The Research on the Effects of Degreasing by Using Enzyme in Liming Process

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Abstract

This study has been conducted for the purposes of contributing to removal of the natural fat in the composition of leather by making use of enzymes in liming process and an increasing the effectiveness of degreasing; decreasing the amount of chemicals used in degreasing, thus lightening the load of water treatment and eventually drawing down the harm that the leather industry could pose on the environment. 56 wet-salted sheep skins of Kıvırcık breed were used in the research. Technical grade of chemicals were applied in processing of the skins. In this context, the enzymes used in the process were selected from among those commercial enzyme preparations that could be easily obtained and used by the leather industry. First, fat quantity of the material was determined. Then, optimum degreasing combination in which natural fat remained in the skin at a level that could determine the activities of the enzymes was investigated. For this purpose, the remaining amount of fat was determined by using combinations of solvents with decreasing ratios of 10-8-6-4-2% and non-ionic emulsifier with a percentage of 2% for each. According to the obtained results, 4% solvent and 2% non-ionic emulsifier combination which gives 5.92% of remained fat in the leather was determined as optimum degreasing combination. During the process of liming, enzymes such as alkali protease and alkali lipase were used alone and in combinations in changing amounts, then optimum degreasing was conducted. Each experiment was processed till the end of tanning and to what extend the used enzymes were effective for degreasing was investigated. Findings suggested that alkali proteases gave the best results and %0.5 alkali lipase and combinations were sufficiently.

Introduction

Basically, the skin or hide is composed of protein, fat, carbohydrate, mineral material and water. On the other hand, processed leather is composed of mainly collagen which is stabilized substantially by tanning materials and others such as fatty materials, retanning and coloring materials added afterwards. In this context some natural components, which are not wanted in the leather, are removed through various methods. Natural fats, that is, lipids that exist in the skin, are desired to be removed and those that might remain in small amounts are wanted to be homogenously distributed. Lipids are present in the skin as sebum in sebaceous gland and hair follicle, as lipid cells distributed among the skin fibers and as fat tissue accumulated among fibers of connective tissue in the reticular and subcutaneous layer. The amount of natural fat in the skin shows difference depending on various factors such as the breed, age, sex, breeding and so on. The amount of fatty material as dry weight base can reach to 2-4% in cattle, 12-15% in goat, and 30% in sheep skin. On the other hand, the amount of fat in the skins of domestic breeds range from 15 to 25%.[19] According to total amount of lipid, sheep skin contains a total 56% triglycerol of which 12% is in epidermis and 44% in dermis, 23% glycerol, 6% phospholipid, 5% cholesterol, and 10% fatty acid.[7] While the amount of lipid changes according to the skin layers, it shows changes according to different regions of the leather as well. According to dry weight, there exists 30-40% fat in the neck and tail regions, 20-30% in the back, 5-10% at the sides, and 1-5% at the flanks.[5]

Natural fats that can not be removed sufficiently during the process prevent the chemicals to be used during leather production from penetrating hydrophilically into the leather and as a result, some defaults with adverse effects on the quality of finished leather occur, such as hardness, fat spew, stained appearance, weak bounding of the finishing layer and also bad odour.

Leather industry, using solvents and emulsifiers or their mixture in degreasing process, tries to attain required product quality.[3,4,7,13,15] But liquid wastes containing both solvents and emulsifiers have negative effects on the health of human beings and environment.

Making use of the recent developments in biotechnology, having new enzyme preparations appropriate for use in leather industry create new opportunities for enzymatic applications in leather industry.[9,16] These products that can be effective on the proteins and lipids present in the composition of skin could hydrolyze the unwanted material in the skin when used under appropriate conditions. Proteases are firstly effective on globular proteins such as albumine, globuline, while lipases hydrolise lipids converting them into free fatty acids and glycerine.[2,17] Thus they help collagen to be isolated. [1,6,8,17]

The current research has been conducted for the purpose of finding out to what extent the natural fats could be degreased by using enzymes during liming process, to what extent the amount of solvents and emulsifiers that traditionally used during the essential process of degreasing could be reduced and thus lightening the load of treatment plant.

Material and Method

Material

The material used in the research was obtained from the cross-breed of "Kıvırcık" sheep, which is known as "Pirit" sheep regionally. The skins were obtained as wet-salted cured. Totally 56 skins were used for the experiments. Since the amount of fat is not the same in all parts of the skins, we preferred to use whole skins and 4 skins were used in each experiment. Commercial grade of chemicals were used in processing of the skins. In this context, the enzymes used in the process were selected from among those commercial enzyme preparations that are easily obtained and used by the leather industry. Dichloromethane was supplied as analytical reagent grade (A).

Method

Previously the percentage of the amount of fat in the skins was determined. For this purpose, total natural fat determination was made using 4 of the skins which were chosen randomly from among the research material. Then, in order to find out optimum degreasing combination, that would allow natural fat to remain in the skin to a degree that is enough for determining the activity of the enzymes to be used in liming process, skins were processed traditionally (Table I).

Table I. Skin processing

Process	Product	Amount (%)	Temperature (^oC)	Duration	Special Note and pH
Wetting	Water	400	20	2hrs	Static, drain
Washing	Water		20	10min	Running water, drain
Soaking	Water NaCl Non-ionic emulsifier (**B**)	400 4 0.5	20	 30min	 +14hrs (5min/h) Drum, drain
Pre-fleshing					
Painting	Na_2S $Ca(OH)_2$ Kaolin			 4hrs	17 $^oBe'$ 26 $^oBe'$ 28 $^oBe'$
Unhairing					
Liming	Water Na_2S $Ca(OH)_2$ Non-ionic emulsifier	200 2 6 0.3	20	1hr 30min	(15min run,15min off) +18hrs Drum (5min/h)
Fleshing-Trimming-Weighing					
Washing	Water	300	35	10min	
Deliming	Water $(NH_4)_2\ SO_4$ Deliming agent (**C**)	100 0.7 0.8	35	 10min 25min	 Control (phenolftalein colorless), drain
Bating	Water Enzyme (**D**)	100 1	37	 45min	 Control ,drain
Washing	Water	300	20	10min	
Degreasing	Solvent Non-ionic emulsifier	X 2		 60min	 Drain
Washing	Water NaCl Non-ionic emulsifier	300 3 0.3	35	 20min	 Drain, 3 x
Washing	Water	300	20	10min	
Pickle	Water NaCl HCOOH H_2SO_4	100 7 0.8 0.7	20	 10min 30min 2hrs	 6-7 $^oBe'$ pH=2.9-3.0
Tanning	Powder chromium (**E**)	10		8hrs	Control
Basification	HCOONa $NaHCO_3$	0.8 0.7		30min 60min	 pH=3.8-3.9
Pile				2days	

X=10,8,6,4,2

At the degreasing stage of process recipe, 5 degreasing trials were realized using solvent in decreasing percentages with 10%, 8%, 6%, 4%, 2% and 2% emulsifier for each (Table I). Keresone was used as solvent. Fat analyses were made on chromium tanned leathers. It was concluded that result of 4% solvent and 2% emulsifier combination was enough to determine the activity of the enzymes. So, it was accepted as the optimum combination of main degreasing.

In order to investigate the effects of enzymes on degreasing in liming, skins were treated with enzymes in 8 experiments; 2 with alkali protease, 2 with alkali lipase, and separately 4 experiments with the mixture of these two

enzymes (Table II). In determining the amounts of enzyme, the limit values recommended by the producer firm were taken into consideration. Following these experiments, deliming, bating, optimum degreasing, pickling and tanning processes were conducted.

Table II. Using enzymes in liming process

PROCESS	EXPERIMENT NO	MATERIAL
ADDITION OF ENZYME INTO THE LIMING BATH	1	0.1% Protease (F)
	2	0.2% Protease
	3	0.025% Lipase (G)
	4	0.5% Lipase
	5	0.1% Protease + 0.025% Lipase
	6	0.1% Protease + 0.5% Lipase
	7	0.2% Protease + 0.025% Lipase
	8	0.2% Protease + 0.5% Lipase

In alkali protease treatments 0.1% and 0.2% alkali protease (F) were added into the bath during liming process and experiments no. 1 and 2 were made.

In order to determine to what degree alkali lipase, when used alone, affects degreasing. 0.025% and 0.5% alkali lipase (**G**) were used and experiments no 3 and 4 were realized.

In order to observe what sort of a change would occur when alkali lipase and alkali protease are used together in liming process, experiments no 5, 6, 7, and 8 were conducted. During liming process: 0.1% alkali protease and 0.025% alkali lipase were used in experiment no. 5; 0.1% alkali protease and 0.5% alkali lipase in no. 6; 0.2% alkali protease and 0.025% alkali lipase in 7; and finally 0.2% alkali protease and 0.5% alkali lipase in experiment 8. Applications were made according to Table I and only enzymes and their ratios were changed in liming process as shown in Table II.

In the analysis of the skins and leathers, some standards were used such as Institution of Turkish Standards TS-4114 “Taking Samples for the Laboratory”, TS-4116 “Preparation of Samples for Chemical Analysis”, TS-4124 “Determination of Dichloromethane Soluble Matter” and TS-4127 “Determination of Volatile Matter” which are identical with IULTCS and SLTC Test Methods.[20] For the evaluation of the results obtained Statistical Package for the Social Sciences (SPSS) Pocket for Windows was applied.[10,14]

Findings

Findings of the Skin Fat and Optimum Degreasing Combination

As a result of the analysis, the quantity of natural fat in the skins of Kıvırcık sheep, which are bred in Uşak region, was found as 15.05% in average.

For the purpose of determining optimum degreasing combination, the results obtained according to the amount of fatty material remaining in the leather after analyses using decreasing amounts of solvents and the efficiency of degreasing are shown in Figure I. and Figure II respectively.

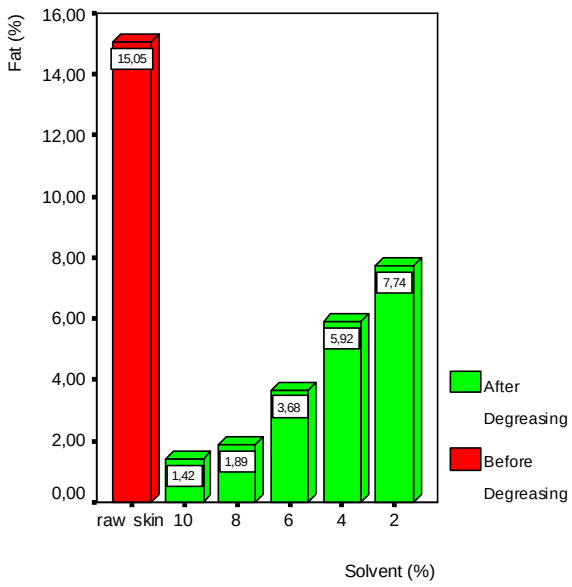

Figure I. Fat quantities of the leathers before and after degreasing

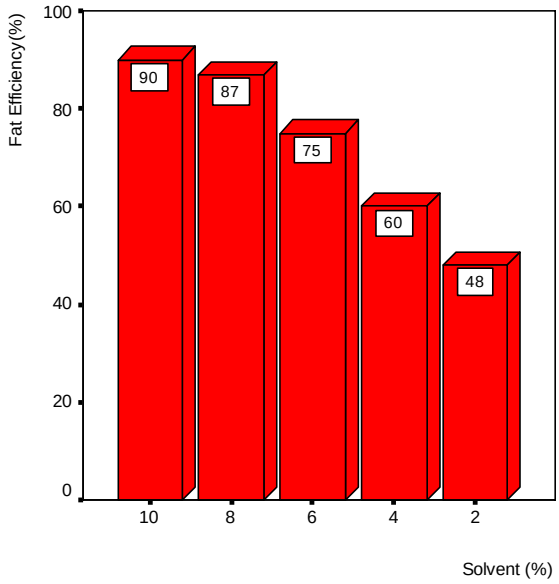

Figure II. Efficiency of degreasing

After experiments that were conducted by decreasing the amount of solvent, a increase was observed in the amount of fat that remains in the leather, thus a decrease in the effectiveness of degreasing. According to the results of the current research, the percentage ratio of fat remaining in the leather after use of 10, 8, 6, 4, and 2% solvent and 2% non-ionic emulsifier were found as 1.42, 1.89, 3.68, 5.92 and 7.74% respectively. Efficiency of degreasing was found as 90, 87, 75, 60 and 48 in same order. According to these findings, it was concluded that 4% solvent and 2% emulsifier combination that allows 5.92% of the fat to remain in the skin with 60% degreasing effectiveness, therefore, it was chosen as optimum degreasing combination.

Findings about the Use of Enzyme in Liming Process

Enzymes of alkali proteases used in liming process are firstly effective on globular or non structured proteins in skin, and they also break the cell membranes of lipid cells. Additionally, alkali medium helps skin fats to turn into soap. On the other hand, alkali lipases are effective on triglycerides, which are skin lipids. Amounts of

dichloromethane soluble matter that remains in the leather, after use of enzymes in liming process, are given in Figure III.

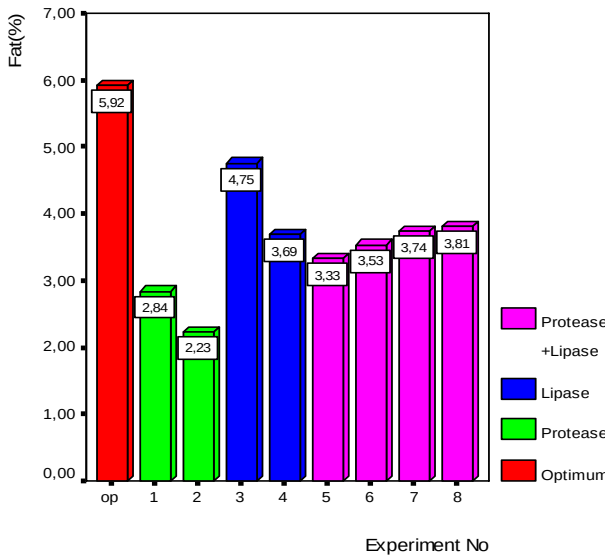

Figure III. Fat quantities of the leathers after enzyme application in liming process

At the end of the analyses realized, the amount of remaining fats in the leathers was determined as 2.84% in experiment 1 and 2.23 % in 2. In experiment 3, as a result of using 0.025 alkali lipase, the average amount of fat was found to be 4.75%. In experiment 4, on the other hand, the amount of alkali lipase was used as 0.5 % and the amount of the fat in the leather was determined as 3.69 %.

Experiments no. 5, 6, 7, and 8 were conducted in which alkali proteases and alkali lipases were used together in liming process. In experiment no.5, 0.1% alkali protease and 0.025% alkali lipase were used. The fat quantity was found in average as 3.33 % in this experiment. In experiment no. 6, however, 0.1% alkali protease and 0.5% alkali lipase were used, and the amount of remaining fat was found as 3.53%. 0.2% alkali protease and 0.025% alkali lipase were used in no. 7 and fat content was found as 3.74%. Finally 0.2 % alkali protease and 0.5 % alkali lipase were used in experiment no. 8 and fat quantity was found as 3.81% in average.

Figure IV exhibits the findings about the efficiency of using combined alkali protease and alkali lipase in liming.

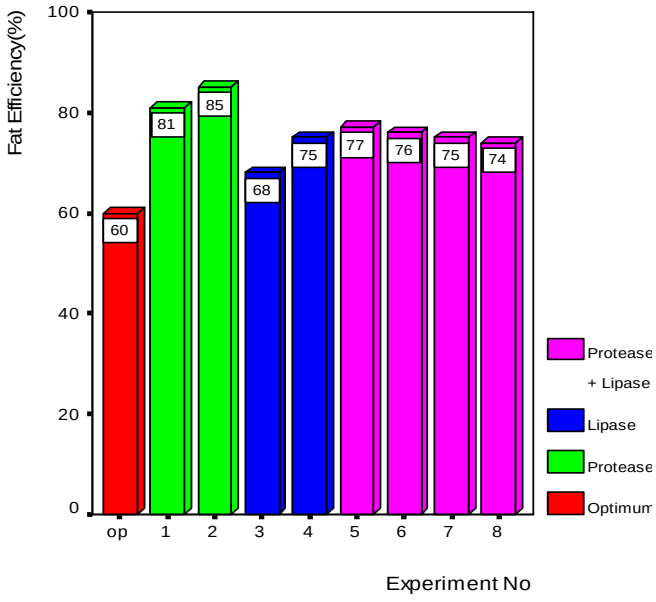

Figure IV. Efficiency of degreasing after enzymes applications in liming processes

The efficiency of degreasing was determined as 81% after using 0.1 % alkali protease in experiment no. 1. This value was found to be 85% when 0.2% alkali protease was used in experiment no. 2. In experiment no. 3, after using 0.025% lipase, the effectiveness of degreasing was determined as 68%, while it was 75% in experiment no. 4. In experiment no. 5, which was conducted with 0.1% alkali protease and 0.025% alkali lipase, the effectiveness of degreasing was determined as 77%, while in experiment no. 6, when 0.1% alkali protease and 0.5% alkali lipase was used, the effectiveness of degreasing was 76 %. In experiment no. 7 after using 0.2% alkali protease and 0.025% alkali lipase, the effectiveness of degreasing was determined as 75% and it was 74% when 0.2 % alkali protease and 0.5 % alkali lipase was used in last experiment.

Results and Discussion

Percentages of fat found in skins of domestic breed which was 15.05 is consistent with the values given by Harmancıoğlu [11] and Sarı and et al.[19]

The fact that the amount of fat remaining in the skin after using 8-10% solvent in our research is found to be low depending on the treatment especially when washing is done well in laboratory conditions. In the experiment realized with 6% solvent, the amount of natural fat in the skin was found as 3.68%. This value is within the limit of values that is considered normal for the purpose of the current study. Afşar [3] declares that the content of natural fat remaining in the skin after degreasing process at 2-4% is enough. The experiment realized with 4% solvent and 2% emulsifier which had 5.92% fat remain in the leather was found to be appropriate as optimum degreasing combination in research conducted with enzymes.

As a result of experiments using enzymes during liming process, while a good degreasing effect is obtained with the use of alkali protease alone, the combination of alkali protease and alkali lipase has proved a sufficient contribution. Just as Ivanova puts it[12], when alkali proteases and alkali lipases are used together, it is known that alkali lipases have an inhibiting effect on alkali proteases. The use of 0.5% alkali lipase provided an acceptable contribution to degreasing, its use in lower amounts was not appropriate.

When the results of statistical analyses of the research conducted using enzymes are taken into the consideration, according to sig. <0.05, a positively significant difference were found between the experiments realized with use of 0.1 % and 0.2% alkali protease with the others. The best result of application was obtained with 0.2% alkali protease.

When examined in terms of the effectiveness of degreasing, it was observed that using enzyme during liming process improves degreasing. Researches conducted by Addy et al [1,2] and Palop et al [17] note that using enzyme during degreasing process has positive effect.

In the current research, an effective degreasing was attained by using 60% less solvent compared to the traditional method in which 10% solvent and 2% non-ionic emulsifier has been. According to the traditional method, 100kg of solvent requirement for degreasing of 1000kg skin will go down to 40kg by using alkali protease during liming process. Compared with 60kg difference in the use of solvent, the amount of enzyme to be used will be 1-2kg. So, using enzyme during liming will bring economical benefits for the tanneries. On the other hand, this application will reduce the expenses of the water treatment systems.

By use of enzymes in leather production processes, while removing a desired amount of lipid from the skin and thus qualified leather production becomes possible, it will be also possible to reduce the negative effects of industry to the environment. Nowadays, as negotiations have begun with European Unity, the importance of environmental friendly production has risen. In this sense, it is obvious that national and international studies should be conducted with similar concerns. We wish that our study would contribute to these activities.

Appendix – chemicals applied

A: Dichloromethane (Carlo Elba)
B: Marlophen NP 9.5 (Degussa)
C: Decaltel AB 25 (BASF)
D: Basozym T 1000 (BASF)
E: Tankrom AB (Kromsan)
F: Erhavit DMC (TFL)
G: Lederzim SG-s (Lamberti)

References

1. **Addy, V.L., Covington, A.D., Langridge, D.A. and Watts, A.**, 2001a, Microscopy Methods to Study Fat Cells, Part 1: Characterisation of Ovine Cutaneous Lipids Using Microscopy, Journal of the Society of Leather Technologists and Chemist, Vol. 85, 6-15.
2. **Addy, V.L., Covington, A.D., Langridge, D.A. and Watts, A.**, 2001b, Microscopy Methods to Study Lipase Degreasing, Part 2: A Study of the Interaction of Ovine Cutaneous Adipocytes With Lipase Enzymes Using Microscopy, Journal of the Society of Leather Technologists and Chemist, Vol. 85, 52-65.
3. **Afşar, A.**, 1986, İşlem Kontrolleri, Giysilik Deri Üretimi ve Sorunları Semineri, Sınai Eğitim ve Geliştirme Merkezi, Ankara.
4. **Bienkiewicz, K.**, 1983 Physical Chemistry of Leather Making, Robert E. Krieger Publishing Company, New York, 441p.
5. **Breitsamer, M., Geissler, R. and Trenkwalder, M.**, 1997, Solvent Free Degreasing of Hides and Skins, World Leather, May, 65-70.
6. **Büyükuslu, N.**, 1998, Deri Sanayinde Enzim Kullanımı, Deri Teknologları Teknisyenleri ve Kimyacıları Derneği Yayın Organı, 1 (6), 19-21.
7. **Christner, J.**, 1992, The Use of Lipases in the Beamhouse Process, Journal of The American Leather Chemist Association, Vol. 87(4), 128-139.
8. **Christner, J.**, 1995, Modern Enzyme Applications in the Beamhouse, World Leather, October, 61-62.
9. **Galante, Y.M.**, 1995, Progress in Enzyme Technology and Applications Within the Tanning Industry, Lamberti SpA, İtaly, Wold Leather, October, 63-64.
10. **George, A.M., Nancy, L.L., Gene, W.G., and Karen, C.B.**, 2004, SPSS For Introductory Statistics, Use and Interpretation, Second Edition, Lawrence Erlbaum Associates, New Jersey.
11. **Harmancıoğlu, M.**, 1998, Deri Kimyası, E. Ü. Zıraat Fakültesi Yayınları, No 533, İzmir, 467s.
12. **İvanova, D.**, 2000, Bating with Protease and Lipase, Leather, November, 35-38.
13. **King, C.**, 1995, Commercial Application of an Aqueous Degreasing System, World Leather, October, 55-59.
14. **Nancy, L.L., Karen, C.B., Karen, C.B. and George, A.M.**, 2005, SPSS For Intermediate Statistics, Use and Interpretation, Second Edition, Lawrence Erlbaum Associates, New Jersey,2005.
15. **Manzo, G.**, 2003, Hot Water Treatment for Degreasing Sheepskins, Leather International, September, 14-18.
16. **Martignone, G., Monteverdi, R., Roaldi, M. and Galante, Y.M.**, 1997, Progress for Leather Makers, Leather, December, 35-40.
17. **Palop, R., Marsal, A. and Cot, J.**, 2000, Optimization of the Aqueous Degreasing Process With Enzymes and Its Influence on Reducing the Contaminant Load, Journal of the Society of Leather Technologists and Chemists, Vol. 84, 170-176.
18. **Roaldi, M., Peratello, S. and Galante, Y.M.**, 2001, Production of Soft Leather for Furniture Upholstery, Leather İnternational, November, 33-38.
19. **Sarı, Ö., Bitlisli, B.O. ve Başaran, B.**, 1998, Su Esaslı Yağ Giderme Yöntemlerinin Teknolojik ve Ekolojik Önemi, Deri Teknologları Teknisyenleri ve Kimyacıları Derneği Yayın Organı, 1 (6) 3-10.
20. **T.S. (4114, 4116, 4124, 4127)** Türk Standardları Enstitüsü, Mamul Deriler Ankara.