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CONNAISSANCE AROMATIQUE DES CÉPAGES  
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QUALITÉ DES VINS

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

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The aromatic fraction of musts and wines, as in other fermented beverages, plays a role of primary sensory importance, despite the fact that it represents one of the minor components, quantitatively.

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 (Günata et al., 1985; Gelsomini et al., 1990; Di Stefano, 1991; Gianotti and Di Stefano, 1991).

Hydrophobic resins, XAD-2 (Rohm and Haas) and C18, 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 must 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.

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. 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.

Unlike the lipophilic resins Amberlite XAD-2 and C18, 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 chromatograph determinations were made for each sample.

### Experimental application

In order to determine the response factors, 300 µL of the internal standard solution (B) were added to 300 µL of standard solution (A) and the volume was adjusted to 3 mL with CH<sub>2</sub>Cl<sub>2</sub> before analysis.

To evaluate the concentration yield, 100 µL of standard solution (A) were diluted to 60 mL with CH<sub>2</sub>Cl<sub>2</sub>. After concentration under N<sub>2</sub>, the sample (0.5 to 1 mL) was quantitatively transferred to a 3 mL volumetric flask containing 100 µL of standard solution (B) and brought to volume with

CH<sub>2</sub>Cl<sub>2</sub>. The determination was repeated five times.

To evaluate the extraction yield, 300 µL 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<sub>2</sub>, brought to 3 mL with CH<sub>2</sub>Cl<sub>2</sub> after addition of 300 µL 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). 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%, except for C3 and C4 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 (tab. 1) and in particular for samples treated with NaCl extraction yields of 2,3-butanediol and fatty acids were higher than for sample not treated. The concentration of esters, on the contrary, is negatively affected by saturation of the sample with NaCl, showing a

yield decreased of as much as 60% for ethyl piruvate. 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 red wine Chianti by Gelsomini et al. (1990). Only the acid concentrations were considerably lower for both "Asti" and red wine in samples extracted by Extrelut resin (tab. 2). C3, C4 and iso-C5 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 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 and 2), 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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	T		
	NT	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
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
Ethyl pyruvate	82.1	20.6	1
Hexyl acetate + 3-Hydroxy-2-butanone	84.6	62	8.8
3-Methyl-1-pentanol	92	96	1.9
Ethyl lactate	90.5	100.1	0.8
1-Hexanol	90.4	94.1	3
trans-3-Hexen-1-ol	90.2	97.8	2.5
3-Ethoxy-1-propanol	89.2	99.6	4.1
cis-3-Hexen-1-ol	91.6	100.4	1.5
Ethyl octanoate	71.4	66.4	1
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
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	3
$\gamma$ -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
Citronellol	94.8	93.3	2
Phenethyl acetate + Nerol	92.7	88	6.5
Hexanoic acid	92.2	102	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
Octanoic acid	0.8	75.4	2.2
Ethyl cinnamate	90.5	90.4	0.4
4-Ethylphenol	96.8	98.4	3.6

Table 1 : 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)

*CU*

	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
trans-3-Hexen-1-ol			31	36	27	28
3-Ethoxy-1-propanol	102	119	89	86	199	173
cis-3-Hexen-1-ol			95	87	147	119
cis-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 2 :** Concentration ( $\mu\text{g/L}$ ) of the volatile fraction components of a vinegar, an "Asti" and a red wine, determined by extraction with solvent and Extrelut resin

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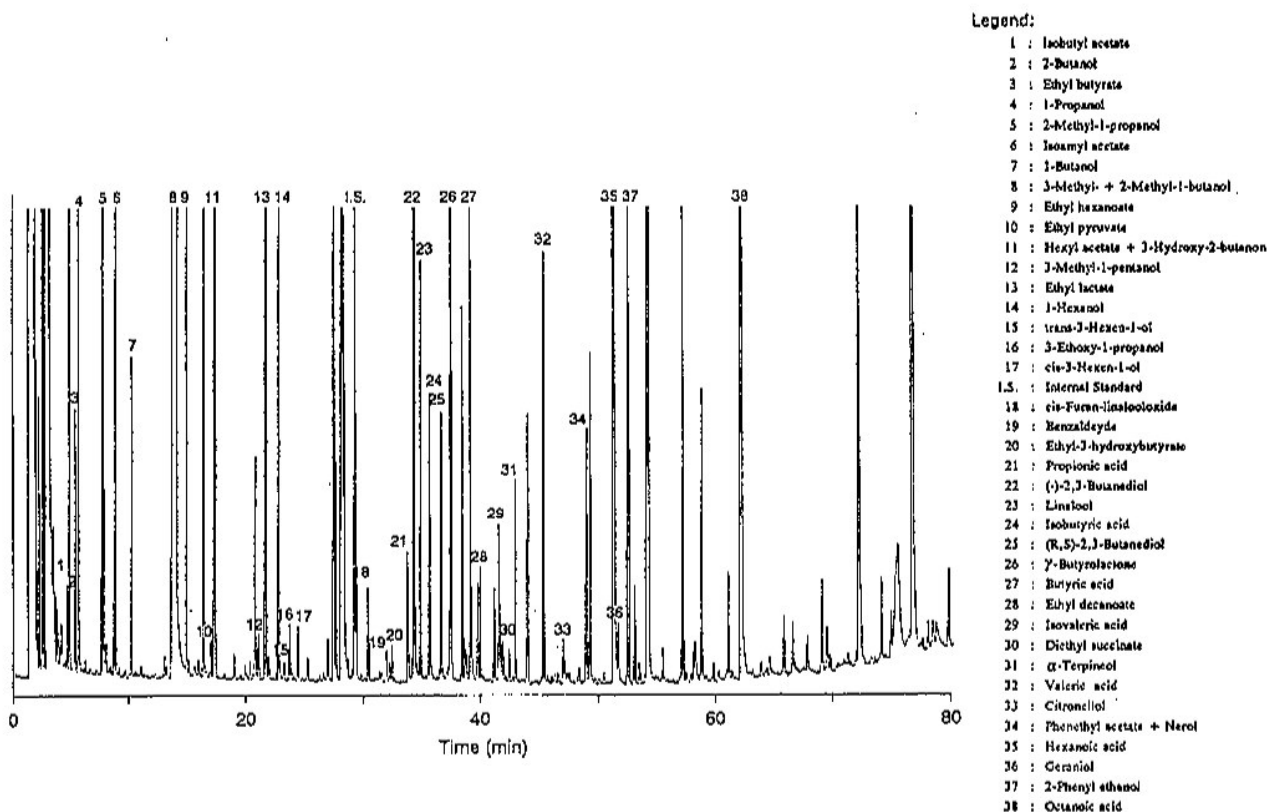


Figure 1 : Gas chromatogram of the aromatic fraction of an "Asti" extracted with Extrelut resin

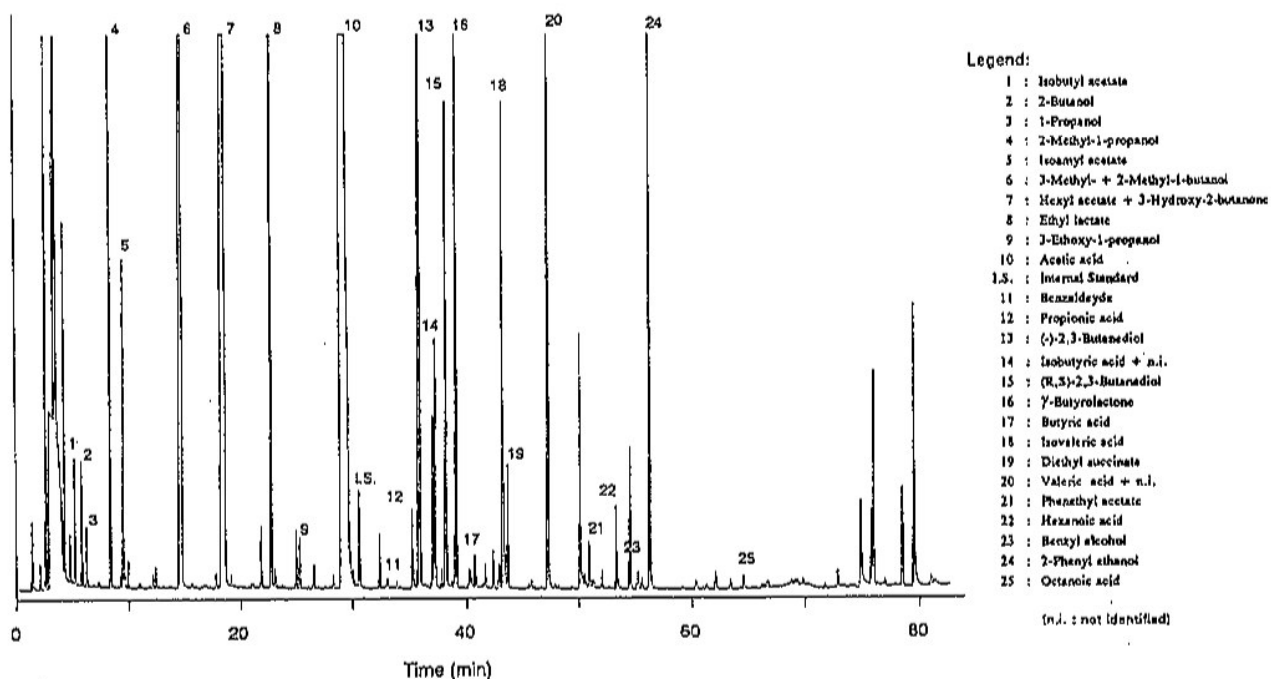


Figure 2 : Gas chromatogram of the aromatic fraction of a white vinegar neutralized with ammonia and extracted with Extrelut resin

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