Innovative Enzyme Applications in Tanning Processes

M. Roig[1], Alice Dall'Ara[2], V. Segarra[1], M. A. Martínez[1], J. Ferrer[1]

[1] Center for Technology and Innovation (INESCOP). Polígono Industrial Campo Alto - 03600 Elda (Spain). e-mail: medioambiente@inescop.es

[2] ENEA. Laboratori di Ricerca Faenza. Via Ravegnana, 186 - 48018 Faenza (Italy)

Abstract

Enzyme products have usually been employed in tanning processes, but in recent years new products and innovative applications have been developed. The use of such products has extended to different operations within the tanning process, thus improving the quality of leather and reducing the overall environmental impact of the process.

One of the most recent innovations has been brought about by the European project LIFE PODEBA "Use of poultry dejection for the bating phase in the tanning cycle", which aims to demonstrate the feasibility of reusing a farming by-product, of proven enzyme activity, in the leather bating stage, thus achieving a reduction in the environmental impact associated to this process stage without compromising leather quality.

Furthermore, some tests are being conducted for the replacement of traditional products (sulphurs) with highly active enzyme products in the liming stage, as well as for the conditioning of semi-processed leather (wet-blue) using acid proteases after long storage periods. Finally, enzyme products have also been used for the treatment of tanned waste by enzyme hydrolysis, this way obtaining two by-products (chrome retanning agent and protein retanning agent) that can be reused in the industrial process.

This paper presents the results obtained by the Center for Technology and Innovation (INESCOP) in the application of the enzyme products tested.

Keywords: enzymes, poultry manure, bating, liming, enzyme hydrolysis.

1. Introduction

The tanning process is carried out over multiple stages in which the tanners seek to optimise the quality and performance of the leather at the same time as attempting to reduce the costs of production and the environmental impact of the process. The adequate development of the first stages of the process (soaking, dehairing, deliming/bating stages) is fundamental in guaranteeing the quality of the finished leather, avoiding problems with wrinkling, grain looseness, low physical resistance, etc.

During the soaking and dehairing stages, enzyme products are frequently used that reduce the processing time and the consumption of chemicals such as surfactants, sulphur etc, maintaining quality, but with a reduced environmental impact on the wastewater of the process.

In the case of the bating stage, the use of enzymes is also inherent, in that only via the use of proteolytic enzymes it is possible to produce a loosening of the collagen structure of the skin, the principle aim of this stage. Furthermore, the enzymes used for bating reduce the alkaline swelling of the skin produced in the dehairing/liming stage, and also enable the elimination of fats, non-fibrous proteins, hair remains etc.

The intensity of the bating depends on the desired type of leather to be obtained as the final product, making it necessary to adequately control the process variables such as the enzyme activity of the product employed, the duration, temperature and pH of the operation, and the mechanical action. If the bating is insufficient due to it not being carried out in optimal conditions, the resulting leather could end up being hard, rough, with loose grain, and with hair-root and epidermis remains. On the contrary, excessive bating could provoke an intense weakening of the fibrous texture and an excessive elasticity and sponginess in the leather, with no fullness and low physical resistance.

The advances in biotechnology have led to the industrial production of enzymes by means of microbial fermentation in aerobic cultures on bacteria and fungi strains [1]. However, for enzyme products used in bating. In this context, the LIFE PODEBA project, which promotes "the use of poultry manure in the enzymatic bating stage of the tanning cycle," has set the demonstration of recycling of poultry manure as its principle objective, which, after having been treated, can be reused in the bating stage of the leather tanning process due to its enzymatic capacity.

The determination of the enzyme activity of poultry manure permits the evaluation of the possibilities of the use of this waste in the bating stage. The characterisation of the enzyme activity of the samples can be carried out by using different methods according to the substrate used as a reference (casein, haemoglobin, etc.), and the method of detection (titration, spectrophotometry, etc.).

In this case, the determination of the enzyme activity was carried out by means of the Lohlein-Volhard method [2]. This method is based on the digestion of a casein substrate by means of the proteolytic activity of enzyme products contained in the sample being tested. Through this method, enzyme activity is measured in Lohlein-Volhard Units per gram (LVU/g), defined as that amount of enzyme which digests 1.725 mg of casein.

Furthermore, the use of poultry manure requires preparation and conditioning beforehand in order to simplify its use and handling, firstly by focusing on the elimination of the problem of bad smells generated by poultry manure. Likewise, it is necessary to determine the possible interference of the deodorising systems in the enzyme activity of poultry manure.

In this study, the results to be presented will be those obtained so far in the tests conducted with cattle hides on a semi-industrial scale using conditioned poultry manure in the bating stage of leather tanning, as an alternative to commercial products normally used. The study offers a description of the characterisation and conditioning of the poultry manure samples, the bating tests realised, the impact of this stage regarding waste effluents and the physicochemical characterisation of the leather obtained.

2. Materials and Methods

Poultry manure has a thick, gluey appearance, with humidity of around 70%, along with an unpleasant smell, because of which it is necessary to carry out previous conditioning of the samples so that their use and handling may be similar to that of commercial products usually applied, reducing the smell factor whilst maintaining its enzyme activity.

The poultry dejections (PD) were obtained from a laying hen intensive livestock farming facility located in Mordano, Emilia-Romagna (Italy); they did not contain litter material. Poultry dejections were collected by manure belts under the cages and dried by Manure Drying System (MDS), one of the most efficient drying systems. With MDS, manure was removed from housing after 24-36 hours by means of manure belts and dried in a tunnel outside the housing in the subsequent 72 hours. MDS, classified as BAT (Best Available Technique) according to BREF (EC, 2003) [3], allows manure to be dried by up to 85%.

Subsequently, a Bio-Treatment was performed directly inside bioreactors, according to the European patented process "A process for maturing and stabilizing biomasses under reduction of smelling emissions" (EP1314710; AMEK, CTI, 2002) [4]. This process describes a complex product, natural enzyme mixtures named VAP (Vegetable Active Principles), prepared from selected plants picked up in their balsamic period, at the time of maximum vegetative development. VAP have been developed in order to speed up bio-oxidation processes, reduce biomass turning and to maintain nitrogen in slow release form. This treatment consists of adding VAP to dried manure inside bioreactors (closed big-bags to avoid recontamination), during filling, in doses of 1 kg/m^3. In the bioreactors, the manure matures by static batch process, which takes up to 120 days; at the end of which it is ready for marketing.

The effectiveness of the conditioning treatment was evaluated via the measurement of VOC, NH_3, CO_2, sulphur, and mercaptan [5] emissions, as shown in Table 1; along with the enzyme activity of poultry manure, both before and after conditioning.

Table 1. Measurement of emissions from poultry manure: parameters and analysis methods

Parameter	Description	Analysis Method
VOCs	Characteristic of poultry manure	Gas-chromatography/ Mass Spectrometry
Ammonia NH_3	Characteristic of poultry manure	Colorimetric method (spectrophotometer)
Carbon dioxide CO_2	Metabolism indicator	Gas-chromatography
Dimethylsulphide $(CH_3)_2S$ Dimethyldisulphide $(CH_3)_2S_2$	Characteristic of poultry manure; indicators of anaerobic conditions; main cause of unpleasant odours	Gas-chromatography/ Mass Spectrometry
Methylmercaptan CH_3SH	Characteristic of poultry manure; indicators of anaerobic conditions; main cause of unpleasant odours	Gas-chromatography

Regarding the determination of the enzyme activity of poultry manure, owing to the granular form of the samples, a procedure was established to prepare the samples for their analysis. This procedure is based upon the standard for waste leaching for its characterisation, and it consists of the grinding of the samples (particle sizes of 4 mm maximum), the leaching of the waste in water being stirred for 24 hours at 50 rpm, and the subsequent vacuum filtration to obtain the aqueous extract to be analysed.

The testing on the utilisation of the conditioned poultry manure in the bating stage of leather tanning was conducted at INESCOP's tanning pilot plants (see figure 1). These tests were completed using tanning drums, measuring 900 and 1200 mm in diameter respectively, and 400 mm in width, and made from bolondo wood, which were equipped with systems for automation, control, and water and reagent dosing.

Figure 1. Tanning drum used for bating tests using poultry manure

In the tests carried out, pelt cattle hides, with a thickness of 2.5 mm, and split into sides (half of a hide) were processed. These hides were subjected to a conventional deliming process through the use of ammonium sulphate, and, following this, the bating process was completed using different poultry manure samples. By way of comparative reference, the bating was carried out using 1% of a commercial standard product with an enzyme activity of 900 LVU/g; consequently, depending on the enzyme activity of each manure sample, the percentage of the sample to be added to the pelt-split hide was recalculated, maintaining the consistency of the operation conditions: i.e. pH value, temperature, rotation speed and duration.

After the bating stage, pickling was done, and, after that, tanning was carried out using basic trivalent Chromium salts. After tanning, the leather hides were sammied and skived to 1.5 mm to proceed with the neutralisation, retanning, dying, and fatliquoring, all using standard procedures.

The formulations used in these tests are shown in Table 2, where, for each of the operations, the table also indicates the product used, the percentage in weight, the temperature, and operation times, as well as the checks conducted (pH, salinity of the bath etc.).

Table 2. Testing formula

Process	%	Product	°C	Rotation time	pH	Remarks
Deliming	200	Agua	30	20’		
(pelt weight)		Drain bath				Rinse twice
	100	Agua	35	20’		
	1.0	Ammonium sulphate		15’		pH
	1.0	Ammonium sulphate		15’		pH
		Rotate		30’ - 45’	< 8	Check cross-section with colourless phenolphtalein
	0.5	Surfactant		20’		
Bating	200	Water	38			
	X	***Poultry manure (*)***		45’ - 60’		
		Drain bath				***Bath sample***
	200	Water	20	5’		Rinse twice
		Drain				
Pickling	50	Water	20			
	8	Salt		10’		Check 6-7ºBe
	1	Sulphuric acid 1:10		30’		
	1	Formic acid 1:5		3 h		Check pH 3.5 in bath and cross-section with bromocresol green
Tanning	8	Chromium salt 33%		60’		Leave in bath for one day
	1	Magnesium oxide (Plenatol SRB2)				Leave in bath overnight
						Check pH 3.8 - 4 and Tc >100 ºC
	0.1	Fungicide - Preventol		30’		
		Drain bath - Unload leathers and pile them up				
Neutralisation	200	Water	20			
(shaved weight)	1.5	Sodium formiate				
	0.5	Sodium bicarbonate		40’	5	Check pH
		Drain and rinse				
Dyeing	2-4	Dye		60’		Check cross-section
	200	Water	50			
	0.5	Formic acid (1:5)		30’		
Retanning	100	Water				
	5	Resin		45’		
Fatliquoring	8	Fatliquor		60’		
	1	Formic acid (1:5)		20’		
	0.5	Formic acid (1:5)				
		Rinse with cold water – Drain bath – Unload leathers				

(*)*Recalculated for each poultry manure sample according to its enzyme activity, so that it is equivalent to 1% (over pelt split weight) of a commercial product of 900 LVU/g*

The leather obtained usually shows a pleasant appearance, fine-grain and adequate smoothness, softness, flexibility, grain firmness, and fullness. An evaluation of the results of the tests was fulfilled via the characterisation of the leather obtained and the resulting effluents, confirming to accepted standards.

3. Results and discussion

3.1. Preparation, conditioning, and characterisation of poultry manure

The results of the characterisation of the aqueous extract of the different poultry manure samples tested are shown in Table 3.

Table 3. Results of the analysis of the enzyme activity of poultry manure.

SAMPLE	Humidity (%)	LVU/g of sample	LVU/g of dry sample
FRESH	69	423	1,364
DRY	20	295	354
AMEK MDS	33	328	438
AMEK V	42	267	456
AMEK G	8	298	323
AMEK PO	20	378	472
C1	16	519	621
C2 (P70)	16	427	511
P120	18	217	489
C4	18	469	575

The results were validated through the testing of a commercial product, the enzyme activity of which was known, obtaining a deviation of between 8 and 15%, coinciding with the error established in the analysis procedure.

The original sample possessed an enzyme activity of 1,364 LVU/g over dry weight, similar to the commercial bating products (1,000-2,000 LVU/g) which, after conditioning treatment, stabilised at around 500 LVU/g.

On the other hand, there was evidence that, through the use of the Bio-Treatment process conducted, there was a reduction in the level of VOC emissions, in accordance to what can be seen in Figure 2, in which the upper line graph corresponds to the conditioned sample, whilst the lower line graph corresponds to the sample that was not subjected to conditioning.

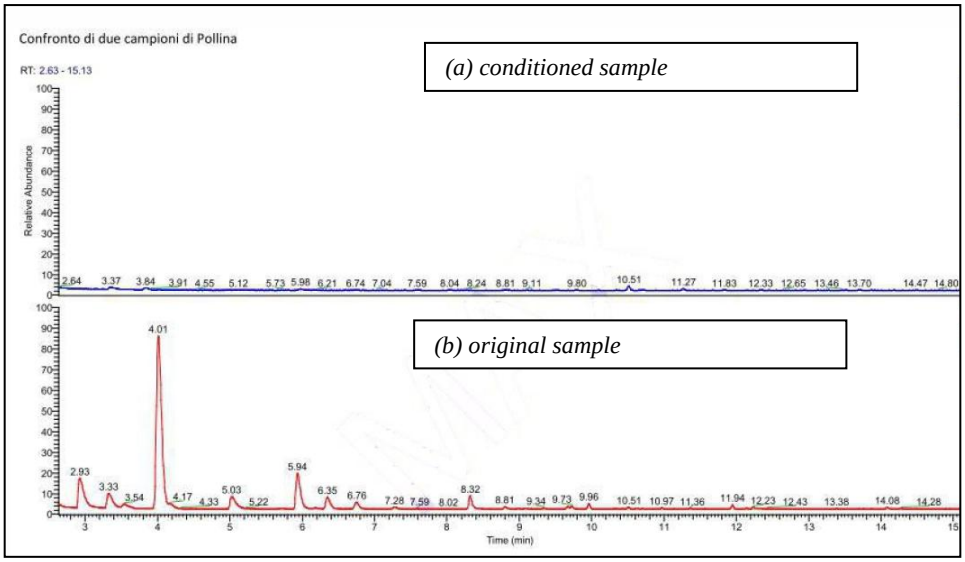

Figure 2. Determination of VOCs in poultry manure samples:

(a) Conditioned sample, (b) Original sample

The absence of peaks in the conditioned sample (top image) in comparison to the original sample (lower image) demonstrates the desodorisation of the poultry manure samples, which simplifies the use of these products in the bating stage of the tanning process.

3.2. Characterisation of the residual baths of the bating stage.

The evaluation of the environmental impact of the use of poultry manure in the bating stage, in substitution of commercial enzyme products, was conducted via the characterisation of the residual baths from deliming/bating resulting from the tests conducted.

In this regard, the most significant parameters, in accordance to international standards, were determined; the results obtained are shown in Table 4.

Table 4. Characterisation of the residual baths of the deliming/bating process.

Sample	pH	Conductivity (µs/cm)	Suspended solids (mg/l)	COD (g O_2/l)	BOD_5 (g O_2/l)	TKN (mg/l)	NH_4^+ (mg/l)	$S^=$ (mg/l)
REFERENCE PRODUCT	8.1	8,870	516	9.8	4.8	1,424	1,090	0.5
AMEK V	7.9	6,842	1,433	14.1	8.4	1,431	1,120	4.1
AMEK G	8.0	5,508	1,829	10.6	6.0	927	725	2.4
AMEK PO	8.2	6,420	1,128	4.9	3.6	819	685	0.1
C2 (P70)	8.1	5,242	1,540	5.4	3.8	740	580	0.1

The characterisation of the residual baths resulting from the deliming/bating process showed a significant reduction in the environmental impacts of those baths where poultry manure was being used, which, in the case of the nitrogen content in the wastewater, measured as

TKN/$NH4^+$, was reduced by more than 50%. On the contrary, the content of solids suspended in the residual baths was higher owing to the very nature of the poultry manure samples.

3.3. Physical characterisation of the leather obtained.

The cattle hide leather samples obtained during the tests on a semi-industrial scale were subjected to different quality control processes according to international standards (EN-ISO) to test their suitability to be used in the manufacture of footwear components. Table 5 shows the results of the physical characterisation of the hides.

Table 5. Physical characterisation of leather (poultry manure bating enzymes)

PARAMETER	MINIMUM	MAXIMUM	AVERAGE	RECOMMENDED VALUES
Thickness (mm)	2.2	2.4	2.3	> 1.1
Tear strength (N)	242	257	246.5	> 50
Tensile strength (N/mm^2)	28.2	29.1	28.7	> 15
Elongation at break (%)	70.2	97.2	83.0	> 40
Grain burst (mm)	>10	>10	>10	> 8

The determination of the physical parameters of leather demonstrated that, in all cases, the recommended values for the manufacture of footwear were fulfilled.

3.4. Chemical characterisation of the leather obtained.

The chemical validation of the leather obtained during the tests, on a semi-industrial scale, was carried out via the chemical characterisation of the leather according to accepted standards (EN. ISO. etc.), as well as the verification of the compliance with the criteria of the European Eco-label for footwear (Decision 2009/563/EC):

- Cr (VI) content in leather: not detected
- As, Cd and Pb content in leather: not detected
- Formaldehyde content in leather ≤ 150ppm
- Pentachlorophenol (PCP) content in leather: not detected
- Tetrachlorophenol (TCP) content in leather: not detected
- Azo-dye content in leather: < 30 ppm

The determination of the chemical parameters of the leather showed its conformity to the limits established in the European Eco-label for footwear.

4. Conclusions

The results show that the use of poultry manure in the bating phase of leather production allows leather to be obtained with a pleasant appearance and good resistance, along with adequate softness, flexibility, grain firmness, and fullness, without witnessing any differences with the results obtained from the use of commercial enzyme products.

In relation to the conditioning of the samples, the drying and deodorising treatment used considerably simplifies the use of these waste products, and, after having optimised it, it does not interfere in the enzyme activity of the poultry manure, which has been established at around 500 LVU/g. This entails a slight increase of the usual dosage by 1%, or prolonging the operation time a little bit.

In relation to the environmental impact, the values obtained in the characterisation of the deliming/bating baths in the different tests conducted show that in all of them, there is a significant reduction in the impact on the environment, mainly regarding the nitrogen content of the wastewater, measured as TKN/NH4+, and COD, observing an increase in the quantity of the suspended solids, owing to the granular nature of the poultry manure samples.

The determination of the physical parameters of the leathers bated with the conditioned poultry manure has demonstrated the fulfilment of the limits established for the manufacture of footwear articles. As regards the chemical characterisation of this leather, the results show the compliance with the limits set by the criteria of the European Eco-label for footwear.

In short, conducting the leather bating process using conditioned poultry manure of proven enzyme activity implies a considerable reduction in the environmental impact of this stage in the process, whilst maintaining the quality of tanned leather.

5. Acknowledgements

The authors thank the European Commission LIFE-Environment Programme for the support to the LIFE Project “Use of poultry dejection for the bating phase in the tanning cycle (PODEBA)” with reference number LIFE10 ENV/It/000365.

6. References

[1] Covington, A. 2009. Tanning Chemistry: The Science of Leather. Chapter 8, 166-176p

[2] Association TEGEWA - Enzyme products working group. 1971. Methode zur bestimmung der wirksamkeit proteolytischer Enzyme nach Löhlein Volhard. Das Leder. 22. 121-126p

[3] EC 2003. Integrated Pollution Prevention and Control (IPPC). Reference document on Best Available Techniques for Intensive Rearing of Poultry and Pigs, pp: 161-184. http://eippcb.jrc.es/pages/FActivities.htm.

[4] European Patent Specification EP 1 314 710 A1. A process of maturing and stabilizing biomasses under reduction of smelling emissions. Cooperativa Trasporti Imola SCRL (CTI Scrl) & AMEK Piccola Soc. Coop. a r.l.. Bulletin 2012/02, 11/01/2012.

[5] Iglesias, A. 1998. Contaminación atmosférica por olores. Química e Industria. Vol 45.