Monitoring of composition of the fats in the leather along the tanning process

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ABSTRACT

A deep characterization of the fats in the leather is fundamental to the knowledge of the properties of the final product and of its merceological features. The amount and the type of fats in the leather affect, for example, physical proprieties as tensile and tear strength, elongation, water, air and air vapor permeability, absorption of water, wettability, thermal and electrical conductivity.
The knowledge of the composition of the fats in the leather is important for the solution of some problems connected to the formation of the characteristic fatty spue too; moreover this information can support tanners for a successful monitoring of all the steps of tanning.

The aim of the present work is the characterization of the fats in the leather in all the steps of the tanning process, from raw material to the finished leather. Many types of finished leathers for different final destinations were analyzed, as clothing, upper, suede, chamois, upholstery, automotive, sole etc. The results obtained from this characterization are shown.
The characterization was possible using of an innovative analytical technique, consisting in an appropriate gas chromatography method, and it was carried out in two steps: the former that allowed to determine the fat components in terms of fatty acids, esters, oils and glycerol esters; the latter that allowed a finer characterization of the fats, consisting in a further separation of the fatty acids in terms of saturated/unsaturated ratio. Then, upon hydrolysis of the extracted fats, it was possible to assess the saturated/unsaturated ratio for the whole fat mixture too.

The analytical investigations carried out was also useful to monitoring the chemical composition of fatty efflorescence (repousse), a typical defect that can be found mainly on goats and sheep skins, mainly characterized by the presence mainly of saturated fats.
Further investigations reveled an increase of the saturated fats during the first tanning phases, due to hydrogenations reactions (reduction of the double bonds C=C), possible cause of the certain fatty efflorescences.
Future developments will be discussed too.

INTRODUCTION

Since today, literature doesn't provide any information about the complete characterization of the fats in the leather in all the steps of the tanning process, from raw material to the finished leather.
On the other hand it is well known that the composition of the fats in the leather influences the success of tanning process, and it is true specially for the degreasing step[(1)].
Furthermore the knowledge of the composition of the fats in the pelt is useful to choose appropriate fatliquors too.

This paper describes an analytical technique, consisting in an innovative gas-chromatographic investigation, specific to the determination of the fatty substances in the leather, that will be fully discussed afterward.

The paper is structured in four parts:

1. in the first part the innovative analytical procedure adopted is described;
2. in the second one some results relative to pelts and leathers on the market are reported;
3. in the third part a specific application of this analytical approach, the study of the phenomenon of the fatty efflorescence, is shown;

4. the last part describes the results obtained by an accurate investigation of the saturated/unsaturated ratio in the pelts, useful to verify the possibility of occurrence of the hydrogenation reactions, as reported in literature. This treatment resulted useful in order to clarify of the origin of the fatty efflorescence, object of the study developed in the third part.

1. PROCEDURE

Extraction of the fats

The extraction of fatty matter from the leather has been executed according to the IUC4 method. It consists of a solid/liquid extraction with dichloromethane using a Soxhlet extraction apparatus.
Then the organic extract has been concentrated by a rotating extractor warming up in a softly thermostatic bath (60°C), in order to remove the solvent without to damage the fats by high temperatures.
However, we have to consider that the solvent can dissolve non-fatty substances too, but the further instrumental analysis we have done to monitor the composition of the fats allows the specific characterization of the only fatty matter.

Instrumental analysis

The gas-chromatographic analysis has been carried out in two steps:

STEP 1) Determination of all the fatty substances contained in the extract
STEP 2) Determination of the only free fatty acids, after removing of the glycerides with an high molecular weigh. In this step it is possible to distinguish carboxylic acids with the same length carbonylic chain but having different saturation ratio (number of double bond "C=C").

STEP 1 METHOD: determination of all the fatty substances in an extract

Instruments and operative conditions

Gas-chromatographic system : GC DANI model GC1000 equipped by:
n.2 inlets: 1) Split/Splitless; 2) PTV Split/Splitless
n.2 detectors: 1) FID; 2) ECD

After opportune preliminary tests on standard solutions of fatty substances and on extracts of fatty matter from several leather samples, we have chosen the most appropriate experimental conditions:

Inlet:

- PTV (Programmable Temperature Vaporizing) with splitting ratio equal to 10
- Gas carrier: He, constant flow (1 mL/min)
- Inlet temperature programming for PTV:

Temperature (°C)	Permanence Time (min)	Ratio (°C/min)
170	0,05	999,90
370	2,00	999,90
250	10,00	

Gas-chromatographic column:

- Capillary column RESTEK model RTX-TG65 (35% dimethyl- 65%diphenyl-poliossane);
- L = 30 m; ID = 0,25 mm; df = 0,1 μm

Oven temperature programming:

Temperature (°C)	Permanence Time (min)	Ratio (°C/min)
170	0	15,0
370	15	

Detector:

- Detector: FID
- Temperature: 370°C
- H_2 pressure: 0,63bar; Air pressure: 1,18bar; auxiliary gas pressure (He): 0,90bar

Qualitative and quantitative analysis

In order to execute the qualitative and quantitative tests on the fatty matter, it has been useful to prepare some standard solutions, by mixing standard fatty substances at different concentration.
In the table1 are given the standard substances tested.

Tab. 1: Standard fatty substances utilized for preliminary GC tests

INTERNAL STANDARD	FATTY ACIDS	ESTERS
methyl palmitate (IS)	palmitic acid(AC C16:0) palmitoleic acid (AC C16:1) stearic acid (AC C16:0) oleic acid (AC C18:1) olenic acid (AC C18:2) cis11-eicosanoic acid (AC C20:0) arachidic acid (AC C20:1) behenic acid (AC C22:0) erucic acid (AC C22:1)	miristyl dodecanoate (E MD_ate) palmityl palmitate (E PP_ate) stearyl stearate (E SS_ate) miristyl beheanate (E MB_ate) oleyl stearate (E OS_ate) oleyl oleate (E OO_ate)
MONOGLYCERIDS	**DIGLYCERIDS**	**TRIGLYCERIDS**
palmitoyl glycerol (M P) stearoyl glyc. (M S) oleyl glyc. (M O)	dilauryl glyc. (D LL) dipalmitoyl glyc. (D PP) palmitoyl stearoyl glyc. (D PS) distearoyl glyc. (D SS) dioleyl glyc. (D OO)	palmitoyl distearoyl glyc. (T PSS) lauryl oleyl palmitoyl glyc. (T LOP) oleyl distearoyl glyc. (T OSS) tripalmitoyl glyc. (T PPP) tristearoyl glyc. (T SSS) trioleyl glyc. (T OOO)

In order to execute a quantitative analysis of the fats, it has been adopted the internal standard method; as internal standard we chosen the methylic ester of the palmitic acid, which is not contained in the leather and whose retention time is lower than the one of the first fatty detectible acids.

In the figures 1 and 2 the gas-chromatograms relative to two standard mix solutions we examined are shown.

Both the chromatograms have been determined under the same operative conditions described for the STEP 1. The choice of divide the detection of the available standards into two different chromatographic runs, depends on the better resolution we could obtain by this way; in the former, “mix 1”, we detected the fatty acids and some triglycerides with an high molecular weight; in the latter, “mix 2”, we detected the glycerides and the esters with an intermediate molecular weight.

Fig 1.: Gas chromatogram of the standard mix solution “mix 1”

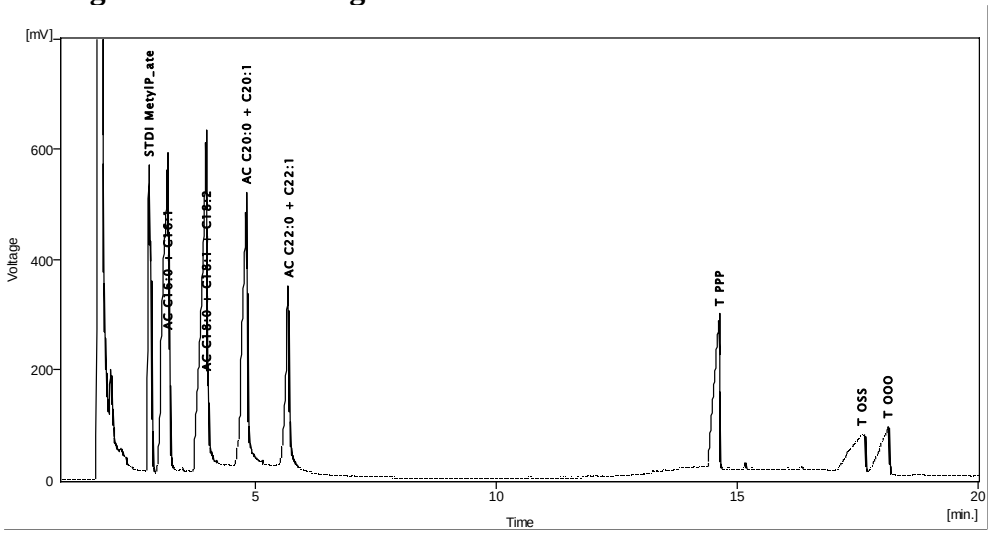
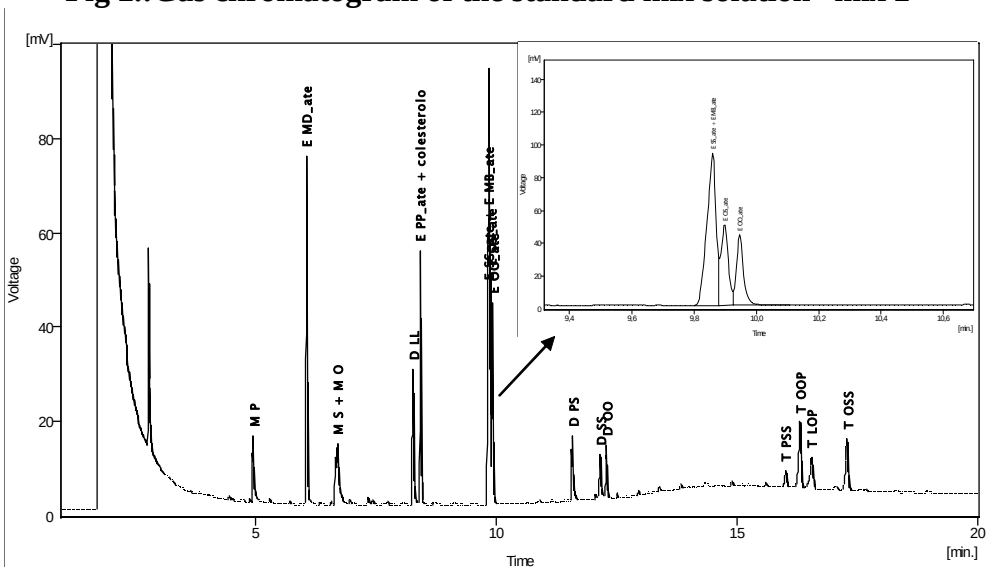


Fig 2.:Gas chromatogram of the standard mix solution “mix 2”



Looking at the chromatograms it is possible to distinguish three different regions:

1. from the beginning to 7 minutes, where you can find the fatty acids peaks.
2. a central region characterized by the presence of peaks of esters of long chain fatty acids with long chain alcohols and esters of glycerol.
3. a final region, from 13 minutes to the end relatives to the triglycerides.

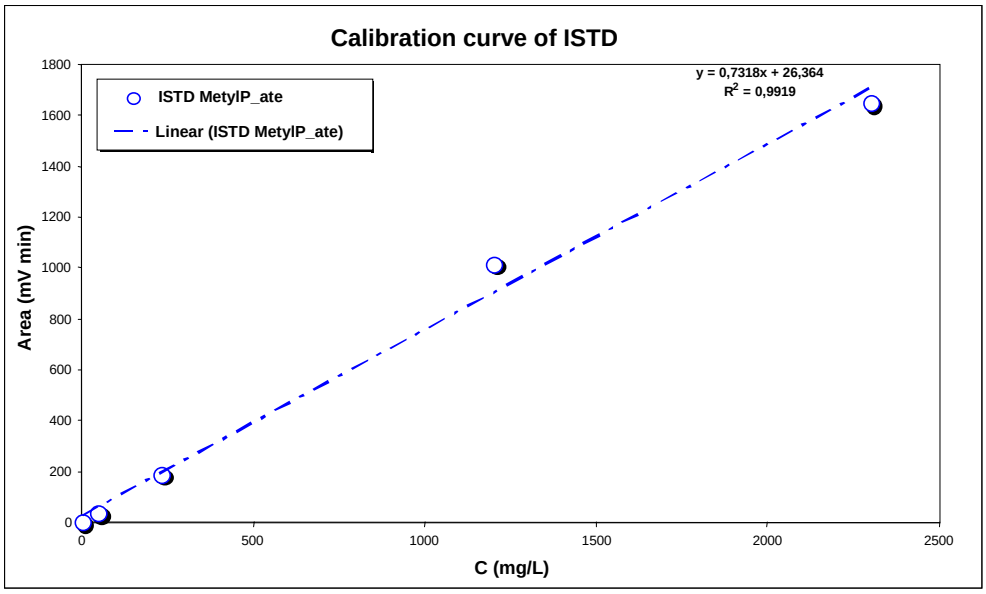
How it is possible to note, it hasn’t been achievable to separate fatty acids characterized by the same chain length (C16, C18, C20 e C22) but with different unsaturation degree (C16:0 from C16:1 and so on). Thus we have chosen to consider the relative signals together, as the sum of saturated and unsaturated acids with the same chain length.
Furthermore, this choice has been done for all the substances whose retention time resulted similar (in table 2 the compounds with this feature are separated by symbol “+”).

Medium relative response factors (RRF_M), characteristic for each standard substance, are given in table 2. These factors have been computed relatively to the internal standard at three concentration levels.

Tab. 2: Medium relative response factor (RRF_M) of the standard substances

	COMPOUND	RRF_M
Fatty acids	AC C16:0 + C16:1	1,26
	AC C18:0 + C18:1 + C18:2	1,39
	AC C20:0 + C20:1	1,43
	AC C22:0 + C22:1	2,09
Esters	E MD_ate	1,41
	E OO_ate	1,27
	E OS_ate	1,23
	E PP_ate	1,27
	E SS_ate + E MB_ate	1,92
Mono glycerides	M P	1,25
	M S + M O	1,8
Diglycerides	D LL	1,4
	D OO	1,38
	D PP	1,46
	D PS	1,53
	D SS	1,92
Triglycerides	T LOP	0,82
	T OOO	1,45
	T OOP	1,41
	T OSS	1,58
	T PPP	1
	T PSS	1,55
	T SSS	1,21

Fig. 3: Calibration curve of the internal standard ISTD (methyl palmitate)



The medium relative response factor is defined as:

$$RRF_i = \frac{C_{STDI} \times A_{Ci}}{C_{Ci} \times A_{STDI}}; \qquad RRF_M = \frac{\sum_i^n RRF_i}{n}$$

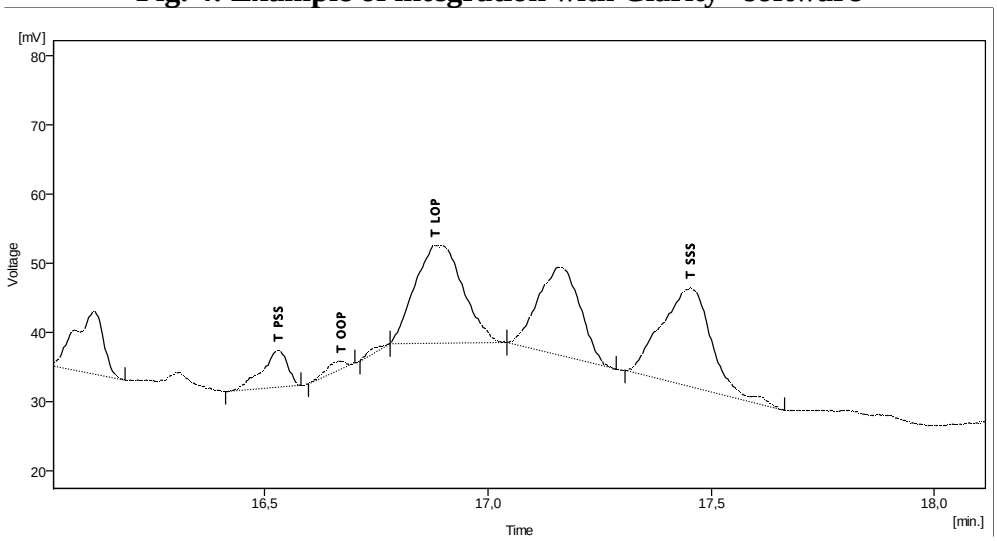
where:

RRF_i = relative response factor for a specific concentration of compound *I;*
RRF_M = medium relative response factor, medium of the RRF_i determined

Calibration curves for each standard substance have been calculated too and show a good linear trend. In figure 3 the calibration curve for the internal standard is shown.

Hereinafter, a slide showing an example of integration of the signals, achieved by the software *Clarity*, is reported (Fig. 4).

Fig. 4: Example of integration with Clarity® software



STEP 2 METHOD: determination of the free fatty acids

Instruments and operative conditions

To begin this step it has been necessary to use an opportune pre-column in which it has been packed glass wool, to remove the glycerides with an high vaporizing temperature.
Minding the low performing temperatures utilized this time, and the adsorbent action of the glass wool, it is necessary serially to regenerate the column.
Also this time the best operative conditions has been given after an optimization work.
The conditions we chosen are shown below:

- PTV with splitting ratio = 10
- Gas carrier: He, constant flow = 5 mL/min
- Inlet temperature programming at PTV:

Temperature (°C)	Holding time (min)	Ratio (°C/min)
100	0	999,90
250	15,00	

Gas-chromatographic column:

- Capillary column RESTEK model Stabilwax – DA (Cross-bond acid-deactivated Carbowax polyethylene glycol); L = 30 m; ID = 0,32 mm; df = 0,25 μm

Oven temperature programming:

Temperature (°C)	Holding time (min)	Velocità (°C/min)
100	0	10,0
250	30	

Detector:

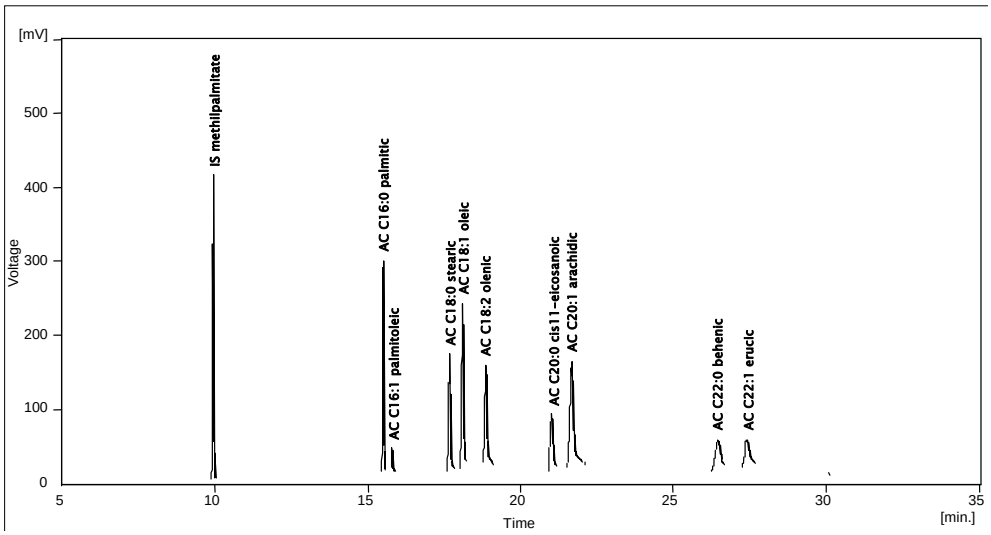
- Detector: FID
- Temperature: 250°C
- H_2 pressure: 0,63bar; Air pressure: 1,18bar; Auxiliary gas pressure (He): 0,90 bar

The chromatogram of the standard solution of fatty acids obtained with Stabilwax-DA column is shown in figure 5.
For the quantitative analysis the internal standard method has been chosen again.
Medium relative response factors (RRF_M), characteristic for each standard fatty acid, are given in table 3.

2. CHARACTERIZATION OF FATTY MATTER IN PELTS AND LEATHER USUALLY ON THE MARKET

The leather and pelt samples on which we have executed the tests are listed in table 3 and 4.
The samples have been codified for typology, on the base of the different origin of the animal, of the step of the tanning process considered and, when known, the corresponding destination.

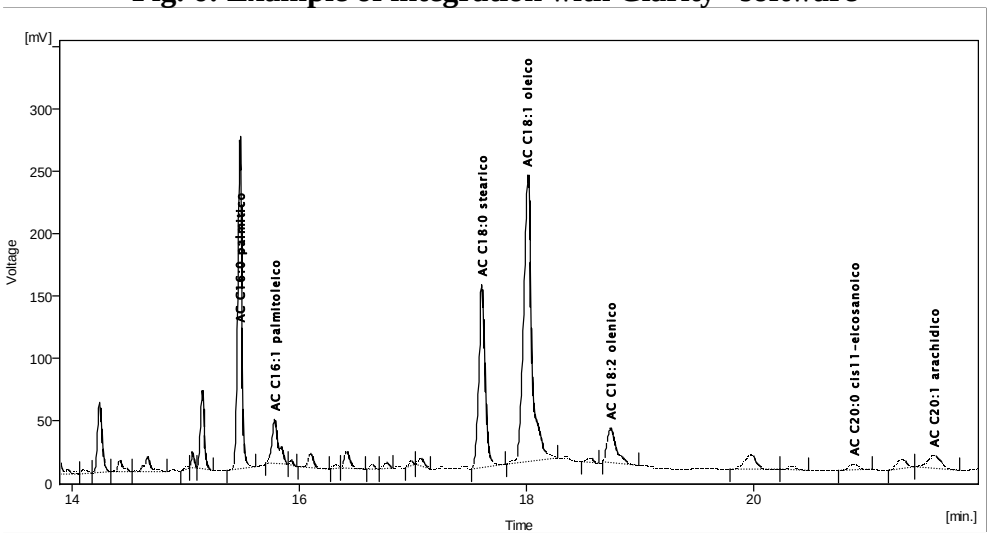
Fig. 5: Gas-chromatogram of the standard solution of fatty acids (Stabilwax-DA column)



Tab. 3.: Medium relative response factor (RRF_M) of the standard substances

FATTY ACIDS	RRF_M
AC C16:0 – palmitic acid	1,0
AC C16:1 – palmitoleic acid	1,1
AC C18:0 – stearic acid	0,8
AC C18:1 – oleic acid	1,0
AC C18:2 – olenic acid	1,0
AC C20:0 – eicosanoic acid	1,4
AC C20:1 – arachidic acid	1,4
AC C22:0 – behenic acid	1,6
AC C22:1 – erucic acid	1,6

Fig. 6: Example of integration with Clarity® software



Tab. 4: Samples delivered from some Italian tanneries

N.	ORIGIN	STEP	DESTINATION	COD
1	ovine	pickel	clothing	**1-ovi.pic.abb**
2	ovine	wet blue	clothing	**2-ovi.wb.abb**
3	ovine	dyed crust	clothing	**3-ovi.cruT.abb**
4	ovine	finished	clothing	**4-ovi.rif.abb**
5	bovine-grain	wet blue	/	**5-bov-F.wb./**
6	bovine-grain	dyed crust	/	**6-bov-F.cruT./**
7	bovine-grain	finished	automotive	**7-bov-F.rif.carroz**
8	bovine-grain	finished	upholstery	**8-bov-F.rif.arreda**
9	bovine-split	wet blue	/	**9-bov-C.wb./**
10	bovine-split	dyed crust	/	**10-bov-C.cruT./**
11	bovine-split	completed	sued	**11-bov-C.fin.scamos**
12	bovine-split	finished	polyurethane	**12-bov-C.rif.polure**
13	bovine	wet white	wet white	**13-WW.ww.wetwht**
14	bovine	finished	wet white	**14-WW.rif.wetwht**
15	ovine	completed	chamois	**15-ovi.fin.chamoi**
16	bovine	finished	vegetable sole	**16-bov.rif.vsuola**

Tab. 5: Samples processed at SSIP

N.	ORIGIN	STEP	GOODS	COD
17	ovine	raw	/	**17-ovi.gre./**
18	ovine	Liming	/	**18-ovi.cal./**
19	ovine	Deliming	/	**19-ovi.dec./**
20	ovine	Pickle	/	**20-ovi.pic./**
21	ovine	wet blue	/	**21-ovi.wb./**
22	ovine	dyeless crust	/	**22-ovi.cruN./**
23	ovine	dyed crust	/	**23-ovi.cruT./**

First, the results relative to samples given in the table 4 will be discussed. Afterward the results obtained from the samples processed by SSIP will be discussed more in detail, in order to better clarify the origin of the transformation of the composition of the fats, that could happen during the tanning process. The results obtained from the samples listed in the table 4 are shown in the following in table 6/a and 6/b.

Tab. 6/a: Results of gas-chromatographic analysis (samples 1 to 8)

	1-ovi.pic. abb	**2-ovi.wb. abb**	**3-ovi.cruT. abb**	**4-ovi.fin. abb**	**5-bov-F.wb./**	**6-bov-F.cruT./**	**7-bovF .rif.carroz**	**8-bov-F.rif.arreda**
n.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
% fats - method IUC 4	**10,9%**	**1,4%**	**9,4%**	**14,2%**	**1,5%**	**14,1%**	**14,8%**	**10,8%**
	Mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**
AC C16:0 + C16:1	1,3	1,4	0,4	0,8	1,4	6,1	1,2	0,6
AC C18:0 + C18:1 + C18:2	1,9	0,7	3,0	9,8	3,5	13,5	2,7	1,6
AC C20:0 + C20:1	-	-	0,1	0,1	-	0,1	0,3	0,2
M P	<0,1	0,2	<0,1	0,2	-	<0,1	-	-
AC C22:0 + C22:1	-	0,3	0,4	0,9	-	0,7	0,7	0,1
E MD_ate	-	-	-	0,2	-	0,1	-	0,1
M S + M O	-	-	-	0,1	-	0,4	0,2	<0,1
D LL	-	-	-	0,3	-	-	0,3	-
E PP_ate + colesterolo	0,2	0,1	-	0,1	0,5	0,1	0,5	0,3
E SS_ate + E MB_ate	-	-	0,1	0,2	-	0,7	0,5	0,4
E OS_ate	-	-	0,7	1,4	-	-	-	-
E OO_ate	-	-	-	-	-	<0,1	-	<0,1
D PP	0,1	-	0,0	0,4	-	0,1	-	<0,1
D PS	-	-	0,2	0,5	0,4	0,8	1,0	0,5
D SS	-	-	0,2	0,5	-	1,4	-	0,3
D OO	<0,1	-	<0,1	0,1	0,3	2,1	0,6	0,3
T PPP	0,3	0,1	<0,1	0,1	0,1	<0,1	0,1	0,1
T PSS	-	0,4	0,1	-	-	-	-	-
T OOP	-	0,3	0,1	-	0,5	1,8	1,3	1,2
T LOP	-	-	-	-	-	0,1	0,3	0,3
T SSS	-	0,1	<0,1	-	-	-	-	-
T OSS	-	0,1	-	-	-	-	-	-
T OOO	-	-	-	-	-	4,2	2,5	2,0
TOTAL								
Fatty acids (AC)	3,2	2,4	3,9	11,6	4,9	20,4	4,9	2,5
Esters (E)	0,2	0,1	0,8	1,9	0,5	0,9	1	0,8
Monoglycerides (M)	<0,1	0,2	<0,1	0,3	<0,1	0,4	0,2	<0,1
Diglycerides (D)	0,1	<0,1	0,4	1,8	0,7	4,4	1,9	1,1
Triglycerides (T)	0,3	1	0,2	0,1	0,6	6,1	4,2	3,6

Tab. 6/b: Results of gas-chromatographic analysis (samples 9 to 16)

	9-bov-C.wb./	10-bov-C.cruT./	11-bov-C.fin.scamos	12-bov-C.rif.polure	13-WW.crustT.wetwht	14-WW.rif.wetwht	15-ovi.fin.chamoi	16-bov.rif.vsuola
n.	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
% fats - method IUC 4	**0,3%**	**18,6%**	**7,3%**	**6,1%**	**16,6%**	**15,8%**	**8,6%**	**7,3%**
	mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**
AC C16:0 + C16:1	-	2,3	-	0,2	3,2	0,9	10,2	0,9
AC C18:0 + C18:1 + C18:2	-	-	-	-	7,5	5,5	9,2	2,0
AC C20:0 + C20:1	-	<0,1	-	-	0,1	0,1	2,2	0,4
M P	-	-	-	-	0,5	0,9	-	-
AC C22:0 + C22:1	-	-	-	0,1	0,5	0,5	4,4	-
E MD_ate	-	-	-	0,2	0,1	<0,1	-	-
M S + M O	-	<0,1	-	-	-	-	-	-
D LL	-	0,3	0,2	-	0,1	0,1	-	-
E PP_ate + colesterolo	-	0,1	0,1	-	0,6	0,6	-	<0,1
E SS_ate + E MB_ate	-	0,4	-	-	-	-	-	-
E OS_ate	-	-	-	-	-	-	-	-
E OO_ate	-	<0,1	-	-	-	-	-	<0,1
D PP	-	<0,1	-	-	0,1	-	-	0,2
D PS	-	-	-	-	0,1	0,2	0,1	-
D SS	-	0,1	-	-	0,2	0,2	0,6	-
D OO	-	0,1	-	-	0,1	0,1	-	0,1
T PPP	-	0,5	0,3	-	<0,1	0,1	0,3	-
T PSS	-	3,5	3,1	-	-	0,1	0,3	-
T OOP	-	1,6	1,4	0,2	-	-	-	-
T LOP	-	0,8	0,3	-	-	-	-	<0,1
T SSS	-	0,3	0,2	-	-	-	-	-
T OSS	-	-	-	-	-	-	0,1	<0,1
T OOO	-	0,1	0,3	-	-	-	-	<0,1
TOTAL								
Fatty acids (AC)	<0,1	2,3	<0,1	0,3	11,3	7	26	3,3
Esters (E)	<0,1	0,5	0,1	0,2	0,7	0,6	<0,1	<0,1
Monoglycerides (M)	<0,1	<0,1	<0,1	<0,1	0,5	0,9	<0,1	<0,1
Diglycerides (D)	<0,1	0,5	0,2	<0,1	0,6	0,6	0,7	0,3
Triglycerides (T)	<0,1	6,8	5,6	0,2	<0,1	0,2	0,7	<0,1

From the observation of the results it is possible to notice that the amount of the fats determined by gas-chromatographic analysis is meaningfully less than the total amount of the fats deriving from the extraction by dichloromethane (according to IUC4).

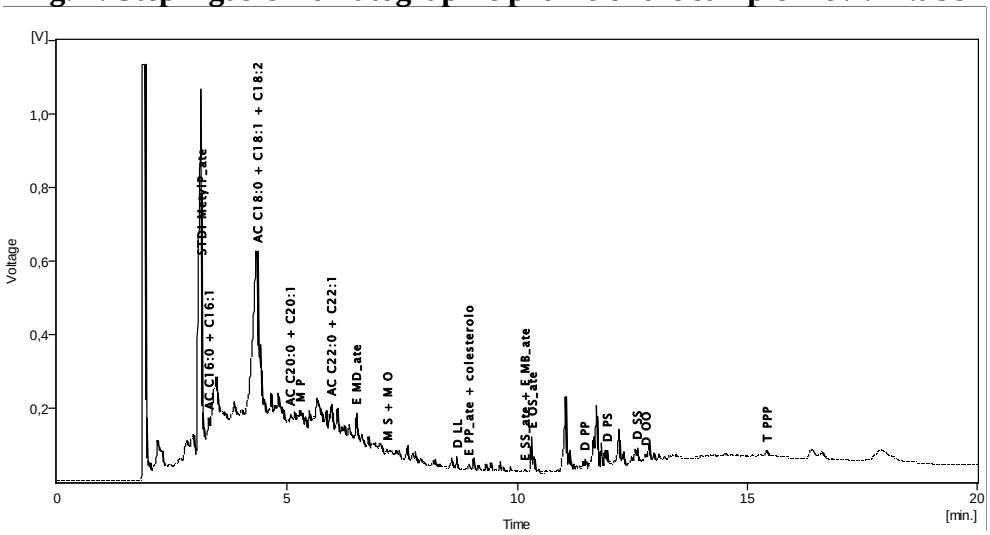
Several are the possible reasons of this evidence:

1. the substances we detected and identified by GC analysis were the only available as standard compounds.
2. as we will show afterward, most of signals reveled refers to complex mixes of substances, as hydrocarbons and waxes, which are not solvable by this GC technique.
3. the fatliquors used during the tanning process contain some functionalized fats too, which may be present in the extract we examined and that are not solvable too by this gas-chromatographic analysis.

Anyway, clarified this experimental evidence, and observing the tables of the results and the chromatograms shown afterward, it is possible to gain some important informations, from the ratios

between the different fatty substances, to the finding of characteristic profiles for classes of fatty extract.
For example, observing the chromatogram in figure 7, obtained according to the Step1of the method described above, relative to the chromatogram of the fats of a sample of clothing leather, it is possible to notice a “bump” at the beginning of the chromatogram, probably due to substances added in the final steps of the tanning process, as mixes of natural or synthetic waxes contained in the chemicals used to finish the leather, that are not solvable by our gas chromatographic technique and also accounts for the partial amount of the fats identified against the total extractable amount.

Fig. 7: Step 1 gas-chromatographic profile of the sample 4-ovi.fin.abb



Hereinafter two chromatograms relative to samples from the same wet-blue bovine leather, grain split and flesh split are shown:

Fig. 8: Step 1 gas-chromatogram of the grain split wet blue (5-bov-F.wb./)

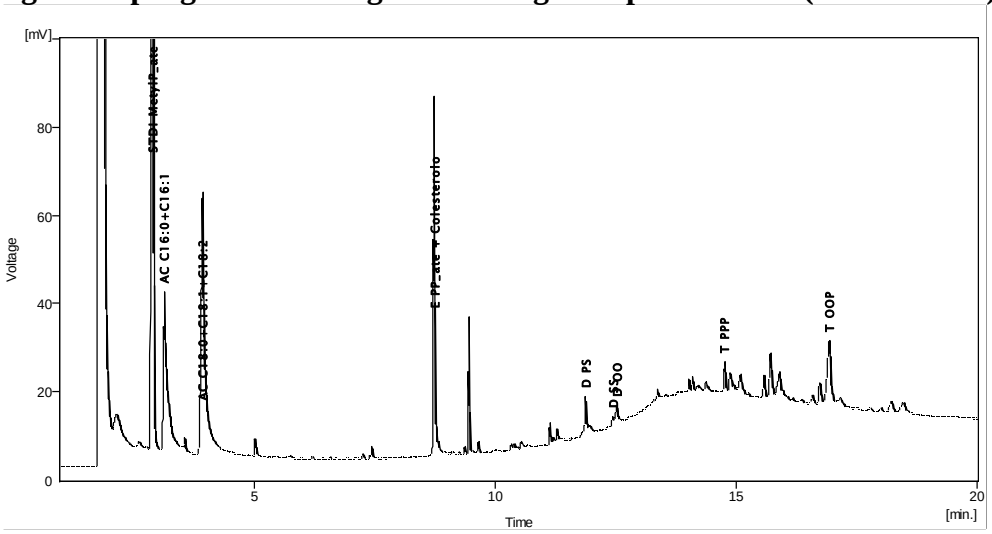
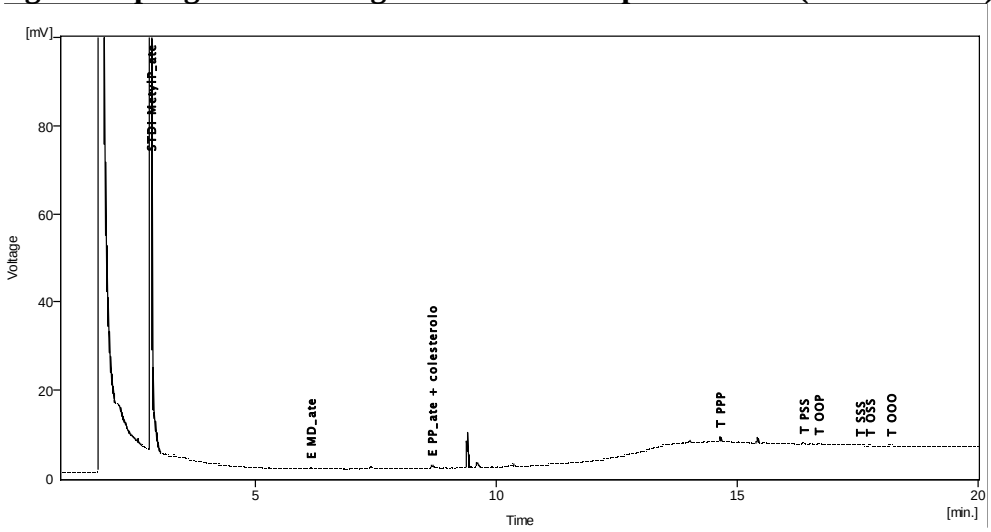


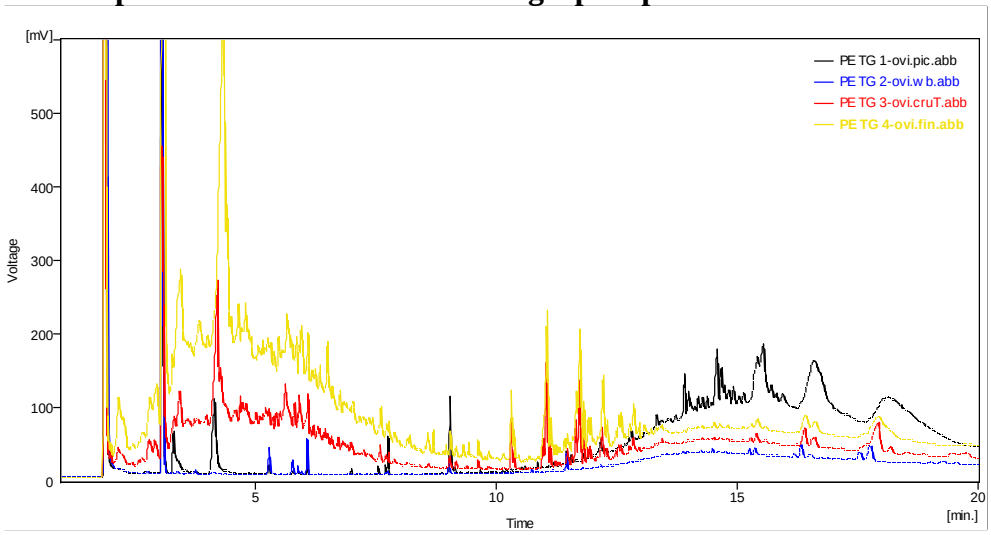
Fig. 9: Step 1 gas-chromatogram of the flesh split wet blue (9-bov-C.wb./)



From the comparison of the two chromatographic profiles it is possible to notice that the flesh split sample gives a more flat chromatogram than the former corresponding to the grain split, how it is confirmed by the results obtained from the gravimetrical amount of the dichloromethane extracted fats (IUC 4). Probably that is why the degreasing operation has been more effective for the flesh split rather that for the grain one, because of the compactness of the structure exhibited by the latter.

In figure 10 is reported the comparison between the chromatographic profiles of samples of the same leather in different tanning steps, obtained using step 1 of the chromatographic method.

Fig. 10: Comparison between the chromatographic profiles of some ovine samples

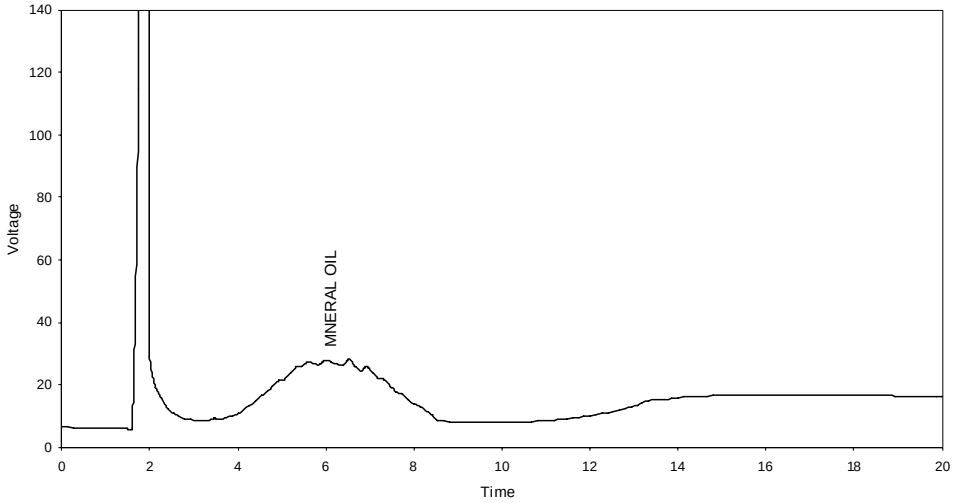


The composition of the fats change from the pickel to the further tanning phases, from one characterized by a great content of glycerides with an high molecular weight to one characterized by fats with a lower molecular weight, as fatty acids and waxes. Furthermore, also in this case it is possible to notice the presence of a “bump” at the beginning of the chromatogram containing substances that are not solvable by our gas chromatographic technique.

The quantification of the chromatographic area of this “bump” has been assessed about ten times greater then the area of all the identified peaks where this area was present.

It is possible to obtain the same chromatographic bump with a miscellaneous of isomeric hydrocarbons, confirming that a miscellaneous of chromatographic not solvable compounds (nor separated peaks) can give this profile. In the figure 11 the chromatographic analysis of a mineral oil mix standard, executed according to the step 1 method (gc-column RTX 65TG),is shown.

Fig. 11: Step 1 gas-chromatographic profile of a mix of hydrocarbons solution (mineral oil)



Results obtained by application of Step 2 of the method

The step 2 of the gas-chromatographic method is useful to discriminate between free saturated and unsaturated fatty acids. In order to show the better resolvability of the single peaks of the fatty acids with step 2 then step 1 of the method, a chromatographic profile relative to a sample of wet blue is reported below (Fig. 12 and 13). On the other hand, it occurs to consider that the analysis obtained by the step 1 of the method for this sample didn't work well to the distinguish between fatty acids with a different unsaturation degree, how it is possible to note in figure 12.

Fig. 12: Step 1 of the method – RTX65TG column (sample cod. 2-ovi..wb.abb)

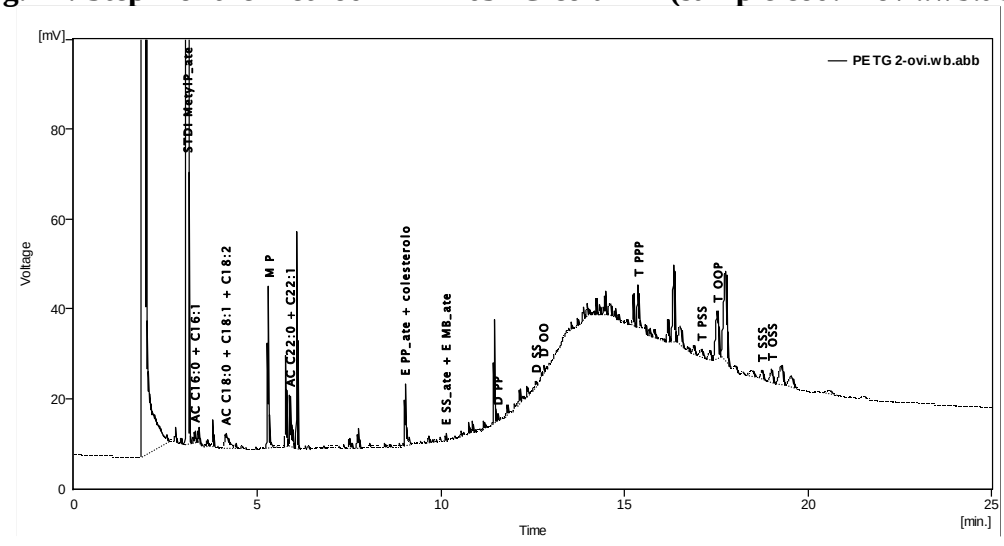
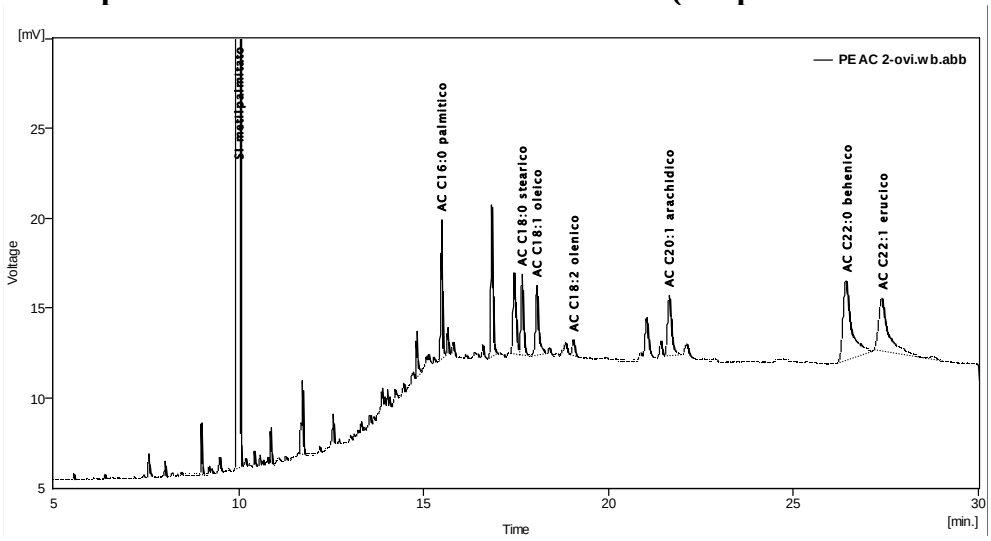


Fig. 13: Step 2 of the method – StabilvaxDA column (sample cod. 2-ovi..wb.abb)



The chromatogram in the figure 13 shows that all the signals of the fatty acids detected are well resolved. Furthermore, the quantitative calculations show that the results obtained by this specific technique are comparable with those relative the other ones obtained from the step 1 of the method.

3. A PARTICULAR APPLICATION: CHARACTERIZATION OF THE FATTY SPUES

The gas-chromatographic technique described, in particular the step 1 of the method, resulted particularly useful to clarify the origin of the characteristic defect of the leather known as fatty efflorescence (repousse) joined to the amount and to the composition of the fats in the leather [(2)].
This application is based on the comparison between the composition of the fats characterizing the fatty efflorescence, taken from the fatty film by a tampon soaked in the solvent, with the composition of the total fats extracted from the whole leather, examined before and after the fat liquoring procedure.
Using this method it is possible to ascertain if the defect detected is determined by an insufficient or an inappropriate degreasing, or if it is due to a bad choice of the chemicals.

We report an example of investigation carried out by SSIP of a leather exhibiting an evident superficial defect due to some fatty spues.
In the specific case, we have carried out the chromatographic analysis of the fats extracted from the leather and of the efflorescence collected in CH_2Cl_2. The efflorescence resulted characterized overall by triglycerides as trioleyl glyc. (T OOO), oleyl distearoyl glyc. (T OSS). tristearoyl glyc. (T SSS), palmitoyl distearoyl glyc. (T PSS), as it is possible to observe in the chromatogram of the figure 14. On the other hand the chromatogram relatives to the extracted fats given a larger range of the signals, and a different composition in triglycerides, as shown in the chromatogram of figure 15. This response indicates that it has occurred a selective migration of the fats from the core of the leather to the surface.
In order to investigate the cause of this migration, we have analyzed the fat liquoring mix adopted in the tanning process. It is resulted composed overall by esters, diglycerides and free fatty acids.
Then, the results obtained suggested that the defect analyzed is due to natural fats contained in the pelt before the fat liquoring step, so that we can conjecture that this defect may be a consequence of a wrong degreasing process.

Fig.14: Step 1 chromatographic profile of the fatty efflorescence

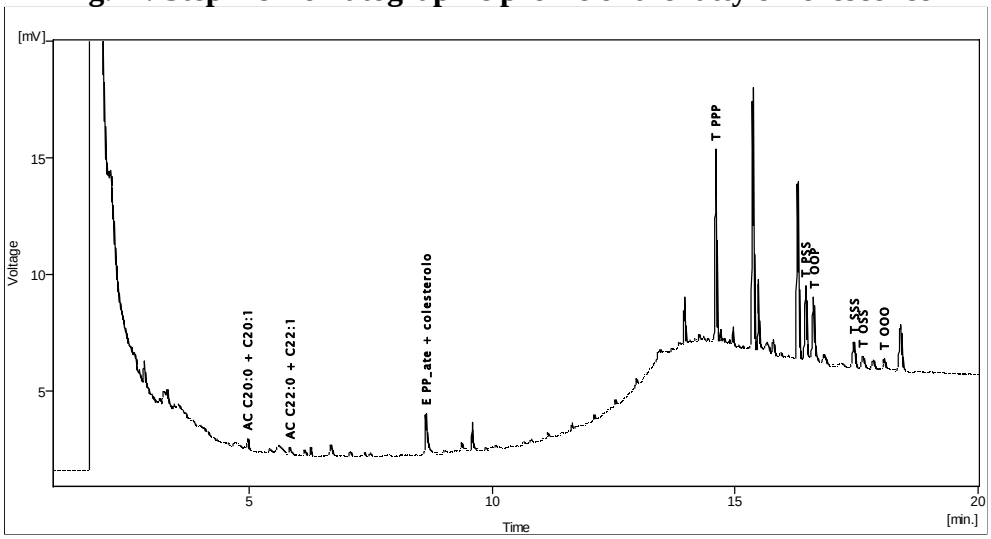
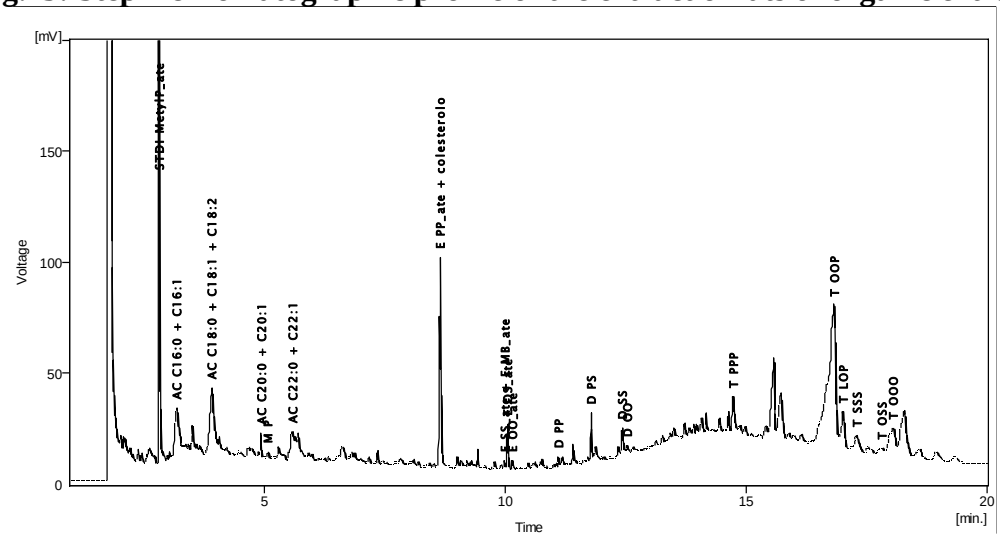


Fig.15: Step 1 chromatographic profile of the extract of fats of organic extract



As it is possible to note by the comparison of the two figures, for the efflorescence we observe a selective migration of the fats, how it is confirmed by many other cases of the fatty efflorescences.
In order to investigate on this phenomenon, we carried out an alkaline hydrolysis of the total fats solution obtained, and then we analyzed the free fatty acids adopting the step 2 of the chromatographic method, specific for this determination.
The prevalence of palmitic and stearic acid and in general of saturated acids in the analyzed efflorescences has often been relieved. Obviously, the detected fatty acids derive both from the hydrolysis of the glycerides and of fatty acid esters and from the free fatty acids present in the efflorescence. This result is also found in the literature; in a previous work[3] the authors declared the possible occurrence of hydrogenation reactions to justify the increase of the saturated fats on unsaturated ones, in particular the formation of stearic acid from the corresponding oleic, verified also in the extractable fats from the leather and not only in the efflorescences.

In the following paragraph we show an investigation work carried out in order to monitor the ratio between saturated and unsaturated fats in the pelt during the tanning steps and to verify the hypothesis reported in the paper above mentioned.

4. MONITORING OF THE RATIO BETWEEN SATURATED AND UNSATURATED FATS IN THE PELT DURING THE TANNING STEPS.

The ability to survey fatty acids, according to the step 2 of the analytical method described, identified both for chain length and for the unsaturation degree (number of double bonds C=C), resulted useful for the monitoring of the ratio of total saturated and unsaturated fats in the leather during the tanning phases.
Then, we have processed some sheep skins in our experimental tannery, from the beamhouse to dyeing and fat liquoring phases.
The raw skin, the unhaired and delimed skins, pickled and wet blue have been analyzed in order to monitor the composition of the fats during the whole tanning process.
In order to reach an accurate determination of the relative variation of the saturated and unsaturated fats, we have carried out an hydrolysis of the fats extracted in dichloromethane and then we have identified most of the fatty acids, both the free ones and the fatty acids present in glycerides and in the esters.
We have also carried out the hydrolysis of the leather or pelt, in order to determine the total fats present in the leather and not only the ones extractable by organic solvent. Indeed, in an other our study still in progress we have obtained that the non-extractable fat percentage is between the 20 and 30 % of the total fat content, depending on the type of semi-finished investigated.
In this last case, the hydrolysis has been achieved in two successive steps:

- the first step has been performed in acid conditions, in order to destroy the leather matrix;
- the second step has been carried out in alkaline conditions, in order to transform all esters and glycerides in free fatty acids.

The results of this deep characterization are reported in the following.
In the table 7 we have shown the quantification of the fatty acids in the hydrolyzed fats extracted by CH_2Cl_2 from the pelts, expressed as grams of fats per 100 grams of dry pelt. In Table 8 we have reported, instead, the increase of these quantification when the analysis is carried out on the fatty acids obtained after the hydrolysis of the pelt, by which we obtain the total fat content.

Tab. 7: Total fatty acids extracted by CH_2Cl_2

	SAMPLES				
FATTY ACID	**RAW G-P1**	**LIMED C-P2**	**DELIMED D-P3**	**PICKLED P-P5**	**WET BLUE WB-P6-A**
	g/100g of skin	g/100g of pelt	g/100g of pelt	g/100g of pelt	g/100g of leather
Total gravimetrical determined fats extracted by IUC 4 (%)	**8,1**	**3,2**	**3,8**	**4,9**	**4,4**
AC C16:0 Palmitic acid	1,216	0,133	0,122	0,154	0,128
AC C16:1 Palmitoleic acid	0,508	0,052	0,049	0,036	0,031
AC C18:0 Stearic acid	0,294	0,018	0,020	0,049	0,044
AC C18:1 Oleic acid	3,200	0,148	0,145	0,099	0,084
AC C18:2 Olenic acid	1,168	0,091	0,089	0,068	0,055
AC C20:0 cis11-eicosanoic acid	0,005	0,001	0,001	0,003	0,004
AC C20:1 Arachidic acid	0,181	0,018	0,016	0,017	0,014
AC C22:0 Behenic acid	0,004	0,002	0,002	0,005	0,004
AC C22:1 Erucic acid	0,179	0,021	0,020	0,014	0,010

Tab. 8: Increase of total fatty acids after hydrolysis compared to the extracted fatty acids

FATTY ACID	SAMPLES				
	RAW G-P1	LIMED C-P2	DELIMED D-P3	PICKLED P-P5	WET BLUE WB-P6-A
	mg/g of pelt	mg/g of pelt	mg/g of pelt	mg/g of pelt	mg/g of pelt
AC C16:0 Palmitic acid	+ 15%	+ 27%	+ 27%	+ 10%	+ 10%
AC C16:1 Palmitoleic acid	+ 21%	+ 28%	+ 23%	+ 24%	+ 21%
AC C18:0 Stearic acid	+ 16%	+ 24%	+ 23%	+ 1%	+ 5%
AC C18:1 Oleic acid	+ 16%	+ 23%	+ 20%	+ 21%	+ 17%
AC C18:2 Olenic acid	+ 10%	+ 22%	+ 17%	+ 20%	+ 20%
AC C20:0 cis11-eicosanoic acid	+ 15%	0%	0%	+ 25%	0%
AC C20:1 Arachidic acid	+ 21%	+ 29%	+ 18%	+ 10%	7%
AC C22:0 Behenic acid	+ 20%	+ 33%	+ 33%	+ 17%	20%
AC C22:1 Erucic acid	+ 20%	+ 13%	+ 18%	+ 18%	23%

Tab. 9: Fatty acid in the CH_2Cl_2, as percentage of single acid in its own group

FATTY ACID	SAMPLES				
	RAW G-P1	LIMED C-P2	DELIMED D-P3	PICKLED P-P5	WET BLUE WB-P6-A
	%	%	%	%	%
AC C16:0 Palmitic acid	69,2%	71,6%	72,4%	78,3%	78,6%
AC C16:1 Palmitoleic acid	30,8%	28,4%	27,6%	21,7%	21,4%
Total C16	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C18:0 Stearic acid	6,4%	7,2%	8,3%	**19,1%**	21,4%
AC C18:1 Oleic acid	70,0%	57,9%	57,5%	48,1%	46,7%
AC C18:2 Olenic acid	23,6%	34,9%	34,2%	32,8%	31,9%
Total C18	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C20:0 cis11-eicosanoic acid	2,6%	3,8%	4,8%	17,1%	21,0%
AC C20:1 Arachidic acid	97,4%	96,2%	95,2%	82,9%	79,0%
Total C20	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C22:0 Behenic acid	2,5%	11,0%	10,9%	26,1%	27,8%
AC C22:1 Erucic acid	97,5%	89,0%	89,1%	73,9%	72,2%
Total C22	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*

Tab. 10: Fatty acid in the hydrolyzed of the pelt, as % of single acid in its own group

FATTY ACID	SAMPLES				
	RAW G-P1	LIMED C-P2	DELIMED D-P3	PICKLED P-P5	WET BLUE WB-P6-A
	%	%	%	%	%
AC C16:0 Palmitic acid	70,5%	71,7%	71,3%	81,1%	80,5%
AC C16:1 Palmitoleic acid	29,5%	28,3%	28,7%	18,9%	19,5%
Total C16	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C18:0 Stearic acid	6,3%	7,2%	7,9%	22,7%	24,0%
AC C18:1 Oleic acid	68,6%	57,5%	57,1%	45,7%	45,9%
AC C18:2 Olenic acid	25,1%	35,4%	35,0%	31,6%	30,1%
Total C18	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C20:0 cis11-eicosanoic acid	2,8%	5,3%	5,9%	14,7%	22,2%
AC C20:1 Arachidic acid	97,2%	94,7%	94,1%	85,3%	77,8%
Total C20	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*
AC C22:0 Behenic acid	2,4%	8,7%	9,1%	26,3%	28,6%
AC C22:1 Erucic acid	97,6%	91,3%	90,9%	73,7%	71,4%
Total C22	*100,0%*	*100,0%*	*100,0%*	*100,0%*	*100,0%*

The percentage of content in the pelt of stearic and palmitic acid increases when raw pelts are processed until to the wet blue state, as it is possible to see in the table 10 (results of the survey on the hydrolyzed of the pelt). The palmitic acid percentage change from 71,7% in the limed pelt to 80,5% in the wet blue, with the relative decreasing of the palmitoleic acid, and the stearic acid from 7,2% in the limed pelt to 24%. In the last case, the amount oleic acid (C8:1, single unsaturation) decrease of 11,6 percentage points and the olenic acid (C8:2, double unsaturation) of 5,3 percentage points.
The profile of the percentages of saturated fats against the total of the fatty acids with the same chain length during the tanning process is shown below.

Fig. 16: Profile of the palmitic acid percentage (C16:0) during the tanning process

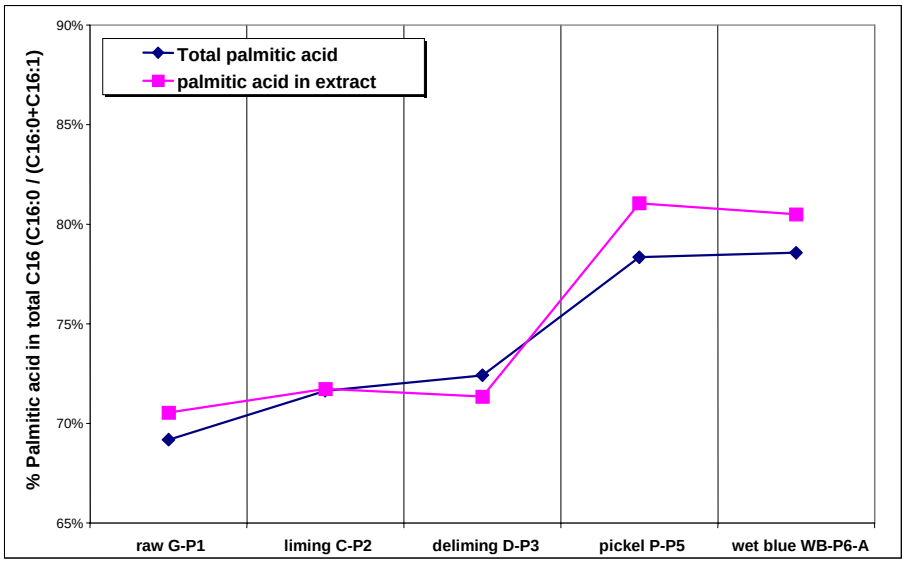
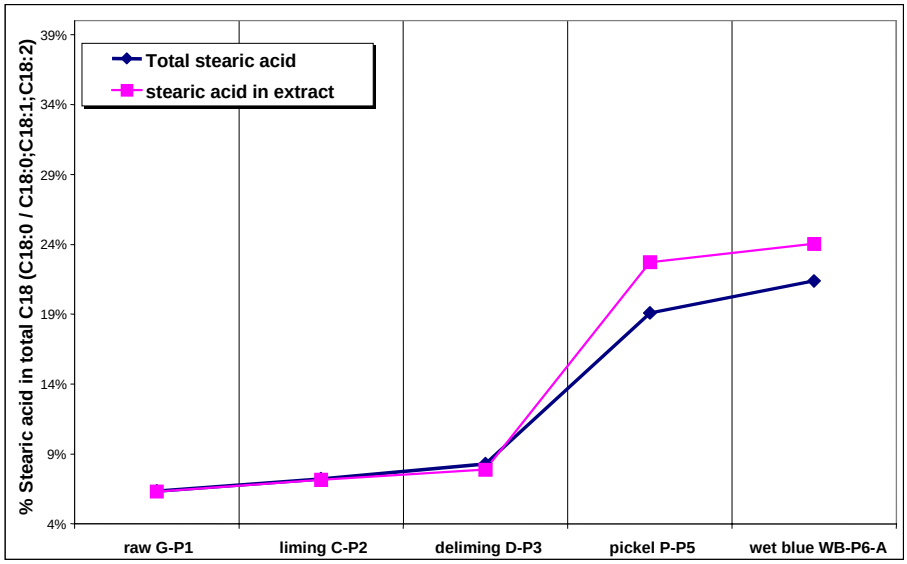


Fig. 17: Profile of the stearic acid percentages (C18:0) during the tanning process



In the table 9 and 10 the high increase of the palmitic and stearic acid in the pickel phase can be observed, specially for the stearic acid varying from 8,3% to 19,1%. This emerges obvious in the Figure 17 too.
Furthermore, it is important to note as the total increase of the saturated acids (Tab. 10) is about the same than the one obtained analyzing the Soxhlet extracted solution (after hydrolysis of the fats). This extract contained the not-bonded fats, then it is possible to consider that the hydrogenation reactions occur a little more for these fats, probably free to react with a some hydrogenation agent.

In order to quantify this phenomenon, we have determined a numeric index that furnishes the course of the unsaturated degree of the fats of the pelt during the tanning process. The index, called unsaturated index (I_{US}), is the amount of ethylenic groups, as C_2H_2 (PM 26), obtained by the equivalents of C_2H_2 relative to unsaturated fats and expressed in term of percentage on total fats of the hydrolyzed pelt solution.

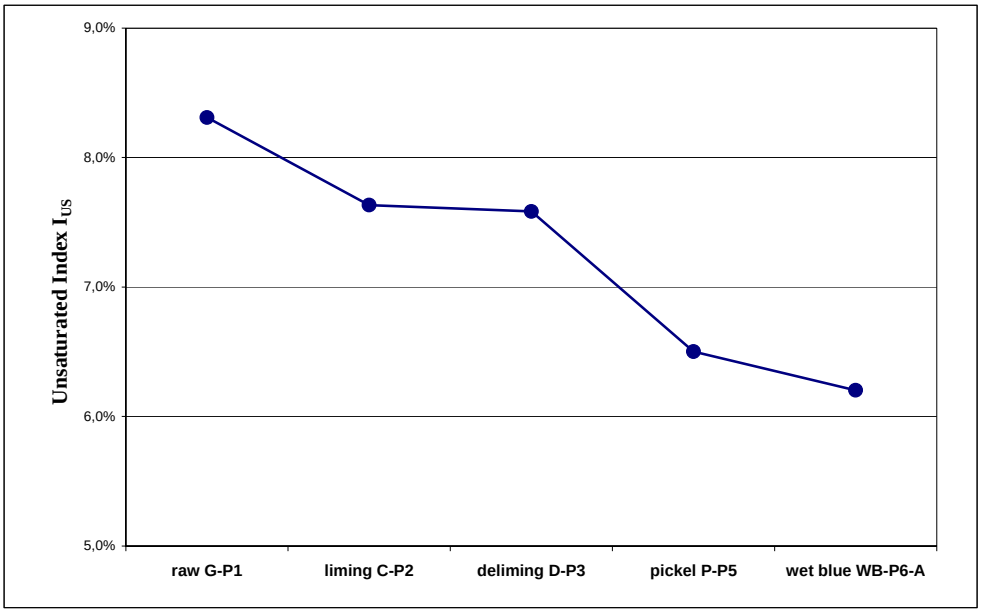
The values of I_{US} have been plotted versus the process steps, from the raw material to the wet blue, as shown in the Table 11.

Tab. 11: Values of C_2H_2 (I_{US}) during the tanning steps

	SAMPLES				
PARAMETERS	**RAW G-P1**	**LIMING C-P2**	**DELIMING D-P3**	**PICKEL P-P5**	**WET BLUE WB-P6-A**
a) **total fatty acid (g/100g of pelt)**	8,015	0,642	0,593	0,523	0,435
b) **total C_2H_2 (g/100g of pelt)**	0,666	0,049	0,045	0,034	0,027
Unsaturated index I_{US} (% C_2H_2 groups)	**8,31 %**	**7,63 %**	**7,58 %**	**6,50 %**	**6,20 %**

The successive figure 18 shows the I_{US} values vs. the tanning steps

Fig. 18: Profile of the ethylenic groups (I_{US} - unsaturated index) during the tanning process



In the Figure 18 it is possible to notice the decreasing profile of the unsaturated index during the tanning processes. In particular, a bigger decrease of the unsaturated fats content in the pelt is relievable in the pickel phase, confirming that this phase achieves the most favourable condition for the hydrogenation reactions.

CONCLUSIONS

The analytical technique we described resulted effective to the characterization of fatty matter extracted from the pelt and the leather, independently from the origin of leather we analyzed and from the relative state of the tanning process in which it was.

The use of a different column, specific for the determination of the only fatty acids, allowed a finer characterization, because of the possibility of separation of the fatty acids with the same length, but with different number of double bound. Furthermore, this technique resulted useful to the specific study of the origin of the defect of the leather known as fatty efflorescence.

Applying this last analytical skill, we have obtained an important result verifying an increase of the content of total saturated compounds in the pelt and leather during the tanning steps, as consequence of the hydrogenations of the unsaturated fats. The saturated fats are often determined as responsible of fatty efflorescences formation or moreover as main components of the efflorescences.
In a previous work[3], indeed, it has been hypothesized that the formation of the fatty spues may be influenced by the formation of saturated fatty acids during some steps of the tanning process, deriving from possible mechanisms of biohydrogenation[4].

In the future, the analytical approach discussed in this work will be applied for further investigations in order to study other pelt-fats systems.

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