(REFEREED RESEARCH)

A RESEARCH ON INCREASING THE EFFECTIVENESS OF DEGREASING PROCESS BY USING ENZYMES

ENZİMLERİN KULLANIMIYLA YAĞ GİDERME ETKİNLİĞİNİN ARTIRILMASI ÜZERİNE BİR ARAŞTIRMA

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ABSTRACT

This research has been conducted for the purposes of enhancing the degreasing activity by making use of enzymes in order to remove natural fats in skins and hides, and to decrease the amount of degreasing chemicals used in the degreasing process, thus to minimize the harm of leather industry on the environment. In leather processing, in the processes of soaking, liming, bating and depickling, protease and lipase type enzymes were used that are appropriate for each step of process and then optimum degreasing treatment was realized. At the end of these processes, the amounts of the residual fats in the leathers were determined. After the completion of the studies, a decrease in the amount of fat remained in leathers processed with enzymes and an increase in the effectiveness in degreasing were observed. 0.2 % use of alkaline protease in liming stage was found the most effective. 0.1 % use of the same enzyme gives the similiar result which is more cost friendly. In addition, the remaining fat contents of the leathers after the applications of 0.5 % alkaline protease at soaking; 0.5 % alkaline lipase and combinations of alkaline protease / lipase at liming; 0.025 % and 0.5 % acid lipase in pickle leathers were found effective.

Key Words: Leather, Enzyme, Natural Fat, Degreasing, Environment.

ÖZET

Bu araştırmada, hamderilerdeki doğal yağın giderilmesinde enzimlerden yaralanılarak işlem etkinliğinin artırılması ve yağ gidermede kullanılan kimyasal niceliklerinin azaltılarak deri sanayinin çevreyi daha az kirletmesi amaçlanmıştır. Deri üretiminin; ıslatma, kireçlik, sama ve depikle işlem aşamalarında uygun enzimler kullanılmış ve deride kalan yağ nicelikleri saptanmıştır. Araştırma sonuçlarına gore; enzimlerin kullanıldığı tüm çalışmalarda, yağ niceliklerinin düşük ve yağ giderme etkinliğinin yüksek olduğu belirlenmiştir. Bulgular arasında; kireçlik işleminde % 0,2 oranında alkali proteaz kullanımının en etkin sonucu verdiği ortaya çıkmıştır. % 0,1 alkali proteaz da benzer sonucu vermekte ve uygulama için daha ekonomik kullanım sağlamaktadır. Yanısıra; ıslatmada % 0,5 alkali proteaz, kireçlikte % 0,5 alkali lipaz ve alkali proteaz/lipaz kombinasyonları ile pikle derilerde % 0,025 ve 0,5 asit lipaz kullanımlarının yağ gidermede etkili olduğu saptanmıştır.

Anahtar Kelimeler: Deri, enzim, Doğal Yağ, Yağ Giderme, Çevre.

Received: 20.06.2008 Accepted: 19.09.2008

1. INTRODUCTION

In processing leather, a degreasing process is needed for pelts with high amount of fat content. Natural fat (lipids) in skin and hide exist in sebaceous gland, hair follicle, randomly between collagen fibers as fat cells and between connective tissue fibers in the subcutaneous layer as deposited fat tissue. The natural fat which is not removed sufficiently prevents the hydrophilic activities of the chemicals, and, therefore, some undesired quality problems occur in the finished goods such as; hardness, fatty spew, stains and bad smells. The industry has been striving to obtain the desired quality by using solvents, emulsifiers (degreasing agents) or their combinations (1-5). However, both solvents and emulsifiers loaded wastes have hazardous effects on human health and the environment.

In recent years, the inventions of leather industry oriented enzyme compositions by making use of the developments in biochemistry have provided new opportunities of enzymatic uses in leather industry (6-11). When used under proper conditions, these products, which are effective on fat and especially globular proteins in the pelts, can be effective in hydrolyzing the undesired matters in the leather (12-15). While proteases are effective on globular proteins in the pelt such as; albumin, globulin, glycoprotein, lipoprotein; lipases hydrolyze triglycerides and convert them into free fatty acids and glycerol (16-19). Since enzymes decompose biologically they will probably be effective on mitigation of environmental pollution caused by the leather industry (20-23).

This study has been carried out in order to verify the use of enzymes in removing natural fat in pelts and throughout the processes of soaking,

liming and bating and also in depickling phase of pickled pelts to enable the minimum use of solvents and emulsifiers at degreasing phase, therefore minimize the harms on the environment and reduce the cost of waste treatment.

2. MATERIAL AND METHOD

The material used in the study is the wet-salted sheepskin of the Kıvırcık breed of western Turkey. In the study, 84 skins were used. The sheepskins were treated according to the garment leather processing methods. In doing so, together with common leather processing chemicals, enzymes such as; alkaline protease, alkaline lipase were used at the processes of soaking, liming and bating and acid lipase for pickled skins. It was taken into consideration that chemical materials and enzymes used in the research can cover the requirements of the industry. 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. Commercial grade of chemicals were used in processing of the skins. In the process; emulsifier, Marlophen NP 925 (Degussa); deliming agent, Decatel AB 25 (BASF); bating enzyme, Basozym T 1000 (BASF); chrome, Tankrom AB (Kromsan) and solvent, kerosene were used. In enzymatic treatments, alkaline protease (Pellvit KAB-P – TFL) for soaking; alkaline protease (Erhavit DMC—TFL) and alkaline lipase (Lederzim SG-s -Lamberti) for liming; alkaline lipase (Foreyzm DG –LFT) for bating and acid lipase (Ledrzim AL – Lamberti) for pickled skins were used. Dichloromethane was supplied as analytical reagent grade (Carlo Elba).

In the research 84 skins were used for 21 experimental groups. Initially, the percentage of natural fat in the skin was determined. Then, in order to bring forward the degreasing combination, which might involve sufficient amount of fat in the skin that would determine the efficiency of the enzymes to be used, experiments and analysis were conducted. The experimental process was carried out on five

Table 1. Process recipe of garment leather

Process	Product	Amount (%)	Temp. (°C)	Duration (min)	Special Note
Washing	Water		20	10	Drain
	Water	400	20		
Soaking	NaCl	4			
	Non-ionic emulsifier (Marlophen NP 9.5 - Degussa)	0.5		30	14 h (5min/h) run, drain
Pre-fleshing					
	Na_2S	17 °Be´			
Painting	$Ca(OH)_2$	26 °Be´			
	Kaolin	28 °Be´		240	
Unhairing					
	Water	200	20		
	Na_2S	2		60	
Liming	$Ca(OH)_2$	6			
	Non-ionic emulsifie	0.3		30	18 h (5min/h)run, drain
Fleshing-Trimming-Weighing					
Washing	Water	300	35	10	
	Water	100	35		
Deliming	$(NH4)_2\ SO_4$	0.7		10	
	Deliming agent (Decaltel AB 25-BASF)	0.8		25	Control (phenolftalein colorless), drain
Bating	Water	100	37		
	Enzyme (Basozym T 1000 - BASF)	1		45	Control ,drain
Washing	Water	300	20	10	
Degreasing	Kerosene	X*			X*= 10- 8-6-4-2
	Non-ionic emulsifier	2		60	
Washing	Water	300	35		
	NaCl	3			Drain. Three times washing
	Non-ionic emulsifier	0.3		20	
Washing	Water	300	20	10	
	Water	100	20		
Pickle	NaCl	7		10	
	HCOOH	0.8		30	
	H_2SO_4	0.7		120	pH=2.9-3.0
Tanning	33 % Basic chromium sulphate (Tankrom AB- Kromsan)	10		480	
Basification	HCOONa	0.8		30	
	$NaHCO_3$	0.7		60	pH=3.8-3.9 Piling for 2 days

Table 2. Depickling recipe for pickled skin

Process	Product	Amount (%)	Tempt. (°C)	Duration (min)	Special Note
Depickling	Water	100	20		
	NaCl	10		10	9 °Be′
	HCOONa	2		30	
	$NaHCO_3$	1		120	pH=5.0
	Acid lipase **(**Lederzim AL-Lamberti**)**	X*		60	Control. X*=0,0-0,025-0,05
Degreasing	Kerosene	4			
	Non-ionic emulsifier (Marlophen NP 9.5 - Degussa	2		60	Drain
Washing	Water	300	35		
	NaCl	3			3 times
	Non-ionic emulsifier	0.3		20	Drain
Washing	Water	300	20	10	Drain
	Water	100	20		
Pickling	NaCl	7		10	6-7 °Be′
	HCOOH	0.8		30	
	H_2SO_4	0.7		120	pH=2.9-3.0
Tanning	33 % Basic chromium sulphate (Tankrom AB-Kromsan)	10		360	Control
Basification	HCOONa	0.8		30	pH=3.8-3.9
	$NaHCO_3$	0.7		60	Piling for 2 days

groups of the material processed according to garment leather process method (Table 1). At the degreasing stage, gradually decreasing amounts of solvent and a constant amount of emulsifier were used for each group. The solvent (kerosene) was used at 10 %, 8 %, 6 %, 4 %, and 2 % respectively and non-ionic emulsifier was used at 2 %. Following the trial process fat analyses were carried. The content of natural fat remaining in the skin after degreasing process at 2-4% is enough in general assent. The results of the analyses were evaluated according to this acceptable residual amount of fat and the optimum combination of degreasing agents, which can cover the remaining amount of fat after enzyme activity was determined and used as the optimum degreasing method after all enzymatic applications.

When pickled leathers were treated, the amounts of common salt and sulfuric acid at the pickle stage in Table 1 were increased by 10 % and 1.5 % respectively, thus quality of pickled skin was obtained. They were then depickled after a 7 day piling period (Table 2).

In the main process, according to the recipe given in Table 1, proper enzyme combinations were used at the stages of soaking, liming, bating and depickling. For enzymatic applications, 15 trials were carried out. In determining the amounts of enzymes, limit values suggested by manufacturers were taken into consideration. Enzyme application stages, their compositions and amounts are given in Table 3.

Table 3. Quntities and qualities of used enzymes at processes

Processes	Enzymes
Soaking	%0.3 Protease
	%0.5 Protease
	%0.1 Protease
	%0.2 Protease
	%0.025 Lipase
	%0.5 Lipase
Liming	%0.1 Protease + %0.025 Lipase
	%0.1 Protease + %0.5 Lipase
	%0.2 Protease + %0.025 Lipase
	%0.2 Protease + %0.5 Lipase
Bating	%1.5 Lipase
	%2.5 Lipase
	%0 Lipase
Depickling	%0.025 Lipase
	%0.5 Lipase

In the analysis of the skins and leathers, related official methods of International Union of Leather Technologists and Chemists (IULTCS) were used such as IUC 2“Sampling”, IUC 3“Preparation of Test Material by Grinding”, IUC 4“Determination of Substances (fats and other solubles) Soluble in Dichloromethane”, IUC 5“Determination of Volatile Matter” (24).

3. RESULTS AND DISCUSSION

Average natural fat content of the subject material, domestic Kıvırcık breed skins, was calculated at 15.05 %. As a result of trials, with reduced amounts of solvents, carried out in order to determine the optimum degreasing level, an increase in the amount of residual fat in the leathers, and, therefore, a decrease in the effectiveness of degreasing activity was observed. After the trials conducted with reduced amounts of solvent in the order of 10 %, 8 %, 6 %, 4 % and 2 %, the remaining fat ratios of the leathers were found as; 1.42 %, 1.89 %, 3.68 %, 5.92 % and 7.74 % in the same order. Results are shown in Figure 1 and degreasing activity in Figure 2.

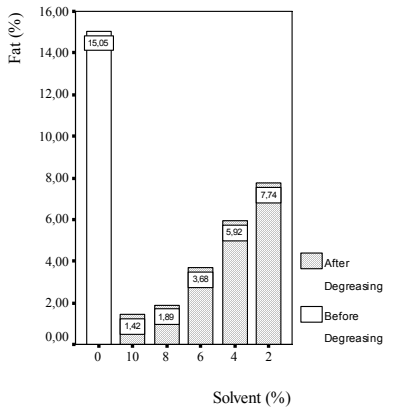

Figure 1. Fat contents of the skins and leathers before and after solvent and emulsifier applications

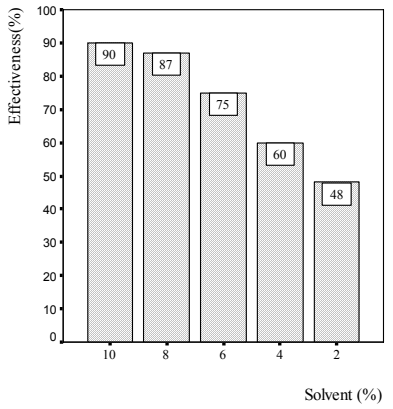

Figure 2. The effectivennes of solvent amount on degreasing

According to the results of these trials, the process of the trial, with 4 % solvent and 2 % non-ionic emulsifier, which resulted in 5.92 % residual fat ratio and 60 % degreasing efficiency, was determined as the optimum degreasing method, through which, enzyme efficiency could be measured. Fat ratio of skin and residual fat ratios after the applications of optimum degreasing and enzymes, trial numbers; enzyme ratios used in the processes and effectiveness of degreasing are given in Table 4.

In leather processing, proper enzymes were used at each stage of soaking, liming, and bating. After enzymatic applications, optimal degreasing method was used at degreasing phase. In order to be able to verify the effects of the enzymes on degreasing in pickled skins, first, skins were preserved by pickling and later at the stage of depickling enzymes were used. Degreasing activity in pickled skins were conducted according to

Table 4. Enzyme usage in processes and their results

Process	No	Material	Fat (%)	Effectivinness of Degreasing (%)
Skin	**0**	Dichloromethane	15.05	100
Optimum Degreasing	**1**	%4 Kerosene and %2 Non- ionik emulsifier	5.92	60
Soaking	**2**	%0.3 Protease	5.43	63
	3	%0.5 Protease	3.46	77
	4	%0.1 Protease	2.84	81
	5	%0.2 Protease	2.23	85
	6	%0.025 Lipase	4.75	68
	7	%0.5 Lipase	3.69	75
	8	%0.1 Protease +%0.025 Lipase	3.33	77
	9	%0.1 Protease +%0.5 Lipase	3.53	76
	10	%0.2 Protease +%0.025 Lipase	3.74	75
	11	%0.2 Protease+%0.5 Lipase	3.81	74
Liming Bating	**12**	%1.5 Lipase	4.89	67
	13	%2.5 Lipase	4.97	66
	14	%0 Lipase	4.64	69
Depickling	**15**	%0.025 Lipase	3.45	77
	16	%0.5 Lipase	3.48	76

optimal degreasing method. Remaining fat ratios in the leathers after enzymatic applications are given in Figure 3 and degreasing effectiveness in Figure 4.

As a result of our study, which aimed at reducing the amounts of chemicals used in degreasing, and therefore, mitigate their harms on the environment, together with producing good quality leather; a decrease in the amount of fat remained in leathers processed with enzymes thus an increase in the effectiveness in degreasing were observed. Between the optimal degreasing application, through which no enzymes were used, and all enzyme applications differences favoring enzyme applications were observed regarding degreasing effectiveness.

After degreasing, 3-4 % residual fat in the skin is acceptable. In our trials to determine the optimal degreasing method, residual fat ratios of the skins following the trials with uses of solvent at 10 %, 8 % and 6 % were found within acceptable limits. The trial with uses of 4 % of solvent and 2 % of non-ionic emulsifier resulted in 5.92 % remaining fat and 60 % degreasing effectiveness was determined as the optimal degreasing method through which the effectiveness was measurable. The comparatively lower residual fat results have been depended on the differences between lab and tannery conditions, especially careful washings. Slightly higher results in tannery conditions are probable.

In soaking, low amounts of alkaline protease use proved ineffective in degreasing but after the increment in the amount of the enzyme from 0.3 % to 0.5 %, the effectiveness of decreasing increased and the result was found acceptable. However, the increment in the amount of enzyme causes a cost problem.

In liming, 8 trials were conducted to investigate effectiveness of separate and combined uses of alkaline protease and alkaline lipase. As a result of trials, application of alkaline protease alone proved well effective on degreasing but the same result was obtained from their combined use. It has been reported that alkaline lipase has an inhibiting effect on alkaline protease (25). Our results also showed similarities to the reports. Our trials in liming showed that there has been no significant difference in degreasing effectiveness between the combinations of 0.1 % alkaline protease/ 0.025 % - 0.5 % alkaline lipase and 0.2 % alkaline protease/ 0.025 % - 0.5 % alkaline lipase. Therefore we can state that there is no necessity for increasing the amount of protease within combinations. The highest level of separate alkaline lipase use (0.5 %) was only able to cover the tolerated remaining fat contents; therefore, separate use of alkaline protease in liming is efficient and economic.

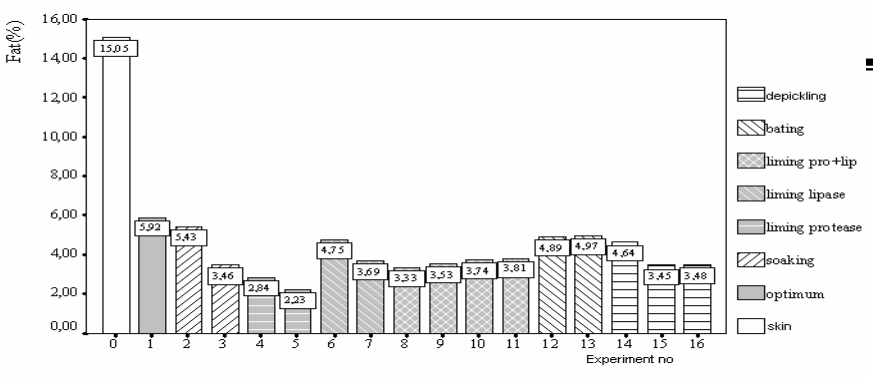

Figure 3 Fat ratios of the leathers after enzymatic applications

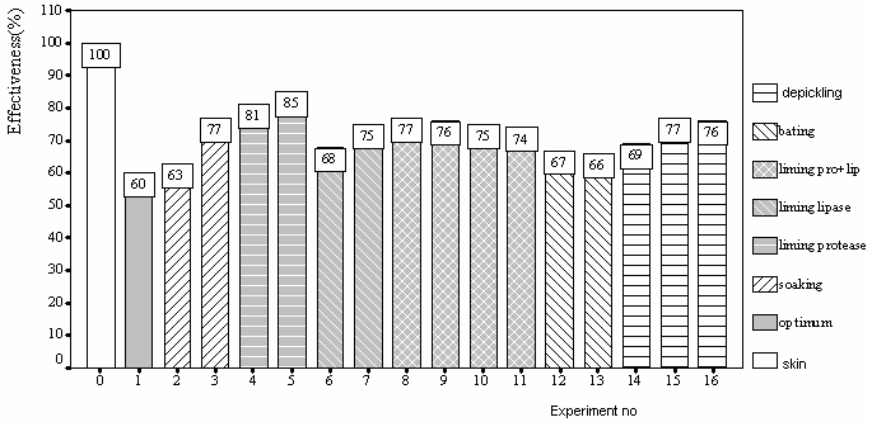

Figure 4 Effectiveness of enzyme use on degreasing activity

Alkaline lipase use at bating phase proved ineffective in terms of degreasing. The inefficiency has been based on inhibiting effects of proteolitic bating enzymes on the efficiency of lipase.

In order to see the effectiveness of lipase on degreasing, at depicle stage of pickled skins, one trial without lipase and two trials with two different dosages of acid lipases were carried out. As a result of the experiments it was observed that even small amounts of acid lipase improved the effectiveness of degreasing, and therefore, sufficiency. In leather industry, degreasing can be practiced either with 10 % solvent / 2 % emulsifier or 4-5 % emulsifier only. With both methods a desired degreasing is achieved. However, in our study, which aims at reducing the amounts of solvents and emulsifiers that have polluting effects on the environment, 0.1 % use of alkaline protease in liming stage was found the most effective. 0.2 % use of the same enzyme gives the same result but it is not cost friendly. In addition, the remaining fat contents of the leathers after the applications of 0.5 % alkaline protease at soaking; 0.5 % alkaline lipase and combinations of alkaline protease / lipase at liming; 0.025 % and 0.5 % acid lipase in pickle leathers, were found below 4 %. And this result shows that these applications are feasible in terms of degreasing. In our trials, degreasing applications were conducted according to the optimal method that requires 4 % solvent and 2 % non-ionic emulsifier. Enzyme applications resulted in effective degreasing requiring 60 % less solvent than the traditional method, in which 10 % solvent and 2 % non-ionic emulsifier is used. 100 kg solvent which is used to degrease 1000 kg leather in the traditional method can be reduced to 40 kg by using alkaline protease in liming. The amount of enzyme that can cover the 60 kg difference in solvent use will be 1-2 kg. Current price of kerosene is 1,81USD/l and the enzyme is 3,48 USD/kg, therefore, use of enzymes in liming will give an economical relief to the tannery. In addition, enzyme applications will reduce purification cost as well.

4. CONCLUSION

By making use of enzymes in eradicating the undesired amount of fat from the leathers in production processes, not only will good quality in leathers be obtained but also possible harms of leather industry to the environment will be mitigated. Importance of environment-friendly technologies is increasing day by day. In this respect, it is obvious that national and international studies will continue with similar concerns. We hope our research will be contributive to such studies.

ACKNOWLEDGEMENTS

The authors wish to thank Ege University- EBILTEM for funding this study as project Bil-022.

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Bu araştırma, Bilim Kurulumuz tarafından incelendikten sonra, oylama ile saptanan iki hakemin görüşüne sunulmuştur. Her iki hakem yaptıkları incelemeler sonucunda araştırmanın bilimselliği ve sunumu olarak ***"Hakem Onaylı Araştırma"*** *vasfıyla yayımlanabileceğine karar vermişlerdir.*