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Studies on the degreasing of skin by using enzyme in liming process

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This study has been conducted for the purpose of making the effective use of two enzymes *viz.* proteases and lipases for the removal of natural fat from the skin and increasing the effectiveness of degreasing; also decreasing the amount of chemicals used in degreasing and reducing the load of water treatment and eventually minimizing the harm that the leather industry poses to the environment. The optimum degreasing combination in which natural fat remains in the pelt at a level that could determine the efficacy of the enzymes was investigated. During the process of liming, enzymes such as alkali protease and alkali lipase were used alone and in combinations in varying amounts, and degreasing was achieved. Each experiment was processed till the end of tanning and effectiveness of the enzymes in degreasing was investigated. Results suggested that best degreasing conditions can be obtained with the use of 0.2% alkali lipase. The alkali lipases and the combinations of alkali proteases and lipases also proved to be satisfactory degreasing agents.

Keywords: Leather, Degreasing, Enzyme, Natural fat, Lipase, Protease

A 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 colouring materials added afterwards. Natural fats, that is, lipids that exist in the skin, are desired to be removed and those that might remain in small amounts need to be homogenously distributed. The amount of natural fat in the skin varies depending on various factors such as the breed, age, sex, breeding and so on. It is approximately 2-4% in cattle, 12-15% in goat, and 30% in sheep skin. Sheep skin lipids contain 56% triglycerol, 23% glycerol, 6% phospholipid, 5% cholesterol, and 10% fatty acids[1]. The amount of lipid varies in different skin layers and in different regions of the skin as well. 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[2]. Natural fats when not removed sufficiently during the process, prevent the chemicals (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, uses solvents and emulsifiers or their mixture in degreasing process, to attain required product quality[1,3,4]. The liquid wastes of leather industry containing significant amounts of both solvents and emulsifiers with other chemicals have negative effects on environment. Recent developments in new enzyme preparations suitable for use in leather production have created new opportunities for enzymatic applications in the leather industry[5,6]. 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 have been found effective on globular proteins such as albumin, globulin while lipases hydrolyze lipids and convert them into free fatty acids and glycerine[6,7]. Thus they help in the isolation of collagen[7,8]. Some studies related to enzyme usage in leather processing such as soaking, unhairing and bating have been reported. Most of them are based on unhairing of skins and hides using a bacterial alkaline protease preparation, which completely eliminates the use of lime and sulphide[9]. Also an eco-friendly dyeing process has been designed using proteolitic enzymes, to achieve increased uptake of dye[10]. To optimize removal of sebaceous grease, combination of a fatty acid ethoxylate with a small amount of proteolytic enzyme, has shown improved degreasing on bovine substrates[11].

The present investigations have been conducted for the purpose of finding out the extent of natural fats that could be degreased by using enzymes during liming process, the amount of solvents and emulsifiers that can be reduced during the process of degreasing thus lightening the load of treatment plant.

Experimental Procedure

Materials

The skins of cross-breed of Kıvırcık sheep used in the experiments were obtained locally. A total of 56 skins were obtained as wet-salted cured skins. Since the amount of fat was not the same in all parts of the skins, it was preferred to use whole skins and 4 skins were used in each experiment. Commercial grade of chemicals were used in processing of the skins. The commercial enzyme preparations that are commonly used by the leather industry (ErhavitDMC-TFL, Lederzym SG-s-LAMBERTI) and dichloromethane supplied as analytical reagent grade (Carlo Elba) was used.

Methods

The mean percentage of fat in the skins was determined using 4 skins chosen randomly. Then the skins were processed traditionally. At the degreasing stage of process recipe, 5 degreasing experiments were conducted using different amount of solvent (10, 8, 6, 4 or 2%) along with 2% emulsifier for each. Keresone was used as solvent.

Fat analyses were made on chromium tanned leathers. Afsar[3] has observed that a 2-4% content of natural fat remaining in the skin after degreasing process is adequate. The experiment conducted with 4% solvent and 2% emulsifier resulted in fat residue of 5.92% in the leather which was assumed to be appropriate degreasing combination for experiments to be conducted to probe the effectiveness of using enzymes in degreasing. So, it was accepted as the optimum combination of degreasing.

In order to investigate the effects of enzymes on degreasing in liming, pelts were treated with individual enzyme/mixture of enzymes. In all, eight experiments were conducted using alkali protease, alkali lipase, and their mixtures in different ratios. In determining the effective amounts of enzyme, the limit values recommended by the producers were taken into consideration. Following these experiments, deliming, bating, optimum degreasing, pickling and tanning processes were performed.

In alkali protease treatments 0.1% and 0.2% alkali protease (Erhavit DMC-TFL) were added into the bath during liming process and experiments were conducted (Exp. No. 1 and 2, respectively). Similarly, 0.025% and 0.5% alkali lipase (Lederzym SG-s-LAMBERTI) were used in two other degreasing experiments (Exp. No. 3 and 4, respectively).

In four other experiments the following combinations of alkali lipase and alkali protease were used together in liming process: (i) 0.1% alkali protease and 0.025% alkali lipase (Exp. no. 5), (ii) 0.1% alkali protease and 0.5% alkali lipase (Exp. No. 6), (iii) 0.2% alkali protease and 0.025% alkali lipase (Exp. No. 7), and (iv) 0.2% alkali protease and 0.5% alkali lipase (Exp. No. 8). Applications were made according to data included in Table 1 and only enzymes and their ratios were changed in liming process.

In the analysis of the skins and leathers, some official standards were used such as: SLC 1, Sampling and chemical testing of leather; SLC 2, Preparation of sample by grinding; SLC 113, Determination of moisture; and SLC 4, Determination of substances (Fats and other solubles) soluble in dichloromethane[12]. For the evaluation of the results Statistical Package for the Social Sciences (SPSS) Pocket for Windows was applied[13,14].

Results and Discussion

Determination of skin fat and optimum degreasing combination

On analysis, the mean quantity of natural fat in the skins of Kıvırcık sheep was found to be 15.05%. Percentage of fat found in skins of domestic breed is consistent with the values previously given by Harmancıoğlu[23] and Sarı *et al*.[16].

The experiments conducted for reducing the amount of solvent used for degreasing, proved that as the percentage of solvent is decreased the amount of fat that remains in the leather increases, indicating a decrease in the effectiveness of degreasing. The percentage of fat remaining in the leather after use of 10, 8, 6, 4 or 2% solvent along with 2% non-ionic emulsifier were found to be 1.42, 1.89, 3.68, 5.92 and 7.74% respectively. Thus efficiency of degreasing was found as 90, 87, 75, 60 and 48 in same order. According to these findings, it was concluded that a combination of 4% solvent and 2% emulsifier allows 5.92% of the fat to remain in the skin with 60% degreasing effectiveness. It was, therefore, chosen as optimum degreasing combination suitable for investigating the effectiveness of enzymes in liming.

Effectiveness of enzyme in liming process

Enzymes like alkali proteases when used in liming process affect the 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.

As per the results included in Table 2, the amount of fats remaining in the leather was found to be 2.84%

Table 1—The recipe for leather processing

Process	Product	Amount (%)	Temp. (°C)	Duration (min)	Special Note
Washing	Water		20	10	Drain
	Water	400	20		
Soaking	NaCl	4		30	14 h (5 min/h), drain
	Non-ionic emulsifier (Marlophen NP 9.5 - Degussa)	0.5			
Pre-fleshing					
	Na_2S				17 °Be′
Painting	$Ca(OH)_2$			240	26 °Be′
	Kaolin				28 °Be′
Unhairing					
	Water	200	20		
Liming	Na_2S	2		60	18 h (5 min/h)
	$Ca(OH)_2$	6		30	
	Non-ionic emulsifier	0.3			
Fleshing-Trimming-Weighing					
Washing	Water	300	35	10	
	Water	100	35		
Deliming	$(NH_4)_2SO_4$	0.7		10	Control
	Deliming agent (Decaltel AB 25-BASF)	0.8		25	(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	Drain
Washing	Water	300	35		
	NaCl	3		20	Drain. Three times washing
	Non-ionic emulsifier	0.3			
Washing	Water	300	20	10	
	Water	100	20		
Pickle	NaCl	7		10	*p*H=2.9-3.0
	HCOOH	0.8		30	
	H_2SO_4	0.7		120	
Tanning	33% Basic chromium sulphate (Tankrom AB- Kromsan)	10		480	
Basification	HCOONa	0.8		30	
	$NaHCO_3$	0.7		60	*p*H=3.8-3.9, horse up(2 days)

Table 2— Using enzymes in liming process

Experiment No	Enzyme product used in liming bath	Amount of fat remained (%)	Effectiveness of fat removal (%)
1	0.1% Protease[1]	2.84	81
2	0.2% Protease[1]	2.23	85
3	0.025% Lipase[2]	4.75	68
4	0.5% Lipase[2]	3.69	75
5	0.1% Protease + 0.025% Lipase	3.33	77
6	0.1% Protease + 0.5% Lipase	3.53	76
7	0.2% Protease + 0.025% Lipase	3.74	75
8	0.2% Protease + 0.5% Lipase	3.81	74

[1]ErhavitDMC-TFL; [2]Lederzym SG-s-LAMBERTI

when 0.1% protease was used in liming bath whereas only 2.23% fat was left when 0.2% protease was used. The use of 0.025% alkali lipase in the liming bath could reduce the fat to the extent of 4.75% while use of 0.5% alkali lipase brought down the amount of the fat in the leather to 3.69%.

The experiments conducted using a combination of different ratios of alkali proteases and alkali lipases together in liming process also gave similar results. When a combination of 0.1% alkali protease and 0.025% alkali lipase was used, the quantity of fat was found to be 3.33% while with a combination of 0.1% alkali protease and 0.5% alkali lipase the amount of residual fat was 3.53%. When a combination of 0.2% alkali protease and 0.025% alkali lipase was used the remaining fat content was found to be 3.74%. On the other hand use of 0.2 % alkali protease and 0.5 % alkali lipase left the 3.81% fat in average.

In terms of fat removal efficiency (degreasing) of the used enzyme products, the best efficiency of 85% was observed with 0.2% alkali protease followed by 81% efficiency exhibited by 0.1% alkali protease product. The efficiency of 0.025% lipase product was only 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 efficiency of degreasing was 77%, while use of 0.1% alkali protease and 0.5% alkali lipase had 76% efficiency of the combination for degreasing. With the use of a combination of 0.2% alkali protease and 0.025% alkali lipase, the effectiveness of degreasing was found to be 75% and it was 74% when 0.2% alkali protease and 0.5% alkali lipase was used. It is evident from the results that when a combination of the two enzyme is used the efficiency of degreasing decreases with the increase of amount of lipase in the combination. This is in accordance with the observation of Ivanova[17], that, when alkali proteases and alkali lipases are used together, the alkali lipases have an inhibiting effect on the effectiveness of alkali proteases.

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 conducted using 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 both the enzymes when used alone or in combination during liming process improve degreasing.

Conclusion

The results of the study prove that the enzymes protease and lipase may be used during liming process. The best degreasing effect (> 81%) is obtained with the use of alkali protease alone. The combination of alkali protease and alkali lipase also gives satisfactory results. The use of 0.5% alkali lipase provided an acceptable contribution to degreasing, its use in lower amounts was not appropriate. In addition, effective degreasing can be attained by using 60% less solvent compared to the conventional method. Using enzyme during liming will bring economical benefits for the tanneries. On the other hand, this application will also reduce the expenses of the water treatment systems.

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