Optimization of degreasing process in cowhide

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Abstract

The aim of this work was optimize the process of cowhide degreasing by selecting the right surfactant to be used alone or with the aid of lipase enzymes.

We started studying the fatty acids composition of the following prepared substrates: tallow (T); tallow treated with enzyme (TE); intradermal fat (IF); intradermal fat treated with enzymes (IFE), intradermal limed fat (ILF); and intradermal limed fat treated with enzymes (ILFE).

The total fatty acids composition from each substrate, after hydrolysis and subsequent derivatization to methyl esters was measured by gas chromatography-flame ionization detection (CG-FID, Shimadzu GC 14B). A standard mixture of fatty acids was used as reference material in the analysis (Supelco 37 FAME MIX). The actual triglyceride composition in each extract was also measured by GC using a specific column (DB-17HT, J & W Scientific).

After analysis, T and IF preparations showed a very similar qualitative composition in triglycerides (98%), and in fatty acids profile. As expected, for TE, IFE, ILF and ILFE, the proportion of free fatty acids found was higher, due to the liming and enzyme treatment.

In the second part, we studied the influence of the degree of ethoxylation (3, 5, 7 and 9 MOE) of a fatty alcohol in the stability of the resulting emulsions with each of the six substrates obtained, including a type of amphoteric surfactant.

Keywords: Degreasing, fatty acids, surfactants

1. Introduction

In cattle leather, fats are mainly distributed in two distinct parts (Sosa Valenzuela 2005; Grapone M. A. 1991)
a) In the so-called tallow; which can be found attached on the outside at the flesh side.

b) The constitutive fats of the skin, which are distributed in the sebaceous glands and in dermal tissue.

Natural fats that cannot 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.
The tallow that remain in the leather during the processes of soaking, unhairing and liming, makes difficult the penetration of water and alkaline products, therefore its emulsification becomes essential to allow a deep and uniform soaking and an effective flesh removing.

Constituent fats from the skin, those that are part of the sebaceous glands should be disposed of in the process of unhairing to facilitate the hair removal; and the components of the dermal tissue, prior to tanning to avoid the formation of chrome soaps.

In the liming process (Thorstermen T. C.1997), free fatty acids are converted into insoluble calcium soaps; after that, in the deliming and bating process the calcium of the soaps is solubilized and extracted in the form of calcium and ammonium salts. In this part of the process are given the right conditions; pH = 8 and temperature of 35-37 ºC, for the elimination of the fatty components.

It has been found (Manzano G. 1991; Adzet J. M. 1991; Ramón M. G. 2005) that the use of lime alone, results in the neutralization of the fatty acids of the epidermis and the corium, but triglycerides are not hydrolyzed in lime wash, using sodium sulphide in the bath liming together with lime, increases the saponifying effect on triglycerides, the saponification is more intense the higher the concentration of sodium sulfide; adding suitable surfactants helps eliminate fat in liming.

2- Objective of the study

Study and compare the composition of the fatty components of the tallow and the intradermal fat, to define which surfactant is more suitable for disposal; and to assess the influence of the use of lipase enzymes in the effectiveness of degreasing.

3- Materials and methods

Sample preparation:
First, as both raw materials (tallow and endodermal tissue) had a certain amount of water, for storage they were placed in a freezer until use.
During the application process, fats are in different conditions, so extractions were carried out by preparing the samples as described below:
Tallows: extraction will be performed as it comes, extracting the fat with the method detailed below. (tallow extract)
Intradermal fat: the extraction of two different forms will be used:

Intradermal fat as it is. (IF extract 1)
Intradermal fat after subjecting it to a similar liming process to a pH: 11-12 (ILF extract 2)

Fat Extraction: Bligh and Dyer Method (Bligh, E. G., et al 1959) an adaptation of Folch Method (Folch, J.et al.1957). The method is based on the homogenization of moist intradermal fat with methanol and chloroform in proportions, to form a single phase thereof miscible with water which, following the addition of chloroform and water, the two phases are separated, with lipid materials in the chloroform layer.
Samples obtained:

- Tallow (T)
- Intradermal fat (IF)
- Intradermal limed fat (ILF)

Treatment of fats with Lipases
A sample of 30 grams of fat, was heated to 40°C (up to liquid state), added 10 ml of ethanol (saw an emulsion), then slowly added 35 ml of "miliRo" water with stirring (always 40 ° C) and the pH adjusted between 10 and 12 (with NaOH).

Once done, the lipase enzyme (diluted 1:10) 1% was added (0.3 ml) and left to react for 2 hours at 40°C with stirring. When the time is finished, it was extracted with hexane in a separating funnel, and carried to the rotary evaporator to extract all the solvent.

Samples obtained:

- Tallows with enzyme (TE)
- Intradermal fat with enzyme (IFE)
- Intradermal Limed fat with enzyme (ILFE)

Determination of fatty acids:
The reference procedure was performed according to Association of Analytical Communities (AOAC) 969.33 (1990). Analysis: Equipment CG Shimadzu 14-B, FID detector Column: DB-Wax 30m x 0,32 mm ID x 25um film. Quantification against a standard solution of fatty acids 37 FAME MIX Supelco.

4-Results and discussion

4.1. Fatty acids melting points (MP °C)

FATTY ACID		MP (ºC)
MYRISTIC	14:00	54,4
PALMITIC	16:00	62,6
STEARIC	18:00	69
MYRISTOLEIC	14:01	-4
PALMITOLEIC	16:01	1
OLEIC	18:01	13
LINOLEIC	18:02	5

Table N°1

4.2. Fatty acids profile

The following tables, shows the total amount of fatty acids in each sample (in grams of fatty acid each 100 grams of sample), the fatty acids profile (in percentage), and the melting point

of each sample given by the contribution of each fatty acid melting point.
In Table N°2, it can be seen that treating tallow with enzymes there is a slight increase in the proportion of fatty acids of high melting point (Myristic, Palmitic and Stearic acids) and a slight decrease of oleic acid with low melting point, raising the extract melting point 1.6 °C.

		TALLOW			TALLOW + ENZIME		
FATTY ACIDS		TOTAL	PROFILE	MP (%)	TOTAL	PROFILE	MP (%)
		(g/100 g)	(%)	ºC	(g/100 g)	(%)	ºC
Myristic	14:00	3,17	5,01	2,72	3,2	5,18	2,81
Myristoleic	14:01	0,81	1,13	--	0,82	1,04	--
Pentadecylic	15:00	0,43	0,63	--	0,45	0,64	--
Pentadecanoic	15:01	0,11	0,18	--	0,12	0,18	--
Palmitic	16:00	21,3	26,69	16,42	21,39	27,87	17,4
Palmitoleic	16:01	2,65	3,34	--	2,75	3,08	--
Margaric	17:00	1,46	1,18	--	1,51	1,14	--
Heptadecanoic	17:01	0,78	0,79	--	0,8	0,66	--
Stearic	18:00	16,97	17,07	11,83	17,13	17,91	12,45
Oleic	18:01	31,05	42,79	5,46	32,05	41,17	5,34
Linoleic	18:02	0,74	1,18	--	0,75	1,12	
TOTAL		**79,52**	**100**	**36,4**	**80,97**	**100**	**38**

Table N°2

In tallow and tallow treated with enzymes, the highest proportion of fatty acids corresponds to: Myristic, Palmitic, Stearic and Oleic.
In Table N° 3, it can be seen that treating the intradermal fat with enzymes also increases fatty acid ratio of high melting point, raising the melting point 1.2 °C, following the same trend as in the tallows.

		INTRADERMAL FAT			INTRADERMAL FAT + ENZIME		
FATTY ACIDS		TOTAL	PROFILE	MP (%)	TOTAL	PROFILE	MP (%)
		(g/100 g)	(%)	ºC	(g/100 g)	(%)	ºC
Myristic	14:00	2,44	3,96	2,15	2,77	4,21	2,29
Myristoleic	14:01	0,78	1,23	--	0,88	1,22	--
Pentadecylic	15:00	0,31	0,49	--	0,36	0,51	--
Pentadecanoic	15:01	0,07	0,1	--	0,08	0,12	--
Palmitic	16:00	20,71	26,6	16,65	23,72	28	17,57
Palmitoleic	16:01	2,82	4,1	--	3,25	4	--
Margaric	17:00	1,32	1,1	--	1,55	1,2	--
Heptadecanoic	17:01	0,9	1	--	1	1	--
Stearic	18:00	14,04	13,8	9,6	15,73	14,5	10,09
Oleic	18:01	30,05	46,5	6,04	33,36	44,2	5,74
Linoleic	18:02	0,46	0,85	--	0,52	0,8	--
TOTAL		**73,9**	**100**	**34,4**	**82,8**	**100**	**35,6**

Table N°3

Table N°4 shows that in the treatment of intradermal fat with lime, Palmitic and Stearic acids (high melting point) decreases, and oleic acid increases (low melting); thereby decreasing the melting point of intradermal limed fat 1.8ºC. Enzyme treatment has no influence on the final melting point.

FATTY ACIDS		INTRADERMAL LIMED FAT			INTRADERMAL LIMED FAT + ENZIME		
		TOTAL	PROFILE	MP (%)	TOTAL	PROFILE	MP (%)
		(g/100 g)	(%)	ºC	(g/100 g)	(%)	ºC
Myristic	14:00	2,89	4,46	2,42	3,32	4,43	2,4
Myristoleic	14:01	1,07	1,52	--	1,22	1,47	--
Pentadecylic	15:00	0,4	0,58	--	0,44	0,55	--
Pentadecanoic	15:01	0,11	0,16		0,13	0,15	--
Palmitic	16:00	21,01	25,7	16,08	24,09	25,52	15,96
Palmitoleic	16:01	4,18	5,27	--	4,72	5,16	--
Margaric	17:00	1,3	1,03	--	1,53	1,05	--
Heptadecanoic	17:01	1,15	1,17	--	1,33	1,17	--
Stearic	18:00	12,05	11,2	7,86	14,32	11,6	8,07
Oleic	18:01	35,6	47,9	6,24	41,23	48,07	6,24
Linoleic	18:02	0,51	0,8	--	0,61	0,82	--
TOTAL		**80,31**	**100**	**32,6**	**92,95**	**100**	**32,7**

Table N° 4

The total amount of fatty acids in intradermal fat goes from 73.9% to 82.8% with the enzyme treatment with an increase of 12% (Table N° 3). When the enzyme treatment preceded liming the total amount of fatty acids increased to 92.95%, which means an overall increase of 25.7% of total fatty acids (Table N° 4).

These small variations in the composition of fatty acids and as a result of the melting points of different substrates are critical in the application processes; as soaking, unhairing and liming are carried out at temperatures between 20-22 °C and bating at 38 °C. The efficiency of surfactant degreasing will be much greater as lower the melting point (intradermal limed fat treated with enzymes.)

4.3. Composition of the extracts

A Thin Layer Chromatography was performed to see the qualitative composition of each of the extracts.

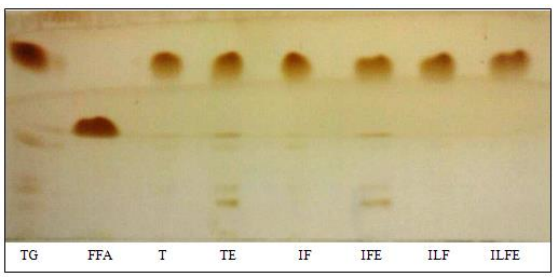

Figure N° 1

TG = Triglycerides (standard reference)
FFA = Free Fatty Acids (standard reference)
T = Tallow
TE = Tallow + Enzyme
IF = Intradermal Fat
IFE = Intradermal Fat + Enzyme
ILF = Intradermal Limed Fat
ILFE = Intradermal Limed Fat + Enzyme

The composition of the extracts coincides with the glycerides, being the proportion of free fatty acids almost null; small differences between the extracts at the bottom of the glycerides are observed, which may be due to small variation of the fatty acids observed in Tables 2, 3 and 4.

4.4. Surfactants

The bibliography (Palop R. 2000) shows numerous studies on bovine leather degreasing; because their fat ranges from 12% in spanish lambs, up to 30% on lambs from Australia and New Zealand; furthermore, the application of degreasing process is performed on skin pickled and in this state is mandatory the use of nonionic surfactants. In the case of ovine leather the bibliography is not abundant and the difference with the sheep leather is due to the amount of natural fat and its distribution in the leather; on the other hand, and from applicative stand of view, in ovine leather degreasing process is performed at soaking, liming and bating, at alkaline or neutral pH, where amphoteric surfactants are stable.

We have selected a fatty alcohol with four degrees of ethoxylation and an amphoteric surfactant (Table N° 5).

Sample Nº	*Type*	*HLB*
1	*Fatty alcohol 3 MOE*	*6,9*
2	*Fatty alcohol 5 MOE*	*10,4*
3	*Fatty alcohol 7 MOE*	*12,8*
4	*Fatty alcohol 9 MOE*	*13,3*
5	*Amphoteric surfactant*	

Table N°5

The methodology followed (Han Key R. A. et al. 2001) has been to assess the stability (values 1 to 5) over time (24 hours) of the emulsions resulting from adding 1g of substrate, 20 ml of a 5% surfactant solution at temperature of 22°C, without and with the addition of 200 □l of pure enzyme.

Table N° 6 shows the stability of emulsions of the tallow with water and the different selected surfactants in various periods of time, from one minute up to twenty four hours, without and with enzymes.

SAMPLE	1 min	5 min	30 min	60 min	120 min	4 hours	24 hours
TALLOW+ H_2O	1	1	1	1	1	1	1
TALLOW+1	3	3	2	2	2	2	2
TALLOW +2	5	4	4	3	3	3	2
TALLOW +3	4	4	3	3	3	2	2
TALLOW +4	4	4	3	3	3	2	2
TALLOW +5	5	5	5	4	4	4	4
TALLOW+ H_2O + ENZ	2	2	2	1	1	1	1
TALLOW+ 1+ ENZ	3	3	3	3	3	3	3
TALLOW + 2+ ENZ	5	5	4	4	4	3	3
TALLOW+ 3+ ENZ	4	4	3	3	2	2	2
TALLOW+ 4+ ENZ	3	3	3	3	2	2	2
TALLOW+ 5+ ENZ	5	5	5	5	5	5	5

Table Nº 6

In tallow without the enzymes, mmaximum stability is obtained with 5 MOE fatty alcohol and the amphoteric surfactant. (Samples 2 and 5).The enzyme treatment increases the stability of surfactants so that the fatty alcohol with 3 MOE also presents a maximum stability. Tallow and tallow with enzymes have similar behavior from surfactants: The Stability decreases slightly over time and considering the fatty alcohol, maximum stability is obtained with 5 moles of ethylene oxide (sample 2). With the amphoteric surfactant, maximum stability is achieved.

Table N° 7 shows the stability of emulsions of the intradermal fat with water and the different selected surfactants in various periods of time, from one minute up to twenty four hours, without an with the addition of enzyme.

SAMPLE	1 min	5 min	30 min	60 min	120 min	4 hours	24 hours
INTRADERMAL LIMED FAT+H20	1	1	1	1	1	1	1
INTRADERMAL LIMED FAT+1	2	2	2	2	2	2	2
INTRADERMAL LIMED FAT+2	5	5	4	4	4	2	2
INTRADERMAL LIMED FAT+3	3	3	3	2	2	2	2
INTRADERMAL LIMED FAT+4	3	3	2	2	2	2	2
INTRADERMAL LIMED FAT+5	5	5	5	4	4	4	4
INTRADERMAL LIMED FAT+H20 + ENZ	2	2	2	1	1	1	1
INTRADERMAL LIMED FAT+1 + ENZ	2	2	2	2	2	2	2
INTRADERMAL LIMED FAT+2 + ENZ	5	5	5	5	5	4	3
INTRADERMAL LIMED FAT+3 + ENZ	3	3	3	2	2	2	2
INTRADERMAL LIMED FAT+4 + ENZ	3	3	3	2	2	2	2
INTRADERMAL LIMED FAT+5 + ENZ	5	5	5	5	5	5	4

Table N° 8

The behavior of intradermal limed fat with surfactants and variables is similar to tallow; that is, greater stability is confirmed with fatty alcohol with 5 moles of ethylene oxide, and maximum stability is obtained with the amphoteric surfactant.

5. Conclusions

After extractions and analysis, it can be seen that the majority composition of bovine intradermal fat and tallow corresponds to glycerides.

Moreover treating tallow extract and intradermal fat extract with enzymes, lime and mixtures thereof varies the fatty acid composition and this results in a variation of the melting point, increasing with the increase of low molecular weight fatty acids content.

As for treatment with surfactants, it is stated that the fatty alcohol ethoxylated with 5 moles of ethylene oxide has a higher stability than those with 3, 7 and 9 moles of ethylene oxide. The maximum stability and therefore maximum efficiency is obtained with the amphoteric surfactant in the two substrates (tallow and intradermal fat) and in all phases of beamhouse.

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