

# RAPID EXTRACTION OF VOLATILE COMPOUNDS IN WINE AND VINEGAR USING EXTRELUT RESIN

ESTRAZIONE RAPIDA DEI COMPOSTI VOLATILI DEL VINO  
E DELL'ACETO MEDIANTE RESINA EXTRELUT

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## ABSTRACT

The aromatic fraction of wine and vinegar has been examined by Solid Phase Extraction (SPE) techniques and in particular by using Extrelut resin (Merck) as an alternative to extraction with solvents. The adoption of appropriate operating procedures permitted very good yields which, coupled with shorter operating times, should allow the described method to be applied routinely.

## RIASSUNTO

Per lo studio della frazione aromatica di vini ed aceti, in alternativa all'estrazione con solvente è stata studiata la possibile applicazione delle tecniche SPE ed in particolare della resina Extrelut (Merck). Adottando opportuni accorgimenti operativi è possibile ottenere ottimi recuperi il che, unitamente ai tempi operativi più contenuti, consente un'applicazione routinaria del metodo descritto.

- Key words: aromatic fraction, Extrelut resin, vinegar, wine. -

## INTRODUCTION

The aromatic fraction of wines and vinegars, as in other fermented beverages, plays a role of primary sensory importance, despite the fact that it represents one of the minor components, quantitatively. To study this fraction, it must be free from the matrix, free of interfering substances and it must be concentrated to a suitable degree for analytical detection.

The innumerable sample preparation methods which have been set up are reviewed by AMATI et al. (1973) and ÉTIEVANT (1987), and are summarized as follows:

- a) Direct injection of the sample (limited to components with high concentrations such as higher alcohols)
- b) Distillation
- c) Headspace analysis
- d) Cryoconcentration
- e) Extraction in inert gas flow
- f) Extraction with solvent
- g) Extraction with resin.

Although extraction methods with solvents are recognised as the most suitable for studying the aromatic fraction, their complexity, inducing high analysis costs and operating time, makes them rather unsuitable for routine use (AMATI et al., 1973).

Resin extraction techniques for extracting components of low concentration from complex matrices are widely known, but they have been utilized only in the last few years in the wine industry (GUNATA et al., 1985; GELSOMINI et al., 1990; DI STEFANO, 1991; GIANOTTI and DI STEFANO, 1991). Hydrophobic resins, XAD-2 (Rohm and Haas) and C<sub>18</sub>, have been widely used because of their power to extract organic compounds from aqueous solutions and to release them selectively by organic solvents. However, the presence of ethanol in fermented products prevents complete recovery of the organic compounds.

The hydrophilus Extrelut resin (Merck) has been modified for drug quantification in biological fluids. Its working principle is the opposite of that of the resins mentioned above. Water is retained by the resin, while low polarity organic compounds of the aromatic fraction (including ethyl alcohol) are eluted with appropriate solvents. Extrelut resin has already been proposed for extraction of the volatile fraction of cider (HUBERT et al., 1990) and wine (GELSOMINI et al., 1990).

The present work is aimed at verifying its possible use for extraction of the volatile fraction of vinegar and sweet sparkling wine. Furthermore extraction yields with this resin were evaluated.

## MATERIALS AND METHODS

### Extraction

Glass columns (ID 2 cm, length 20 cm) fitted with glass terminal taps were used. Each contained 25 g of Extrelut resin (Merck) supported by a frit glass. Several grams of anhydrous Na<sub>2</sub>SO<sub>4</sub> were placed between the resin and the frit to dehydrate the extract in the percolating phase, thus preventing a subsequent dehydrating phase.

Two standard solutions, A and B, in absolute ethanol at known concentrations, contained, respectively, a mixture of standard volatiles and the internal standard, 0.083 g/L 2-Octanol (Table 1). They were utilized to determine the extraction yields of some of the more important compounds in the aromatic fraction of the oenological products.

The sample (20 mL) was poured into the column and percolated through the resin until complete absorption. Volatiles were eluted by gravity with 80 mL of dichloromethane. After 30 min of elution, 60 mL of dehydrated solution were collected.

Table 1 - Composition of standard solution (A).

	g/L
γ - Butyrolactone	.112
α - Terpineol	.093
1 - Butanol	.081
1 - Heptanol	.082
1 - Hexanol	.081
1 - Octanol	.083
1 - Pentanol	.081
2 - Phenyl ethanol	.102
1 - Propanol	.080
2,3 - Butanediol	.099
2,3 - Butanedione	.098
2 - Butanol	.081
2 - Methyl-1-butanol	.081
2 - Methyl-1-propanol	.080
3 - Ethoxy-1-propanol	.090
3 - Hydroxy-2-butanone	.091
3 - Methyl-1-butanol	.081
3 - Methyl-1-pentanol	.082
4 - Ethylphenol	.057
Benzaldehyde	.104
Benzyl alcohol	.104
Butyric acid	.096
cis-3-Hexen-1-ol	.085
Citronellol	.086
Diethyl succinate	.105
Ethyl butyrate	.088
Ethyl cinnamate	.105
Ethyl decanoate	.086
Ethyl hexanoate	.087
Ethyl lactate	.104
Ethyl octanoate	.088
Ethyl pyruvate	.106
Ethyl-3-hydroxybutyrate	.102
Furfuryl alcohol	.114
Geraniol	.089
Hexanoic acid	.093
Hexyl acetate	.088
Isoamyl acetate	.088
Isobutyl acetate	.087
Isobutyric acid	.095
Isovaleric acid	.094
Linalool	.087
Nerol	.088
Octanoic acid	.091
Phenethyl acetat	.103
Propionic acid	.099
trans-3-Hexen-1-ol	.082
Valeric acid	.094

Unlike the lipophilic resins Amberlite XAD-2 and C<sub>18</sub>, Extrelut cannot be re-used. Disposable plastic columns are available and contain the amount of resin necessary to absorb a 20 mL sample. Concentration was carried out under nitrogen until a final volume of 0.5 to 1 mL.

A mod. 3400 Varian gas chromatograph equipped with a J&W DBWAX-30 N (30 m; ID 0.25 mm) column connected to a Spectra-Physics integrator (mod. 4290) was used for the gas chromatographic analysis. Operative conditions were as follows: injector and detector temperature 250°C; split ratio 1:20; carrier gas, H<sub>2</sub> 1 mL/min; oven temperature program, 5 min at 60°C then 2°C/min up to 180°C and finally 10 min at 180°C; volume injected, 1 μL.

Three gas chromatographic determinations were made for each sample.

#### Evaluation of extraction yields

##### - Theoretical principles

The method of HUBERT et al. (1990) was used in part to evaluate the extraction yields. It may be assumed, for analytical procedures with three successive phases (extraction, concentration, analysis), that for each compound the overall yield is given by

$$RG_x = RC_x \times RE_x$$

where:

RG<sub>x</sub> : overall yield for compound x  
 RC<sub>x</sub> : yield of the concentration phase  
 RE<sub>x</sub> : yield of the extraction phase

##### - Calculation of the concentration yield

Operating in internal standardization, it is necessary to calculate the response factor by means of the internal standard procedure because it is not possible to

determine exactly the reproducibility of the sample injection:

$$RF_x = \frac{A_{is}}{A_x} \times \frac{C_x}{C_{is}}$$

where:

- $RF_x$  : response factor for compound x
- $A_x$  : chromatographic peak area corresponding to compound x
- $C_x$  : concentration of compound x
- $A_{is}$  : area of the chromatographic peak of the internal standard
- $C_{is}$  : concentration of the internal standard

Using the internal standard method, the unknown concentration of a compound x in a solution is given by

$$C_x = RF_x \times \frac{A_x}{A_{is}} \times \frac{IS}{SA} \quad (1)$$

where:

- $C_x$  : concentration of compound x
- $RF_x$  : response factor for compound x
- $A_x$  : area of the chromatographic peak of compound x
- $A_{is}$  : area of the chromatographic peak of the internal standard
- IS : quantity of internal standard added to the sample
- SA : volume of sample analysed.

If the solution examined has been subjected exclusively to a process of concentration, the concentration yield for compound x will be given by

$$C_x : C_{theor} = RC_x : 100$$

where:

- $C_x$  : concentration of compound x calculated with (1)

$C_{theor}$  : theoretical concentration of compound x

- Calculation of the extraction yield

The extraction process yield is given by

$$C_x : C_{theor} = RG_x : 100$$

where

$$RG_x = RC_x \times RE_x$$

obtaining

$$RE_x = \frac{RG_x}{RC_x}$$

- Experimental application

In order to determine the response factors, 300  $\mu$ L of the internal standard solution (B) were added to 300  $\mu$ L of standard solution (A) and the volume was adjusted to 3 mL with  $CH_2Cl_2$  before analysis.

To evaluate the concentration yield, 100  $\mu$ L of standard solution (A) were diluted to 60 mL with  $CH_2Cl_2$ . After concentration under  $N_2$ , the sample (0.5 to 1 mL) was quantitatively transferred to a 3 mL volumetric flask containing 100  $\mu$ L of standard solution (B) and brought to volume with  $CH_2Cl_2$ . The determination was repeated five times.

To evaluate the extraction yield, 300  $\mu$ L of standard solution (A) were diluted to 20 mL with malate buffer (pH 3.5, 10% in ethyl alcohol) and extracted with Extrelut resin.

The eluate was concentrated under  $N_2$ , brought to 3 mL with  $CH_2Cl_2$  after addition of 300  $\mu$ L of standard solution (B) and then analyzed. This determination was carried out four times. Three of the samples were saturated with NaCl before extraction.

## Qualitative and quantitative evaluations

In order to evaluate possible applications of the proposed extraction method, trials were performed on red wine produced by carbonic maceration, on vinegar and on sweet sparkling wine ("Asti"). Samples were also extracted by means of liquid-liquid extraction.

### Extraction with resin

Extraction was performed on untreated samples of red wine and "Asti", after addition of standard solution (B), while in the case of vinegar, a preliminary neutralization with MgO was necessary in order to reduce acetic acid interference (KAHN et al., 1972). In order to allow determination of organic acids, neutralization must be limited to pH 6 to 6.1. MgO was compared to ammonia for neutralization to test its influence on the detectability of organic acids in one red and two white vinegar samples.

Extraction was carried out following the procedures indicated above.

### Liquid-liquid extraction

40 g/L NaCl (demulsifying function) and standard solution (B) were added to red wine and the "Asti". The vinegar was neutralized with MgO to pH 6 to 6.1 and standard solution (B) was added. Samples of 500 mL for both wines and vinegar were treated in a continuous extractor using 200 mL of a 7:3 pentane: dichloromethane mixture for 72 hours (VERSINI, 1985 modified). Concentration under N<sub>2</sub> continued until a final volume of 0.5 to 1 mL.

## RESULTS AND CONCLUSION

The yields of the concentration phase for the compounds under consideration were more than 80% (Table 2), except for

Table 2 - Mean yields and coefficients of variation calculated for the concentration phase with N<sub>2</sub> (compounds in order of retention time).

	Mean (%)	CV (%)
2,3 - Butanedione	121.1	14.4
Isobutyl acetate	89.3	5.5
2 - Butanol	54.6	1
Ethyl butyrate	87.9	5.2
1 - Propanol	34.4	5.8
2 - Methyl-1-propanol	59.4	1.3
Isoamyl acetate	91.0	4.9
1 - Butanol	73.2	4.2
3 - Methyl-1-butanol + 2-Methyl-1-butanol	83.2	4.7
Ethyl hexanoate	94.0	4.5
1 - Pentanol	88.6	4.8
Ethyl pyruvate	110.6	7.4
Hexyl acetate + 3-Hydroxy-2-butanone	90.6	1.4
3 - Methyl-1-pentanol	91.4	3.5
Ethyl lactate	95.5	1.9
1 - Hexanol	93.8	5.3
<i>trans</i> -3-Hexen-1-ol	93.4	2.8
3 - Ethoxy-1-propanol	96.2	3.7
<i>cis</i> -3-Hexen-1-ol	93.5	2.6
Ethyl octanoate	96.9	6.9
Furfuryl alcohol	126.3	11.7
1 - Heptanol	94.6	4.5
Benzaldehyde	152.7	17.9
Ethyl-3-hydroxybutyrate	96.3	3.9
Propionic acid	85.6	17.1
(-)-2,3-Butanediol	90.0	3.7
Linalool	95.9	5.3
1 - Octanol + Isobutyric acid	94.4	1.8
(R,S)-2,3-Butanediol	102.9	7.3
γ - Butyrolactone	97.5	3.8
Butyric acid	89.5	16.2
Ethyl decanoate	96.6	5
Isovaleric acid	89.9	15.1
Diethyl succinate	98.6	6.2
α - Terpineol	93.8	3.9
Valeric acid	86.1	16.1
Citronellol	94.9	1.6
Phenethyl acetate + Nerol	98.5	5.7
Hexanoic acid	88.1	16.4
Geraniol	93.8	3.3
Benzyl alcohol	97.1	7
2 - Phenyl ethanol	95.5	3.5
Octanoic acid	84.0	13.9
Ethyl cinnamate	95.1	3.5
4 - Ethylphenol	92.0	2.5

Table 3 - Yield (%) of the extraction phase by passage through Extrelut resin of samples not treated (NT) or treated (T) preliminarily with NaCl (compounds in order of retention time).

	NT	T	
		Mean (%)	CV (%)
2,3 - Butanedione	64.4	61.7	13.5
Isobutyl acetate	81.7	70.4	1.3
2 - Butanol	104.7	126.8	7.0
Ethyl butyrate	83.6	74.4	7.2
1 - Propanol	121.1	163.5	6.7
2 - Methyl-1-propanol	102.5	124.8	4.5
Isoamyl acetate	83.3	64.5	5.4
1 - Butanol	95.9	112.6	3.8
3 - Methyl-1-butanol + 2-Methyl-1-butanol	103.3	100.3	4.8
Ethyl hexanoate	82.5	62.2	4.6
1 - Pentanol	91.7	100.5	3.0
Ethyl pyruvate	82.1	20.6	1.0
Hexyl acetate + 3-Hydroxy-2-butanone	84.6	62.0	8.8
3 - Methyl-1-pentanol	92.0	96.0	1.9
Ethyl lactate	90.5	100.1	0.8
1 - Hexanol	90.4	94.1	3.0
<i>trans</i> -3-Hexen-1-ol	90.2	97.8	2.5
3 - Ethoxy-1-propanol	89.2	99.6	4.1
<i>cis</i> -3-Hexen-1-ol	91.6	100.4	1.5
Ethyl octanoate	71.4	66.4	1.0
Furfuryl alcohol	143.9	138.4	7.4
1 - Heptanol	90.7	89.8	1.9
Benzaldehyde	143.7	107.8	7.3
Ethyl-3-hydroxybutyrate	90.7	102.9	2.7
Propionic acid	0	54.4	1.3
(-) -2,3-Butanediol	0.6	45.6	6.0
Linalool	89.5	81.1	5.9
1 - Octanol + Isobutyric acid	64.6	82.2	2.1
(R,S)-2,3-Butanediol	0.3	26.0	3.0
$\gamma$ - Butyrolactone	90.3	97.1	2.3
Butyric acid	0.4	89.1	1.2
Ethyl decanoate	64.5	79.4	7.3
Isovaleric acid	49.3	106.6	3.8
Diethyl succinate	90.3	94.1	3.9
$\alpha$ - Terpineol	92.7	91.4	5.4
Valeric acid	61.1	104.1	3.0
Citronellol	94.8	93.3	2.0
Phenethyl acetate + Nerol	92.7	88.0	6.5
Hexanoic acid	92.2	102.0	1.3
Geraniol	95.4	96.2	5.7
Benzyl alcohol	92.1	96.1	3.3
2 - Phenyl ethanol	93.3	98.9	3.0
Octanoic acid	0.8	75.4	2.2
Ethyl cinnamate	90.5	90.4	0.4
4 - Ethylphenol	96.8	98.4	3.6

C<sub>3</sub> and C<sub>4</sub> alcohols; this is in agreement with results reported by HUBERT et al. (1990).

Saturation of samples with NaCl before extraction seems to have improved the extraction yields (Table 3) and in particular for samples treated with NaCl, extraction yields of 2,3-butanediol and fatty acids were higher than for untreated samples. It may be assumed that the presence of high amounts of salts in solution may shift the solution equilibria, thus facilitating dissolution in the organic solvent of molecules with polarity centers. The concentration of esters, on the contrary, is negatively affected by saturation of the sample with NaCl, showing a yield decrease by as much as 60% for ethyl pyruvate.

The extraction yields were generally high (from 70% to 100%) for the other compounds examined as already shown by HUBERT et al. (1990).

The results of extracting the volatile fraction from the red wine, "Asti" and vinegar, by Extrelut resin or by the liquid-liquid extractor, demonstrate that the two procedures agree well as already shown for the Chianti red wine by GEL-SOMINI et al. (1990). Only the acid concentrations were considerably lower for both "Asti" and red wine in samples extracted by Extrelut resin (Table 4). C<sub>3</sub>, C<sub>4</sub> and iso-C<sub>5</sub> acids were absent in vinegar samples. Valeric and isobutyric acids were not detected due to the presence of undetermined substances in the unresolved peaks of these acids. Lower concentrations of acids in wines when resin was used may be justified because samples were not saturated with NaCl before analysis. The absence of acids in the vinegar samples is due to neutralization with MgO. It is very difficult to obtain the desired pH exactly using MgO because the neutralization process is slow and continues to pH 7 and more, thus causing salification of other acids in addition to acetic acid. The use of

Table 4 - Concentrations ( $\mu\text{g/L}$ ) of the volatile fraction components of a vinegar, an "Asti" and a red wine, determined by extraction with solvent and Extrelut resin.

	Vinegar		"Asti"		Wine	
	Solvent	Extrelut	Solvent	Extrelut	Solvent	Extrelut
2,3 - Butanedione					2540	2646
Isobutyl acetate	1937	2290	63	53	101	443
2 - Butanol	593	798	303	799	310	690
Ethyl butyrate			296	265	200	240
1 - Propanol	560	729	6672	11424	10235	23091
2 - Methyl-1-propanol	11736	10142	11379	16741	27207	64100
Isoamyl acetate	2760	3088	872	1180	1495	1896
1 - Butanol			629	766	627	1074
3 - Methyl- + + 2-Methyl-1-butanol	15914	18149	29816	53796	46123	142313
Ethyl hexanoate			691	564	480	327
Ethyl pyruvate			232	254	38	208
Hexyl acetate + + 3-Hydroxy-2-butanone	33537	39407	3753	6294	3291	4196
3 - Methyl-1-pentanol			42	59	105	126
Ethyl lactate	1332	1390	6957	8775	7273	10099
1 - Hexanol			1292	1263	1389	1268
<i>trans</i> -3-Hexen-1-ol			31	36	27 *	28
3 - Ethoxy-1-propanol	102	119	89	86	199	173
<i>cis</i> -3-Hexen-1-ol			95	87	147	119
<i>cis</i> -Furan-linalooloxide			72	62		
Benzaldehyde	266	97	237	47	227	50
Ethyl-3-hydroxybutyrate			47	44	395	272
Propionic acid			233	177	410	206
(-) -2,3-Butanediol	4893	12666	7116	28858	10839	42747
Linalool			199	168		
Isobutyric acid (+ n.i. in vinegar)	2236	2263	459	294	870	462
(R,S) - 2,3-Butanediol	3156	4148	2515	7805	4051	9391
$\gamma$ - Butyrolactone	3725	4894	1665	2001	2340	3855
Butyric acid			898	492	516	201
Ethyl decanoate			122	319	123	192
Isovaleric acid			216	142	587	149
Diethyl succinate	267	269	346	328	516	227
$\alpha$ - Terpineol			463	392		
Valeric acid (+ n.i.)	14701	13332	385	461	1034	1304
Citronellol			50	42		
Phenethyl acetate + Nerol	93	154	199	147	3914	6490
Hexanoic acid	294	85	5275	4338	1578	984
Geraniol			28	35		
Benzyl alcohol	1146	1198			759	832
2 - Phenyl ethanol	20998	22442	12114	14367	26511	45613
Octanoic acid	774	561	7104	8805	2569	2143
n.i. : not identified						

Table 5 - Concentration ( $\mu\text{g/L}$ ) of acids from three vinegars (1= red; 2 and 3= white) neutralized with magnesium oxide or ammonia before extraction with Extrelut resin.

	1		2		3	
	Magnesium oxide	Ammonia	Magnesium oxide	Ammonia	Magnesium oxide	Ammonia
Propionic acid	0	939	0	590	0	792
Butyric acid	0	322	0	924	0	298
Isovaleric acid	0	4638	0	21380	0	4582
Hexanoic acid	117	855	63	2397	126	832
Octanoic acid	321	422	846	1347	28	130

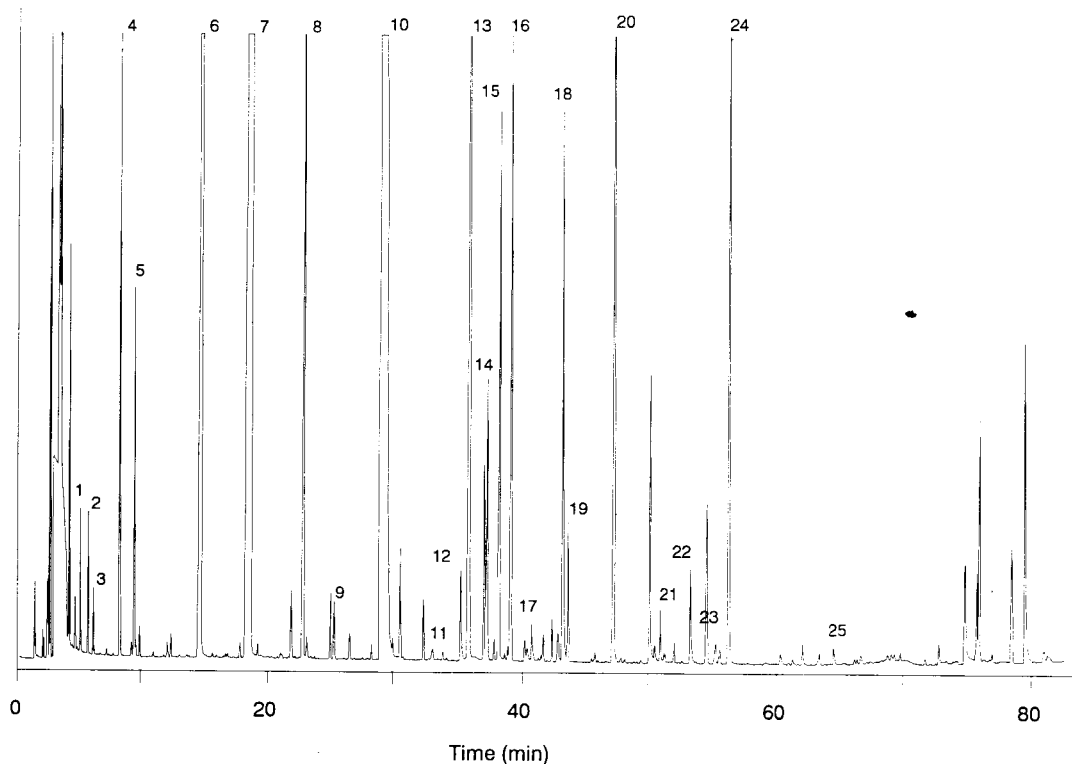


Fig. 1 - Gas chromatogram of the aromatic fraction of a white vinegar neutralized with ammonia and extracted with Extrelut resin.

Legend:

- |  |                             |                         |
|--|-----------------------------|-------------------------|
| 1: Isobutyl acetate                          | 8: Ethyl lactate            | 18: Isovaleric acid     |
| 2: 2-Butanol                                 | 9: 3-Ethoxy-1-propanol      | 19: Diethyl succinate   |
| 3: 1-Propanol                                | 10: Acetic acid             | 20: Valeric acid + n.i. |
| 4: 2-Methyl-1-propanol                       | 11: Benzaldehyde            | 21: Phenethyl acetate   |
| 5: Isoamyl acetate                           | 12: Propionic acid          | 22: Hexanoic acid       |
| 6: 3-Methyl- +<br>+ 2-Methyl-1-butanol       | 13: (-)-2,3-Butanediol      | 23: Benzyl alcohol      |
| 7: Hexyl acetate +<br>+ 3-Hydroxy-2-butanone | 14: Isobutyric acid + n.i.  | 24: 2-Phenyl ethanol    |
|  | 15: (R,S)-2,3-Butanediol    | 25: Octanoic acid       |
|  | 16: $\gamma$ -Butyrolactone |                         |
|  | 17: Butyric acid            | (n.i.: not identified)  |



ammonia for neutralization may allow for more precise pH control. This is confirmed by the results of three extraction trials with Extrelut resin on vinegars (Table 5) where fatty acid concentrations were higher in samples neutralized by ammonia.

The concentrations obtained with the Extrelut method were higher for some compounds (alcohol, esters) than those obtained by the liquid-liquid extraction. Both methods may allow the extraction of numerous other substances in addition to those reported here (Fig. 1), some of them identified (not reported for the sake of brevity) and others not yet identified.

Provided the sample is saturated with NaCl before analysis, extraction by passage through Extrelut resin, due to its simplicity and quantitative accuracy, may therefore be considered a valid alternative to long, laborious extractions with solvents.

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