Africa Leather and Leather Products Institute (ALLPI)

Journal of Africa Leather and Leather Products Advances
ISSN: 2410-0838 2023, Vol. 7, No. 1

EVALUATION OF THE EFFECTIVENESS OF DIFFERENT ENZYME FORMULATIONS IN THE UNHAIRING PROCESS OF GOATSKINS

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Abstracts

Due to the high levels of pollution load produced in the leather industry, many studies are being conducted to replace most of the hazardous chemicals used. One of the options is to use enzymes which are biodegradable and considered environmentally friendly. Hides and skins contain many non-collagenous substances that require specific enzymes to remove. The aim of the study was to evaluate the effectiveness of different enzyme formulations in the unhairing process of goatskins. To achieve this, four types of enzymes were purchased from Jian Grace Industries which include amylase (100,000 U/g), protease (200,000 U/g), keratinase (200,000 U/g), and lipase (100,000 U/g). All possible combinations of one, two, three and four enzymes per formulation were prepared by mixing equal amounts per formulation. Parameters such as total solids, dissolved solids and protein content were determined on the affluent by standard procedures. In addition, the percentage weight gain, residual fat content and organoleptic test of the pelt were evaluated. Data were analysed using SPSS statistical packages version 21. ANOVA and t-test was used to test the level of significance ($p \leq 0.05$). Although several formulations gave complete unhairing, a formulation of keratinase, protease, and lipase (KPL) was selected as the best formulation. This formulation gave a completely unhaired pelt with a residual fat content of 6.4%. The organoleptic tests of all the unhaired pelts had a rating of 7-9. In conclusion, application of more than one enzyme can be a better option in removing non-collagenous components and in the replacement of the use of sodium sulphide in unhairing process.

Keywords: Enzymes, Pollution, Unhairing, Formulations, Goatskin

Introduction

Environmental pollution has been reported to be the main cause of many diseases and premature deaths worldwide. Pollution can cause three times more deaths than malaria, tuberculosis and AIDS (Landrigan *et al*., 2018). Many countries are now raising awareness of the impact of pollution and putting strict measures to reduce pollution. Despite the global intervention towards environmental pollution, the impacts are still being felt due to their severe long-term consequences (Ukaogo *et al*., 2020). The leather industry is among the most polluting agro-based industries in the world and has come under serious public and government criticism due to the release of untreated wastewater (Saxena *et al*., 2016). This pollution is due to the use of chemicals used in the sequential processing of the hides and skins. There are four main stages involved in the production of finished leather which include: beamhouse/pre-tanning, tanning, post-tanning, and finishing operations. Beamhouse involves the cleaning and removal of interfibrillar matters that may hinder the penetration of the tanning agents. The tanning process is the main process that helps stabilize the collagen structure by converting the putrescible hides and skins into non-putrescible and hydrothermal stable products. (Hansen *et al*., 2020). Post-tanning and finishing processes involve improving the performance and sensory properties of leather (Hansen *et al*., 2020). Pre-tanning and tanning processes contribute to almost 80–90% of the total pollution from the tanneries (Saran *et al*., 2013). Unhairing and liming are the main sources of pollution in the beamhouse process. This is mainly due to the use of sulfide and lime in the hair burning process. This produces effluent with high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS) Total Solids (TS) sulfide etc (Andrioli *et al*., 2015). The wastewater also has an alkaline pH, a dark brown color and an unpleasant odor (Saxena *et al*., 2016). At pH levels lower than 10, there is a likelihood of hydrogen sulfide gas production which is toxic. There have been reported deaths in Kenyan tanneries due to production of this gas at the effluent treatment plants. The presence of sulfide also reduces the efficiency of tannery effluent treatment plants (Kanagaraj *et al*., 2016). Even in low concentration (200 ppm), hydrogen sulfide which is produced during the unhairing process has proven to be deadly (Galarza *et al*., 2012). In order to reduce the pollution associated with these tanneries much emphasis should be put in place on using these technologies and techniques. Some of the suggested methods to reduce pollution in the unhairing/liming process include recycling of unhairing lime–sulfide liquors, a hair-saving approach, and replacing sulfide with amines, hydrogen peroxide and nickel carbonate (Jian *et al*., 2011). However, using enzymes is the most promising and effective approach to replacing sodium sulfide.

Different types of enzymes are used in leather processing because enzymes are specific, act quickly, and often save raw materials, chemicals, water and energy compared to conventional processes (Jegannathan, and Nielsen, 2013). These enzymes help remove various skin components such as albumin, globulins, mucoid, hair, fat, dung, and dirt. There are several enzymes that have been suggested to perform the following functions such as amylase, protease, keratinase and lipase. Most studies have evaluated the effectiveness of different enzymes in a single process. Keratinase and protease have been reported to be used in soaking, unhairing, and degreasing (Never *et al.,* 2019). The enzyme α-amylase has been reported to work effectively in the soaking process by opening up the fiber structure through the removal of proteoglycans

(Shanmugavel *et al*., 2011). The use of lipase enzyme has been reported in many applications such as the degreasing of hides and skins, leather, fur, crepe, and gelatin. A residual fat content of 2% has been reported after the application of 10% lipase in combination with commercial degreasing formulations (Kavitha, 2019). It is clear that the use of enzymes can help remove most of the non-collagenous components of the skin. The main concern is the quality of the resulting leather and whether enzymes will remove the non-collagenous substances as intended: It is in the view of these studies that combinations of enzymes may have a synergetic effect that can aid in the rapid removal of non-collagenous substances and also produce a pelt with better quality. Also, most of the documented studies have examined the use of a single enzyme in a single processing step. In this regard, this study evaluated the effectiveness of single and combined enzyme formulations for use in unhairing process of goatskins.

Materials and Methods

Sample collection

Four different enzymes with known activity were purchased from Jinan Grace Industry Co Ltd China and used in the study. The enzymes were amylase (100,000 U/g), protease (200,000 U/g), keratinase (200,000 U/g) and lipase (100,000 U/g). The wet salted goatskins used in this experiment were obtained from curing premises near Dagoretti slaughterhouse.

Formulation of enzymes

The effectiveness of enzymes was evaluated using a single enzyme, combinations of two enzymes, a combination of three enzymes and a combination of all the four enzymes. All possible trials are listed in Table 1. The formulations were made by mixing equal amounts of enzymes per formulation.

Table 1. All possible formulations of enzymes

Formulation type	Possible formulations
Single enzymes	Amylase
	Protease
	Lipase
	Keratinase
Two enzyme combinations	Amylase + keratinase (MK)
	Lipase + keratinase (LK)
	Lipase + amylase (LA)
	Amylase + protease (AP)
	Lipase + protease (LP)
	Keratinase + protease (KP)
Three enzyme combinations	Keratinase +protease+ amylase (KPA)
	Keratinase + protease + lipase (KPL)
	Protease + amylase + lipase (PAL)
	Amylase + lipase + keratinase (ALK)
Four enzymes' combinations	Keratinase + amylase + protease+ lipase (KAPL)

To evaluate the effectiveness of enzymes in the unhairing process, 8 goatskins were used in the study. Ten grams were sampled from the butt area as recommended by the Society of Leather Technologies and Chemists [1996]. For the unhairing process, 5% of the enzyme's formulations and 200% water based on the weight of the skins were used for a period of 24 hours. The samples were placed in a 1-liter beaker and shaken slowly using a horizontal laboratory shaker. All pieces were observed periodically for hair looseness and weight gain. The effectiveness of the formulations for the degreasing process was evaluated by the residual fat content after soaking the skins for twenty-four hours. Several variables were evaluated, such as percent weight gain and fat content of the residual samples. The total solids, suspended solids and protein content were also assessed on the liquor. Total solids and suspended solids were evaluated according to APHA, AWWA, and WPCF (1989). For each setup, a control blank (without enzymes) was used for comparison purposes.

Total solids

A clean dish was heated in a drying oven at 105 ^{o}C for one hour and stored in a desiccator until use. The dish was weighed and 25 ml of well-mixed samples were added. The temperature in an oven was reduced to about 2 ^{0}C below the boiling point to avoid splattering. The dried samples were evaporated in an oven for at least 1 hour in an oven at 103-105 ^{o}C. The dish was cooled in a desiccator and weighed after cooling. The cycles of drying and cooling in the desiccator were repeated until the weight loss of the previous weight was less than 0.5 mg (APHA, AWWA, and WPCF, 1989).

Calculation:

$$\text{Mg total solids /L} = = \frac{(A-B)\times 1000}{\text{Sample volume,(mL)}}$$

Where A=Weight of dried residue + dish

B= weight of dried residue

Total suspend solids

The filtering apparatus and the filter were assembled. The filter was wetted with a small volume of distilled water to seat it. Twenty-five milliliters of the samples were filtered with glass fiber filter paper. It was then washed with three successive 10 mL volumes of distilled water allowing complete drainage between washing and continuous suction for about three minutes after filtration was complete. The filter was removed from the filtration apparatus and transferred to an aluminum planchet as support. The residue was dried in an oven at 103 ^{o}C and cooled in a desiccator. The dying and cooling cycles were repeated until a constant weight of less than 0.5 mg was obtained (APHA, AWWA, and WPCF 1989).

Calculation

$$\text{Mg total suspended solids/l} = \frac{(A-B)\times 1000}{\text{Sample volume,(mL)}}$$

A= weight of the filter paper + dried residue,

B= weight of the filter (mg).

Protein content

The protein content was determined according to the Association of Official Analytical Chemists (1995). A sample of 25 mL was placed into an 800 mL Kjeldahl flask and 100 mL of digesting solution was added. It was boiled until fumes evolved and the solution becomes colorless or pale yellow. It was cooled and diluted with 300 mL of water. NaOH-NaS_2O_3 solution was slowly added down the neck of the tilted flask underlay acid solution in an amount sufficient to make the final solution strongly alkaline as indicated by phenolphthalein. The flask was connected to the condenser, with the tip of the condenser immersed in 50 mL 2% H_3BO_3 Solution in a 500 mL glass-stoppered Erlenmeyer flask. Three drops of the mixed indicator were added to the distillate and titrated with 0.02 N H_2SO_4 matching the endpoint against the blank containing

the same volume NH_3-Free H_2O, H_3BO_3 Solution indicator. The protein content was obtained by multiplying the nitrogen content obtained by a factor of 5.62.

Determination of fat

Determination of fats was done according to the official methods of analysis by the society of leather technologies and chemist SLC 4 (IUC4; BS 1309:4) (1996). The wet samples were dried in an oven at a temperature of 50 ^{0}C and then conditioned in an atmosphere with a temperature of 20± 2 ^{0}C and relative humidity of 65±2% for 24 hours before grinding. The samples for fat analysis were ground using a cutter mill, with knives rotating at 700-1000 revolutions per minute and with a sieve of 4mm diameter mesh. Ten grams of ground samples were weighed then pressed evenly into the filter paper thimble and covered with a thin layer of cotton wool. The extraction flask with two glass beads in it was dried by heating at 102 ±2 ^{0}C for half an hour. It was weighed after cooling in a desiccator. The continuous extraction process began with dichloromethane for at least 30 solvent changes. Dichloromethane from the flask containing the extract was distilled. The extracts were dried in an oven for four hours at 102 ± 2 ^{0}C. It was cooled in a desiccator for 30 minutes and weighed. Drying, cooling and weighing were repeated until further weight loss was less than 10 mg. it was calculated using the following formula.

Percentage fat content= 100 M_1/M_*

Where: M_1= mass of the extracts

M_*= mass of the sample used

Assessment of the Organoleptic tests of unhaired and degreased pelts

After 24 hours, the parameters such as thumb imprint, softness, appearance, flexibility, grain firmness, and cleanliness were assessed on the pelts for formulations that had a complete unhairing by rating them from a rate of 0-10 (very poor- very good) according to Unango *et al*., (2019).

Data analysis

The data were analysed using the statistical package for social science (SPSS) version 21. The results were presented using descriptive statistics such as means, standard deviation, and graphs. ANOVA and t- test were used to test the level of significance and post hoc test was also performed using Duncan multiple comparison tests to identify the means that were significantly different ($p \leq 0.05$).

Results and Discussion

There are many parameters that can be used to assess the effectiveness of enzymes in the unhairing process. Apart from hair removal, water content is a very important parameter as water acts as a carrier of chemicals in and out of the pelt. Reduction in the amount of fat is also very important as fat hinders the penetration of water and other chemicals. Other parameters that were evaluated include TS, DS, protein and unhairing. Different formulations (single enzyme, two enzymes, three and four enzymes' formulations) gave different results for the variables measured during the unhairing process as shown in Table 2.

Table 2: Unhairing parameters

ENZYMES COMBINATIONS	Test variables					
Single enzymes	**% Weight gain (24 Hours)**	**Fat content (%)**	**Total solids (mg/l)**	**Suspended solids (mg/l)**	**Protein content (%)**	**Unhairing conditions**
Keratinase	58.55 ± 2	15.84 ± 0.3	67.43 ± 5	12.29 ± 2	0.48 ± 0.04	Complete unhairing

Lipase	55.65 ± 2	8.30 ± 2	75.24 ± 0.98	20.49 ± 5	0.44 ± 0.02	No unhairing
Protease	55.91 ± 4	14.89 ± 4	73.81 ± 5	22.7 ± 3	0.8486 ± 0.02	Hair looseness
Amylase	46.68 ± 3	16.8 ± 2	66.1 ± 1	12.2 ± 1	0.28 ± 0.002	No unhairing
Blank	53.45 ± 5	17.14 ± 2	64.08 ± 2	11.04 ± 1	0.3 ± 0.01	No unhairing
P values	0.05	p< 0.001	0.21	0.02	p< 0.001	
Two enzymes' combinations						
Amylase+Keratinase	60.34 ± 6	14.32 ± 1	70.68 ± 3	8.11 ± 2	0.5 ± 0.08	Incomplete
Lipase+Keratinase	65 .65 ± 8.5	9.78 ± 0.7	74.69 ± 6	8.5 ± 4	0.65 ± 0.11	Complete
Lipase+Amylase	55.59 ± 1	10.95 ± 1	88.89 ± 9	7.3 ± 3	0.35 ± 0.04	No unhairing
Amylase+Protease	71.08 ± 7	14.41 ± 0.7	113 ± 3	9.8 ± 2	0.73 ± 0.1	Incomplete
Lipase+Protease	77.49 ± 3	10.5 ± 1	97.08 ± 16	11.05 ± 3	0.64 ± 0.07	Incomplete
Keratinase+Protease	73.89 ± 5	14.82 ± 0.75	86.00 ± 17	9.3 ± 1	0.74 ± 0.1	Complete
Blank	51.61 ±7	15.42 ± 5	80.48 ±7.3	6.05	0.29 ± 0.01	No unhairing
P values	< 0.0001	< 0.0001	< 0.0001	0.54	< 0.0001	
Three enzymes' combinations						
Keratinase+Protease+ Amylase	61.82 ± 5	10.58 ± 4	68.34 ± 6.4	9.34 ± 6	0.918 ± 0.1	Complete
Keratinase+Protease+ Lipase	72 ± 2	6.4 ± 1	70.28 ± 5	10.28 ± 5	0.92 ± 0.2	Complete
Protease+Amylase+ Lipase	71.9 ± 3	11.56 ± 2	67.90 ± 0.46	9.73 ± 0.7	1.03 ± 0.23	Incomplete
Amylase+Lipase+ Keratinase 1	72. 61 ± 11	9.5 ± 0.5	65.71 ± 0.7	8.04 ± 0.4	0.8144 ± 0.06	Incomplete
Blank	55.52 ± 5	11.54 ± 2	60.30 ± 3	8.01 ± 3	0.793 ± 0.3	No unhairing
P Values	< 0.001	0.001	0.1	0.9	0.74	
Four enzymes' combinations						
Keratinase+Lipase+ Protease+Amylase	74.67 ± 5	10.64 ± 0.7	112.82 ± 16	18.38 ± 2	1.705 ± 0.7	Incomplete unhairing
Blank	74.47 ± 3	14.61 ± 0.6	79 ± 15	11.90 ± 0.8	0.688 ± 0.03	No unhairing
P Values	0.42	0.264	0.538	0.104	0.08	

When the percentage weight gain was assessed for single enzymes, keratinase enzymes had the highest weight gain (58.55 ± 2 %) followed by protease (55.91 ± 4), although the difference was not statistically significant (p= 0.05). For two enzymes' formulations (Lipase + Protease) and (Amylase + Protease) had the highest weight gain (77.49 ± 3 %) and (71.08 ± 7 %) respectively. The difference in percentage weight gain for formulations with three enzymes combinations was highly significant (p< 0.001) and a combination of Amylase, Lipase and Keratinase (ALK) and Keratinase, Protease and Lipase (KPL) had the highest weight gain of (72. 61 ± 11 %) and (72 ± 2 %) respectively. A comparison of the blank and four enzymes' formulations did not reveal much differences and the difference in weight gain was not statistically significant (p > 0.05). A combination of enzymes or enzymes and surfactants has been reported to have a positive change when used in the soaking process. Choudhary *et al*., (2004), reported a complete absorption of water after 5 hours when a combination of lipase and surfactants, protease and surfactant, and protease and lipase was used. In addition, the author indicated that these combinations lead to a 45% reduction in the

time required for the complete absorption of water as compared to conventional methods. Another study by Queirós *et al*., (2018), assessing the optimization of the bovine leather soaking process found a mixture of wetting agent and a traditional degreaser to have a percentage weight gain of 41.1 % while a mixture of lipase and protease had 41.6% (Choudhary *et al*., 2004).

All the formulations had a significant change in the residual fat content of the pelt ($p < 0.05$). For each category, the following are the residual fat content of the most effective formulation in fat reduction: Lipase (8.30 ± 2 %), Lipase and Keratinase (9.78 ± 0.7%), Keratinase, Protease and Lipase (KPL) (6.4 ± 1%) and Keratinase, Lipase, Protease and Amylase 10.64 ± 0.7 %. A combination of lipase and protease has been reported to be very effective in removing fat from hides and skins. A good degreasing effect has been reported when the combination of bacterial lipase and protease are used (Mhya and Mankilik, 2015). The fat cells are easily accessible by lipase when protease enzymes open up the membranes surrounding the fat cells (Mhya and Mankilik, 2015).

The total solids, total proteins and suspended solids were also evaluated on the liquors after 24 hours. For single enzyme application, lipase and protease had the highest level of total solid and protease also had the highest total suspended solids (22.7 ± 3 mg/l) and protein content was (0.8486 ± 0.02 %). Keratinase enzyme was the second enzyme that produced liquors with higher protein content (0.48 ± 0.04 %). Keratinase and protease enzymes act on different proteins and this is the main reason why their liquors had a higher level of proteins. The opening of the fiber bundles is directly proportional to the extent of interfibrillar substance removal (George *et al*., 2014). The presence of these substances hinders the penetration of various agents during leather making process (George *et al*., 2014).

For two enzymes, combination of Lipase and Protease had a higher content of total solids (97.08 ± 16 mg/l) and suspended solids (11.05 ± 3 mg/l) whereas a mixture of Keratinase and Protease had the highest protein content. For three enzyme formulations, Keratinase, Protease, and Lipase had the highest content of total solids (10.28 ± 5 mg/l) and suspended solids (0.92 ± 0.2 mg/l). This was lower as compared to other conventional methods. A study by Quandary *et al*., (2014), comparing hair-saving methods using thiol compounds and hair pulping sulphide methods of unhairing found the suspended solids to be 4.7 and 6.2 mg/l respectively. The variations of these variables may vary from one study to another depending on the type, condition and number of hides and skins used in the experiments.

A combination of Protease, Amylase and Lipase enzymes had the highest protein content (1.03 ± 0.23%) under three enzymes combination but was not statistically different from the others ($p = 0.74$). In addition, a combination of all the enzymes showed no significant difference ($P=0.08$) in the protein content of the liquors.

Other variables that were evaluated during the soaking process were unhairing and the appearance of the pelt. A mixture of lipase and protease, keratinase and Protease and amylase and protease enzymes had 100% complete unhairing but the resulting pelts were very soft. A similar study by Mamun *et al*., (2015), found that a mixture of 2.5% of keratinase and 2.5% of protease enzymes were able to unhair 85% of the pelt area for a period of 26 hours. The addition of calcium oxide to keratinase and protease enzymes improved the performance of the enzymes and the pelt area was unhaired 100% for a period of 24 hours (Mamun *et al*., 2015). Several formulations gave a complete unhairing as indicated in Table 3.

Assessment of the Organoleptic tests of enzymatic unhaired pelts

Journal of Africa Leather and Leather Products Advances
ISSN: 2410-0838 2023, Vol. 7, No. 1

Organoleptic tests such as thumb imprint, softness, general appearance, flexibility, grain firmness, and cleanliness were performed on all the samples that had a complete unhairing. These include: Keratinase (K), combination of (Lipase and keratinase), (Keratinase and Protease), (Keratinase, protease and amylase) and (Keratinase, protease and lipase) and the results are presented in Fig 1.

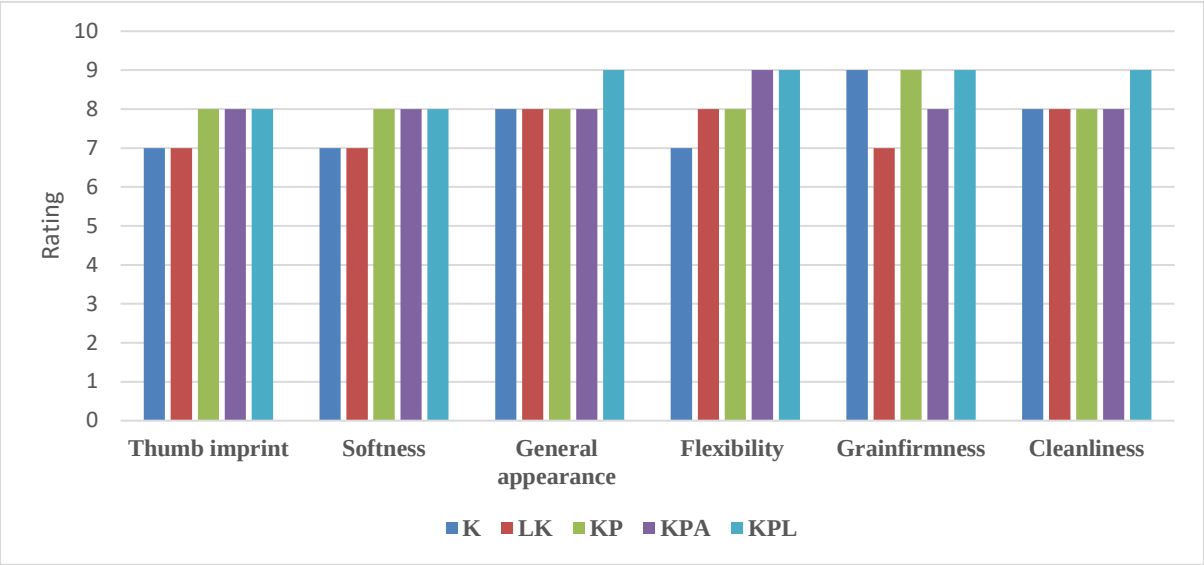

Figure 1: ***Assessment of the organoleptic tests of enzymatic unhaired pelts***

(**K**-Keratinase, **LK**- Lipase and keratinase, **KP**- Keratinase and Protease, **KPA**- Keratinase, protease and amylase and **KPL**- Keratinase, protease and lipase).

All the unhaired pelt had a rating of 7-9, meaning they were within the required range. A formulation of KPL had the best rating for general appearance and cleanliness with a rating of 9. It can be seen from the results that this combination had the best properties. A similar study by Ranjithkumar *et al*., (2017), comparing the visual characteristics of enzymatic processed leather reported a rating of 8-9 which was within the range obtained in this study.

Conclusion

Combination of enzymes seemed to be effective in the unhairing process as compared to use of single enzymes. Removal of several skin components is facilitated by use of different enzymes. Although several formulations gave complete unhairing of the skin, a combination of Keratinase, protease, and lipase (KPL) was selected as the best for unhairing purposes. The pelt had a residual fat content of 6.4 ± 1% and the resulting pelts were clean with a firm grain. It also had a good weight gain of 72% compared to most of the formulations. In additional it had the highest protein content in the effluent among the formulations that had a complete unhairing and this is a good indication that a lot of nonfibrous proteins were removed. Therefore, application of more than one enzyme can be a better option in removing non-collagenous components and in the replacement of use of sodium sulphide in unhairing process.

Acknowledgments

The authors acknowledge the Department of Public Health, Pharmacology and Toxicology and the Department of Animal Production of the University of Nairobi for their kind support that made this research work possible.

Conflicts of interests

The authors declare that there are no competing interests.

References

Andrioli, E., Petry, L., and Gutterres, M. (2015): Environmentally friendly hide unhairing: Enzymatic-oxidative unhairing as an alternative to use of lime and sodium sulfide. *Process safety and environmental protection*, *93*, 9-17.

[AOAC] Association of Official Analytical Chemists. (2005): AOAC official method 973.48. Nitrogen (Total) in water-Kjeldahl method. *Official methods of analysis of AOAC Intl*.

APHA., AWWA., WPCF. 1989: Standard methods for the examination of water and waste water.17th edition. Washington, DC: American public health association.

Choudhary R.B., Jana A.K., Jha M.K., (2004): Enzyme technology applications in leather processing. *Indian Journal of Chemical Technology*.11: 659-671.

Galarza B., Cavello I. A., Garro M. L., Gortari M. C., Hours R. A., and Cantera C. (2012): Evaluation of increase at the production of keratinolytic enzymes. *Journal of Aqeic*, *63.*

George N., Chauhan P.S., Kumar V., Puri N., and Gupta N. (2014): Approach to eco-friendly leather: Characterization and application of an alkaline protease for chemical free dehairing of skins and hides at pilot scale. *Journal of Cleaner Production* 79: 249–257.

Hansen É., Aquim P. M., Hansen A. W., Cardoso J. K., Ziulkoski A. L., and Gutterres M. (2020): Impact of post-tanning chemicals on the pollution load of tannery wastewater. *Journal of Environmental Management*, *269*, 110787.

Jegannathan K. R., and Nielsen, P. H. (2013): Environmental assessment of enzyme uses in industrial production–a literature review. *Journal of cleaner production*, *42*, 228-240.

Jian S., Wenyi T., and Wuyong., C. (2011): Kinetics of enzymatic unhairing by protease in leather industry. *Journal of Cleaner Production*, *19*(4), 325-331.

Kanagaraj, J.; Panda, R.C.; Senthilvelan, T. (2016): Green remediation of sulfide in oxidative dehairing of skin and correlation by mathematical model: An eco-friendly approach. *Process Safety and Environmental Protection*, 100, 36–48.

Kavitha M., (2019): Cold active lipase from Pseudomonas sp. VITCLP4 as a degreasing agent in leather processing. *Indian Journal of Chemical Technology (IJCT)*, *25*(5), 482-488.

Landrigan P. J., Fuller R., Acosta N. J., Adeyi O., Arnold, R., Baldé A. B., and Zhong M. (2018): The Lancet Commission on pollution and health. *The lancet*, *391*(10119), 462-512.

Mamun M.A., Hosain M.A., Ahmed S., Tuj-Zohra F., Sultana K., Zerin M., Khan S.N., Hoq M.M. (2015): Development of an Alternative Enzyme-assisted Dehairing Method of Animal Skins using Proteases from *Bacillus licheniformis* MZK05M9. *Bangladesh J Microbiol.*32(1&2) :33.

Mhya D.H., Mankilik M. (2015). Bacterial Enzymes: A Good Alternative to Conventional chemicals in Leather Processing. *International Journal of Biosciences and Nanoscience's*. 2 (1): 20-23.

Never Z., Cecilia C., Suleimain, M., & Madundo, M. (2019): Dehairing of animal hides and skins by alkaline proteases of Aspergillus oryzae for efficient processing to leather products in Tanzania. *African Journal of Biotechnology*, *18*(20), 426-434.

Quadery A.H., Uddin M. T., Azad A. K., Chowdhury M. J., Deb A.K., Nazmul M., (2014) Development of the hair-save unhairing method using organic thiol compounds in pre-tanning

stages of leather production. *International Journal of Scientific & Engineering Research*. 5 (12): 376.

Queirós A., Silva F.V., Santos T., Crispim A., (2018): optimization of bovine leather soaking process. *Leather and Footwear Journal*.;18: 2.

Ranjithkumar, A., Durga, J., Ramesh, R., Rose, S., & Muralidharan, C. (2017). Studies on alkaline protease from Bacillus crolab MTCC 5468 for applications in leather making. *Journal of the American Leather Chemists Association*, 112(07), 232-239.

Saran S., Mahajan R. V., Kaushik R., Isar J., and Saxena R. K. (2013): Enzyme mediated beam house operations of the leather industry: A needed step towards greener technology. *Journal of cleaner production*, *54*, 315-322.

Saxena G., Chandra R., and Bharagava R. N. (2016): Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. *Reviews of Environmental Contamination and Toxicology Volume 240*, 31-69.

Shanmugavel M., Premalatha A., Dayanandan A., Ramesh R., Gnanamani A., Sounderraj, L and Rajakumar G. S., (2011). Enzyme consortium of Aspergillus tamarii MTCC5152 for greener beam house processing of hides. In *31st IULTCS Congress.*

Society of leather technologies and chemists. Official methods of analysis. 1996; SLC 1 (IUC/ 1; IUC/2; BS1309:0).

Ukaogo P. O., Ewuzie U., and Onwuka C. V. (2020): Environmental pollution: causes, effects, and the remedies. In *Microorganisms for sustainable environment and health* (pp. 419-429). Elsevier.

Unango F.J., Duraisamy R., Ramasamy, KM., Preparation and characterization of eco-friendly ash salts for Goat skins preservation. Int*. J. Innov. Technol. Explor. Eng*. 2019 8:184-90.