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ENZYMES IN WET-BLUE NEUTRALIZATION: EFFECT ON PROCESSES AND LEATHER PROPERTIES

RENATA BIŠKAUSKAITĖ-ULINSKĖ, VIRGILIJUS VALEIKA

Department of Polymer Chemistry and Technology, Kaunas University of Technology, Radvilėnų pl. 19,50299 Kaunas, Lithuania, renata.biskauskaite@ktu.edu, virgilijus.valeika@ktu.lt

Enzymes are biocatalysts that have wide applications in many industries. Traditionally, they are employed in leather industry for bating to remove non-collagenous proteins and open-up the fibres for better penetration of chemical substances. Due to growing environmental concerns, more studies are done to use enzymes in other leather processing steps as auxiliaries in soaking, liming-unhairing of hides/skins or for bating of chromed leather (wet-blue). However, enzyme application in post-tanning is still a new field; there is limited information if enzymes can be employed in leather finishing. The aim of this research was to investigate neutralization with enzyme preparations and evaluate the influence on further wet-finishing operations and its effect on the qualities of the semi- and finished product. In the study two enzyme preparations were used: Novo Bate WB and Vilzim PRO N. After enzymatic process the shrinkage temperature and the amount of removed collagen were evaluated; after wet finishing the dye and tannin exhaustion, the penetration of the dye, the matter soluble in dichloromethane were determined. Using enzymes led to greater collagen removal compared to conventional neutralization; however, shrinkage temperature after the neutralization as well as after wet finishing did not show any significant differences. Moreover, only dye penetration through grain side was markedly better when using enzyme preparations. The obtained results showed that some characteristics improved while others got worse or remained similar during further processing. Because of this, it is crucial to analyze and optimize usage of each new enzyme preparation to produce leather with desired qualities.

Keywords: neutralization, enzyme, chromed leather.

INTRODUCTION

Leather is pliable and strong material produced by tanning cattle hide or other animals' rawhides. Even though leather is by-product of meat industry, during manufacturing high amount of solid and liquid waste is produced (Kolomaznik *et al*., 2008). Tannery wastewater is highly polluted in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, sulphate, sulphide, chromium and other chemicals used in the processing (Dandira *et al*., 2012). Due to growing environmental concerns researchers study new technologies to reduce negative impact of leather industry and employment of enzymes could be one of the solutions.

With advancements in development, purification and improvement, enzymes have wide applications in many industries. Conventionally, in leather industry these biocatalysts are used in bating process to remove non-collagenous proteins and open-up the fibres for better penetration of chemical substances (Gallego-Molina *et al*., 2013; Zhang *et al*., 2021). Studies show that enzymes can be used in all beam house processes and have even entered the tan house providing advantages for high-quality leather (Khambhaty, 2020). Ferments in beam house operations can be use as auxiliaries in soaking (Valeika *et al*., 2019), liming-unhairing of hides/skins (Sujitha *et al*., 2018), degreasing (Palop *et al*., 2000).

However, enzyme application in post-tanning is still a new field; there is limited information about enzymes usage in leather finishing. Recent studies showed new possibilities of enzyme use in wet blue rebating; after enzymatic rebating chrome and/or other

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chemicals exhaustion increases (Biškauskaitė *et al*., 2023; Jayakumar *et al*., 2019). Also, ferments were studied in leather dyeing. Song *et al*. (2017) findings suggest better fastness properties against rubbing and better dyed absorption in treated leather. Nevertheless, there are no information about enzyme use in neutralization process. Effective neutralization adding enzyme preparations (EP) would allow to exclude additional enzymatic bating processes after chroming to obtain best quality of leather. The aim of this research was to investigate the neutralization with enzyme preparations and evaluate the influence on further wet-finishing operations and its effect on the qualities of the semi- and finished product.

MATERIALS AND METHODS

Materials

Bovine chromed leather (wet blue) was purchased from tannery “TDL Oda”, Lithuania. Samples for experiments were cut into 6x8 cm series of samples in such a way that all leather parts would be presented in each experiment.

The chemicals used for the analysis were of analytical grade. Analytical and technical grade materials were used for the technological processes.

The EP Novo Bate WB (acid protease, optimal pH 4.5 – 7.0, Novozymes, Denmark) and Vilzim PRO N (neutral protease, optimal pH 6.5 – 7.5, Baltijos enzimai, Lithuania) were used for the neutralization process study.

Enzymatic Neutralization

To study enzymatic neutralization process samples were treated as follows:

1) $NaHCO_3$ 1.5%; H_2O 150%, drum ran for 30 min;
2) NaCOOH 2%, drum ran for 10 min;
3) EP 1% or 5%, drum ran for 85 min.

   For conventional process (control) neutralization was performed without EP. All percentages are based on wet blue leather.

Analysis Methods

The amount of collagen proteins removed was estimated from the amount of hydroxyproline in the neutralization solution using a photo colorimetric method (Zaides *et al*., 1964) using GENESYS-8 (Spectronic Instruments, UK).

For non-collagen proteins removal determination Lowry method was used (Lowry *et al*., 1951).

The shrinkage temperature of wet blue after neutralization and wet-finishing was determined as described in the literature using special equipment and replacing the distilled water with glycerol (Golovteeva *et al*., 1982).

Matter soluble in dichloromethane and pH of aqueous leather extract were determined according to standards (Standard ISO 4048, 2018; Standard ISO 4045, 2018).

For pH measurements pH-meter WTW 526 (WTW Xylem brand, UK) was used.

Dye exhaustion was estimated by colorimetric method by measuring the absorbance of the dye solution at a 495 nm wavelength. Dye consumptions were calculated using the calibration curve.

Tannin exhaustion was measured by gravimetric analysis.

The penetration of dye through the hide was evaluated using a special optical microscope with scale (magnification 15 times) MPB-2 (Izyum Instrument Making Plant, Izyum, Ukraine).

RESULTS AND DISCUSSION

Neutralization is performed after chroming to increase wet blue pH, in order to avoid the surface fixation of negatively charged post-tanning chemicals on the positively charged chromium cross linked matrix (Saravanabhavan *et al*., 2006). Neutralization helps to achieve a better and more uniform distribution of retanning, dyestuff and fat liquors substances (Sundar *et al*., 2001). To study enzymes influence in the neutralization process, four experimental variants were tested and after the process they were compared to conventional method. Firstly, pH values of neutralization solution and aqueous leather extract were measured (Table 1). The results show slightly higher pH of the neutralization solution when using Novo Bate WB; lowest pH value was obtained with Vilzim PRO N. However, only sample processed with lower concentration of Vilzim PRO N resulted in higher aqueous leather extract pH, that may suggest better neutralization process, values of other measured samples were alike. Furthermore, differences between determined shrinkage temperature were negligible, indicating that EP did not have effect on shrinkage temperature.

Table 1. pH and shrinkage temperature after neutralisation

	pH of neutralization solution	pH of aqueous leather extract	Shrinkage temperature, ºC
Control	6.11±0.21	4.33±0.15	118.00±0.50
1% Novo Bate WB	6.36±0.15	4.43±0.15	118.17±0.47
5% Novo Bate WB	6.53±0.30	4.30±0.17	118.27±0.63
1% Vilzim PRO N	5.81±0.20	5.23±0.20	117.93±1.18
5% Vilzim PRO N	5.80±0.20	4.35±0.15	119.00±0.50

Secondly, after neutralization amount of removed non-collagenous and collagenous proteins were evaluated (Fig. 1). Even though, shrinkage temperature after process was not affected, using EP led to higher protein removal compared to conventional method. The amount of removed proteins increased with increasing EP concentrations. Results show that EP acted differently on proteins; highest effect on collagenous proteins had Vilzim PRO N, while on non-collagenous proteins Novo Bate WB. Similar shrinkage temperature, despite that addition of enzymes leads to higher removal of collagen proteins, presumably be due to EP act on telopeptide regions in collagen structure which do not have critical role in stabilizing reactions or preparative processes (Covington, 2009).

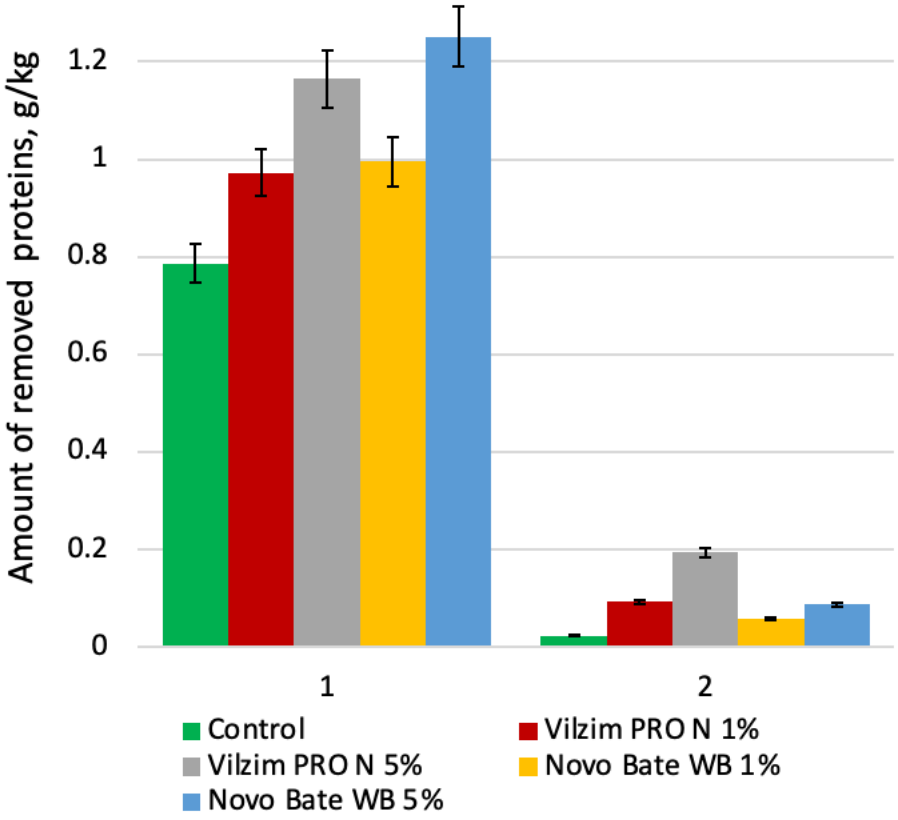

Figure 1. Influence of enzyme on the amount of removed non-collagenous (1) and collagenous (2) proteins during neutralization

After neutralization wet-finishing processes such as re-tanning, dyeing and fat-liquoring were performed. To determine the effectiveness of them and the quality of leather, tannin and dye exhaustion, matter soluble in dichlormethane and shrinkage temperature were evaluated (Table 2). Study findings indicated that during re-tanning only using 5% of Novo Bate notably improved tannins exhaustion, other experimental samples resulted in lower tannin consumption than conventional process or just slightly better. Furthermore, dye exhaustion after enzymatic neutralization did not show improved process; control process was the most effective. Shrinkage temperature after wet-finishing as well as after the neutralization were similar between the samples, only using 1% of Vilzim Pro N temperature increased slightly.

Table 2. Influence of enzymatic treatment in wet-finishing processes

	Tannin exhaustion, %	Dye exhaustion, %	Matter soluble in dichloromethane, %	Shrinkage temperature, ºC
Control	58.20±1.75	84.37±1.31	5.46±0.10	120.33±0.66
1% Novo Bate WB	58.29±1.61	79.81±0.89	5.69±0.11	119.75±0.40
5% Novo Bate WB	65.24±2.02	82.35±1.15	5.45±0.17	119.00±0.50
1% Vilzim PRO N	54.91±1.14	83.05±1.50	5.47±0.21	121.57±0.47
5% Vilzim PRO N	47.96±1.21	83.22±0.97	6.27±0.18	120.00±0.50

Even though dye exhaustion did not show improvement while using enzymes, dye penetration enhances with EP (Fig. 2). From results it was observed that using EP led to significantly better dye diffusion through grain layer compared to control samples. Usually, greater penetration of dyes is achieved on the flesh side, due to well-known structural differences between the two sides; flesh side has more open structure fiber that helps dye penetrate better (Haroun, 2015). Using these EP led to better dye diffusion on grain side almost in all cases; wet blue affected by neutralization with enzymes behaved differently during dyeing than control sample. Overall, total dye exhaustion was better with EP; using 1% Novo Bate WB led to best dye diffusion.

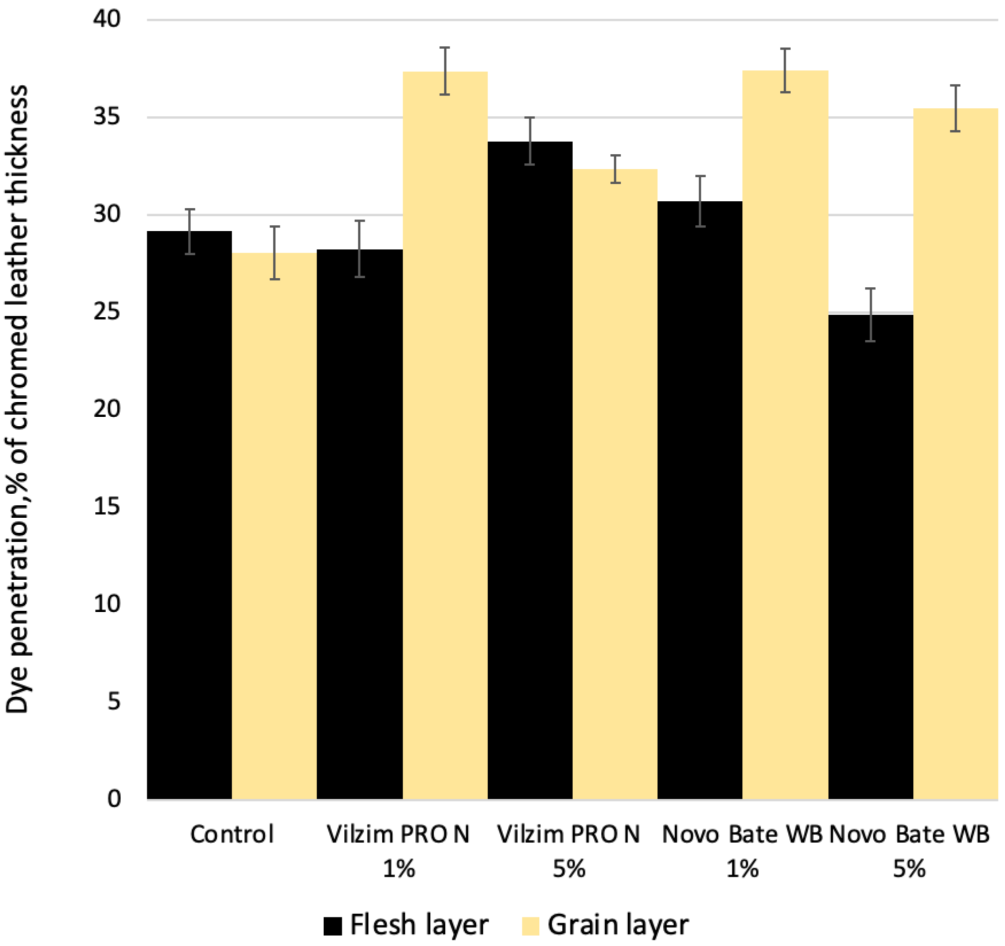

Figure 2. Depth of dye penetration into the chromed leather

CONCLUSIONS

Results obtained during the research show that with the use of enzymes more collagenous and non-collagenous proteins are removed from wet blue. However, shrinkage temperature after neutralization and wet-finishing did not show any significant differences between the samples. Also, dye exhaustion values indicated better dye process of conventional neutralization. Nevertheless, enzymes in neutralization process led to better dye penetration through grain side. Finding show that some characteristics may improve while others may worsen or remain constant after enzymatic processing. Because of this, it is crucial to analyse and optimise the use of each new enzyme preparation to produce leather with desired qualities. However, from an economical point of view enzymes in neutralization do not have many advantages.

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Standard ISO 4045:2018. Leather — Chemical Tests — Determination of pH and Difference Figure

Standard ISO 4048:2018. Leather — Chemical Tests — Determination of Matter Soluble in Dichloromethane and Free Fatty Acid Content

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