4.66(±0.3)	20.37(±0.5)	-4.66(±0.9)	-0.43(±0.3)	19.90(±0.5)	20.45(±0.6)
5 %	75.17(±0.8)	-4.13(±0.1)	20.89(±0.6)	-6.39(±0.8)	0.10 (±0.1)	20.42(±0.6)	21.41(±0.8)

Where: L: lightness / brightness (L=0 black, L=100 white), a: red/green color (+a red, -a green), b: yellow/blue color (+b yellow, -b blue)
and dL, da, db and dE the changes in color compared to the control sample.



	Before Ageing	(80°C for 1 day)	(80°C for 7 days with UV light)
🟦 Control Sample	4.1	4.8	73.3
🟧 1%	1.7	3.8	43.0
⬜ 2.5%	1.3	2.0	11.4
🟨 5%	1.0	1.7	1.8

Figure 1. Comparison of Cr(VI) levels for leather samples retanned with different amount of pomegranate peel extracts.

no remarkable alteration in the color and shade of the retanned leathers by ageing.

Cr(VI) contents of leather samples: In order to determine Cr(VI) formation preventing effect of pomegranate peel extract use in retannage, the Cr(VI) contents in control samples and the leather samples retanned with pomegranate peel extract were measured before and after ageing treatments (Figure 1).

Considering the values regarding the Cr(VI) content of the leathers before and after pre-ageing (80°C for 1 day); the decrease in the Cr(VI) content of the samples in parallel with the increasing use of pomegranate peel extract drew attention. However, the most striking result emerged in the data obtained after ageing under drastic conditions. As a matter of fact, 73.3 ppm of Cr(VI) resulting from drastic ageing in the control sample decreased to 43, 11.4 and 1.8 ppm in the leather samples retanned with 1%, 2.5% and 5% of pomegranate peel extract respectively. Thus, it was determined that the use of pomegranate peel extract in retanning, effectively suppressed/prevented the formation of Cr(VI) in the leathers via its strong antioxidant effect.

Conclusions

In the present study, pomegranate peels which arise as waste in fruit juice enterprises were investigated in terms of total phenolic contents and antioxidant powers as well as the pomegranate peel tannins' retanning performances and Cr(VI) preventing effect. Considering the analysis results, while the highest phenolic content was found to be 208.8 mg GAE/g using a water:methanol extraction, the highest antioxidant powers were found to be 885.5 and 577.3 mg TE/g using a water:acetone extraction both for ABTS and FRAB methods, respectively.

Regarding pomegranate peel tannins' retanning and Cr(VI) preventing performances; the increase in the thicknesses (5, 8 and 10.6%) of the leathers which were retanned with 1, 2.5 and 5% of pomegranate peel extract can be regarded as a remarkable solo performance for retanning. Considering the data obtained from the experiments/analysis on Cr(VI) prevention; even in mild ageing conditions the decrease in the Cr(VI) content of the leather samples, in parallel with the increasing use of pomegranate peel extract drew attention. However, the suppression of very high amounts of Cr(VI) - such as 73.3 ppm arising under drastic ageing conditions - revealed that the use of pomegranate peel extract in retanning strongly suppressed/prevented the formation of Cr(VI) in the leathers.

The findings revealed that pomegranate peels can be utilized in leather production as a valuable new source of tannins with their high phenolic content, antioxidant powers and significant Cr(VI) inhibition effects.

References

1. China, C.R., Maguta, M.M., Nyandoro, S.S., Hilonga, A., Kanth, S.V., Njau, K.N.; Alternative tanning technologies and their suitability in curbing environmental pollution from the leather industry: A comprehensive review. *Chemosphere* **254**, 126804, 2020. https://doi.org/10.1016/j.chemosphere.2020.126804
2. Kilicarislan Ozkan, C., Ozgunay, H., Kalender, D.; Determination of antioxidant properties of commonly used vegetable tannins and their effects on prevention of Cr(VI) formation. *J Soc Leather Technol Chem* **99(5)**, 245-249. 2015.
3. Khalil, I., Yehye, W.A., Etxeberria, A.E., Alhadi, A.A., Dezfooli, S.M. Julkapli, N.B.M., Basirun, W.J., Seyfoddin, A.; Nanoantioxidants: Recent trends in antioxidant delivery applications. *Antioxidants* **9(1)**, 24, 2020. https://doi.org/10.3390/antiox9010024
4. Dong, Z., Xu, F., Ahmed, I., Li, Z., Lin, H.; Characterization and preservation performance of active polyethylene films containing rosemary and cinnamon essential oils for Pacific white shrimp packaging. *Food Control* **92**, 37-46, 2018. https://doi.org/10.1016/j.foodcont.2018.04.052
5. Figueroa-Lopez, K.J., Vicente, A.A., Reis, M.A.M., Torres-Giner, S., Lagaron, J.M.; Antimicrobial and antioxidant performance of various essential oils and natural extracts and their incorporation into biowaste derived poly(3-hydroxybutyrate-co-3-hydroxyvalerate) layers made from electrospun ultrathin fibers. *Nanomaterials* **9(2)**, 144, 2019. https://doi.org/10.3390/nano9020144
6. Zhang, Y., Wang, B., Lu, F., Wang, L., Ding, Y., Kang, X.; Plant-derived antioxidants incorporated into active packaging intended for vegetables and fatty animal products: A review. *Food Addit Contam Part A,* **38(7)**, 1237-1248, 2021. https://doi.org/10.1080/19440049.2021.1885745
7. Frederik, H., Romer, A., Nadine, S., Susan, I., Bonnet, D., David, R., Dan, V.; Tanning fingerprint in vegetable tanned leather by solid state NMR spectroscopy and comparison with leather tanned by other processes. *Molecules* **16(2)**, 1240-1252, 2011. https://doi.org/10.3390/molecules16021240
8. Isam, E., Christina, Y.; Determination of tannins of three common acacia species of Sudan. *J Adv Chem* **9**, 25-30, 2014. http://doi.org/10. 1155/2014/192708
9. Mohammed, S.A., Rames, C., Madhan, B., Demessie, B.A.; Extraction of bio-active compounds from Ethiopian plant material Rumex abyssinicus (mekmeko) root-A study on kinetics, optimization, antioxidant and antibacterial activity. *J Taiwan Inst Chem Eng* **75**, 228-239, 2017. https://doi.org/10.1016/j.jtice.2017.03.004
10. Mohammed, S.A., Naisini, A., Madhan, B., Demissie, B.A.; Rumex abyssinicus (mekmeko): A newer alternative for leather manufacture. *Environ Prog Sustain Energy* **39(6)**, e13453, 2020. https://doi.org/10.1002/ep.13453
11. Badessa, T.S., Hailemariam, M.T., Ahmed, S.M.; Greener approach for goat skin tanning. *Cogent Eng* **9(1)**, 2018959, 2022. https://doi.org/10.1080/23311916.2021.2018959
12. Kahramanoglu, I., Usanmaz, S.; Pomegranate Production and Marketing, first ed. Boca Raton, USA. CRC Press. 2016. DOI:10.1201/b20151
13. Singh, B., Singh, J.P., Kaur, A., Singh, N.; Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: A review. *Food Chem* **261**, 75–86, 2018. https://doi.org/10.1016/j.foodchem.2018.04.039
14. Cam, M., Icyer, N.C., Erdogan, F.; Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT-Food Sci Technol* **55(1)**, 117-123, 2014. https://doi.org/10.1016/j.lwt.2013.09.011
15. Singh, B., Singh, J.P., Kaur, A., Singh, N.; Antimicrobial potential of pomegranate peel: a review. *Int J Food Sci Technol* **54(4)**, 959-965, 2019. https://doi.org/10.1111/ijfs.13964
16. Zhao, X., Yuan, Z., Fang, Y., Yin, Y., Feng, L.; Characterization and evaluation of major anthocyanins in pomegranate (Punica granatum L.) peel of different cultivars and their development phases. *Eur Food Res Technol* **236(1)**, 109-117, 2013. https://doi.org/10.1007/s00217-012-1869-6
17. Venkataramanamma, D., Aruna, P., Singh, R.P.; Standardization of the conditions for extraction of polyphenols from pomegranate peel. *J Food Sci Technol* **53(5)**, 2497-2503, 2016. https://doi.org/10.1007/s13197-016-2222-z
18. Amri, Z., Zaouay, F., Lazreg-Aref, H., Soltana, H., Mneri, A., Mars, M., Hammami, M.; Phytochemical content, fatty acids composition and antioxidant potential of different pomegranate parts: Comparison between edible and non edible varieties grown in Tunisia. *Int J Biol Macromol* **104**, 274-280, 2017. https://doi.org/10.1016/j.ijbiomac.2017.06.022
19. Sestili, P., Martinelli, C., Ricci, D., Fraternale, D., Bucchini, A., Giamperi, L., Curcio, R., Piccoli, G., Stocchi, V.; Cytoprotective effect of preparations from various parts of Punica granatum L. fruits in oxidatively injured mammalian cells in comparison with their antioxidant capacity in cell free systems. *Pharmacol Res* **56**, 18-26, 2007. https://doi.org/10.1016/j.phrs.2007.02.003
20. Aviram, M., Volkova, N., Coleman, R., Dreher, M., Reddy, M.K., Ferreira, D., Rosenblat, M.; Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: Studies in vivo in atherosclerotic apolipoprotein E-deficient (Eo) mice and in vitro in cultured macrophages and lipoproteins. *J Agric Food Chem* **56(3)**, 1148-1157, 2008. https://doi.org/10.1021/jf071811q
21. Zahin, M., Aqil, F., Ahmad, I.; Broad spectrum antimutagenic activity of antioxidant active fraction of Punica granatum L. peel extracts. *Mutat Res* **703(2)**, 99-107, 2010. https://doi.org/10.1016/j.mrgentox.2010.08.001
22. Malviya, S., Arvind, Jha, A., Hettiarachchy, N.; Antioxidant and antibacterial potential of pomegranate peel extracts. *J Food Sci Technol* **51**, 4132-4137, 2014. https://doi.org/10.1007/s13197-013-0956-4
23. Akhtar, S., Ismail, T., Fraternale, D., Sestili, P.; Pomegranate peel and peel extracts: Chemistry and food features. *Food Chem* **174**, 417-425, 2015. http://dx.doi.org/10.1016/j.foodchem.2014.11.035

24. Karimi, M., Sadeghi, R., Kokini, J.; Pomegranate as a promising opportunity in medicine and nanotechnology. *Trends Food Sci Technol* **69**, 59-73, 2017. https://doi.org/10.1016/j.tifs.2017.08.019
25. SLC-113, Determination of Moisture, Society of Leather Technologists and Chemists, 1996.
26. Sivakumar, V., Ravi Verma, V., Rao, P.G., Swaminathan, G.; Studies on the use of power ultrasound in solid-liquid myrobalan extraction process, *J Clean Prod* **15**, 1813-1818, 2007. https://doi.org/10.1016/j.jclepro.2006.06.006
27. SLC 114, Determination of Total Solids, Society of Leather Technologists and Chemists Official Methods of Analysis, 1996.e
28. SLC 115, Determination of Total Solubles, Society of Leather Technologists and Chemists Official Methods of Analysis, 1996.
29. SLC 116, Determination of Non-tannin Constituents, Society of Leather Technologists and Chemists Official Methods of Analysis, 1996.
30. SLC 117, Determination of Tannin Matter Absorbable by Hide Powder, Society of Leather Technologists and Chemists Official Methods of Analysis, 1996.
31. Swain, T., Hillis, W.E.; The phenolic constituents of Purmus domestica. I. The quantitative analysis of phenolic constituents. *J Sci Food Agric* **10**, 63-68, 1959. https://doi.org/10.1002/jsfa.2740100110
32. Benzie, I.F.F., Strain, J.J.; Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* **239(1)**, 70-76, 1996. https://doi.org/10.1006/abio.1996.0292
33. Ozgen, M., Rees, R.N., Tulio, A.Z., Scheerens, J.S., Miller, A.R.; Modified 2,2'-azino-bis 3-ethylbezothiazoline – 6-sulphonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'- diphenil-1-picrilhydrazyl (DPPH) methods. *J Agric Food Chem* **54(4)**, 1151-1157. 2006. https://doi.org/10.1021/jf051960d
34. Miller, N.J., Riceevans, C., Davies, M.J., Gopinathan, V., Miller, A.; A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* **84**, 407-412, 1993. https://doi.org/10.1042/cs0840407
35. ISO 2589 [IULTCS/IUP 4], Leather - Physical and mechanical tests - Determination of thickness, 2016.
36. ISO 10195 [IULTCS/IUC 41], Leather - Chemical determination of chromium (VI) content in leather - Thermal pre-ageing of leather and determination of hexavalent chromium, 2018.
37. ISO 17075-1 [IULTCS/IUC 18-1], Leather - Chemical determination of chromium (VI) content in leather - Part 1: Colorimetric method, 2017.
38. Orak, H.H., Yagar, H., Isbilir, S.S.; Comparison of antioxidant activities of juice, peel, and seed of pomegranate (Punica granatum L.) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. *Food Sci Biotechnol* **21(2)**, 373-387, 2012. https://doi.org/10.1007/s10068-012-0049-6
39. Abid, M., Yaich, H., Cheikhrouhou, S., Khemakhem, I., Bouaziz, M., Attia, H., Ayadi, M.A.; Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. *J Food Sci Technol* **54(9)**, 2890-2901, 2017. https://doi.org/10.1007/s13197-017-2727-0
40. Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., Ferchichi, A.; Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *J Med Plants Res* **6(32)**, 4724-4730, 2012. https://doi.org/10.5897/JMPR11.995

An Investigation of the Ethiopian Tanning Industry's Competitiveness

by

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Abstract

Ethiopia's leather development plan has prioritized the tanning industry in order to accelerate the sector's growth. The aim of this study is to identify the factors that allow and prohibit Ethiopian tanning industries from coping with competitive challenges, as well as the competitive advantages and drawbacks that the tanning industry faces.

The study followed a primary and secondary data collection methods, which included both qualitative and quantitative approaches. In the study, 13 functioning tanneries in the country involved in leather production from 2020 to 2021 were investigated. The study data were collected through distributing structured questionnaire and having interviews with tanning industry top management body (i.e. managers, directors, and heads).

The study results are presented in forms of the mean and standard deviation. The study findings revealed that the tanning industry competitiveness is low due to different determinates factors. With the exception of the country's natural resource endowment in hides and skins and low labor cost availability, all determinants of competitiveness (raw material, infrastructure, skilled labor, industry and institution linkage, capital and managerial capability, and government policy) are found to be insignificant.

Similarly, the tanning industry's ability to use its cost advantage in raw hides and skins and maintain its market share on the domestic and international market is limited by poor quality of locally supplied raw material, lack of strong supporting institutions and association's, lack of infrastructure, capital and managerial capability, poor industry and institution linkage as well as factors such as scarcity of skilled labor and raw materials (chemicals and spare parts). In order to enhance the competitiveness of the tanning industry in the country, the industry, government and stakeholders need to work in collaboration in creating strong supporting industries, fulfilling infrastructure, providing investment capital, and supplying experienced managers and skilled workers.

Introduction

Leather and leather products are one of the world's most commonly traded and widely used commodities. In this case, Africa is fast emerging as one of the most promising future markets for sourcing high-quality leather and skins for the world's booming leather industry.[1] (Abtew and Research, 2015).

The Ethiopian Leather industry is centered on its livestock resources, which include 63.2 million cattle, 31.8 million sheep, and 34 million goats, making it one of the world's largest livestock populations.[2-3] Taking into account the country's resource advantage, the countries government identified the leather and leather products sector as one of seven priority areas with potential for industrialization and employment creation opportunities.[4-5]

Despite the country's government initiatives and special consideration, the leather sector's performance was far below the government's and stakeholders' expectations.[6] The tanning industry is dynamic, with distinct characteristics that will have varying effects on the leather sector competitiveness.[7] In this context, assessing organizational competitiveness and establishing competitive strategies has been a major concern in leather processing and marketing, leading to an increase in the number of studies on the subject.

The main objective of this research was to identify the major factors that affect the Ethiopia's tanning industry's competitiveness and to reveal the competitiveness positions of the main and sub factors impacting the industry, as well as to make suggestions for what could be done to improve the sector's competitiveness.

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Competitiveness

The term "competitiveness" refers to an entity's economic strength in comparison to its competitors, and it includes the perspectives of the country, industry, and enterprise.[8] Similarly, most experts use the term "competitiveness" to describe a firm's or industry's advantage over its local or worldwide competitors.[9]

According to Porter 198, gaining a competitive edge is critical for improving corporate, industrial, and economic performance[10] Competitive advantage, is a company's ability to manufacture items that provide more value to customers than competitors' products, resulting in greater sales and profits.[11-12]

In line with this, project management, competitive strategy, partnerships, supply, marketing, technical and technological skills, as well as financial capabilities are all aspects that may be employed to improve the competitiveness of the Ethiopian tanning industry. Many factors may influence the country's tanning industry's competitiveness. It is critical to understand which aspects are most important in this respect.

Methodology

Data Collection:
The study employed primary and secondary data collection methods. The primary data was collected through semi structured closed ended questionnaire questions and face-to-face interviews, while the secondary data were collected through reviewing existing tanning industries reports and different literatures. The questionnaires were divided into two sections, the first section were prepared to gather respondent respondents' profile as well as overall image of the tanneries. The second section of the questionnaire were prepared to identify the constraints that impact the tanning industry competitiveness.

Result and Discussion

Method of data processing and analysis:
The study data collected via questionnaire were filled in SPSS and different data processing and analysis techniques including descriptive and inferential statistics technique were used to analyze the total and mean score of each competitiveness factors.[13]

General information of respondent:
The authors selected the industries top management to respond the questionnaire, because it is expected that most of the respondent will have wide information about their tannings industry, which allows to get a fairly balanced information about the tanning industries. As shown in Table I, managers, directors, and heads were among the respondents who filled out the questionnaires.

General Information of the Tanning Industry:
The ownership structure, and place of the industry has been in operation were all factors considered in the study.

Table I
Respondent position in the tanning industries

Respondent Position	Frequency	Percent	Valid Percent	Cumulative Percent
Deputy Chief Managing Director for Tannery	1	7.7	7.7	7.7
Technical Manager	1	7.7	7.7	15.4
Factory Manager	1	7.7	7.7	23.1
Production Manager	5	38.5	38.5	61.5
Quality Division Head	1	7.7	7.7	69.2
Environmental Head	1	7.7	7.7	76.9
Quality Control and R&D Head	1	7.7	7.7	84.6
Human Resource Director	1	7.7	7.7	92.3
Deputy Manager	1	7.7	7.7	100.0
Total	13	100.0	100.0	

Source: Research Data (2022)

Table II

Name and place of the Tanning Industries available

	Place/City	Frequency	Percent	Valid %	Cumulative %
Batu Tannery	Addis Ababa	1	7.7	7.7	7.7
Ethio Leather Industry (ELICO)	Addis Ababa	1	7.7	7.7	15.4
Bahir Dar Tannery	Bahir Dar	1	7.7	7.7	23.1
Habesha Tannery	Bahir Dar	1	7.7	7.7	30.8
George Shoe Ethiopia Tannery	Modjo	1	7.7	7.7	38.5
Colba Tannery	Modge	1	7.7	7.7	46.2
Modjo Tannery	Modjo	1	7.7	7.7	53.8
Friendship Tannery	Modjo	1	7.7	7.7	61.5
Addis Ababa Tannery	Addis Ababa	1	7.7	7.7	69.2
Farida Tannery	Modge	1	7.7	7.7	76.9
Jianxin Zhang Tannery	Modjo	1	7.7	7.7	84.6
Hodaoche Tannery	Modjo	1	7.7	7.7	92.3
Ethiopian Tannery	Modjo	1	7.7	7.7	100.0
Total		13	100.0	100.0	

Source: Research Data (2022)

Table III

Ownership of Tanning Industry

Tannery	Frequency	Percent	Valid Percent	Cumulative Percent
Government Owned	0	0	0	0
Privately owned	13	100.0	100.0	100.0

Source: Research Data (2022)

Initially, the authors planned to gather data from the country's 20 active tanning industries. However, due to various constraints, data were collected only from 13 active tanning industries. This allowed the research to cover 65% of the country's targeted industries, which is higher than the average. As a result, meeting the target group of 65% was adequate for statistical analysis, resulting in representative findings from the study. During data collection, it was observed that some tanning industries have been closed due to different constraints. As shown in Table II, most of tanning industries are concentrated in one place and which will be a constraint to utilize the countries raw hide and skin.

Tanning Industry Ownership status

As shown in Table III, all (100%) of the targeted tanning industries are privately owned, with government owned industries having limitations. As the country's government is the main concerned body in the development of different policies and facilitation of import material supplies for the tanning industries, limitation of government owned tanning sector may have influence in development of the tanning industry.

Analysis of determinant factors of competitiveness

The degree of mean scores based on the criteria for class interval scale rating were shown in Table IV.

Table IV

The degree of mean scores based on the criteria for class interval scale rating

Range	Degree
1.29-1.99	Most unfavorable
2.00-2.74	Unfavorable
2.75-3.49	Average
3.5-4.24	Favorable
4.25-5.00	Most Favorable

Raw material determinant factors of competitiveness:
As illustrated in Table V, locally produced raw material quality and supply were unfavourable with mean scores of 2.46 and 2.76, respectively. Due to this, the tanning industries are dependent on imported raw materials with a mean scores 3.07. This indicated that, the tanning industry are forced to import most of raw materials (chemicals etc.), from abroad, which result in a negative impact on the availability of foreign currency in the country. On the other hand, locally produced raw material fulfils the international standard to some extent and the cost is moderately favourable with the mean scores of 3.00 and 3.30 respectively.

In general, the average mean score (3.18) and standard deviation (0.66) showed that the raw material factors contribution to competitiveness of tanning industry in Ethiopia is low to a moderate extent. In line with this, the study finding revealed that the tanning industry competitiveness were influenced by raw material quality and supply related constraints. In order to enhance the competitiveness of the tanning industry in the country, the industry, government and stakeholders need to work in collaboration for producing good quality raw materials that substitute imported raw materials.

Infrastructure determinant factors of competitiveness:
The study further aimed to investigate the availability of infrastructure for the tanning industry. From the findings shown in the Table VI, electricity, communication network, water, port transport facility and port handling and customs processes were moderately available for the tanning industries with the mean score of 3.0, 3.07, 3.46, 3.46, and 2.9231 respectively. This implies that the availability of infrastructure not enough for the industries to be competitiveness in the national and international markets. In line with this, working to provide required physical infrastructure clearly plays an important role in enhancing the productivity, performance and competitiveness of the tanning industries.

Industry and institution linkage determinant factors of competitiveness:
In the study, the existence of related and supported industries in the countries has been considered. Table VII, demonstrated that coordination between institutes and associations, frequent communication with suppliers and customers were moderately favourable with the mean scores of 3.30, 3.23 and 3.15, respectively. However, supporting institutions and association's contribution

Table V

Raw material determinant factors of competitiveness

Descriptive Statistics			
Statements	**N**	**Mean**	**Std. Deviation**
Local raw materials supply (chemicals, etc)	13	2.7692	1.09193
Local raw materials quality	13	2.4615	.77625
Local raw materials cost	13	3.3077	.75107
Dependency on imported raw materials	13	3.0769	1.32045
Local raw material fulfil international standards	13	3.0000	1.08012
Average	13	3.1846	.66564

Source: Research Data (2022)

Table VI

Infrastructure determinant factors of competitiveness

Descriptive Statistics			
Statement	**N**	**Mean**	**Std. Deviation**
Adequate supply of electricity	13	3.0000	1.22474
Adequate supply of water	13	3.4692	.72501
Adequate supply of communication network	13	3.0769	.86232
Sufficient transport to/and from port	13	3.4923	.75107
Fair cost of transport to/and from port is	13	3.2308	.83205
Port handling and customs processes	13	2.9231	1.11516
Average	13		

Source: Research Data (2022)

Table VII

Industry and institution linkage determinant factors of competitiveness

Descriptive Statistics			
Statement	**N**	**Mean**	**Std. Deviation**
Supporting institutions and associations	13	2.6923	0.85485
Coordination between institutes and associations	13	3.3077	0.75107
Frequent communication with suppliers	13	3.2308	0.72501
Frequent communication with customers	13	3.1538	0.68874
Average	13	3.0962	0.40232

Source: Research Data (2022)

Table VIII

Skilled labor determinant factors of competitiveness

Descriptive Statistics			
	N	**Mean**	**Std. Deviation**
Supply of skilled labor force	13	2.4615	0.87706
Experienced and export oriented marketing personnel	13	2.3846	0.65044
Continuous training	13	2.5385	1.12660
Capability to handle new technology	13	3.0000	1.08012
Average	13	2.5962	0.57317

Source: Research Data (2022)

for the tanning industry were unfavourable with mean scores of 2.69.

However, the grand mean score (3.09) and standard deviation (0.4) showed that the industry and institution linkage contribution to competitiveness of tanning industry is to moderately favourable (average). In order to improve the industry and institution linkage, the tanning industries, government and stakeholders need to work closely. The presence of effective and efficient related and supported industries will provide benefits such as technology up gradation, innovations, quick information flow and shared new technology, etc.

Skilled labor determinant factors of competitiveness:

Human resources relates to the people that make up an organization's workforce. It is a valuable asset for every organization and a source of competitive advantage for any businesses. It provides organizational capacities and intellectual capital by converting resources like money, machines, processes, and materials into products or services. Human resource competence, which refers to the skills and knowledge of workers and others in an organization's network, remains critical.

The study further requested the respondents to indicate the labor force characteristics within their tanneries. As shown in Table VIX, skilled labor and export oriented marketing personnel, and training were unfavorable to a high extent with mean scores of 2.46, 2.38 and 2.53 respectively. However, capability to handle new technology were favorable to some extent with mean scores of 3.0. In the study, the average mean score of (2.59) and standard deviation (0.57) showed that the skilled labor factors' contribution to competitiveness of tanning industry is unfavorable and implementing different enhancement strategies are necessary. The tanning industries need to work on enhancing their workforce competence and skill through creating different training and experience sharing opportunities.

Government Policy Determinant factors of competitiveness: Government policy as a factor

Porter stated that the governments play role of catalyst and challenger to companies to raise their aspirations.[14] (Porter, 1996b). As illustrated in Table IX, the country's government's policy on infrastructure, science & technology, business start-up and industry regulation were favorable to some extent with the mean scores of 3.91, 3.58, 3.58and 3.5385, respectively. Similarly, the county's

Table IX

Government Policy Determinant factors of competitiveness

Descriptive Statistics

	N	Mean	Std. Deviation
Government policy on human resources	13	3.3846	1.04391
Government policy on Science & Technology	12	3.5833	0.90034
Government policy on infrastructure	12	3.9167	1.08362
Government policy on demand stimulation	12	3.4167	1.08362
Government policy on business start-up	12	3.5833	0.90034
Government policy on protectionism	13	2.5385	0.96742
Government policy on taxes	13	3.0769	1.03775
Government policy on industry regulation	13	3.5385	1.05003
Political environment	13	2.4615	1.39137
Average	12	3.2427	0.79925

Source: Research Data (2022)

government policy on demand stimulation, human resources and taxes contribution to the competitiveness of the tanning industry were moderately favorable with mean scores of 3.41, 3.38 and 3.07, respectively. However, the government policy on protectionism and political environment were unfavorable with the mean scores of 2.53 and 2.46, respectively. The study grand mean score (3.24) and standard deviation (0.79) showed that the government's policy contribution to competitiveness of tanning industry is too moderate and requires attention.

Capital and Managerial capability Determinant factors of competitiveness:

Table X indicated that the tanning industries are facing lack of financing to acquire loans which is due to the complexity of loan processing procedures and collateral requirements from banks and other lending institutions. The tanning industry relatively have skilled and experienced manager's that have a capability in planning and making effective decisions for the success of the firm.

Table X

Capital and Managerial capability Determinant factors of competitiveness

Descriptive Statistics

	N	Mean	Std. Deviation
Financial institution give priority to facilitate the required loan	13	3.3077	0.94733
The collateral requirement for lending institutions is one of the hindrances to obtaining a loan	13	3.5385	0.66023
Loan processing procedures of banks and other lending institutions are too complicated and time consuming	13	3.5385	0.77625
There is lack of financing to acquire new technology because it requires huge investment	13	4.0769	0.86232
Educational readiness and capability of managers to help them in planning and making effective decisions for the success of the firm	13	3.3077	0.75107
There are well skilled and experienced managers available in your organization	13	3.1231	0.86232
Your organization manager has a world market idea or exposure	13	3.2231	0.75955
Average	13	3.6593	0.27688

Source: Research Data (2022)

Table XI

Competitiveness measure factors

Descriptive Statistics	N	Mean	Std. Deviation
Due to export competitiveness your company maintains its comparative advantage	13	3.6154	0.76795
My company derives sufficient income from domestic and international markets	13	3.6923	0.85485
My company maintains, or expands, a place in international markets	13	3.6154	1.19293
My company has access to new technology, new ideas and subsequent productivity growth	13	4.0000	0.81650
Ethiopian leather product earns a higher price in the international market than its competitors	13	3.0000	1.00000
Ethiopian leather is preferred over its competitors in the international market	13	3.6923	0.85485
Average	13	3.6026	0.50249

Source: Research Data (2022)

Competitiveness Measure factors

Table XI, demonstrated that the mean scores for the six items under the competitiveness ranges from 3.0 to 4.0. All means fall above the mean score of three on a five point Likert scale. As the entire mean scores fall on the average to a moderate extent range (3.0 to 4.0). However, the grand mean score of 3.6 (SD = 0.5), this shows that all the factors under competitiveness measure are favorable to high extent to the competitiveness of the industries under this study.

Conclusion

The study results revealed that the competitiveness of the tanning industry in the country is overwhelmed by several challenges. Based on the study findings, most influential competitiveness factors include lack of strong supporting industries, infrastructure, capital access, managerial capability, poor industry and institution linkage as well as factors such as scarcity of skilled labor and raw materials (chemicals and spare parts). With these constraints, it is unable to compete in the worldwide market owing to its inability to produce high-quality products. This study also provided recommendations on how to improve the competitive performance of the tanning industry.

Competing interests

The authors declare that they have no competing interests.

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References

1. Abtew, M. A.; Revealed comparative advantage of Ethiopian leather industry with selected African economies. *International Journal of Business and Economics Researc*h, 4**,** 229-237, 2015.
2. Fetahı, T. J.; Eutrophication of Ethiopian water bodies: a serious threat to water quality, biodiversity and public health. *African Journal of Aquatic Science*, 44**,** 303-312, 2019.
3. Areda, D. B., Muwonge, A. & Dıbaba, A. B. 2019. Status of Bovine Tuberculosis in Ethiopia: Challenges and Opportunities for Future Control and Prevention. *Tuberculosis in Animals: An African Perspective*. Springer.
4. Muchıe, M.; Leather processing in Ethiopia and Kenya: lessons from India. *Technology in Society*, **22,** 537-555, 2000.
5. Brautıgam, D., Weıs, T. & Tang, X. J.; Latent advantage, complex challenges: Industrial policy and Chinese linkages in Ethiopia's leather sector. *China Economic Review*. **48,** 158-169, 2018.
6. Teklay, A., Gebeyehu, G., Getachew, T., Yayneshet, T., Sastry, T.; Quantification of solid waste leather generation rate from the ethiopian leather sector-a contributing perspective to waste management approach. *Innovative Energy & Research*, **7**(2), 2018.

7. Mbate, M. J. J. O. A. T. 2016. Structural change and industrial policy: A case study of Ethiopia's leather sector, *Journal of African Trade*, **3,** 85-100.
8. Rast, S., Touranı, A., Aslan, A. S. J. I. J. O. B. & Management 2015. Effect of organizational factors on university-industry collaboration: a conceptual model. *International Journal of Biometrics*, **10,** 188.
9. Shafaeı, R. J. J. O. F. M. & Journal, M. A. I. ; An analytical approach to assessing the competitiveness in the textile industry. *Journal of Fashion Marketing and Management*, **13**, 2009.
10. Porter, M. E. & Mıllar, V. E. 1985. How information gives you competitive advantage. *Harvard Business Review Reprint Service.*
11. Porter, M. E.; What is strategy? *Harvard Business Review.* 1996.
12. Hemmatfar, M., Salehı, M., Bayat, M.; Competitive advantages and strategic information systems. *International Journal of Biometrics*, **5,** 158, 2010.
13. Maskey, R., Feı, J., Nguyen, H.-O.; Use of exploratory factor analysis in maritime research. The Asian Journal of Shipping and Logistics, **34**(2)**,** 91-111.
14. Porter, M. E. J. I. R. S. R. 1996b. Competitive advantage, agglomeration economies, and regional policy. *International Regional Science Review*, **19,** 85-90.

Studies on the Fabrication of Hydrophobic Coating Incorporating Bentonite Clay and its Effect on the Physical Properties of the Finished Leather

by

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Abstract

One of the most important properties that can be added to leather-based products is its ability to self-clean as leather cannot be washed frequently like fabrics. A simple and direct method to achieve this is to introduce a hydrophobic coating on the top of the leather, which can minimize the adhesion of foreign particles and thereby improve stain resistance. Additionally, this also increases the economic, aesthetic and functional values of the material. Generally, a hydrophobic surface on the leather is created by coating a polymer based on fluorine or silicone, which has the tendency to repel the water droplets. In this paper, the effect of hydrophobic coating on the physical properties of the leathers was studied. It was observed that the hydrophobic coating had a positive effect on the rub fastness properties improving the rating of 3 to 4. Water vapor permeability of the FCP-A alone coated leather reduced from 8.7 to 4.4 mg/cm^2/hr in comparison to crust leather, owing to the sealing of pores on the grain surface due to the film formed by these polymers. However, incorporation of bentonite clay improved the water vapor permeability of the leathers from 3.4 to 12.9% in comparison to that of the FCP-A alone coated leathers owing to the pores created in the finish film. In addition, the contact angle of the leather surface was also increased from 98° to 121° due to the introduction of roughness induced by the clay particles. Hence, the proposed work can aid in the development of easy-care and smart leathers.

Introduction

Garment and shoe industries rely to a large extent on leather for the production of footwear and clothing, which would impart good health and comfort. Leather being a heterogenous matrix with the right combination of three-dimensional structure along with required physical and chemical properties makes it a versatile material for its usage in the fashion industry. One drawback of such a breathable matrix is its poor resistance to stains and thus keeping it clean and hygienic is a challenging task.[1] Some of the methods available to overcome this problem includes a special selection of leathers that are free from the usage of non-ionic surfactants. In addition, proper selection of neutralization and dispersing agents is very critical in bringing about the washability property in leather. Moreover, use of silicone-based or fluoropolymer-based fatliquors, followed by metal capping results in washable leathers with the desired properties.

On the other hand, several researchers have focused on the production of textiles and leathers with superhydrophobicity[2–5] (i.e. those with high Water Contact Angle (CA) >150°). Owing to their unique self-cleaning, anti-contamination and anti-sticking properties, the need for washing can be reduced.[6–8] In order to realize this property, two major routes followed are i) reducing the surface free energy or ii) modifying the surface morphology of the materials. The superhydrophobic nature of lotus leaves, famously identified as the "Lotus leaf effect" confirms this phenomenon. The high surface roughness and minimal contact area between the leaf and the liquid is attributed to the unique micro-nano-binary structure on the lotus leaves. The high roughness causes a layer of air to become trapped under the water droplet, which acts as a barrier enabling the droplet to roll off from the surface.[9] Inspired by these natural structures, numerous superhydrophobic surfaces have been produced by means of a variety of techniques including plasma etching,[10] lithography,[11–13] selfassembly,[14] chemical vapor deposition (CVD),[15] electrospinning,[16] layer-by-layer deposition[17] and sol-gel processing,[18–20] generally followed by further chemical modification. The disadvantage of these techniques is that they implicate tedious and multistage processes, which restrict their large-scale application.

Recent advances in micro- and nano-fabrication techniques has diversified the fabrication methods and materials employed in leather processing varying from inorganic nanoparticles to bulk polymeric materials.[21,22] When incorporated into leather, polymeric materials improve the surface roughness and lower the surface free energy resulting in a superhydrophobic surface. Amongst the various available polymer-based materials, fluoropolymers are being increasingly employed as water repelling agents in textile industries. Water-based fluoropolymer coatings have some attractive properties, such as exceedingly low surface energy, low friction coefficients, repellency to both oil and water and relatively low permeability to

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most gases.[23–25] Even though there are several works carried out in this field, the impact of the hydrophobic or superhydrophobic coating on the physical properties of the finished leathers has not been studied in detail.

Thus, in the present work, a commercially available aqueous fluoropolymer-based binder was mixed with bentonite clay particles and used to impart hydrophobicity to the leathers. The prepared hydrophobic coating materials were then applied on the leather surface by spray coating technique. The hydrophobicity of the coated surface was measured in terms of contact angles. Eventually, the impact of polymeric coating on the leather properties such as water and air permeability, fastness and optical behavior were studied and presented in this research article.

Materials

Goat natural crust leathers were chosen as raw material. Bentonite clay with average particle size ranging between 15 to 20 microns was procured from M/S Sigma-Aldrich Pvt. Ltd. Two water-based fluorocarbon polymers (FCP-A and FCP-B), obtained from M/S ICAP leather chemicals, were chosen as waterproofing agents and used for the leather finishing trials. The chemicals procured were used as such without any further purification.

Application of fluorocarbon polymers for leather finishing trials

To initially identify the suitable fluorocarbon polymer out of the chosen, FCP-A and FCP-B, a neat coat of the polymer alone was sprayed and checked for its water repellency. Accordingly, 20% solution of the fluorocarbon polymers was directly sprayed on to the leather (crust) and the required tests were conducted based on the standard procedure as mentioned in each sub section. A 0.5 mm spray nozzle with a standard operating pressure of 50 to 70 psi was used to spray the samples from a working distance of 30 cm. Care was taken to ensure uniform spraying of the polymer in order to avoid excess deposition on the leather surface.

Preparation of water-based hydrophobic finish solution

Initially, required amount of bentonite clay (0 to 1.0 g at an interval of 0.25 g) was dispersed in deionized water and sonicated for 15 min using a Labman digital ultrasonicator with a working frequency of 20 kHz to obtain a uniform dispersion of the nanoparticles in the aqueous medium. After sonication, the chosen fluorocarbon polymer solution was added dropwise under vigorous mechanical stirring over a course of 5 min to ensure proper mixing. The obtained bentonite/fluorocarbon polymer composite BFCP-A-X (X implies the amount of bentonite used i.e. from 0 to 1.0 g) as shown in Table I. The prepared solution was then applied to the leather following the spray coating technique. The spray coated samples were then aged for 24 hours and taken up for further testing. The dry add-on of finish solution applied on the leather varied from 4.0 to 5.0 g/sq.ft depending upon the amount of bentonite used in the finish formulation.

Water contact angle (CA) measurement and surface morphology studies

Wettability characterization of the spray-deposited coating was done by measuring the contact angle values by the sessile drop method, wherein 5 μL of water were dispensed through a flat tip needle placed near the substrate. The images of the droplets were captured using a HO-IAD-CAM-01B contact angle meter from M/S Holmarc Opto-Mechatronics Ltd India. Each measurement was an average of three drops. The captured images were processed using ImageJ software along with Low-Bond Axisymmetric Drop Shape Analysis plugin for contact angle measurement.[26] To study the changes in the morphology of the leather before and after the coating process, the leather samples were visualized under a Scanning Electron Microscope (SEM). Samples from leathers were cut from the official sampling position. The surface microstructure was studied after gold sputtering using Bruker S-3400N sputter coater. All microscopic images were taken at the same magnification and compared against each other.

Air permeability and water vapor permeability (WVP) assessment

A capillary flow porometer from M/S Porous Materials Inc, USA was used to measure the pore size and distribution using leather

Table I

Solution preparation for different spray coating trials using bentonite combination with fluorocarbon polymer

Experiment	Bentonite clay added (g)	Fluorocarbon polymer (g)	Pigment (g)	Solution make up volume with water (g)
BFCP-A-1	1.00	20	5	100
BFCP-A-0.75	0.75	20	5	100
BFCP-A-0.5	0.50	20	5	100
BFCP-A-0.25	0.25	20	5	100
FCP-A alone	0 (Control)	20	5	100

samples of 20 mm diameter. Initially, a non-reacting gas was sent through the experimental samples followed by wetting with liquid of known surface tension (Calwick, surface tension 15.9 dynes cm^{-1}). The changes in flow rate were measured as a function of pressure for both dry and wet processes. WVP of the leathers were determined by following the standard method described in ISO 20344:2022-6.6. This test method was intended to determine the ability of water vapor (steam vapor) to permeate through the leather in terms of milligram per unit area and for a specified period of time. This test method works on the principle of absorption and permeation of moisture vapor between two distinct humidity levels between the grain & flesh surfaces of the leather.

Fastness properties of the finished leather

The fastness properties of the finished leather were studied by evaluating the color changes after circular rubbing action following ISO 17700:2019-Method B. This is mainly used to assess the degree of damage and transfer of a material's surface color or finish onto the rubbing felt during mild dry and wet rubbing. A specimen of finished leather is rubbed in a circular fashion using a white wool felt of 25 mm diameter under force. After a specified number of cycles of rubbing, the extent of damage to the color/finish and color transfer to the rubbing felt are assessed with grey scales.

Results and Discussion

Wetting behavior of the finished leathers

The wetting behavior of any surface plays a major role in the development of hydrophobic coatings that are capable of repelling water droplets upon contact. The hydrophobicity of any surface is generally classified based on the contact angle (CA) that a water droplet makes with the surface and is classified as hydrophilic when CA is lesser than 90° and hydrophobic if CA is 90 to 150° and superhydrophobic if CA is greater than 150°. To create a hydrophobic surface, as explained in the introduction section, a go-to system is through combination of low surface energy coatings along with micro- to nano-scale roughness. Thus, in this study, two fluorocarbon polymers (FCP-A and FCP-B) were chosen to create a non-polar low energy surface and used contact angle measurements as a tool to evaluate the fabricated surface. Though the contact angle method can help in understanding the hydrophobicity of the leather samples, the method has some drawbacks. Due to the fibers sticking out from the surface of the sample, the determination of the baseline of the water droplet is more difficult, which may in turn lead to possible underestimation of the CA data.[27–29]superhydrophobic surfaces should be able to form a composite interface with air pockets in the valleys between asperities (pillars Additionally, the protruding fibers may exhibit forces on the water droplet making it difficult to yield accurate values for advancing and receding CAs. Thus, in this work, we only measure and report static CAs for 5 µL water droplets.

The contact angle of the crust leather was at 45° which could be mainly attributed to the capillary action of the hair pore present on the grain surface and thus leading to the immediate absorption of water droplet within few seconds. The contact angle of the 20% fluorocarbon polymer only coated leather increased to 98° for FCP-A and 67° for FCP-B polymers as shown in Figure 1. This increase in the CA could be attributed to the fluorine present in the polymeric backbone, leading to a creation of low polar surface and the water droplet stayed on the surface without being absorbed in case of FCP-A coated leather while the water droplet was absorbed within 30 seconds in case of FCP-B coated leather. Thus, it can be seen that the FCP-A coated leather was in the hydrophobic region in terms of contact angle and thus improving hydrophobicity was attempted further by incorporating roughness to the polymer coated surface using bentonite clay particles. But, in case of FCP-B, there was not much improvement from the control leather and the contact angle still remained in the hydrophilic region and thus the FCP-B polymer was not further considered for this work.

Bentonite clay particles are known to improve the chemical resistance apart from introducing the roughness in polymer nanocomposites. Additionally, bentonite being an inert material can

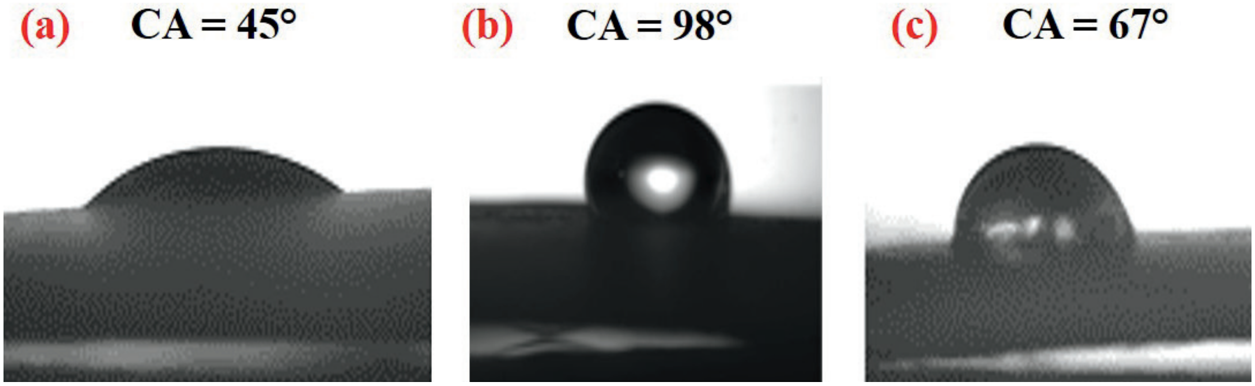

Figure 1. Water droplet contact angle images of **(a)** untreated crust leather (control), **(b)** leather coated with FCP-A polymer alone and **(C)** leather coated with FCP-B polymer alone by spray method.

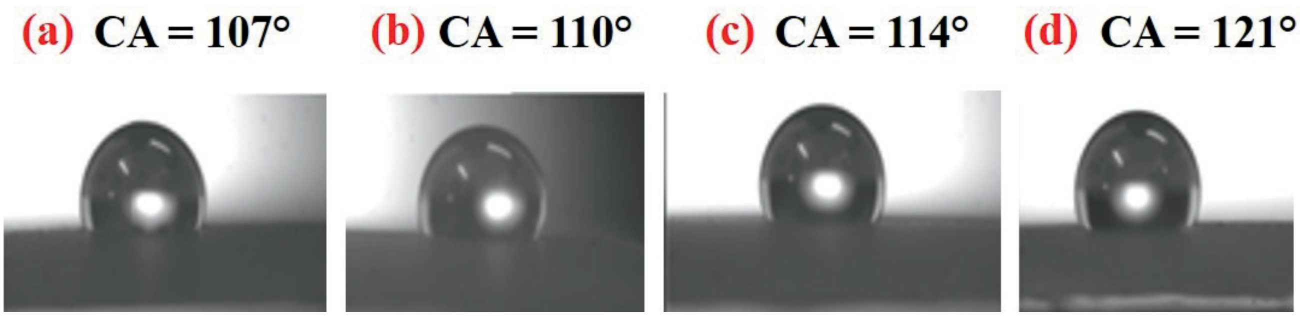

Figure 2. Contact angle of leather surfaces coated with **(a)** BFCP-A-0.25, **(b)** BFCP-A-0.50, **(c)** BFCP-A-0.75 and **(d)** BFCP-A-1.

also act as filler and its alkaline nature aids in uniform dispersion and improves its compatibility with the anionic charged binders used in leather finishing process.[30] Accordingly, the bentonite clay particles were added to the finish solution ranging from 1.25 to 5% (w/w) based on the weight of fluorocarbon polymer. It was found that the contact angle of the coated surfaces after incorporating the bentonite clay particles increased as expected, which could be attributed to the formation of micro- and nano-roughness along with the low surface energy of the fluorocarbon polymer. As a result, this synergistic effect increased the contact angle from 98° degrees of FCP-A only coated leather to 121° for BFCP-A-1 coated leather as shown in Figure 2. With respect to the BFCP-A-0.25 to 1.0, the measured contact angles were 107°, 110°, 114° and 121°, respectively as shown in Figure 2. But increasing the bentonite concentration further did not increase the contact angle and this could be attributed to the agglomeration of clay particles leading to reduction in the roughness created on the surface. This could be attributed to the larger particle size of bentonite clay leaving a very small window to achieve the maximum loading without agglomeration. But the loading efficiency without agglomeration could be maximized by using nano sized particles instead of micro particles to create the surface roughness of micro-nano scale that can even enhance the hydrophobicity of the surface.[5,31] Therefore, it can be concluded that by introducing roughness on the surface using bentonite clay particles along with the polymer of low surface energy, it is possible to drastically increase the hydrophobicity of the coated surfaces.

Surface morphology of the finished leathers

To study the influence of the morphology of the surface on the wetting behavior, SEM images of leather before and after coating with FCP-A alone and BFCP-A-1 were captured and compared. From the SEM images of uncoated crust leather, it could be seen that grain structure of the sample was fibrous and clean without any damage. Besides, the hair pores of leather were markedly large, while microstructures of leathers were denser and homogeneous in morphology. It can be seen from Figure 3 (a & b), that the surface of the uncoated leather showed grooves and cracks. In contrast, in case of the SEM micrographs of the FCP-A only coated leather, such morphological characteristics were covered by the film formed on the surface. The surface was found to be smooth due to the uniform continuous film that is formed by the fluorocarbon polymer as shown in Figure 3 (c & d). With the addition of the bentonite into the finishing solution, a uniform distribution of clay particles could be clearly seen on the surface of the leather. This deposition created the roughness required for improving the hydrophobicity of the coating on the leather surface which could be clearly seen in Figure 3 (e & f). Further, the equal distribution of bentonite particles on the leather surface was ascertained using EDAX mapping technique. In the Figure 3 (g, h & i), it can be clearly seen that the aluminum (1.486 keV) and silicon (1.740 keV) are equally distributed throughout the leather surface.

Air and water vapor permeability of the finished leathers

In order to study the effect of the finish coat on the breathability property, water vapor and air permeability tests were conducted and compared in case of polymer only coated leather along with bentonite incorporated polymer coated leather. Measurement of air permeability can be determined rapidly by Capillary flow porometry (CFP) technique.[25] In this regard, CFP measurements were carried out for crust leather, FCP-A only coated leather and BFCP-A coated leather. Generally, the permeability of leathers gets reduced after the finishing process mainly due to the formation of a continuous film on the surface, which hinders the movement of water molecules. Accordingly, as shown in Figure 4, the flow rate of the gas increased to the maximum with respect to the increase in pressure in case of crust leather (without finishing coat) in comparison to the other polymer coated leathers. Interestingly, the air flow rate was higher for the bentonite incorporated finishing system in comparison to the polymer alone coated leather. This could be attributed to the creation of pores/in-continuity in the finish film upon the addition of bentonite as shown in Figure 3 (e & f) leading to the formation of channels that can allow the air to diffuse through the finish film easier.[32] Even though the air flow rate increased for BFCP-A-1 coated leather in comparison to that of the FCP-A only coated leather, it was rather not much significant enough to be quantified and compared against the crust leather. Thus, to further quantify the breathability of the BFCP-A-1 coated leather and FCP-A only coated leather as

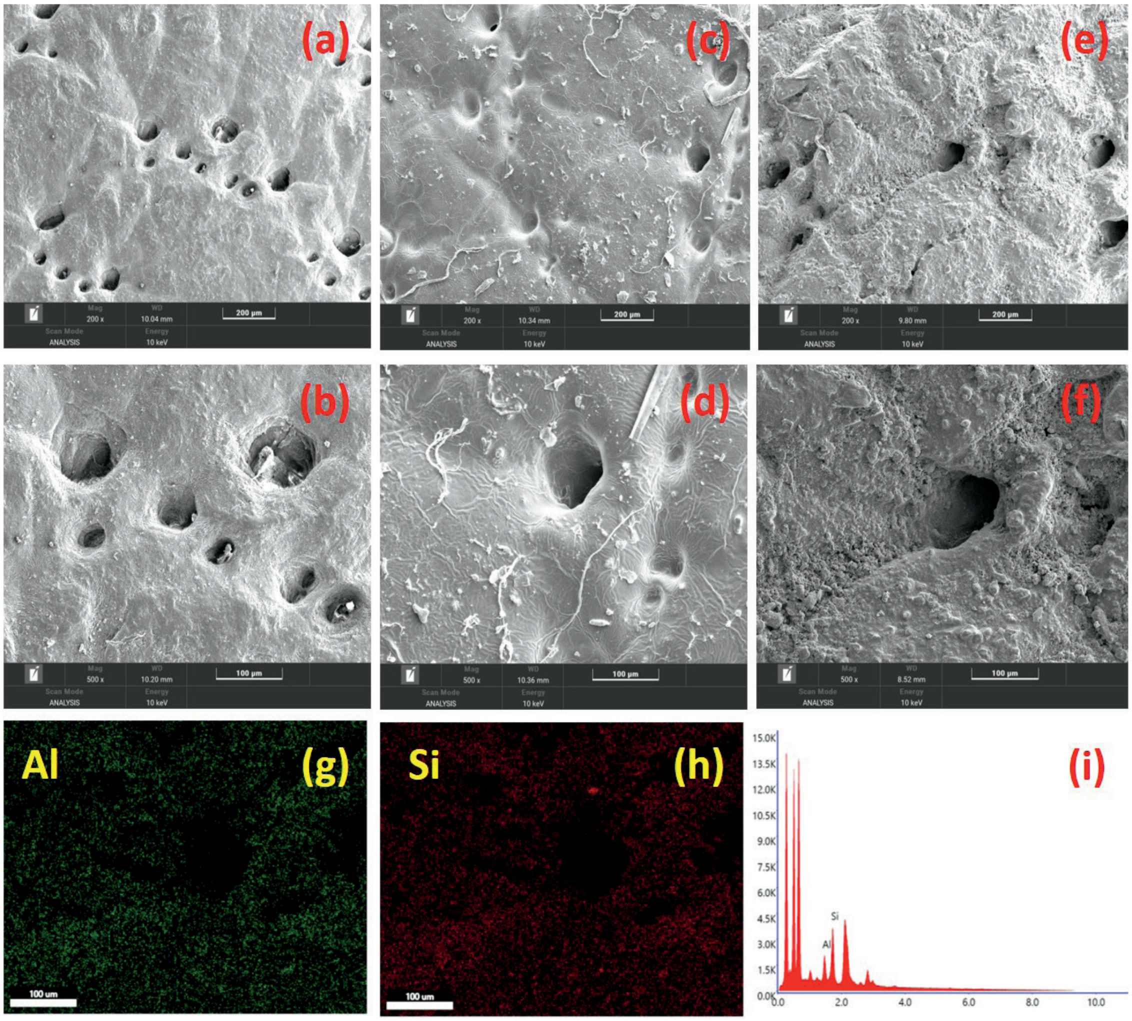

Figure 3. SEM images of untreated leather **(a & b)**, FCP-A only coated Leather **(c & d)** and BFCP-A-1 coated leather **(e & f)** at 200 µm and 100 µm respectively. EDAX - Elemental mapping and spectra of bentonite particles on leather surface **(g, h & i)**.

against the control crust leather, the water vapor permeability of the leathers was measured and compared.

The water vapor permeability of the crust leather and the polymer coated leathers were measured and the results of the measurement is tabulated in Table II. As expected, the water vapor permeability (WVP) of the crust leather was found to be highest at 8.7 mg/cm^2/hr. Meanwhile, the polymer coated leathers showed decreased WVP. It could be seen that the WVP values increased approximately from 3.4 to 12.9% in comparison to that of the FCP-A alone coated leathers. Similar to the air permeability studies, it was found that the WVP of the leathers coated with bentonite incorporated polymers were higher than the polymer alone coated leathers thus confirming the proposed hypothesis. Additionally, it was found that with the increase in the bentonite concentration, the WVP values also increased confirming that in the presence of higher amounts of clay particles, the increase in the porosity/in-continuity of film helps in increasing the movement of water vapors through the film

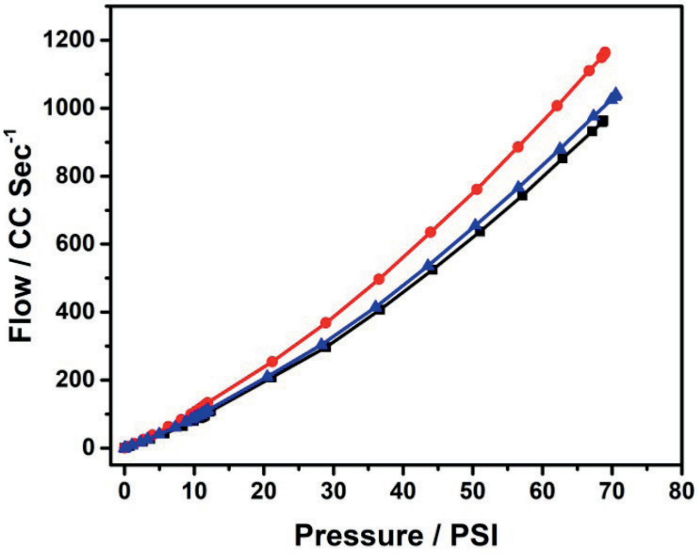

Figure 4. Capillary flow porometry analysis of crust and polymer coated leather samples - (red circles) Crust leather, (blue triangles) BFCAP-A-1 coated leather and (black squares) FCP-A only coated leather

Table II

WVP of the crust leather and polymer coated leather

Sample name	WVP (mg/cm^2/hr)	% Retention in WVP as against crust leather	% Increase in WVP values as against FCP-A leather
Crust leather	8.7	-	-
FCP-A alone coated leather	4.4	50.6	-
BFCP-A-0.25	4.7	54.0	+ 3.4
BFCP-A-0.50	5.0	57.5	+ 6.9
BFCP-A-0.75	5.5	63.2	+ 12.6
BFCP-A-1.00	5.7	65.5	+ 12.9

thus enhancing the water vapor permeability values. Still, from the results, it could be seen that the WVP values were much higher than the required standard of 2.0 mg/cm^2/hr. Thus, even though a polymeric finish coat decreases the breathability of the leather due to the continuous nature of film formed, it can be improved by incorporating clay like particles which can mimic the action of pore-forming agents and in turn enhance the required properties.[33]

Fastness properties of the finished leather

In order to study the effect of the hydrophobic coating on the fastness properties of the leather, the light fastness, wet and dry rub fastness of the crust and polymer coated leather samples were assessed. It was found that the polymer coating enhanced the rub fastness values which could be attributed to the formation of a protective film by the polymeric binder. However, it was observed that as the hydrophobicity of the surface increased, the fastness properties especially the wet rub fastness improved, which could be mainly attributed to the increase in the contact angle of the leather leading to a disruption in the interaction between the water and dye molecules present in the leather.[34] Thus, the fastness characteristics of leather treated with polymer combined with bentonite clay showed an increase as compared with the leather treated with polymers alone as shown in Table III.

Optical properties of the finished leather

Though the presence of clay-incorporated hydrophobic coating on the leather surface helped enhance the permeability of air and water vapor, and fastness properties of the leather, it imparted some negative effects on the optical properties of the same. First, a mild yellow tint was observed with the addition of the bentonite clay particles upon the formation of finish film. Thus, to check this, the CIELAB L*, a* and b* values of the finished leathers coated only with the polymer and polymer-bentonite combinations were measured and compared as shown in Table IV.

Table III

Results of fastness characteristics

Sample name	Dry 512 rubs	Wet 256 rubs
Crust leather	4	3
FCP-A alone coated leather	4	3/4
BFCP-A-0.25	4	4
BFCP-A-0.50	4	4
BFCP-A-0.75	4/5	4
BFCP-A-1.00	4/5	4

Table IV

Color coordinates of crust leather and finished leathers.

Sample name	L*	a*	b*
Crust leather	79.02	-2.70	7.66
FCP-A alone coated leather	77.43	-2.53	9.56
BFCP-A-0.25	77.31	-2.02	10.25
BFCP-A-0.50	76.58	-2.11	10.87
BFCP-A-0.75	76.65	-2.05	11.64
BFCP-A-1.00	76.87	-2.00	12.38

Here, the L* represents lightness value measured from 0 to 100 wherein, the higher the number the lighter the color is, while a* represents the balance between red (positive) and green (negative) and b* represents the balance between yellow (positive) and blue (negative). Though the L* value and a* values were not much affected, the b* value increased from 7.66 for crust leather to 12.38 for BFCP-A-1 coated leather in the positive side. This could be attributed to the inherent cream-colored nature of the bentonite clay particles. Though the color is light, it could be masked with proper pigment mixtures. However, its effect on white colored leathers could be a bit challenging.

Conclusions

The treatment of leather surfaces using selected fluorocarbon polymer and bentonite clay mixture can impart hydrophobicity character to the leathers. The treatment of leather with the polymer containing relatively higher concentrations of bentonite clay showed better contact angle values than the polymer alone coated leather due to the increase in surface roughness. The hydrophobic coating increased the rub fastness, and air and water vapor permeability properties of the leather while it had a negative impact on the optical properties of the leather, mainly the color. Still, this method to impart hydrophobicity to leather surfaces is a relatively simple procedure and has venues for commercial applications in future to fine tune the properties required for a specific set of leather finishing system.

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References

1. Ramesh, R. R., Murali, S and Jonnalagadda, R. R.; Research Advances in the Fabrication of Biosafety and Functional Leather: A Way-Forward for Effective Management of COVID-19 Outbreak. *Journal of Cleaner Production* 127464, 2021.
2. Ma, J., Zhang, X., Bao, Y and Liu, J. A.; Facile Spraying Method for Fabricating Superhydrophobic Leather Coating. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **472**, 21–25, 2015.
3. He, X., Huang, Y., Xiao, H., Xu, X., Wang, Y., Huang, X and Shi, B.; Tanning Agent Free Leather Making Enabled by the Dispersity of Collagen Fibers Combined with Superhydrophobic Coating. *Green Chem.* **23**, 3581–3587, 2021.
4. Silvestre, C. R., Blasco, M. P. C., López, S. R., Aguilar, H. P., Limiñana, M. Á. P., Gil, E. B., Calpena, E. O and Ais, F. A.; Hydrophobic Leather Coating for Footwear Applications by a Low-Pressure Plasma Polymerisation Process. *Polymers* **13**, 3549, 2021.
5. Ramesh, R. R and Jonnalagadda, R. R.; Fabrication of Zirconium Based Coordination Polymers for Fluorine/Silane Free Superhydrophobic Coatings. *Chemical Engineering Journal* **431**, 134262, 2022.
6. Ivanova, N. A and Zaretskaya, A. K.; Imparting Waterproof Properties to Cotton Surface. *Prot Met Phys Chem Surf* **47**, 369–371, 2011.
7. Erasmus, E and Barkhuysen, F. A.; Superhydrophobic Cotton by Fluorosilane Modification. *IJFTR* **34**, 377-379, 2009.
8. Xu, L., Zhuang, W., Xu, B and Cai, Z.; Fabrication of Superhydrophobic Cotton Fabrics by Silica Hydrosol and Hydrophobization. *Applied Surface Science* **257**, 5491–5498, 2011.
9. Barthlott, W and Neinhuis, C. Purity of the Sacred Lotus, or Escape from Contamination in Biological Surfaces. *Planta* **202**, 1–8, 1997.
10. Tropmann, A., Tanguy, L., Koltay, P., Zengerle, R and Riegger, L. Completely Superhydrophobic PDMS Surfaces for Microfluidics. *Langmuir* **28**, 8292–8295, 2012.

11. Pozzato, A., Zilio, S. D., Fois, G., Vendramin, D., Mistura, G., Belotti, M., Chen, Y and Natali, M.; Superhydrophobic Surfaces Fabricated by Nanoimprint Lithography. *Microelectronic Engineering* **83**, 884–888, 2006.
12. Öner, D and McCarthy, T. J.; Ultrahydrophobic Surfaces. Effects of Topography Length Scales on Wettability. *Langmuir* **16**, 7777–7782, 2000.
13. McHale, G., Aqil, S., Shirtcliffe, N. J., Newton, M. I and Erbil, H. Y.; Analysis of Droplet Evaporation on a Superhydrophobic Surface. *Langmuir* **21**, 11053–11060, 2005.
14. Ke, Q., Fu, W., Jin, H., Zhang, L., Tang, T and Zhang, J. Fabrication of Mechanically Robust Superhydrophobic Surfaces Based on Silica Micro-Nanoparticles and Polydimethylsiloxane. *Surface and Coatings Technology* **205**, 4910–4914, 2011.
15. Ma, M., Mao, Y., Gupta, M., Gleason, K. K and Rutledge, G. C.; Superhydrophobic Fabrics Produced by Electrospinning and Chemical Vapor Deposition. *Macromolecules* **38**, 9742–9748, 2005.
16. Pan, S., Kota, A. K., Mabry, J. M and Tuteja, A.; Superomniphobic Surfaces for Effective Chemical Shielding. *J. Am. Chem. Soc.* **135**, 578–581, 2013.
17. Zhao, N., Shi, F., Wang, Z and Zhang, X. Combining Layer-by-Layer Assembly with Electrodeposition of Silver Aggregates for Fabricating Superhydrophobic Surfaces. *Langmuir* **21**, 4713–4716, 2005.
18. Zhang, X., Ye, H., Xiao, B., Yan, L., Lv, H and Jiang, B.; Sol–Gel Preparation of PDMS/Silica Hybrid Antireflective Coatings with Controlled Thickness and Durable Antireflective Performance. *J. Phys. Chem. C* **114**, 19979–19983, 2010.
19. Hikita, M., Tanaka, K., Nakamura, T., Kajiyama, T and Takahara, A.; Super-Liquid-Repellent Surfaces Prepared by Colloidal Silica Nanoparticles Covered with Fluoroalkyl Groups. *Langmuir* **21**, 7299–7302, 2005.
20. Latthe, S. S., Imai, H., Ganesan, V and Venkateswara Rao, A.; Porous Superhydrophobic Silica Films by Sol–Gel Process. *Microporous and Mesoporous Materials* **130**, 115–121, 2010.
21. Kopp, V. V., Agustini, C. B., Gutterres, M and dos Santos, J. H. Z.; Nanomaterials to Help Eco-Friendly Leather Processing. *Environ Sci Pollut Res* **28**, 55905–55914, 2021.
22. Wang, X., Wang, W., Liu, X and Wang, Y.; Amphoteric Functional Polymers for Leather Wet Finishing Auxiliaries: A Review. *Polymers for Advanced Technologies* **32**, 1951–1964, 2021.
23. Hayakawa, Y., Terasawa, N., Hayashi, E and Abe, T.; Synthesis of Novel Polymethacrylates Bearing Cyclic Perfluoroalkyl Groups. *Polymer* **39**, 4151–4154, 1998.
24. Park, I. J., Lee, S.B and Choi, C. K.; Surface Properties of the Fluorine-Containing Graft Copolymer of Poly((Perfluoroalkyl) Ethyl Methacrylate)-g-Poly(Methyl Methacrylate). *Macromolecules* **31**, 7555–7558, 1998.
25. Thomas, R. R., Anton, D. R., Graham, W. F., Darmon, M. J., Sauer, B. B., Stika, K. M and Swartzfager, D. G.; Preparation and Surface Properties of Acrylic Polymers Containing Fluorinated Monomers. *Macromolecules* **30**, 2883–2890, 1997.
26. Stalder, A. F., Melchior, T., Müller, M., Sage, D., Blu, T and Unser, M.; Low-Bond Axisymmetric Drop Shape Analysis for Surface Tension and Contact Angle Measurements of Sessile Drops. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **364**, 72–81, 2010.
27. Boinovich, L. B and Emelyanenko, A. M.; Hydrophobic Materials and Coatings: Principles of Design, Properties and Applications. *Russ. Chem. Rev.* **77**, 583-601, 2008.
28. Nosonovsky, M.; Multiscale Roughness and Stability of Superhydrophobic Biomimetic Interfaces. *Langmuir* **23**, 3157–3161, 2007.
29. Patankar, N. A.; On the Modeling of Hydrophobic Contact Angles on Rough Surfaces. *Langmuir* **19**, 1249–1253, 2003.
30. Ruamcharoen, J., Ratana, T and Ruamcharoen, P. Bentonite as a Reinforcing and Compatibilizing Filler for Natural Rubber and Polystyrene Blends in Latex Stage. *Polymer Engineering & Science* **54**, 1436–1443, 2014.
31. Jia, L., Wang, L., Li, J., Xiang, J., Chen, Y and Fan, H.; Silica-Embedded Polyurea Microspheres with Rough Surface for Matte Leather Finishing. *JALCA* **117**(5), 184- 194, 2022.
32. Bao, Y., Feng, C., Wang, C., Ma, J and Tian, C.; Hygienic, Antibacterial, UV-Shielding Performance of Polyacrylate/ZnO Composite Coatings on a Leather Matrix. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **518**, 232–240, 2017.
33. Chen, W., Su, Y., Zhang, L., Shi, Q., Peng, J and Jiang, Z. In Situ Generated Silica Nanoparticles as Pore-Forming Agent for Enhanced Permeability of Cellulose Acetate Membranes. *Journal of Membrane Science* **348**, 75–83, 2010.
34. Wang, Z., Sun, Z., Chen, X., Zou, W., Jiang, X., Sun, D and Yu, M. Color Fastness Enhancement of Dyed Wood by Si-Sol@ PDMS Based Superhydrophobic Coating. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **651**, 129701, 2022.

Removal of Proteoglycans in Animal Hides by Glycosidase to Promote Opening-Up of Collagen Fiber Bundles: Optimization of Glycosidase and its Application in the Soaking Process

by
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Abstract

The degree of proteoglycan removal from animal hides is closely related to the opening-up of collagen fibers and the properties of leather, but conventional leather-making processes are not very effective at removing proteoglycans. By rationally selecting and utilizing glycosidases during the leather-making process, proteoglycans can be efficiently removed without causing collagen damage due to glycosidases specifically hydrolyzing glycosidic bonds. In spite of this, glycosidases are highly specific and come in a wide variety of types. To solve the blindness of optimizing glycosidases and its application in the soaking process, in this study, seven typical commercial glycosidases capable of hydrolyzing β-glycosidic bonds were selected according to the types of major glycosidic bonds in animal hide proteoglycans. The basic properties of the selected glycosidases and its effectiveness in removing proteoglycans from the hide during the soaking process were investigated. Then, the effects of the dosage of the two optimal glycosidases on the dissolution of polysaccharide, total soluble protein and hydroxyproline, as well as the properties of leather during the soaking process were further studied. The results showed that although the optimal pH of the selected glycosidases was between pH 5 - 7, they maintained more than 50% of their activity under weak alkaline conditions and could be applied to the soaking process. The removal of proteoglycan by different glycosidases is closely related to their specificity for glycosidic bonds, and the penetrability of the enzyme proteins, namely the molecular weight and isoelectric point of the enzymes. Among them, β-Mannase-AKT and Xylanase-AKT are more effective in removing proteoglycans. When the dosage of β-Mannase-AKT and Xylanase-AKT reached 47 U/mL, almost all the glycosaminoglycan was removed from the hide, the opening-up of collagen fibers and the softness and physical properties of the crust leather were significantly improved without damage to the structural proteins.

Introduction

Proteoglycans (PG) are primarily found between the epidermis, hair follicles and collagen fibers. Although its content is not high, they play a significant role in the growth of animals. Once the raw hides lose moisture, glycoconjugates will adhere the collagen fibers together, negatively affecting the penetration of water and other chemical materials into the hides, as well as the opening-up of collagen fibers and the softness of the leather.[1-3] Therefore, proteoglycans should be removed as early as possible in the leather-making process. Since proteoglycans are chemically stable, only half of them can be removed in strongly liming processes.[4] Although the application of proteases during the soaking process can effectively remove inter-fibrillary substances, it is important to realize that the use of proteases may lead to the destruction of structural proteins in the leather, resulting in loose and damaged grain.[5]

Glycosidase can specifically hydrolyze glycosidic bonds and the sugar chain, thus removing the proteoglycan from the hides. Studies showed that the use of glycosidases, such as galactosidase, amylase, glucosidase and cellulase, in the soaking, enzymatic dehairing and bating processes is beneficial for the removal of proteoglycan and the improvement of leather properties.[6,7] In addition, glycosidases do not hydrolyze collagen and can be properly selected and used in the leather-making process to achieve efficient removal of proteoglycans without causing damage to collagen.[8,9] Therefore, glycosidases have great application potential in the leather making process.

There are a wide variety of glycosidase enzymes, each of which has a specific substrate specificity. The cleavage sites and action mechanisms to proteoglycan chains of different glycosidases differ considerably, and their optimal conditions of use also vary considerably. Additionally, animal hides are primarily composed of collagen fibers, which are formed in a three-dimensional network. Consequently, there is some mass transfer resistance to the penetration of enzyme molecules into the hide, which is influenced by the molecular size and charge state of the enzyme molecules. Therefore, the removal effect of glycosidase on proteoglycan depends on the specificity of the glycosidic bond, the molecular weight and the isoelectric point of the enzyme, as well as the conditions of application. Although there are some reports regarding the application of glycosidases in the leather-making process, however, there has been a lack of in-depth research and blindness in the

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optimization of glycosidases and the development of application technologies.

In this paper, seven typical commercial glycosidases were selected to hydrolyze animal hides β-glycosidic bonds according to the glycosidic bond specificity and the types of main PG glycosidic bonds in animal hide. The hydrolytic properties of the selected glycosidases were investigated by using proteoglycan-containing hide powder as substrate, and the molecular weight and isoelectric point of the enzyme molecule were also determined. Based on this, proteoglycan removal effects of different glycosidases in the soaking process were compared, the optimal enzymes were screened out and the relationship between the dosage of enzyme and the degree of proteoglycan removal and leather properties were investigated. This study attempts to provide methods and a basis for the development of glycosidase application technologies in the leather making processes.

Materials and Methods

Materials

Wet-salted cattle hides were purchased from the local market in Chengdu, China. Alison Blue, 1,6-dimethyl methylene blue and bovine serum protein was purchased from Sigma, USA. Proteoglycan-containing hide powder was made by our laboratory.[10] Glycosidases were all commercial grade and supplied by Qactive Bio-products Co. Ltd. and Longda Bio-products Co. Ltd, China. Leather chemicals were all industrial grade and supplied by Sichuan Dowell Science and Technology Co., Ltd, China. Other analytical grade reagents were purchased from local suppliers in Chengdu, China. The weight of leather making chemicals used for soaking was based on the weight of the wet-salted hide.

Assay of glycosidase activity

The activity of glycosidases was determined through our laboratory-established polysaccharide-containing hide powder substrate method.[10] In detail, 2.000 g ± 0.001 g of hide powder was accurately weighed and added to a 150 mL conical flask. 40 mL of Britton-Robinson buffer (0.1 mol/L, pH 7.5) was added and stirred at 30°C for 2 h. Then, 2 mL of enzyme solution was added to the experimental group and stirred at 30°C for 2 h, relatively, 2 mL of hyperthermia (100°C) inactivated enzyme solution was added to the control group under the same conditions. The reaction was terminated by adding 2 mL of sulfuric acid (6 mol/L) and the mixture was filtered with a qualitative filter paper. The concentration of total sugar in the filtrate was determined by the phenol sulfate method[11] and the total sugar content in the reaction liquor was calculated.

One unit of glycosidase activity is defined as the amount of enzyme required to produce 1 μg of glucose per hour for the hydrolysis of polysaccharides in hide powder at specific conditions.

Application of glycosidase in the soaking process

After pre-soaking and fleshing according to the conventional method, wet-salted cattle hides were symmetrically sampled and weighed. The soaking process was carried out in 100 % (w/w) of water at 25°C, a certain amount of glycosidase was added to the experimental groups and 0.2% of soaking auxiliary SWA was added to the control group. The drum was rotated continuously for 1 h at the beginning of the soaking; then, rotated for 30 min and stopped for 30 min 2 times; then, rotated for 10 min and stopped for 50 min 2 times; finally, soaked for 13 h overnight at room temperature. After 30 min of rotation, the produced amount of soluble protein, total sugar, hydroxyproline and glycosaminoglycan at the end of the soaking process were measured, respectively. The residual sugar in the hide was observed after being adjacently and symmetrically sampled from the hides and stained by the periodate-Schiff-Alixin blue staining method.

Soaked samples were treated according to the conventional leather making procedures. The softness, physical properties and organoleptic properties of the crust leather were evaluated as per the standard methods.

Analysis of soluble protein content in the soaking liquors

The soaking liquors were centrifuged at 3500 r/min for 10 min and the content of soluble protein in the supernatant of the soaking liquor was measured by Lowry's method.[12] The absorbance of the colored mixture was determined at 680 nm to measure the concentration of soluble protein using bovine serum protein as the standard substrate.

Analysis of total sugar content in the soaking liquors

The content of total sugar in the supernatant of the soaking liquor was determined by the sulfuric acid-phenol method.[11] In detail, 1 mL of the supernatant and 0.5 mL of phenol solution (6 %) were mixed in a test tube; then, 2.5 mL of concentrated sulfuric acid was added and reacted at 90°C for 30 min; finally, the mixture was cooled in cold water for 3 min and the absorbance of the mixture was measured at 490 nm. The content of total sugar was calculated using glucose as the standard substrate.

Analysis of glycosaminoglycan content in the soaking liquors

The content of glycosaminoglycan (GAG) in the supernatant of the soaking liquor was determined by the DMB method.[13] In detail, 100 μL of the supernatant and 2.5 mL of DMB reagent were mixed in a colorimetric tube and the absorbance was measured at 525 nm, immediately. The content of GAG was calculated using chondroitin sulfate as the standard substrate.

Analysis of hydroxyproline content in the soaking liquors

The content of hydroxyproline (Hypro) in the supernatant was determined by the Chloramine-T oxidation method.[14,15] The absorbance of the colored mixture was determined at 560 nm and the content of Hypro was calculated using analytical grade Hypro as the standard substrate.

Histological staining of polysaccharides

Samples were fixed in a neutral formaldehyde solution (10%, v/v) for 24 h. Sections of 12 μm thickness were obtained using a CM1950 freezing microtome (Leica, Germany) and stained according to the Periodic acid-Schiff-Alcine blue method.[16] In detail, sections were stained in Alcine Blue staining solution for 40 min, followed by thorough washing with deionized water. Then, samples were oxidized in a periodic acid solution for 8 min and washed with deionized water and stained with Schiff's staining solution for 8 minutes. Next, the sections were dehydrated by 70% alcohol, 95% alcohol, 100% alcohol, xylene-ethanol solution (1:1), and pure xylene for 3 min each, respectively, and sealed with a neutral resin. The residue of polysaccharides was observed with a Nexcope NE900 optical microscope (Ningbo Yongxin Optics Co. Ltd., China) and the stained polysaccharide was in red.

Molecular weight and isoelectric point of the glycosidases

The molecular weight (Mr) and isoelectric point (pI) of the selected glycosidases were determined through SDS-PAGE and IEF-PAGE methods, respectively, and stained by the Coomassie Brilliant Blue method.[17,18]

Stereoscopic microscope and scanning electron microscope analysis of the crust leather

The grain surface and reticular layer fibers of the crust leather were observed using a stereoscopic microscope and a scanning electron microscope (SEM), respectively.

Pore diameter of the crust leather

The pore diameter of the crust leather was determined using a Gemini VII 2390 Surface Area Analyzer (Micrometrics Instrument Gorp., USA) after cutting the samples into approximately 0.125 cm^3 pieces.

Physical properties of the crust leather

Crust leather was sampled in the adjacent and symmetrical parts and conditioned as per the IUP method (IUP 2, 2000). The physical properties such as tear strength, tensile strength and bursting strength were tested as per the standard procedures (IUP 8, 2000; IUP 6, 2000; IUP 9, 2000). The softness of the crust leather was tested using a GT-303 Leather Softness Tester (Gotech Testing Machines Inc., China).

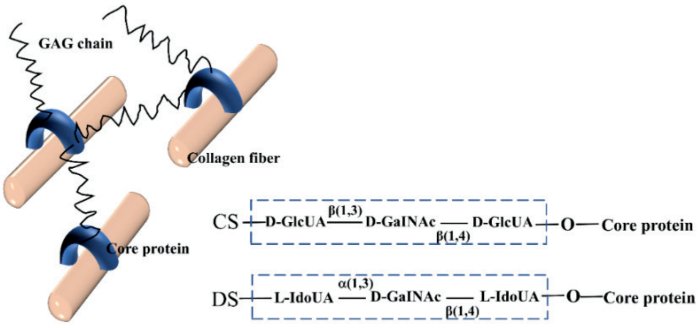

Figure 1. Schematic diagram of proteoglycan structure in cattle hide[30]

Results and Discussion

Performance of glycosidase on proteoglycan

There are various types of glycosidases and the substrate specificity is very strict. Therefore, the substrates for different types of glycosidase activity assessment are different. For example, starch, xylan, and barley dextran are usually used as the substrates for amylase, xylanase, and dextranase activity assessment, respectively.[19-21] However, the glycosidic bonds in these substrates are different than that of the proteoglycans in cattle hide. Enzyme activities measured by these methods can not reflect their actual effects on the glycosidic bond in animal hide proteoglycan (PG). The proteoglycan in cattle hide is diverse and structurally complex. PG consists of a core protein chain and one or more glycosaminoglycan (GAG) chains. The GAG chain of cattle hides PG is mainly composed of disaccharide structural units of chondroitin sulfate (CS) and dermatan sulfate (DS), namely the glycosyl groups are mainly connected by β-glycosidic bonds, such as β-1,3 glycosidic bonds and β-1,4 glycosidic bonds (as is shown in Figure 1).[22]

Based on the above analysis, several commercial glycosidases (including β-Mannase-AKT, Cellulase-AKT, Hyaluronate lyase-AKT, β-Glucosidase-AKT, Xylanase-AKT, Xylanase-LKT and β-Glucanase-AKT) capable of hydrolyzing β-glycosidic bonds were selected based on the types of major glycosidic bonds in cattle hide PG (Table I). The hydrolysis effect on the PG glycosidic chain was investigated by using a glycosidase activity assessment method, which was established by our laboratory using PG-containing hide powder as the substrate.[10]

Basic properties of typical glycosidases

Effect of pH on the activity of glycosidases

Most of the optimum pH of glycosidases is acidic, and the pH of the main treatment in the beamhouse of leather making is in an alkaline environment, except for the pickling process. Therefore, the variation of several glycosidases' activity with pH was investigated in the pH range of 4 - 9 using the hide powder substrate method, and the results are shown in Figure 2.

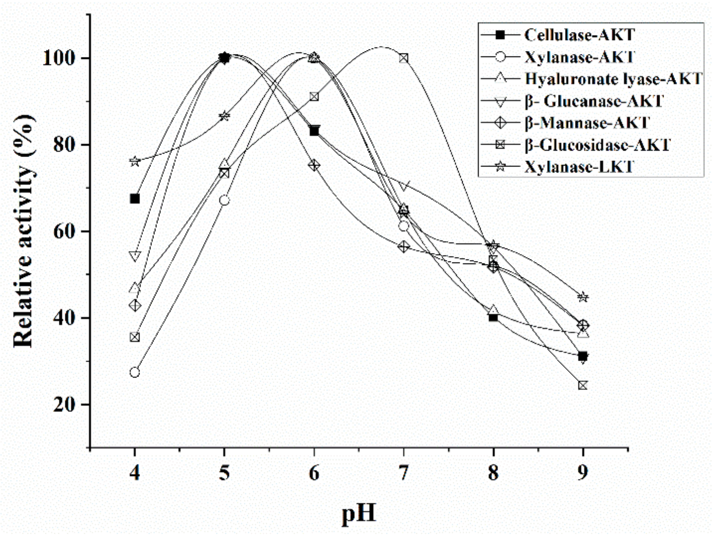

Figure 2. Effect of pH on the glycosidase activity (30°C)

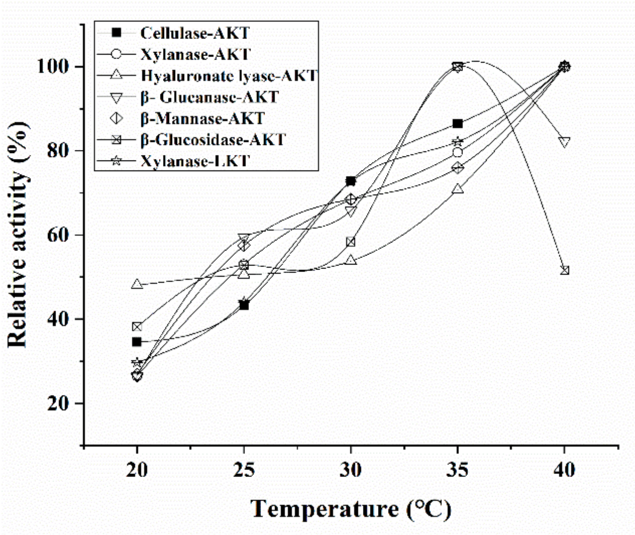

Figure 3. Effect of temperature on the glycosidase activity (pH 7.5)

The result in Figure 2 shows that, for hydrolyzing proteoglycans in the hide powder substrate, the optimum pH of β-Mannase-AKT, β-Glucanase and Cellulase-AKT is around 5.0, the optimum pH of Xylanase-LKT, Hyaluronate lyase-AKT, and Xylanase-AKT is around 6.0, β- Glucosidase has the highest activity at pH 7.0. Although the optimum pH of the selected glycosidases is acidic (pH 5 - 7), most of the enzymes still maintain more than 50 % of activity in the pH range of 7 - 8. Therefore, they have the potential to be applied in weakly alkaline processes, such as soaking and bating.

Effect of temperature on the activity of glycosidases

Collagen in rawhide is sensitive to heat, and high temperature can easily cause collagen denaturation. Therefore, the treatment temperature of the beamhouse generally does not exceed 40°C. The variation of the selected glycosidase activity with temperature was investigated in the range of 20°C - 40°C, and the results are shown in Figure 3.

As is shown in Figure 3, the thermal resistance of β-Glucanase-AKT and β-Glucosidase-AKT was poor, and their optimum temperature was 35°C, which decreased sharply when the temperature was increased further. The activity of other glycosidases was increased with the increase of temperature in 25°C - 40°C. Remarkably, Hyaluronate lyase-AKT shows little activity change from 20°C to 30°C. The activity of glycosidases changes greatly with temperature, so its dosage should be adjusted according to the temperature of different leather making processes. For example, the dosage of enzyme used in the soaking process at a lower temperature (25°C) should be higher than that of bating process at a higher temperature (35°C).

Table I

Molecular weight, isoelectric point and activity of glycosidases

Glycosidase	**Mr (kDa)**			**pI**		**Activity* (U/g)**
	M_1	**M_2**	**M_3**	**P_1**	**P_2**	
β-Mannase-AKT	40.9	-	-	4.40	-	5546 ± 57
Cellulase-AKT	64.3	56.1	45.2	4.85	7.50	2888 ± 30
Hyaluronate lyase-AKT	80.4	-	-	4.60	-	2788 ± 35
β-Glucosidase-AKT	123.3	-	-	4.85	-	2599 ± 28
Xylanase-AKT	11.3	-	-	6.72	-	2580 ± 33
Xylanase-LKT	70	60.5	17.7	4.80	8.30	2484 ± 16
β-Glucanase-AKT	25.7	-	-	4.55	-	2253 ± 23

*30°C, pH 7.5

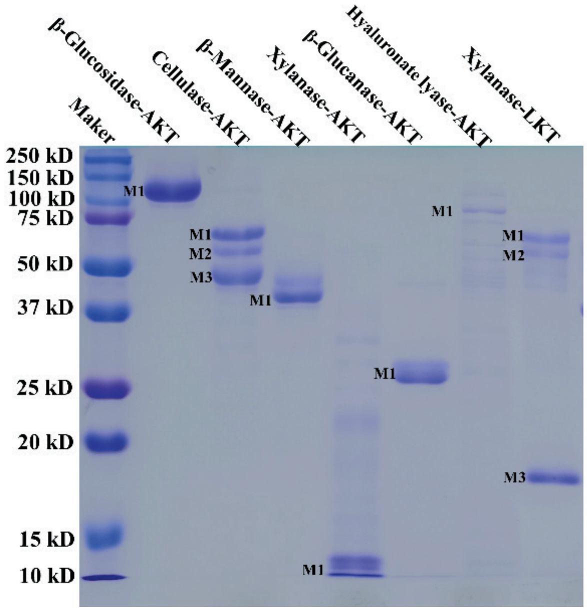

Figure 4. SDS-PAGE electropherogram of glycosidases

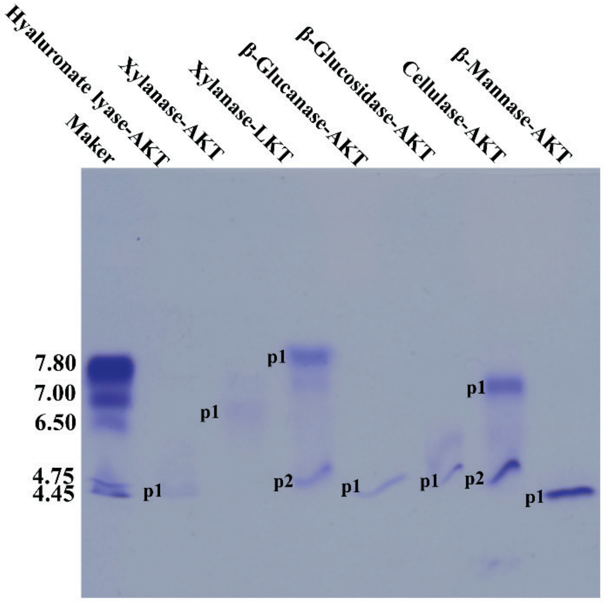

Figure 5. IEF-PAGE electropherogram of glycosidases

Usually, the soaking temperature is below 30°C and the pH is in the range of 8 - 10, however, the optimum pH of the selected glycosidases is close to acidic. Considering the effective utilization of enzyme activity, the glycosidase-based soaking stage should be controlled at pH 7 - 8 and 30°C. Table I shows that β-Mannase-AKT possesses of highest enzyme activity to proteoglycan, and other glycosidases show similar activity.

Molecular weight, isoelectric point and activity properties of the selected glycosidases

The penetration of glycosidase protein molecules into the hides is very important during the treatment process. The penetration property of enzymes mainly depends on their molecular weight and charge state. Therefore, the molecular weight (Mr) and isoelectric point (pI) of the selected glycosidases were analyzed using SDS-PAGE and IEF-PAGE methods, and the results are shown in Figure 4, Figure 5 and Table I, respectively.

As is shown in Figure 4 and Table I, except for Cellulase-AKT and Xylanase-LKT, most of the glycosidases contain only one enzyme protein component. The Mr of these glycosidases is different. The Mr of β-Glucosidase (123.3 kDa) is the largest, and Xylanase-AKT has the smallest Mr (11.3 kDa). The Mr of β-Mannase-AKT, β-Glucanase-AKT and Hyaluronate lyase-AKT are 40.9 kDa, 25.7 kDa and 80.4 kDa, respectively. Cellulase-AKT contains three different protein components in the range of 45.2 - 64.3 kDa. Xylanase-LKT contains three different protein components with the Mr of 70.0 kDa, 60.5 kDa and 17.7 kDa, it's worth noting that the smallest component (17.7 kDa) doesn't have activity.

As is shown in Figure 5 and Table I, Xylanase-LKT and Cellulase-AKT contain two different pI components. The pI of Xylanase-LKT is 8.3 and 4.8, and the pI of Cellulase-AKT is 7.5 and 4.85. The other five glycosidases only have one protein component with a pI in the range of 4 - 5, except Xylanase-AKT has a relatively higher pI of 6.72.

The penetration of enzyme molecules into cattle hide is closely related to its molecular size. Among the selected glycosidases, β-Glucosidase-AKT has a larger molecule weight and may have an obvious penetration barrier. Furthermore, the penetration of enzyme molecules is also influenced by the charge state of hide collagen and enzyme proteins. When it takes unlike charge at a certain pH value, a strong bond on the surface of hides between collagen fiber and enzyme may affect the penetration of enzyme proteins.

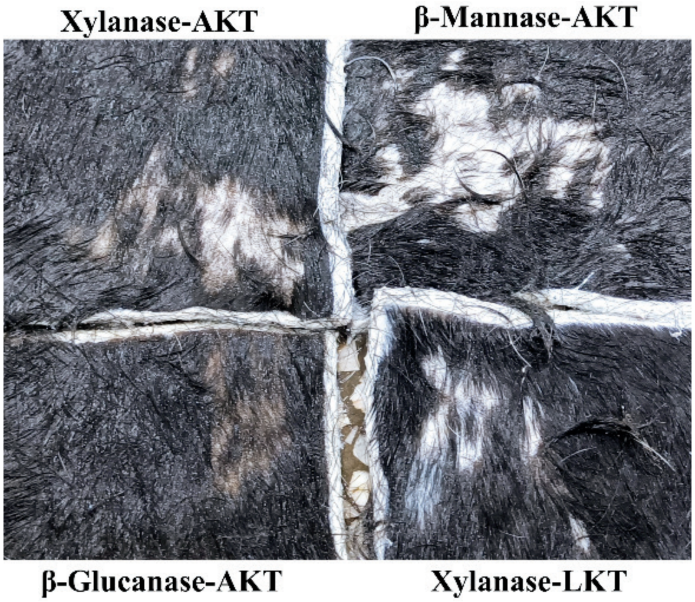

Figure 6. Residue of the epidermis after the soaking process

Comparison of the proteoglycan removal effect by different glycosidases

Effect of glycosidase on the epidermis

Seven kinds of glycosidases were applied in the soaking process with the same dosage of enzymes (0.6%, w/w), and the removal effect of the epidermis at the end of soaking is shown in Figure 6.

The results showed that the hair root of β-Mannase-AKT, Xylanase-AKT and Xylanase-LKT treated hide were loosed and easily pulled out by hand. Most of the epidermis was removed, especially for the β-Mannase-AKT treated sample. However, the hair and epidermis were still tightly bound after being treated with β-Glucanase-AKT, Cellulase-AKT, Hyaluronate lyase-AKT and β-Glucosidase-AKT.

The inner layer of the hair follicle is the inner root sheath, which is formed by the depression of keratinized epidermis cells; Its outer layer is the outer root sheath, which consisted of the basal layer of the epidermis; The outermost layer is connective tissue sheath; Hair bulbs and hair papilla are bonded mainly by proteoglycan.[31] The epidermis, hair papillae, hair root sheath and connective tissue sheath contain a large number of proteoglycans, and the action of glycosidase on proteoglycans leads to the destruction of the epidermis and the loosening of the hair root. Due to the high substrate specificity of glycosidase, different glycosidases have different hydrolysis properties to the glycosidic bond of the specific proteoglycan, GAG. The above experimental results show that β-Mannase-AKT, Xylanases-AKT and Xylanases-LKT have stronger proteoglycans removal effects on the epidermis and hair follicle.

Produced amount of total sugars and glycosaminoglycans in soaking liquors

At the end of the soaking, the produced amount of total sugar in the soaking liquor was used to characterize the removal effect of polysaccharides by glycosidase. The content of total sugar and GAG

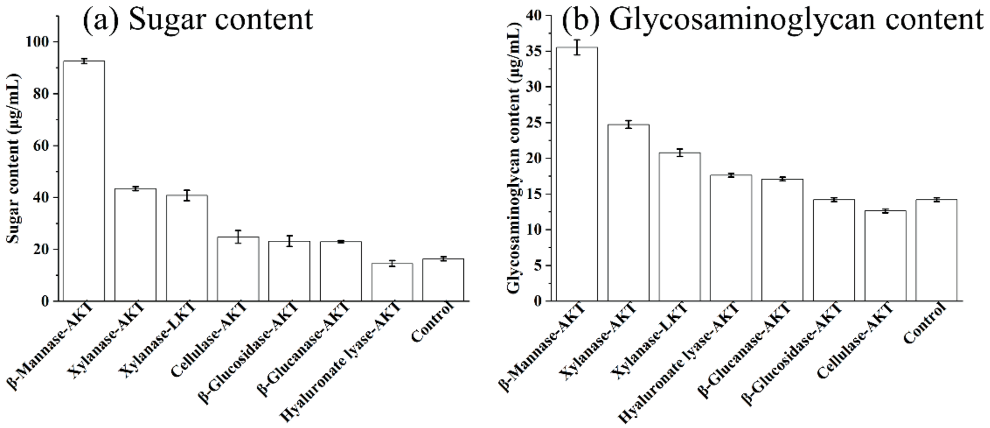

Figure 7. Total sugar and glycosaminoglycan content in the soaking liquors

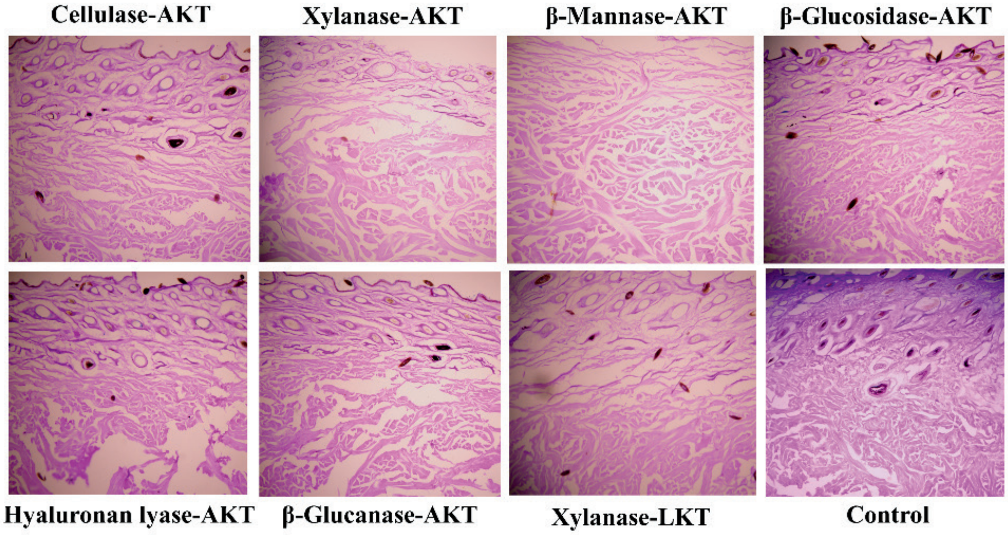

Figure 8. Residues of polysaccharides in the soaked hide (40 X)

treated with the same dosage (0.6 %, w/w) of enzymes were shown in Figure 7.

In the soaking process, glycosidases specificity hydrolyzes the glycosidic bond of the polysaccharide chain and destroys the polysaccharide structure, then, small sugar chains are dissolved from the hide. As is shown in Figure 7, β-Mannase-AKT has the highest activity and produced the highest content of total sugar and GAG. Although there is a little difference in the activity of other glycosidases to the proteoglycan-containing hide powder substrate, the produced amount of total sugar shows a significant difference. Xylanase-AKT and Xylanase-LKT produced a higher amount of total sugar and GAG. β-Glucosidase-AKT and Hyaluronate lyase-AKT have poorly effective in the removal of proteoglycan.

The types and structures of proteoglycan in the cattle hide are complicated, and the hydrolysis specific to glycosidic bonds of glycosidases is very high. Therefore, different glycosidases have significantly different performances on the proteoglycan. In addition, the effect of glycosidases on hides proteoglycan is also influenced by its penetration ability in the hides. Figure 4 and Table I showed that β-Glucosidase-AKT, Hyaluronate lyase-AKT and Cellulase-AKT have relatively larger molecular weights, which may have penetration barriers, exhibiting poor polysaccharide removal effect. Furthermore, the solubility, agglomeration and charge state of enzyme molecules also affect their penetrability. Figure 5 and Table I showed that the pI value of the major component of Cellulase-AKT was 7.5, its solubility is poor under pH 7 - 8, and enzyme proteins may be agglomerated together. The molecular weight of Xylanase-LKT is significantly higher than that of Xylanase-AKT. Moreover, Xylanase-LKT (pI 8.3) and collagen fiber (pI 7) take an unlike charge at pH 7 - 8, causing a strong affinity on the surface of the hide. All of these make the penetration of Xylanase-LKT not easy. All of these indicated that, although Xylanase-AKT and Xylanase-LKT have almost a similar activity on the hide powder substrate, the proteoglycan removal effect of Xylanase-LKT is not good as Xylanase-AKT. Furthermore, β-Mannase-AKT has the highest activity, small molecular weight and acidic isoelectric point, which make it easy to penetrate the cattle hide and exhibit a higher proteoglycan removal effect.

Staining results of polysaccharides

To further observe the effect of glycosidase on the removal of proteoglycan, the residue of polysaccharides in the hides was stained using the Periodic acid-Schiff and Alcine blue method (PAS-AB), and the results are shown in Figure 8.

Acidic glycosaminoglycan can be colored blue using the PAS-AB staining method, other sugars with red color, and purple color represent the presence of both types of polysaccharides. As is shown in Figure 8, the epidermis, basement membrane and hair follicle of the control group showed a deep purple color, indicating that it contains a large amount of GAG. The hides treated with different glycosidases showed lighter color than the control group, indicating that parts of the acidic GAG were removed. β-Mannase-AKT and Xylanase-AKT treated hides showed the lightest color, and the color is uniform throughout the vertical section and without any purple color, which means that all of the GAG has been removed. The above staining results are consistent with the quantitative results shown in Figure 7.

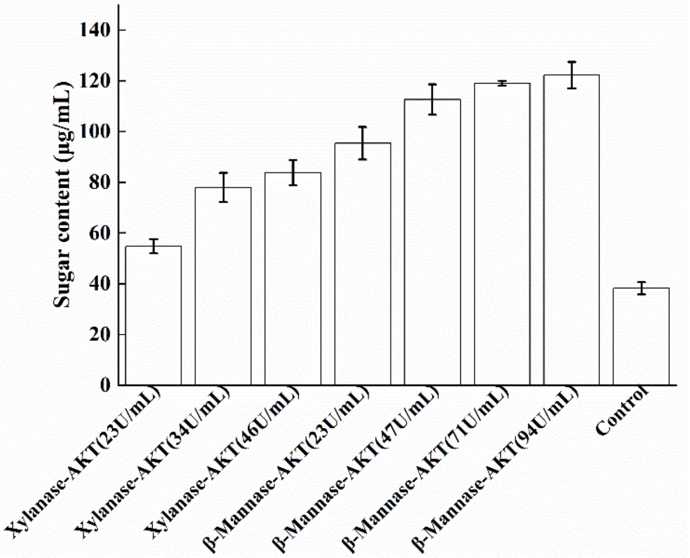

Figure 9. Content of total sugar in the soaking liquors

GAG was almost completely removed from the epidermis, basement membrane and hair follicle after being treated with β-Mannase-AKT and Xylanase-AKT. In particular, β-Mannase-AKT treated hides almost have no epidermis, basement membrane, hair follicle and hair root (Figure 8). These results are consistent with the results shown in Figure 6. It proved that the presence of proteoglycans in the hair follicle has a binding effect on the hair root, and the hair root could be loose after proteoglycans are destroyed. Although the GAG in other glycosidases treated hides showed a different extent of degradation, the hair follicle is still clearly visible, and an obvious proteoglycan circle around the hair root is observed around the hair follicles.

Meanwhile,β-Mannase-AKT and Xylanase-AKT treated hides showed the lightest color on the dermis and the reticular layer, indicating that it contains less GAG. The removal of proteoglycan can eliminate the adhesion of collagen fibers, making hides more open and uniform.

Effect of glycosidase dosage on the soaking

The above result shows that β-Mannase-AKT and Xylanase-AKT have better proteoglycan removal effects in the soaking process. Therefore, the effect of the dosage of these two glycosidases on the soaking and the properties of crust leather was further studied.

Produced amount of total sugar in the soaking liquors

Glycosidases hydrolyze insoluble large sugar chains into soluble small sugar, therefore, the content of total sugar in the soaking liquor can be used to characterize the effect of glycosidases on the removal of proteoglycan from the hide. After being treated with different dosages of glycosidase, the produced amount of total sugar at the end of the soaking is shown in Figure 9.

The result in Figure 9 shows that the concentration of total sugar in the soaking liquors increased with the increase of enzyme dosage. However, the concentration of total sugar was close to the maximum value when the dosage of β-Mannase-AKT and Xylanase-AKT reached 47 U/mL and 34 U/mL, respectively. Staining results of polysaccharides also showed that almost all of the GAG has been removed when the concentration of β-Mannase-AKT reached up to 47 U/mL. Furthermore, at the same concentration of glycosidase activity, β-Mannase-AKT is more effective than Xylanase-AKT in removing polysaccharides from cattle hide. The reason for this is that the enzyme molecule of β-Mannase-AKT has a low isoelectric point, which makes it dissolve well at pH 7 - 8 and it possesses a weak affinity for collagen fibers, making it easier to penetrate the cattle hide.

Produced amount of soluble protein and hydroxyproline in the soaking liquors

The produced amount of soluble protein and Hypro in the soaking liquors after being treated with different amounts of glycosidases are shown in Figure 10.

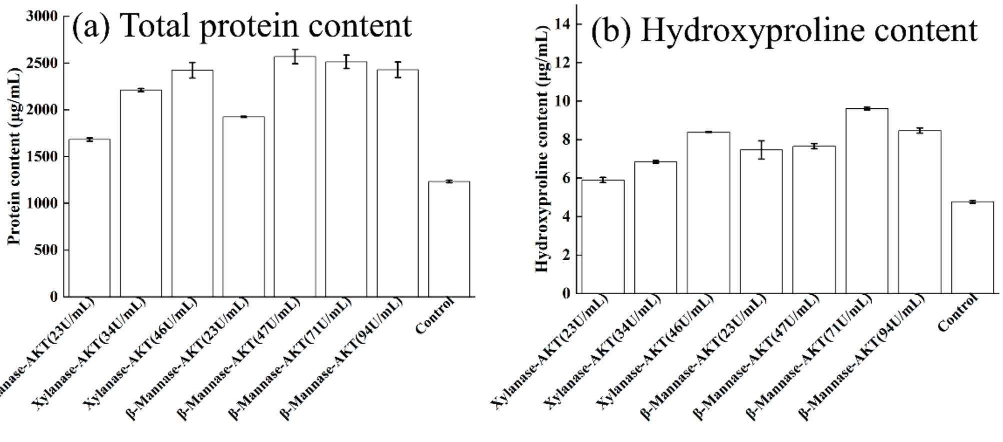

Figure 10. Content of soluble protein and hydroxyproline in the soaking liquors

The result in Figure 10 (a) shows that the content of soluble protein in the glycosidases-soaked liquors was significantly higher than that of the control. The hydrolysis of sugar chains in the proteoglycans by glycosidase can break the proteoglycan structure, which is a benefit for the solubilization of core proteins and increases the soluble protein content in the soaking liquor.[23] The content of soluble protein in Xylanase-AKT treated liquor increased with the increase of enzyme dosage. However, when the concentration of β-Mannase-AKT reached up to 47 U/mL, the content of soluble protein in the soaking liquor was close to maximum value because the GAG in the cattle hide was almost all removed by β-Mannase-AKT. The result in Figure 10 (b) shows that the content of Hypro in both glycosidases treated liquors was slightly higher than that of the control and has not increased with the increase of glycosidase dosage. Overall, the concentration of Hypro in the soaking liquor was low (less than 10 μg/mL), indicating that glycosidases just have a weak effect on collagen.

The grain surface of the wet blue was observed after being soaked with different dosages of glycosidases and conducted the conventional leather making procedures, and the results are shown in Figure 11.

The result in Figure 11 shows that there was no obvious difference in the grain surface of the wet blue after being soaked by glycosidase when compared to the control. Even with a higher glycosidase dosage and longer duration time (94 U/mL of β-Mannase-AKT for 18 h), the grain of the crust leather was still full, intact and undamaged. These results proved once again that the application of glycosidases in the soaking process is a safe and efficient method for raw hides to rehydrate and open the fibers because it has nearly no effect on collagen fibers compared to proteases.

Opening up of collagen fibers

SEM result of the reticular layer fibers in Figure 12 shows that, after being treated with glycosidase, the opening-up of collagen fibers significantly improved, especially, when the dosage of glycosidases reached 47 U/mL, the opening-up of collagen fibers was more uniformly. Oppositely, collagen fibers of the crust leather treated without glycosidase (Figure 12, Control) showed obvious adhesion and poor opening up effect.

Generally, the opening up of collagen fibers is closely related to the pore diameter of the leather. The result in Table II shows that the average pore diameter of the crust leather treated by glycosidases was larger than that of the control. The average pore diameter of 23 U/mL β-Mannase-AKT (52.8 nm) treated sample was significantly larger than that of 46 U/mL higher dosage of Xylanase-AKT (43.5 nm). When the dosage of β-Mannase-AKT reached 47 U/mL, the average pore diameter of the crust leather reached maximum value and did not increase with further increase of the glycosidase dosage.

The opening up degree of the collagen fibers and the average pore diameter of the crust leather is consistent with the results of the amount of sugar dissolved in the soaking liquor, namely the higher the amount of sugar dissolved from the cattle hide, the higher the degree of collagen fiber opening up and average pore diameter. All of these results further demonstrated that glycosidases can break the proteoglycans and eliminate the bonding between collagen fibers, which could promote the opening up of collagen fibers and improve the softness of the crust leather. Furthermore, at the same concentration of glycosidase activity, β-Mannase-AKT can remove more proteoglycan and preferably opening up collagen fibers.

Physical and organoleptic properties of the crust leather

The physical and organoleptic properties of the crust leather are shown in Table III. The result shows that, compared with the

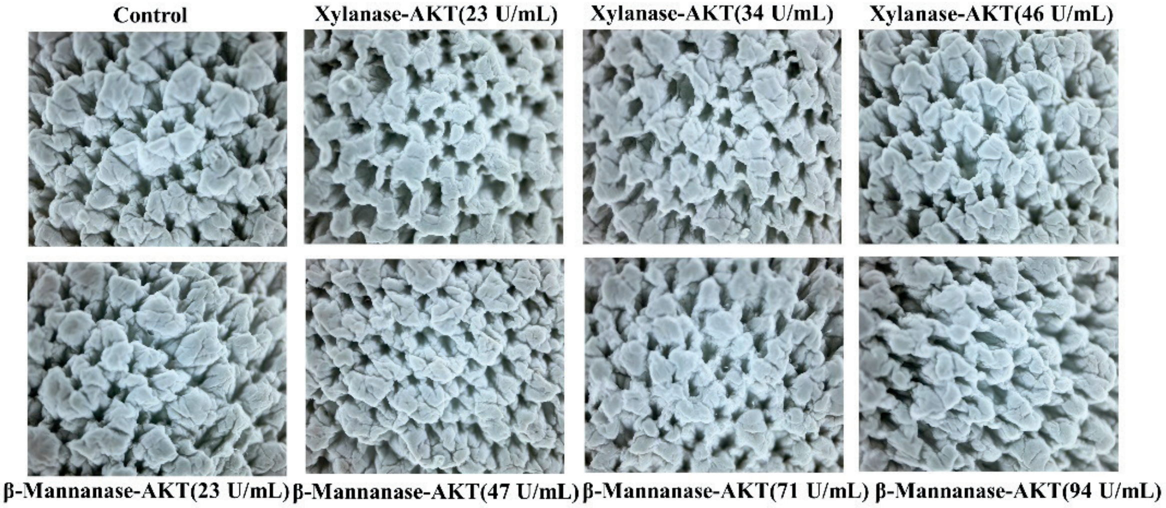

Figure 11. Grain surface of wet blue leather (100 X)

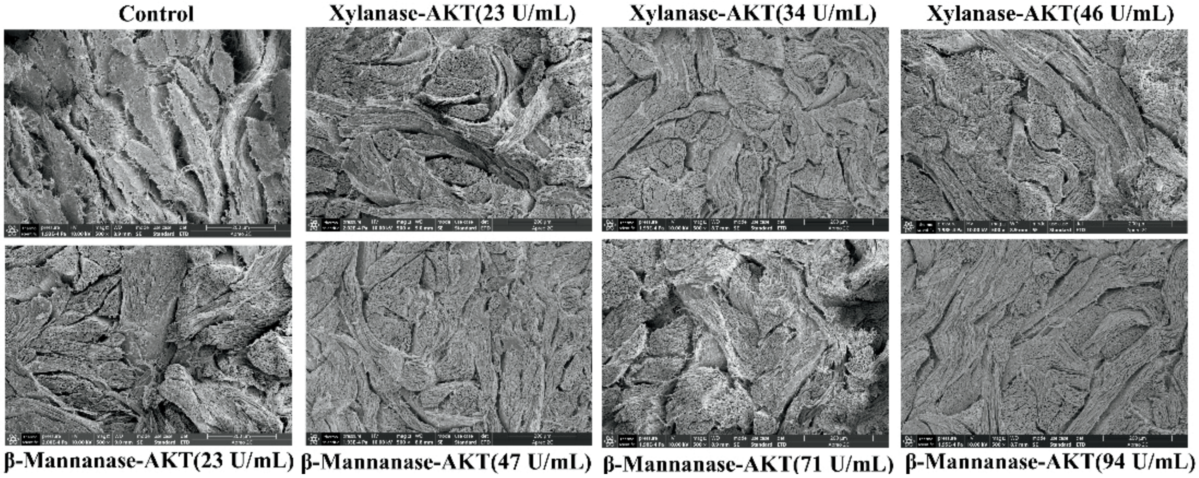

Figure 12. SEM images of the reticular layer of crust leather (500 X)

Table II

Average pore diameter of the chrome tanned crust leather

Glycosidase	Average pore diameter (nm)
Xylanase-AKT (23 U/mL)	38.3 ± 0.24
Xylanase-AKT (34 U/mL)	42.2 ± 0.41
Xylanase-AKT (46 U/mL)	43.5 ± 0.18
β-Mannase-AKT (23 U/mL)	52.8 ± 0.27
β-Mannase-AKT (47 U/mL)	67.9 ± 0.30
β-Mannase-AKT (71 U/mL)	66.2 ± 0.48
β-Mannase-AKT (94 U/mL)	68.1 ± 0.25
Control	34.3 ± 0.39

control, the softness of the crust leather was significantly improved after being treated with glycosidase. Moreover, the main physical properties of the crust leather were also improved. The improvement of softness and physical properties of the crust leather was caused by the removal of proteoglycan and better opening up of the collagen fibers after being treated with glycosidases in the soaking process. The properties of the crust leather were obviously improved when the concentration of β-Mannase-AKT reached up to 47 U/mL; however, the physical properties of the crust leather decreased when the enzyme activity reached 94 U/mL, which is probably caused by the excessive opening up of the collagen fibers.

Conclusion

Based on the types of major PG glycosidic bonds in animal hide, seven typical commercial glycosidases capable of hydrolyzing β-glycosidic bonds were selected and their basic properties and the effect on the removal of proteoglycans in soaking were investigated. Glycosidase β-Mannase-AKT and Xylanase-AKT with good proteoglycan removal effect were selected to investigate the relationship between the dosage of enzyme and the dissolution value of polysaccharide, total soluble protein and hydroxyproline, as well as the overall performance of the leather. The results showed that the seven glycosidases were able to maintain more than 50% of their activity under weak alkaline conditions and have the potential for application in the soaking process. The molecular weight, isoelectric point and specificity to the glycosidic bonds of glycosidases affected the penetration of the enzymes into the hides and the removal of proteoglycans. Among them, β-Mannase-AKT and Xylanase-AKT were more effective in removing proteoglycans, and almost all glycosaminoglycan was removed from the hides when the enzyme dosage reached 47 U/mL. The removal of proteoglycans by glycosidase can significantly improve the opening-up of collagen fibers and the pore diameter of the crust leather, the softness and physical properties of the crust leather were also improved. The

Table III

Physical and organoleptic properties of the crust leather

Enzyme	Tensile strength (N/mm^2)	Breaking strength (N/mm)	Breaking height (mm)	Tear strength (N/mm)	Softness (mm)
Xylanase-AKT (23 U/mL)	9.64 ± 0.21	246.07 ± 0.41	15.79 ± 0.38	46.73 ± 0.71	7.51 ± 0.38
Xylanase-AKT (34 U/mL)	10.02 ± 0.23	267.49 ± 0.77	20.10 ± 0.45	52.36 ± 0.83	7.57 ± 0.32
Xylanase-AKT (46 U/mL)	9.42 ± 0.30	257.97 ± 0.25	15.48 ± 0.26	51.17 ± 0.71	7.40 ± 0.24
β-Mannase-AKT (23 U/mL)	9.52 ± 0.31	281.10 ± 0.46	18.64 ± 0.32	51.05 ± 0.43	7.27 ± 0.14
β-Mannase-AKT (47 U/mL)	10.36 ± 0.24	329.60 ± 0.62	17.15 ± 0.51	63.20 ± 0.60	7.72 ± 0.33
β-Mannase-AKT (71 U/mL)	10.72 ± 0.17	338.67 ± 0.17	19.26 ± 0.52	61.38 ± 0.46	7.84 ± 0.23
β-Mannase-AKT (94 U/mL)	9.56 ± 0.23	254.84 ± 0.34	16.91 ± 0.38	54.06 ± 0.25	7.54 ± 0.31
Control	9.26 ± 0.18	238.36 ± 0.89	17.94 ± 0.42	46.61 ± 0.71	6.93 ± 0.13

use of glycosidase in the soaking process does not cause excessive damage to the structural proteins due to the weak effect on the structural proteins. Therefore, through the rational selection and application of glycosidases in leather making process, it is possible to safely and effectively remove proteoglycans from the hides, promote the opening of collagen fibers and improve the softness and overall properties of the leather.

Acknowledgements

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References

1. Cheng, W. Y., Li, G. Y.; Tanning chemistry. China Light Industry Press, 2011.
2. Liu, L., Hu, Y., Dan, W. H., et al.; Molecular mechanism in process of fiber opening-up. *China Leather* **42**, 10-12, 2013.
3. Queiros, A., Silva, V. F. M., Santos, T., et al.; Optimization of bovine leather soaking process. *Leather and Footwear Journal* **18**, 117-122, 2018.
4. Alexander, K. T. W., Haines, B. M., et al.; Influence of proteoglycan removal on opening-up in beamhouse. *JALCA* **81**,85-100, 1986.
5. Peng, B. Y., Yu, Z. M.; Action of proteolytical enzymes to the hide components and application of proteases in leather-making process. *China Leather* **36**, 60-65, 2007.
6. Khambhaty, Y.; Applications of enzymes in leather processing. *Environmental Chemistry Letters* **18**, 747-769, 2020.
7. Dettmer, A., Anjos, P. and Gutterres, M.; Special review paper: enzymes in the leather industry. *JALCA* **108**, 146-158, 2013.
8. Jayanthi, D., Victor, J. S., Chellan, R., et al.; Green processing: minimising harmful substances in leather making. *Environmental Science and Pollution Research* **26**, 6782-6790, 2019.
9. Schlake, T.; Determination of hair structure and shape. *Seminars in Cell & Developmental Biology* **18**, 267-273, 2007.
10. Luo, F. X., Zhong, X., Zhang, C. X., et al.; A novel approach for quantitative characterization of hydrolytic action of glycosidases to glycoconjugates in leather manufacturing. *JALCA* **115**, 263-270, 2020.
11. Dubois, M., Gilles, K. A., Hamilton J. K., et al.; Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350-356, 1956.
12. Seevaratnam, R., Patel, B. P., Hamadeh, M.J.; Comparison of total protein concentration in skeletal muscle as measured by the Bradford and Lowry assays. *Journal of Biochemistry* **145**, 791-797, 2009.
13. Farndale, R. W., Buttle, D. J., Barrett, A. J.; Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethyl methylene blue. *Biochim Biophys Acta* **883**, 173-177, 1986.

14. Jr, W. J.; The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry & Biophysics* **93**, 440-447, 1961.
15. Zhang, X., Bu, D. Y., Tiang, Y. X., et al.; Optimization of method for determination of hydroxyproline in enzymatic hydrolysate of collagen fibers. *Leather Science And Engineering* **29**, 25-30, 2019.
16. Yamabayashi, S.; Periodic acid - Schiff - Alcian Blue: A method for the differential staining of glycoproteins. *The Histochemical Journal* **19**, 565-571, 1987.
17. Li, X. Y., Zhu, L., Li, X. X.; Determination of relative molecular weight and content of allinase by SDS-PAGE method. *Journal of Xinjiang Medical University* **41**, 751-755, 2018.
18. Xie, Y. F., Liang, Y. L., Zhang, Y.; The improvement on the Method of Electrophoretic Focusing. *Biotechnology Newsletter* **1**, 70-71, 2006.
19. Zhang, X. J., Tian, H., Lue, C. Y., et al.; Determination of the activity of a-Amylase by visible spectrophotometry. *Chemistry & Bioengineering* **37**, 65-69, 2020.
20. Wu, Y. B., Feng, X. Y.; Study on the method of determination of xylanase enzyme activity. *China Feed* **4**, 34-36, 2008.
21. Wang, Z. G., Zhang, H. F., He, R. G.; Research on the determination of xylanase and β-glucanase activity for feeding. *Feed Wide* **15**, 21-24, 2005.
22. Luo, F. X., Zhong, X., Gao, M. C., et al.; Progress and mechanism of breaking glycoconjugates by glycosidases in skin for promoting unhairing and fiber opening-up in leather manufacture, A review. *Journal of Leather Science and Engineering* **2**, 2-16, 2020.
23. Ma, J. Z., Hou, X. Y., Gao, D. G., et al.; Greener approach to efficient leather soaking process: role of enzymes and their synergistic effect. *Journal of Cleaner Production* **78**, 226-232, 2014.

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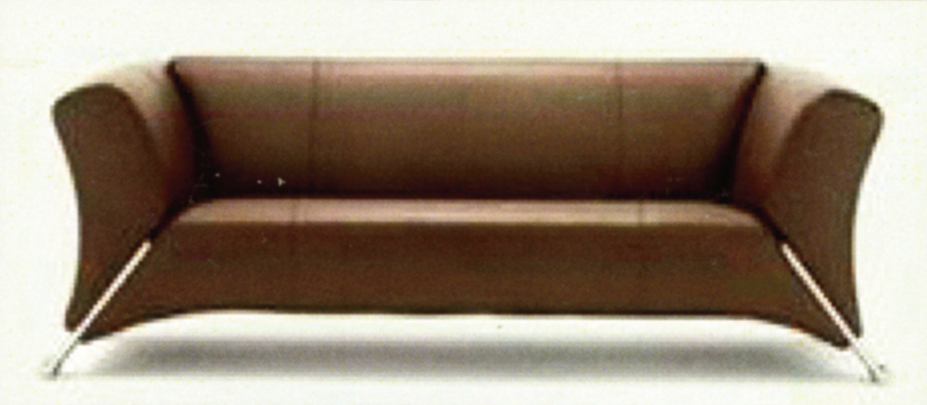

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Lifelines

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Fengxiang Luo see *JALCA* **115**, 263-269, 2020)

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Grand Geneva Resort & Spa, Lake Geneva, WI
June 20-23, 2023

If you have recently completed or will shortly be completing research studies relevant to hide preservation, hide and leather defects, leather manufacturing technology, new product development, tannery equipment development, leather properties and specifications, tannery environmental management, or other related subjects, you are encouraged to present the results of this research at the next annual convention of the Association to be held at the Grand Geneva Resort & Spa, Lake Geneva, Wisconsin, June 20-23, 2023.

Abstracts are due by April 1, 2023
Full Presentations are due by June 1, 2023

They are to be submitted by e-mail to the
ALCA Vice-President and Chair of the Technical Program:

John Rodden
Union Specialties, Inc.
3 Malcom Hoyt Dr.
Newburyport, MA 01950
E-mail: johnrodden@unionspecialtiesinc.com

The **Abstract** should begin with the title in capital letters, followed by the authors' names. An asterisk should denote the name of the speaker, and contact information should be provided that includes an e-mail address. The abstract should be no longer than 300 English words, and in the Microsoft Word format.

Full Presentations at the convention will be limited to 25 minutes. In accordance with the Association Bylaws, all presentations are considered for publication by *The Journal of the American Leather Chemists Association.* They are not to be published elsewhere, other than in abstract form, without permission of the *Journal* Editor. For further paper preparation guidelines please refer to the *JALCA* Publication Policy on our website: leatherchemists.org

Full Presentations are to be submitted by e-mail to the *JALCA* editor:

Steven D. Lange, *Journal* Editor
The American Leather Chemists Association
E-mail: jalcaeditor@gmail.com
Mobile Phone (814) 414-5689

117th ALCA
ANNUAL CONVENTION
June 20-23, 2023
Grand Geneve Resort & Spa
Lake Geneva, Wisconsin

Featuring the 62nd John Arthur Wilson Memorial Lecture

Retelling "Viewing Leather Through the Eyes of Science"

A Century On

By Mike Redwood, Leather Naturally,
Teacher at University of Bath School of Management,
and Trustee of the U.K. Leather Conservation Centre

Tentative Schedule

Tuesday, June 20
Golf Tournament, Opening Reception and Dinner

Wednesday, June 21
John Arthur Wilson Memorial Lecture
All Day Technical Sessions, Fun Run, Dinner
Boat Cruise

Thursday, June 22
All Day Technical Sessions, Annual Business Meeting
Activities Awards Luncheon
Social Hour, Awards Banquet

Visit us at www.leatherchemists.org for full details
under Annual Convention as they become available


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