Effect of Enzymatic Soaking on Properties of Hide and the Leather Produced

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Summary

The use of enzymes for soaking has influences on rehydration levels of hide and the amount of collagenous and non-collagen proteins removed. The removal depends on the EP (enzyme product) used and on the amount. The increase of the amount of EP in a soaking solution affects faster rehydration, higher content of removed non-collagen proteins but, unfortunately, has an effect on the collagen of hide not only during the soaking but during subsequent liming as well, this can be the reason for defects in the finished leather. The duration of enzymatic soaking has an influence on chroming and chromed leather properties also: too short an enzymatic soak leads to weakened grain and to decreased relative elongation of the leather.

A properly chosen EP preparation and its amount can result in higher rehydration and removal of non-collagen materials from the derma during soaking when compared with the treatment without enzyme. On the other hand, such a use of EP has not caused any observable influence on the exploitation properties of the finished leather.

INTRODUCTION

The use of enzymes is one of the most promising ways to develop the technology of leather processing. Nowadays, no one leather technologist can imagine the bating of leather without use of enzymes: enzymatic bating has become the classical method. Recently, enzymes are very widely applied in various unhairing systems, completely or partly replacing sodium sulphide[1,2,3,4] and for degreasing process.[5,6] Investigations have been presented describing the application of enzymes for pickling,[7,8] for vegetable tanning[9,10] or even for dyeing.[11,12,13]

There is one more technological stage for which enzymes are recommended[14,15] – the soaking process. According to the literature, various enzymes can be employed as soaking auxiliaries: proteases, lipases, amylases, chondroitinases, amidases, phospholipases *etc*.[16] Every enzyme acts very individually and its exploitation depends on the targets which should be reached during the soaking process.

As the advantages of the use of enzymes for soaking have been mentioned – faster rehydration, removal of interfibriliary materials *etc*. Technologists use numerous ways to facilitate and to accelerate soaking, preferentially by action of the substances that decrease surface tension or by enzyme action.[17] Mhya and Mankilik[18] mention that the benefits observed in using bacterial enzymes in soaking include loosening of the scud, initiation of the opening of the fibre structure and production of leather with a less wrinkled grain.

Overall, there is little scientific literature describing the action of enzymes during soaking process and the influence of the enzymatic soaking on the run of subsequent processes and the properties of the processed leather.

Cantera *et al*.[3] prepared the protocol of the analysis to characterise enzymatic preparations allowing determination of their different behaviours. It was stated that the other components used in the soaking and unhairing processes have various influences on the proteolytic activity of the various enzyme preparations. Xu *et al*.[19] reported about investigation of commercial proteases widely used in soaking, unhairing, liming and bating, for assaying elastin activity. It was established that most commercial alkaline and neutral proteases exhibit elastase activity, while acid proteases show very low activity against elastin. The authors concluded that care is essential to select proteases for leather-making processes to avoid grain loosening and damage from the excessive removal of elastin.

A protease secreted by *Pseudomonas aeruginosa*[20] when used in a soaking process showed increased water penetration because of hydrolysis of albumin and elastin proteins as indicated by opened fibres in histopathological sections. These findings suggest a possible use of this protease in the soaking operation of leather processing for minimising use of harmful dehairing chemicals and processing time.

Ma *et al*.[21] investigated a simultaneous action of few enzymes in soaking. The results indicated that the combination of commercial enzymes TanG and LimeG had a good synergistic effect during the soaking. The authors propose that it is better to choose a combination of larger and smaller molecule weight

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enzymes and thus utilise the diffusion differences of enzyme in the skin matrix.

De Souza and Gutterres[11] presented data about the effect of enzyme action on organic matter removal and amount of soluble proteins after the soaking process. They established that the co-enzymatic processes operate faster than the chemical processes. The enzyme concentration in the tests does not exhibit a linear relationship with the organic matter removal, as excess enzyme did not lead to a greater removal of this material.

Summarising the literature review: unfortunately, it can be stated that the conclusions proving the advantages of the use of the enzymatic soaking are not supported by any experiments showing the influence of the enzymatic action on produced leather quality.

The aim of the current research was to determine the influence of soaking with enzymes on the run of subsequent processes, such as liming and chroming, and on properties of produced leather.

EXPERIMENTAL PROCEDURES

Raw material

Salted cattle hide was used as raw material for this study (mass 24kg, moisture content 40.8%, NaCl 29.8%). The hide was cut into pieces 5 x 10cm and a series of samples were prepared from the pieces.

Chemicals

The chemicals used for the technological processes and for the analysis were mainly of analytical grade. Some chemicals used for technological processes were of commercial grade. The enzyme preparations (EP) used in the soaking process and their activity were (activity presented at pH of medium is 10.5, temperature 20°C) EP Aquaderm A isolated from *Aspergillus* (Novo Nordisk, Denmark), 174U/g; EP BAP isolated from *Bacillus lichenifornis* (UAB Biosinteze, Lithuania), 23U/g and EP Vilzim PRO ALK isolated from *Bacillus lichenifornis* (UAB Baltijos Enzymai, Lithuania), 920U/g. All EP are characterised as alkaline proteases.

Other technical products were EP Codeymac 5.0 M for bating (Codyeco s.p.a., Italy); Cromeco Extra (chromium extract – 25% of Cr_2O_3 and 33% basicity) by Gruppo Chimico Dalton (Italy) and Neutragene MG-120 (for increasing the chromium compounds' basicity) by Codyeco s.p.a. (Italy).

Parameters of processes

Soaking: water 100%, temperature 20 ± 1°C, Na_2CO_3 1.4%, duration and amount of EP were variable.

Washing: water 200%, temperature 20 ± 1°C, duration 1 hour, run continuously.

Liming-unhairing: H_2O 40%, temperature 20-22°C, $Ca(OH)_2$ 2.3%, Na2S(100%) 1.2%, 1 hour run continuously, $Ca(OH)_2$ 2.3%, 1 hour run continuously, water 100%, 2 hours run continuously, later 5 minutes every 3 hours (% are based on fresh hide weight); total duration 24 hours.

Deliming-bating: water 40%, temperature 37 ± 1°C, $(NH_4)_2SO_4$ 2.2%, 30 minutes, $(NH_4)_2SO_4$ 1.5%, 30 minutes; water 100%; enzyme preparation Codyemac 5.0M 0.05%, 1 hour. Regime: run continuously.

Pickling: water 40%; temperature 20-22°C, NaCl 5.5%, 15 minutes; HCOONa 1%, 20 minutes; H_2SO_4 0.5%, 15 minutes; H_2SO_4 0.5%, 15 minutes; H_2SO_4 0.5%, 5 hours. 15 minutes. Regime: run continuously.

Chroming (in used pickling solution): temperature 20-22°C, Chromeco 6%, 20 hours; Neutragene MG-120 0.35%, 2 hours, water 100%; temperature 40-42°C, 2 hours. Regime: run continuously.

Washing: water 100%, temperature 40-42°C, 1 hour. Regime: run continuously.

Note: Amounts of materials for the processes are based on fresh hide (soaking – liming) or limed pelt (bating – washing after chroming) weight.

Analysis methods

The EP proteolytic activity was determined using the Anson method.[22] Sodium caseinate was used as a substrate.

The amount of collagen proteins removed from hide was estimated from the amount of hydroxyproline in the soaking and unhairing solutions. The amount of hydroxyproline was determined using a photocolorimetric method.[23] The total amount of protein was estimated by employing Kjeldahl's method.[24] The amount of removed non-collagen proteins was calculated as the difference between the total proteins and the collagen proteins in the treatment solutions.

Contents of chromium oxide, volatile matter and matter soluble in dichloromethane, and strength properties of leather were determined according to standard methods.[25,26,27,28]

RESULTS AND DISCUSSION

Soaking is very important process because insufficient and uneven rehydration of the derma leads to non-qualitative run of subsequent processes: liming-unhairing, deliming *etc*. Soaking would lead to removal of interfibre materials having the aim to increase the permeability of derma tissue to chemicals.

Therefore, the first step was to establish an influence of the EP addition on kinetics of rehydration of hide. The results are presented in Figure 1.

Evidently, the addition of EP influences the character of the hide's moisture change during soaking. The influence is very specific and depends on amount and type of EP. For sample, addition of BAP 0.48% (Fig. 1, curve 1) or Vilzim PRO ALK 0.2% (curve 2) had practically very similar influences despite the fact that amount of 'EP active units' which took part in the soaking process, were different: the level of units was about 10 times higher in the case of EP Vilzim PRO ALK. On the other hand, the use of EP Aquaderm A 0.005% (when the amount of 'EP active units' passed into soaking solution is about 200 times less than in the

case of Vilzim PRO ALK 0.2%) leads to faster rehydration over 2-8 hours compared with soaking without enzymes. It allows the conclusion that the use of each EP for soaking must be explored thoroughly. Knowing the origin and activity of an EP is not sufficient for direct application of any EP in soaking due to the specific action of the EP.

By the way, the differences of rehydration level are negligible when duration of the soaking is long (18 hours).

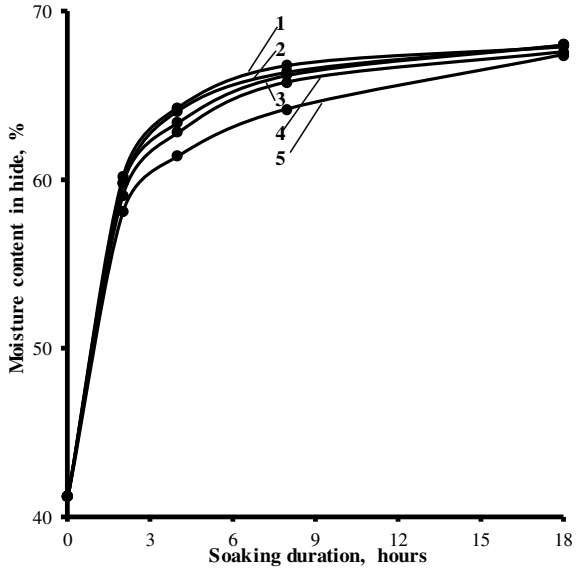

Figure 1. Change of moisture content in hide dependent on soaking duration:
1 – BAP – 0.48%; **2** – Vilzim PRO ALK - 0.2%;
3 – BAP– 0.24%; **4** – Aquaderm A 0.005%; **5** – without EP.

Figure 2 presents a kinetic of non-collagen proteins removal during the soaking process. All the EPs added had an influence on non-collagen removal. By the way, the influence of the added EP becomes observable only after 4 hours of soaking. Only after 8 hours of soaking do the differences of EP action become distinctly seen. Herewith, the use of EP for soaking increases the removal of interfibrous proteins but the action is very individual and depends on the EP applied. For sample, EP Vilzim PRO ALK has a proteolytic activity about 40 times higher than EP BAP but the use almost the same amounts of both EP does not lead to significant different values of removed non-collagen proteins (Figs. 1, 2 and 3 curves).

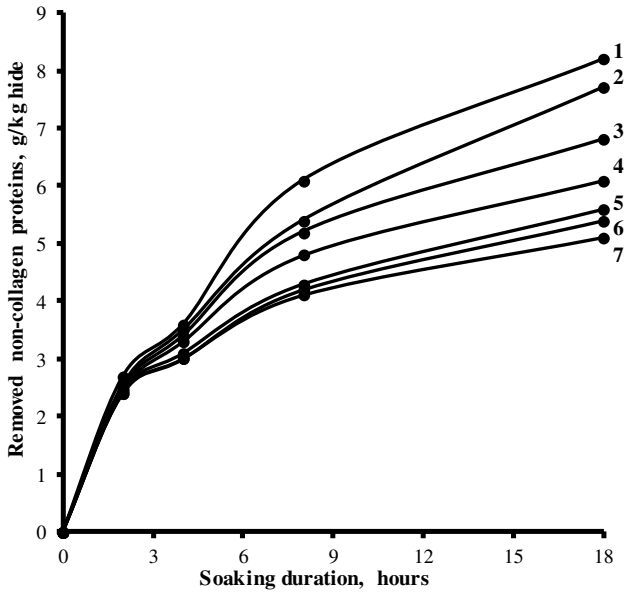

Figure 2. Kinetic of removal of non-collagen proteins during enzymatic soaking:
1 – BAP – 0.48%; **2** – Vilzim PRO ALK - 0.2%;
3 – BAP – 0.24%; **4** – Aquaderm 0.005%; 5 – BAP – 0.12%;
6 – BAP – 0.06%; **7** – without enzyme preparation.

Since proteolytic enzymes are commonly used for the soaking, they act not only on non-collagen proteins but on collagenous proteins as well. Too strong an action on collagen is not desirable due to the possibility of appearance of defects such as loose grain.

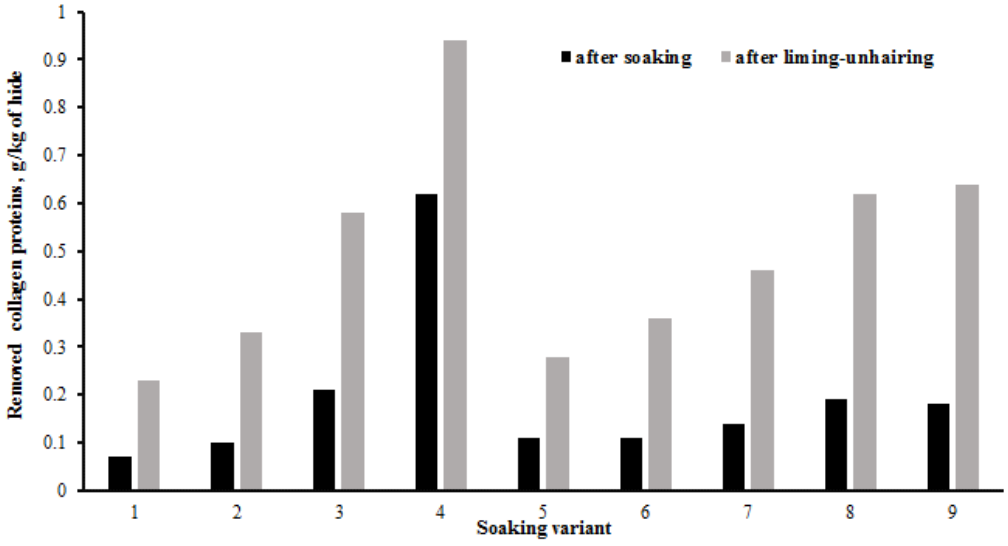

Figure 3. Removal of collagenous proteins during enzymatic soaking and subsequent liming dependent on EP used for the soaking:
1 – without enzyme preparation; **2** – Aquaderm 0.005%; **3** – Aquaderm 0.05%; **4** – Aquaderm 0.5%; **5** – BAP 0.06%;
6 – BAP 0.12%; **7** – BAP 0.24%; **8** – BAP 0.48%; **9** – Vilzim PRO ALK 0.2%.

Herewith, the effect on hide during soaking should have an influence on collagen behaviour during subsequent process – liming-unhairing. Accordingly, the next experiment was carried out with the aim to assess the action on collagen during enzymatic soaking (duration 18 hours) and subsequent liming-unhairing (Fig. 3).

The results obtained (Fig. 3) allows the conclusion that effect on collagen which occurred during soaking with enzymes is related closely with the effect during subsequent liming. Usually, liming-unhairing acts more strongly on collagen that is more strongly affected during soaking. On the other hand, the specificity of EP is also observed. As an example, EP Vilzim PRO ALK (0.2%) is characterised by a weaker action on collagen during soaking and stronger during liming compared with the use of EP Aquaderm 0.05% or BAP 0.48% when the action on collagen is stronger when soaking and slightly weaker when liming.

Usually, the amount of removed collagenous proteins varies in the range 0.2-0.5 g/kg of hide[29,30] after conventional unhairing-liming when soaking without enzymes. Accordingly, it can be concluded that using Aquaderm 0.05% or BAP 0.06-024% for 18 hours soaking is appropriate because after such soaking the removal of collagenous proteins during liming-unhairing does not exceed 0.5g/kg of hide.

Since the rehydration level of hide when soaking with addition of enzymes after 4 hours was practically the same as after 4 hours when soaking without EP (Fig. 1), a dependence of the removal of collagenous proteins on soaking duration was explored. Correspondingly, the removal of collagenous proteins during liming depending on the soaking duration was also tested. The soaking was carried out with the addition of EP Aquaderm 0.005% (Fig. 4).

Hence, the amount of removed collagenous proteins which increases during soaking depends on process

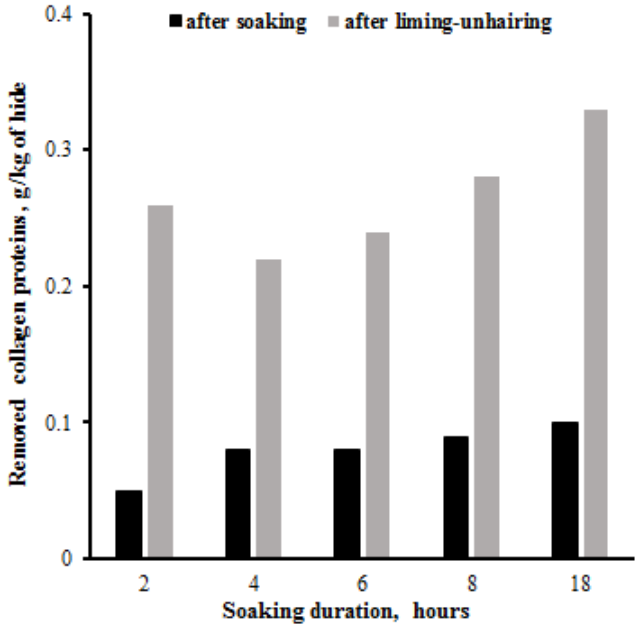

Figure 4. Removal of collagenous proteins during enzymatic soaking and subsequent liming-unharing dependent on soaking duration. Soaked with EP Aquaderm 0.005%.

duration. Unexpected results were obtained when determining the amount of removed collagenous proteins after liming-unhairing. The data show that more collagen proteins are removed during liming when hide soaking is shorter (duration 2 hours) compared with when the hide before liming is soaked for a longer time: 6 hours. The reason may be because, when soaking is too short, the (rehydrated) layers of hide are affected unevenly. Due to this, the liming solution penetrates badly into the inner layer of hide and, accordingly, the alkali concentration in external layers becomes higher and therefore collagen proteins in these layers are more affected.

To verify this assumption, the influence of enzymatic soaking duration on chroming run and chromed leather properties was investigated. Hide pieces were soaked for 2, 4, 6 or 8 hours with a supplement of EP Aquaderm 0.005% and for 18 hours without EP. After soaking, the hide samples were limed-unhaired, delimed-bated, pickled and chromed according to conventional leather processing technology (see Experimental procedures). After chroming, the chromed leather samples were washed, fixed by dehydration in acetone[31] and tested (Table I).

TABLE I
Influence of enzymatic soaking duration on chromed leather properties

Index	Soaking duration, hours				
	2	4	6	8	18*
Amount of Cr_2O_3 in leather, %	4.52	4.45	4.47	4.35	4.42
Shrinkage temperature, °C	108	112	112	113	114
Tensile strength, N/mm^2	24.5	22.2	21.2	20.8	20.2
Grain strength, N/mm^2	16.6	19.2	20.8	20.8	19.6
Relative elongation at a strain 10N/mm^2, %	46.0	57.0	66.0	65.8	66.4

* Soaking without addition of EP

Obviously, the duration of soaking with an EP supplement had some influence on chromed leather properties. The results obtained (Table I) allow conclusions about the appropriateness of the soaking duration: too short a soaking leads to lower shrinkage temperature and low grain strength despite a high content of Cr_2O_3 in leather and its high tensile strength. Probably, the reason is, as mentioned above, uneven rehydration during soaking and accordingly, uneven opening up of derma during liming-unhairing. Prolonging the soaking allows making a leather characterized by higher shrinkage temperature, by slightly lower tensile strength but having a resistant grain. Assessing the results it can be proposed that soaking with EP during six or 8 hours, or soaking without EP during 18 hours leads to processing of chromed leather with very similar properties. Therefore, 6 hours soaking when adding EP is appropriate to produce a quality chromed leather.

Industrial trials of shoe upper leather were performed in the joint-stock company 'Kedainiu Oda' (Lithuania). Salted cow hide (weight 22kg) was cut along the

backbone. The right side (control) was soaked during 8 hours according to the conditions presented in the *Experimental procedure* section. The left side (experimental) was soaked in the same way as the control side but with addition of EP Aquaderm 0.005%. Further, both sides were processed together according to the shoe-upper leather manufacturing technology used in the enterprise.

The quantitative properties of the produced leather were determined and presented in Table II.

TABLE II
Characteristics of shoe upper leather depending on method of soaking

Indexes	Soaking	
	with EP (experimental)	without EP (control)
Moisture content, %	13.2	13.4
Cr_2O_3 content, %	4.03	4.08
Shrinkage temperature, °C	116	116
Amount of matter soluble in dichloromethane, %	4.64	4.49
Tensile strength of leather, N/mm^2	16.8	17.0
Grain strength, N/mm^2	16.8	17.0
Relative elongation of leather at the strain 10N/mm^2, %	40.6	39.5

An assessment of the leather produced allows us to propose that the change of soaking process conditions (with addition of EP or without) is not reflected in the chemical and strength characteristics of the processed leather. An organoleptic estimation of the both leather sides has not revealed any differences as well.

CONCLUSIONS

The use of enzymes for soaking hides leads to faster rehydration of the hide and to faster removal of non-collagen proteins. The differences of rehydration level of hide depend on the EP used and are distinct when the duration of the soaking is short (4-8 hours) but practically not observable when duration is long (18 hours). Prolongation of soaking has a direct influence on the amount of removed interfibrous proteins: longer soaking leads to higher level of non-collagen proteins removal. The removal depends on the EP used and on its amount.

The increasing the amount of EP in soaking results in faster rehydration, higher content of removed non-collagen proteins but, unfortunately, has a stronger effect on collagen of hide during soaking and subsequent liming as well. What can be the reason for the defects in the finished leather.

The duration of enzymatic soaking has an influence on chroming and chromed leather properties: too short an enzymatic soak leads to weakened grain and to decreased relative elongation of leather.

A properly chosen EP preparation and its amount results in higher rehydration and removal of non-collagen materials from derma during soaking. Herewith, such use of EP has no observable influence on the exploitation properties of the finished leather

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