MULTI-ENZYMATIC BEAMHOUSE OPERATION: INFLUENCE OF CLEANER TECHNOLOGY IN LEATHER MANUFACTURE

Jeyas Kandhan Sudhagar[1], Jayakumar Gladstone Christopher[2]

1. Department of Leather Technology, AC Tech Campus, Anna University, Chennai-600025, Tamil Nadu, India. Email ID: jeyasuthakar@gmail.com
2. Biochemistry and Biotechnology Lab, CSIR-Central Leather Research Institute, Chennai-600020, Tamil Nadu, India & Department of Leather Technology, AC Tech Campus, Anna University, Chennai-600025, Tamil Nadu, India Email ID: jaykumar.clri@csir.res.in

ABSTRACT

The emerging technological interventions in leather manufacture are widely focussing on complete bioprocess manufacturing methods considering the future prognosis and industrial sustainability of leather sector. Enzyme assisted leather processing is a cleaner bioprocessing technology being practised in various leather processing stages. Leather industry predominantly focuses on three hydrolase enzymes viz., protease, amylase and lipase, which are used for unhairing, fiber opening and defleshing applications respectively. The process design for the single step enzymatic beamhouse operation with all the three enzymes application together for their specific action on the skin matrix is the way forward technology. This multi-enzyme application as biomimicking solution from human intestinal mechanism will significantly reduce the processing time, energy and effluent discharge. This paper focuses on utilizing multi-enzyme system (Protease, Amylase and Lipase – PAL) for performing single step beam house operation. The behavioural pattern of the enzyme in the presence of counter enzyme were understood using enzyme kinematics. The enzyme kinematics studies show that proteases activity were negligible on the counter enzymes viz., amylase and lipase. While a significant increase in the protease and lipase activity in PAL system were recorded. The PAL system processed goat skin were observed with complete unhairing and defleshing. The physical properties such as tensile strength with 21.91 N/mm^2 and tear strength with 56.04 N/mm found to meet standard norms. Thus, the multi-enzyme beamhouse operation is the futuristic cleaner technology in leather manufacturing.

Keywords: Enzyme; Protease; Amylase; Lipase; Sustainability.

1. Introduction

Cleaner technologies are the way forward in the manufacturing industries to achieve sustainability. Leather manufacturing involves chemical intensified processing which continuously explores cleaner technologies (De Souza and Gutterres 2012). Enzymatic beam house operation is an impetus area of research in leather process engineering. The hydrolase enzymes majorly used in leather processing are proteases, amylases and lipases. The use of protease and amylase enzymes in the leather industries are well established. These enzyme act at their specific site in the skin viz., protease at proteoglycans, amylase at glycosaminoglycans and lipase at adipose tissues, finding application for dehairing, fibre opening and defleshing respectively in leather processing (Senthilvelan et al. 2012 & Yasmin 2020). The application of protease, amylase and lipase enzyme in the leather processing could replace lime, sodium sulphide, bate and ammonium salts used in beam house operations of leather manufacture. Moreover, these enzymes could complete the beam house operations in less than 24 h which usually take minimum of 2 to 3 days and even longer duration depending on the type of articles (Md Jawad et al. 2022). Enzymatic leather processing using protease and amylase enzyme subsequently through monopot system (using one enzyme at a time) makes it more time intensified process and increases the possibilities of putrefaction. Performing dehairing, fibre opening and defleshing simultaneously with a multi enzyme feed (tripot system) is a future technology to be explored. This study focuses on understanding the enzyme-enzyme interaction between protease, amylase and lipase enzymes when used in a single step process.

Enzymes are protein which catalysis a biological process thus interaction between enzymes is a protein-protein interaction. Major investigation of protein-protein interaction is carried out as *in vivo* studies where these interaction plays a vital role in the regulation of cellular metabolism (Srivastava and Bernhard 1986). Protease-Amylase-Lipase enzyme system (PAL system) used for this elemental analysis is an *in vitro* multi enzyme system. The interaction within the PAL system is a heterologous enzyme interaction where all three enzymes have different specific function and mechanism to perform upon their respective substrate (Hess and Boiteux 1972).

2. Material and Methods

Protease, amylase and lipase enzymes used were of laboratory grade form SRL chemicals. The leather processing chemicals used were of commercial grade. Wet salted goat skin was used for the enzymatic treatment. Enzymatic leather processing using pasting methods is shown in Table I. Different combination of enzymatic leather pre-tanning processing has been performed such as protease and amylase enzyme combination as trial 1 (PA enzyme system); protease and lipase enzyme combination as trial 2 (PL enzyme system) and protease, amylase and lipase enzyme combination as trial 3 (PAL

enzyme system). The enzymes concentration used for the trials are shown in Table II. Conventional vegetable tanning process was followed as a tanning method (Table I).

Table I. Process design for enzymatic leather processing

Process	Chemical	% Offer	Time (min)
Soaking	Water	300	120′
	Water	300	180′
Enzyme Treatment	Water	30	Overnight
	Protease	X*	
	Amylase	Y*	
	Lipase	Z*	
Dehairing and Defleshing			
Pickling	Water Salt Formic Acid Sulfuric Acid	100 10 1 0.5	 10′ 10′ x 3 10′ x 2 + 15′ pH = 4.5-5
Tanning	Wattle Myrobalan	20 5	60′*2 60′
Fixing	Water Formic Acid	50 3	 10′ x 3 + 30′
Wash/Drain/Pile			

* Concentration of X, Y and Z for trial 1, 2 and 3 are shown in table II.

Table II. Concentration of enzymes for different trials used in leather processing

Trial	Protease Concentration (X) (%)	Amylase Concentration (Y) (%)	Lipase Concentration (Z) (%)
1	4	2	0
2	4	0	5
3	4	2	5

2.1. Hydrothermal stability measument

The vegetable tanned leather was analysed for its hydrothermal stability by shrinkage temperature analysis using thesis shrinkage tester (McLaughlin and Theis 1945). The experimental vegetable tanned leather was exposed to a boiling glycerol-water combination (3:1). The temperature at which the leather shrinks is recorded as shrinkage temperature. The shrinkage temperature was correlated as the hydrothermal stability of the processed leather.

2.2. Physical characterization

The enzymatically processed upper crust leather was analysed for tensile strength, tear strength and lastometer ball burst test analysis. Testing was carried out following SATRA TM 43:2021 procedure for tensile strength, SATRA TM 162:2017 procedure for tear strength using Universal Testing Machine.

3. Results and Discussion

Enzymes are next generation chemicals which are explored for its best utilization in reducing the limitations of the conventional processing methods. Enzymes are known for its substrate specificity which makes it ideal for centric applications in various industrial sector (De Souza and Gutterres 2012). Use of hydrolase enzymes in leather processing have been well established. However, the optimization of enzyme applications in leather manufacture that could aids in reducing the do-undo ecological concepts and accelerating the processing steps are yet a thrust area of research. The application of the hydrolase enzymes for a single step beam house operation is the area of study which directly reduces the carbon footprints from the conventional leather processing. The leather processed with the PA, PL and PAL system have shown a complete dehairing and defleshing from grain and flesh side respectively. This signifies the effect of protease and lipase action upon proteoglycans and adipose tissues respectively in the skin.

3.1. Enzyme activity studies

Protease enzyme hydrolysis the peptide bond present in proteins. Enzyme being a protein there is the possibility of enzyme degradation of amylase and lipase by protease in a PAL system. Enzyme complexing is the other possible aspects which regulates the catalytic activity. Thus, to understand the behavioural pattern of the protease, amylase and lipase in a multi enzyme system catalytic activity analysis was carried out.

Table III. Protease activity in different enzyme system

System	Substrate	Activity (Units/mg)
Protease	Casein	109.41
Protease-Amylase	Casein	121.70
	No Substrate	0
Protease-Lipase	Casein	121.72
	No Substrate	0
Protease-Amylase-Lipase	Casein	122.27
	No Substrate	0

Table IV. Amylase activity in different enzyme system

System	Substrate	Activity (Units/mg)

Amylase Standard	Starch	3594.67
Protease-Amylase	Starch	3046.33
	No Substrate	0
Amylase-Lipase	Starch	2760.00
	No Substrate	0
Protease-Amylase-Lipase	Starch	2803.33
	No Substrate	0

Table V. Lipase activity in different enzyme system

System	Substrate	Activity (Units/mg)
Lipase Standard	Olive oil	1400
Protease-Lipase	Olive oil	1200
	No Substrate	0
Amylase-Lipase	Olive oil	34 00
	No Substrate	0
Protease-Amylase-Lipase	Olive oil	1600
	No Substrate	0

3.1.1. Specificity of protease towards amylase and lipase

Proteolytic activity of protease on amylase and lipase shows that protease has negligble catalytic activity upon amylase and lipase. This shows that protease may have a least possibility for a binding site in amylase and lipase. While protease shows a marginal increase in the catalytic activity in the presence of amylase and lipase (Table III).

3.1.2. Specificity of amylase towards protease and lipase

The catalytic activity of amylase on proteasae and lipase shows that activity of amylase is nil in the presence of protease and lipase as susbtrate. Since, protease and lipase is devoid of glycosidic bond for amylase activity. While the presence of protease and lipase considerably decrease the activity of the amylase. Moreover, the presense of Lipase in amylase system, reduces the amylase activity, this is probably that lipase may act as an inhibitor in the enzyme system (Table IV).

3.1.3. Specificity of lipase towards protease and amylase

Lipolytic activity of lipase on protease and amylase shows that lipase has no catalytic activity in the presence of protease and amylase because of the absence of ester bond. Though, there is significant increase in the lipolytic activity in the AL and PAL system. PAL enzyme system complexing may provide lipase with increased active site through protein folding (Table V).

3.2. Discussion

Protein folding and enzyme complexing are the major factors governing the change in catalytic activity of the protease, amylase and lipase in the PAL system. From the enzyme activity studies it is clear that amylase and lipase is not degraded by the protease into small peptides and amino acids. The significant increase in the protease and lipase activity in the PAL system can be defined based on the reported literatures a) The combined activity of the enzmye upon the substrate simultaneously facilitate the specific substrate to bind with the respective enzyme effectively (Gunavadhi and Sreeram 2020) and b) Enzyme complex formation which alters the protein folding of the respective enzyme causing increase in the availabity of binding site to respective substrate (Angelo et al. 1997). The reduction in the amylase activity in the presence of protease can be explained by the limited proteolysis of amylase into large fragment which will not hinder the catalytic activity (Khomaini et al. 2008 & Khosro et al. 2006). While lipase act as inhibitor to the amylase activity by blocking the active sites of the substrate to bind with the enzyme. Lipase is reported to bind carbohydrate substrate which acts as the inhibitor for the amylase enzyme (Zoy et al. 2019).

3.3. Hydrothermal stability

The shrinkage temperature analysis of the experimental leather is shown in table VI. Varied enzyme combination treated experimental leather were kept in the boiling glycerol and water mixture to measure the temperature at which leather shrinks. The experimental leather shown comparatively similar shrinkage temperature with respect to conventionally processed leather. This implies that the single step enzymatic pre-tanning treatment have not damaged the fibre orientation and reduced the strength of the fibre. Thus, making the skin matrix suitable for the tanning operation.

Table VI. Shrinkage temperature of the veg. tanned leather

Sample	Ts (°C)
PAL	79
PA	80
PL	78

3.4. Physical characteristics

The physical strength of multi-enzymatically processed crust leather has been tested. The tensile strength was recorded as 21.91 N/mm^2. The experimental leather withstands a maximum load of 453.50 N with 53.45% of elongation at break. While the tear strength of experimental leather was found to be 60.36 N/mm. The overall physical strength characteristics was found to meet with the standard norms.

4. Conclusion

Enzyme technology is a futuristic aid for an environmentally friendly leather processing. Single step enzymatic beam house operation is the aim of the leather sector to achieve a sustainable zero waste emission processes. The present study focused on understanding the behavioural mechanism of *in vitro* multi enzyme system for its application in the leather processing. This study has revealed that protease activity upon the amylase and lipase is negligible. Protease and lipase activity in the tripot system (PAL system) has significantly increased. Combined multi enzymes activities and enzyme complexing are the facilitating parameters for the enhancement in the efficiency of tripot enzyme system. The hydrothermal stability of the experimental leather is similar to the conventional processed leather. Thus, a tripot system of enzyme application can be used for achieving single step enzymatic beam house operation.

5. Acknowledgement

Authors would like to thank the Director, CSIR-CLRI and CLRI-CATERS for the testing facilities. Authors also acknowledge the financial support from SMEP. Sudhagar Jeyas Kandhan thanks the financial support from Anna Research Fellowship, Centre for Research, Anna University.

6. Reference

1. Angelo, F. Patrizia, P.L., Vincenzo, D.F., Elena, S., Marcello, Z., Probing the partly folded states of proteins by limited proteolysis, Folding & Design, 2(2), 17-26, 1997.
2. De Souza, F.R., Gutterres, M., Application of Enzymes in Leather Processing: A Comparison Between Chemical and Coenzymatic Processes, Brazilian Journal of Chemical Engineering, 29(03), 473-481, 2012.
3. Gunavadhi, M., Sreeram, K.J., Nano-biocatalyst: Bi-functionalization of Protease and Amylase on Copper Oxide Nanoparticles, Colloids and Surfaces B: Biointerfaces. 197, 111386, 2020.
4. Hess, B., Boiteux, A., Heterologous Enzyme-Enzyme Interactions. In: Jaenicke, R., Helmreich, E. (eds) Protein-Protein Interactions, 23, 271-297, 1972.
5. Khomaini, H. Wangsa, T.I. Idar, K. Yandi, A. Susanti, K. Safri, I. Toto, S., Soetijoso, S., Proteolysis of α-amylase from Saccharomycopsis fibuligera: characterization of digestion products, Biologia, 63(6), 1044—1050, 2008.
6. Khosro, K., Maryam, M.S., Mohsen Asghari, S., Fatemeh, M., Atiah, G., Mehdi, S., Bijan, R., Saman, H., Sara, G., Hossein N.M., Acidic and proteolytic digestion of α-amylases from Bacillus licheniformis and Bacillus amyloliquefaciens: Stability and flexibility analysis, Enzyme and Microbial Technology, 38, 422–428, 2006.

7. McLaughlin, G. D., Theis, E.R., The chemistry of leather manufacture, Reinhold Publishing Corp., New York, 133, 1945.
8. Md Jawad, H., Papia, H., Mohammed, M.R., Protease enzyme based cleaner leather processing: A review, Journal of Cleaner Production, 365, 1-14, 2022.
9. Senthilvelan, T., Kanagaraj, J., Mandal, A.B., Application of enzymes for dehairing of skins: cleaner leather processing, Clean Techn Environ Policy, 14, 889–897, 2012.
10. Srivastava, D.K., Bernhard, S.A., Enzyme-Enzyme Interactions and the Regulation of Metabolic Reaction Pathways, Current Topics in Cellular Regulation, 28, 1-68, 1986.
11. Tony, T., Johanna, C., David, Y.G., Pancreatic enzyme replacement therapy for pancreatic exocrine insufficiency in the 21st century, World J Gastroenterol, 20(33), 11467-11485, 2014.
12. Yasmin, K. Applications of enzymes in leather processing, Environmental Chemistry Letters, 18, 747–769, 2020.
13. Zoy, N., Dildar, A., Hafiz, M.R., Muhammad, T.Q., Matheus, F., Sarfraz A., Muhammad U.M., In Vitro Antidiabetic, Anti-Obesity and Antioxidant Analysis of Ocimum basilicum Aerial Biomass and in Silico Molecular Docking Simulations with Alpha-Amylase and Lipase Enzymes, Biology, 8(4), 92, 2019.