

# Identification of Volatile Constituents in Wines from *Vitis vinifera* var Vidadillo and Sensory Contribution of the Different Wine Flavour Fractions

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**Abstract:** A wine made with vidadillo, a very old Spanish variety, has been continuously extracted and the extract further fractionated in a silica gel column with successive portions of hexane/dichloromethane/ether mixtures following an elutropic order. Ten fractions showing different odours were isolated and analysed by HRGC-MS and their olfactory and flavour threshold determined via triangular tests. More than 200 compounds belonging to different biosynthetic pathways have been identified. It is important to note the large number of  $\gamma$ - and  $\delta$ -lactones and of compounds derived from the phenylpropanoid metabolism present. From a sensory point of view, all the fractions showed aroma values below 100, meaning that there are no clear impact compounds. Three fractions scored aroma values higher than 10, and therefore play an important role in the perception of the different flavour and aroma nuances of the wine. Compounds that may play an outstanding role are fusel alcohol acetates in the fruity notes,  $\gamma$ - and  $\delta$ -lactones in the peach notes, and some unidentified compounds in the liquorice notes. The silica gel prefractionation method leads to a class separation, and compounds showing similar aromatic notes are eluted together, allowing an easy interpretation of the role of the different groups of flavours.

**Key words:** wine flavour, aroma, HRGC-MS, sensory analysis.

## INTRODUCTION

The *Vitis vinifera* cultivar vidadillo, has been grown in certain areas of Spain for several centuries (Asso 1798; Pacottet 1918). However, specific technology has never been applied to make wine from these grapes, since most of the wine produced is sold in bulk. Only in recent years has it been found that the wine made with this variety possesses a particular flavour quite different from the other wines made in this area, being distinguished by an intense black-fruit bouquet, and a peachy, creamy and truffle-like flavour. From the technological and scientific points of view the necessity of having a comprehensive knowledge of its flavour composition emerges.

Although wine flavour composition has been studied extensively and a large amount of information dealing with the wine's qualitative and quantitative flavour composition is available (Maarse and Visscher 1989; Schreier 1979), it is not easy, at present, to correlate this composition with the observed flavour (Etievant 1991). Therefore, a simultaneous chemical and sensorial approach should be used. There are traditionally two different approaches to achieve this goal: the determination of flavour or aroma thresholds of individual compounds (Meilgaard 1975), or the determination of aroma values via aroma extract dilution analysis (Acree *et al* 1984; Schieberle and Grosch 1987; Miranda-López *et al* 1992; Linssen *et al* 1993), but both methods have several drawbacks. The former is time consuming, very expensive, requires the previous identification of all the important flavours, and is limited by the availability

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of pure standards. The latter often leads to different conclusions depending on the data treatment chosen (Abbot *et al* 1993) and cannot give a realistic idea of the behaviour of the compounds in the original matrix. A more pragmatic approach is that proposed by MacNamarra *et al* (1990) for the study of whisky flavour composition. It consists of the fractionation of flavour components in a distillation system, and the further study of the sensory properties of the isolated fractions over the original matrix. However, the distillation of wine is more problematic than that of a spirit, and as a result, wine extract fractionation over silica gel has been chosen. The results of this research are presented in this paper.

## MATERIAL AND METHODS

### Reagents

All the reagents used were of analytical grade. Solvents were purchased from Merck (Darmstadt, Germany). All the solvents used were previously redistilled in a 60 cm Vigreux column. Most of the reference samples were purchased from Aldrich (Wisconsin, USA), others were obtained from a local flavouring company.

### Wine

Red wine was made from hand-harvested vidadillo grapes (10000 kg) grown in Almonacid de la Sierra (Zaragoza, Spain) and was fermented in the cellars owned by Covinca in the same village following standard wine processing in inert tanks. Grapes were harvested in 1991, and after fermentation the clarified wine was stored in glass until analysis in 1992. No contact with wood took place during this process. The wine sample was a young wine having the characteristic vidadillo bouquet.

### Flavour extraction

Several wine samples of 500 ml were added to 210 g of ammonium sulphate and were then continuously extracted with 150 ml of Freon 11 and 24 h. The aroma extract was then concentrated in a 60 cm Vigreux column to 20 ml, and then to 1 ml in a three-ball Snyder column.

### Flavour fractionation

A similar method to the one described by Etievant and Bayonoue (1983) was used to fractionate the aroma extract. Ten different flavour fractions showing different odours were collected and then concentrated under N<sub>2</sub>

at ambient temperature to a final volume of 50 µl. The fractions to be analysed were injected directly into the chromatograph, the fractions to be sensory tested were put in the top of a 200 mg silica minicolumn and the solvent was evaporated by passing a weak stream of N<sub>2</sub> through it. No significant losses were detected in this process. The volatiles were recovered with 0.5 ml of ethanol. A second 0.5 ml of ethanol was added to test if the recovery was total.

### Gas chromatography

An HP 5890 series II gas chromatograph fitted with an automatic sampler HP7673A was used. Column, Supelcowax 10, 60 m × 0.32 mm; film thickness, 0.5 µm; carrier gas, H<sub>2</sub> at 3.0 ml min<sup>-1</sup>; injection splitless (3 min), split gas flow 27 ml min<sup>-1</sup>; initial temp, 40°C held for 5 min, then 2°C min<sup>-1</sup> gradient until 200°C; detector, FID; injected volume, 1 µl.

### Gas chromatography-mass spectrometry

An HP 5890 Series II chromatograph fitted with a 5971A electronic impact detector was used. The signal was registered and processed with MS Chemstation Series II software fitted with the Wiley MS library. Conditions: injected volume, 2 µl; mode splitless (3 min); carrier, He at 50 kPa; initial temperature 40°C, held for 5 min and then raised at 2°C min<sup>-1</sup> to 220°C; column, the same as before.

### Sensory analysis

Triangular tests were performed in the following way: 2, 20, 60 and 200 µl of each of the 10 ethanol-extracted fractions were added separately to 200 ml volumes of a neutral red wine (ethanol 12.5%; pH 3.3), corresponding to dilutions of 1:100, 1:10, 1:3.3 and 1:1 (v/v), respectively. Similarly 2, 20, 60 and 200 µl of the 10 ethanol extracted fractions from a blank fractionation were added to 200 ml volumes of the same red wine to act as blank samples.

The tasting panel was composed by seven experienced individuals. Each panellist was presented with one 25 ml cup of each of the flavoured wine samples and two 25 ml cups of each of the blank samples. Each test was performed twice. Only the aroma was judged.

## RESULTS AND DISCUSSION

The chemical composition of the wine extract can be seen in Table 1. The extract was composed of more than 350 substances, and of these, 205 were identified. The references cited in Table 1 are not exhaustive but

**TABLE 1**  
Composition of the flavour extract of the vidadillo red wine

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
<i>Carbonylic compounds</i>					
2,3-Butendione	a	0, 4	1	2	Maarse and Visscher (1989)
3-Methyl-3-buten-2-one	a	6	0	3	Schreier <i>et al</i> (1976)
4-Methylpentanone	a	5	0	2	Tressl <i>et al</i> (1978)
2,3-Pentanodione	a	0, 4	1	2	Maarse and Visscher (1989)
1(1-Ciclohexen-1-il)-ethanone	c	9	0	1	
2-Heptanone	a	5	0	2	Etievant and Bayonove (1983)
3-Octanone	a	4	0	1	Boidron and Ribéreau-Gayon (1967)
2-Octanone	a	5	0	1	Schreier <i>et al</i> (1980)
3-Hydroxybutanone	a	0, 8	1	3	Shinohara <i>et al</i> (1979)
Cyclohexanone	c	6	0	tr	
4-Hydroxy-4-methylpentanone	a	8-10	0	3	Ter Heide <i>et al</i> (1978)
2-Nonanone	a	4, 5	0	2	Schreier <i>et al</i> (1980)
Decanal	a	7	0	1	Bertuccioli and Viani (1976)
Furfural	a	6-8	0	1	Maarse and Visscher (1989)
2-Decanone	a	4, 5	0	1	Schreier <i>et al</i> (1980)
Undecanal	a	8	0	1	Drawert and Rapp (1968)
3-Methyl-2-cyclopentenone	b	7	0	tr	
Benzaldehyde	a	0, 4	1	3	Maarse and Visscher (1989)
2-Methyltetrahydro-2-thiophenone	a	5	0	4	Schreier <i>et al</i> (1980)
5-Methyl-2-furfural	a	6	0	1	Schreier <i>et al</i> (1976)
2-Undecanone	a	4	0	1	Drawert and Rapp (1968)
p-Tolualdehyde	c	2, 3	0	1	
Phenylacetaldehyde	a	0, 3	tr	1	Maarse and Visscher (1989)
Acetophenone	a	3-5	0	3	Bertuccioli and Viani (1976)
4-Hydroxybenzaldehyde	b	10	0	tr	Laub and Woller (1974)
2-Dodecanone	a	3, 4	0	1	Ter Heide <i>et al</i> (1978)
4-Hydroxymethylhexanone	c	9, 10	0	1	
m-Methylacetophenone	c	4, 5	0	1	
p-Methylacetophenone	a	4, 5	0	2	Ter Beide <i>et al</i> (1978)
Unknown 2-ketone		4	0	tr	
5-Methyl-5-phenyl-2-hexanone	b	5	0	tr	
Ethyl pyroglutamate	c	8	0	tr	Güntert <i>et al</i> (1986)
Ftalide	b	8	0	tr	Schreier and Drawert (1974)
5-Hydroxymethylfurfural	a	8	0	tr	Amerine and Roessler (1976)

TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
Diphenylethanone	a	4	0	2	Dourtoglou <i>et al</i> (1994)
3-Methylumbeliferone	b	6	0	1	
ms similar to 5-hydroxymethyl-2-furfural	c	10	0	tr	
Vanillin	a	0, 6, 7	tr	1	Schreier <i>et al</i> (1976)
Acetovanillone	a	0, 6, 7	1	2	Etievant (1981)
Propiovanillone	a	0, 6, 7	1	2	Etievant (1981)
<i>Esters</i>					
Ethyl propanoate	a	0	1		Maarse and Visscher (1989)
Isobutyl acetate	a	0, 4	1	3	Maarse and Visscher (1989)
Ethyl butyrate	a	0, 3, 4	2	4	Maarse and Visscher (1989)
Ethyl isovalerate	a	3	0	2	Maarse and Visscher (1989)
Butyl acetate	a	4	0	2	Maarse and Visscher (1989)
Isoamyl acetate	a	0, 4	3	5	Maarse and Visscher (1989)
Ethyl valerate	a	3	0	1	Maarse and Visscher (1989)
Ethyl-2-butenolate	a	4, 5	0	1	Schreier <i>et al</i> (1976)
Amyl acetate	a	4		tr	Maarse and Visscher (1989)
Methyl hexanoate	a	3	0	1	Etievant and Bayonove (1983)
Cyclohexyl acetate	b	4	0	tr	Schreier <i>et al</i> (1976)
Ethyl hexanoate	a	0, 3	3	5	Maarse and Visscher (1989)
Hexyl acetate	a	0, 4	2	3	Maarse and Visscher (1989)
Ethyl c-3-hexenoate	a	4	0	tr	Schreier <i>et al</i> (1980)
<i>l</i> -3-Hexenyl formate	b	4	0	1	Schreier <i>et al</i> (1976)
<i>c</i> -3-Hexenyl acetate	a	4	0	1	Schreier <i>et al</i> (1980)
Ethyl heptanoate	a	3	0	1	Schreier <i>et al</i> (1980)
Ethyl <i>l</i> -2-hexenoate	a	4	0	2	Schreier <i>et al</i> (1980)
Ethyl lactate	a	0	3		Maarse and Visscher (1989)
Heptyl acetate	a	4	0	2	Schreier <i>et al</i> (1976)
Methyl octanoate	a	3	0	2	Schreier and Drawert (1974)
Ethyl octanoate	a	0, 3	4	5	Maarse and Visscher (1989)
Isoamyl hexanoate	a	3	0	1	Schreier <i>et al</i> (1980)
Ethyl 4-methyl 2-Hydroxypentanoate	b	6	0	tr	Schreier and Drawert (1974)
Ethyl 3-hydroxybutyrate	a	0, 8, 9	1	2	Schreier <i>et al</i> (1980)
Ethyl nonanoate	a	3	0	1	Schreier <i>et al</i> (1980)
Ethyl 2-hydroxy 4-methylbutyrate	a	6, 7	0	2	Schreier <i>et al</i> (1980)
Methyl decanoate	a	3	0	1	Schreier and Drawert (1974)
Ethyl 2-furancarboxylate	a	5	0	2	Schreier <i>et al</i> (1980)
Methyl ethyl succinate	a	5	0	tr	Schreier <i>et al</i> (1980)
Ethyl decanoate	a	0, 3	3	4	Maarse and Visscher (1989)

TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
Diethyl malonate	a	6	0	1	Schreier and Drawert (1974)
Isoamyl octanoate	a	3	0	1	Schreier <i>et al</i> (1980)
1,3-Propanediol diacetate	b	0, 7	1	2	Schreier and Drawert (1974)
Citronellil acetate	b	4	0	1	
Ethyl benzoate	a	3	0	1	Schreier <i>et al</i> (1980)
Diethyl succinate	a	0, 6	4	5	Maarse and Visscher (1989)
Ethyl 3-hydroxyhexanoate	c	8	0	1	Borea Carnacini <i>et al</i> (1980)
Ethyl 9-decenoate	a	3, 4	0	3	Schreier and Drawert (1974)
c-3-Hexenyl hexanoate	c	8	0	1	
Benzyl acetate	a	5	0	1	Schreier and Drawert (1974)
1,3-Propanediol monoacetate	a	0, 7	1	3	Schreier and Drawert (1974)
Diethyl glutarate	a	6, 7	0	1	Schreier <i>et al</i> (1980)
Ethyl phenylacetate	a	0, 4, 5	tr	2	Schreier <i>et al</i> (1980)
Phenylethyl acetate	a	0, 4, 5	2	3	Maarse and Visscher (1989)
Ethyl nicotinate	a	8	0	tr	Tressl <i>et al</i> (1978)
Ethyl laurate	a	0, 3	tr	2	Maarse and Visscher (1989)
Phenylethyl propanoate	a	5	0	1	Tressl <i>et al</i> (1978)
Ethyl isobutyl succinate	a	6	0	1	Schreier <i>et al</i> (1980)
Phenylethyl butyrate	a	5	0	tr	Schreier and Drawert (1974)
Isobutyl laurate	b	3	0	tr	Connel and Strauss (1974)
Ethyl isoamyl succinate	b	6	0	1	Schreier <i>et al</i> (1980)
Diethyl malate	a	8	0	2	Schreier <i>et al</i> (1980)
Ethyl 3-Hydroxyoctanoate	a	7	0	2	Borea Carnacini <i>et al</i> (1980)
Ethyl phenylethylsuccinate	c	10	0	1	Baumes <i>et al</i> (1986)
Diethyl azeleate	b	6	0	1	Ter Heide <i>et al</i> (1978)
Ethyl palmitate	a	3	0	1	Maarse and Visscher (1989)
Ethyl cinnamate	c	3	0	tr	Schreier <i>et al</i> (1976)
Diethyl ftalate	a	0, 6, 7	tr	1	Schreier and Drawert (1974)
Diisobutyl ftalate	b	0, 6, 7	tr	1	
<i>Alcohols and phenols</i>					
2-Methyl-2-butanol	a	10	0	2	Drawert and Rapp (1968)
1-Propanol	a	0, 10	4	5	Maarse and Visscher (1989)
Methyl butenol	c	9	0	1	Liebich <i>et al</i> (1970)
Isobutanol	a	0, 7	5	5	Maarse and Visscher (1989)
2-Methyl-2-pentanol	c	9	0	1	
3-Pentanol	a	6	0	1	Schreier <i>et al</i> (1980)
2-Pentanol	a	5, 6	0	1	Schreier <i>et al</i> (1980)
1-Butanol	a	0, 6	tr	2	Maarse and Visscher (1989)

TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
3-Penten-2-ol	b	6, 7	0	tr	
2-Methoxyethanol	b	6, 7	0	2	
3-Methyl-1-butanol	a	0, 7	5	5	Maarse and Visscher (1989)
2-Methyl-1-butanol	a	0, 7	2	5	Maarse and Visscher (1989)
1-Pentanol	a	0, 7	tr	2	Maarse and Visscher (1989)
2-Methyl-1-pentanol	a	5	0	1	
4-Methyl-1-pentanol	a	0, 6	tr	1	Schreier <i>et al</i> (1980)
3-Methyl-1-pentanol	a	0, 6	1	2	Schreier <i>et al</i> (1980)
1-Hexanol	a	0	4		Maarse and Visscher (1989)
<i>l</i> -3-Hexenol	a	0, 6	1	2	Baumes <i>et al</i> (1986)
3-Ethoxy-1-propanol	a	0, 10	tr	2	Baumes <i>et al</i> (1986)
<i>c</i> -3-Hexenol	a	0	2		Maarse and Visscher (1989)
2-Butoxyethanol	a	8	0	1	
<i>l</i> -2-Hexenol	a	0, 7	tr	1	Maarse and Visscher (1989)
2-Octanol	a	6	0	tr	Schreier <i>et al</i> (1980)
1-Octen-3-ol	a	6	0	2	Schreier <i>et al</i> (1976)
1-Heptanol	a	0, 6	tr	1	Schreier <i>et al</i> (1980)
2-Ethylhexanol	a	0, 6	tr	1	Schreier <i>et al</i> (1980)
<i>c</i> -2-Heptenol	b	0, 7	tr	1	Etievant and Bayonove (1983)
3,3,5-Trimethylhexanol (?)	c	0, 6	tr	2	
<i>L</i> -2,3-Butanediol	a	0, 6	3	4	Maarse and Visscher (1989)
1-Octanol	a	0, 6	1	2	Maarse and Visscher (1989)
<i>meso</i> -2,3-Butanediol	a	0, 6	2	3	Maarse and Visscher (1989)
1-Propoxy-2-propanol	c	0	tr		
1-Nonanol	a	6	0	1	Schreier <i>et al</i> (1980)
Furfural	a	0, 7	tr	1	Maarse and Visscher (1989)
1-Decanol	a	0, 6	tr	1	Schreier <i>et al</i> (1980)
2-Dibutoxyethoxy ethanol	b	10	0	1	
Benzyl alcohol	a	0, 7	1	3	Maarse and Visscher (1989)
$\beta$ -Phenylethylalcohol	a	0, 8	5	5	Maarse and Visscher (1989)
Phenol	a	5	0	1	Etievant (1981)
Phenylpropyl alcohol	a	7	0	2	Boidron and Ribèreau-Gayon (1967)
<i>p</i> -Cresol	a	6	0	1	Etievant (1981)
<i>m</i> -Cresol	a	7	0	2	Etievant (1981)
Tetradecanol	a	6	0	1	Drawert and Rapp (1968)
<i>p</i> -Ethylphenol	a	0, 7	tr	1	Etievant (1981)
Methoxytyrosol	c	8	0	tr	

TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
<i>Terpenols</i>					
<i>trans</i> -Furanlinalool oxide	b	7	0	1	Etievant and Bayonove (1983)
Nerol oxide	c	7	0	tr	Etievant and Bayonove (1983)
Camphor	c	5	0	1	Dourtoglou <i>et al</i> (1994)
Linalool	a	6	0	1	Etievant and Bayonove (1983)
$\alpha$ -Terpineol	a	6	0	1	Etievant and Bayonove (1983)
Carvone	c	4, 5	0	1	Dourtoglou <i>et al</i> (1994)
<i>trans</i> -Pyranlinalooloxide	c	9	0	1	Etievant and Bayonove (1983)
$\beta$ -Citronelol	a	6	0	1	Etievant and Bayonove (1983)
Nerol	a	6	0	1	Etievant and Bayonove (1983)
Geraniol	a	6	0	1	Etievant and Bayonove (1983)
Dihydrofarnesol	c	6	0	tr	
Farnesol	a	6	0	2	Dourtoglou <i>et al</i> (1994)
<i>Lactones</i>					
$\gamma$ -Pentalactone	a	0, 7	tr	1	Schreier <i>et al</i> (1980)
$\gamma$ -Butyrolactone	a	7, 8	0	3	Maarse and Visscher (1989)
$\gamma$ -Hexalactone	a	0, 6	tr	1	Etievant and Bayonove (1983)
4-Hydroxy 4-methyl D-pentalactone	c	6	0	tr	
$\gamma$ -Heptalactone	a	6	0	1	Tressl <i>et al</i> (1978)
$\gamma$ -Octalactone	a	0, 6	tr	2	Tressl <i>et al</i> (1978)
$\delta$ -Octalactone	b	7	0	tr	Etievant and Bayonove (1983)
<i>trans</i> -Oak lactone	a	6	0	tr	Schreier <i>et al</i> (1980)
$\gamma$ -Nonalactone	a	0, 6	1	3	Maarse and Visscher (1989)
$\delta$ -Nonalactone	b	6, 7	0	1	Maarse and Visscher (1989)
Unknown 5-hydroxy $\delta$ -lactone		6	0	tr	
$\gamma$ -Decalactone	b	6, 7	0	3	Etievant and Bayonove (1983)
$\delta$ -Dodecalactone	c	6	0	1	Liebich <i>et al</i> (1970)
<i>Acids</i>					
Acetic acid	a	0	1		Maarse and Visscher (1989)
Propanoic acid	a	0	1		Maarse and Visscher (1989)
Isobutyric acid	a	0	2		Maarse and Visscher (1989)
Butyric acid	a	0	1		Maarse and Visscher (1989)

TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
Isovaleric acid	a	0	2		Maarse and Visscher (1989)
Hexanoic acid	a	0	3		Maarse and Visscher (1989)
Octanoic acid	a	0	3		Maarse and Visscher (1989)
4-Hexyl-2,5-dihydro-2,5-dioxo-3-Furanacetic acid	c	0	2		
Decanoic acid	a	0	1		Maarse and Visscher (1989)
Dodecanoic acid	a	0	2		Maarse and Visscher (1989)
<i>Amides</i>					
<i>N,N</i> -Dimethyl formamide	a	6	0	tr	Tressl <i>et al</i> (1978)
<i>N,N</i> -Diethyl formamide	c	2	0	tr	
Isobutyl acetamide	a	0	1		Etievant and Bayonove (1983)
Isoamyl acetamide	a	0	3		Etievant and Bayonove (1983)
Phenylethyl acetamide	a	0	2		Etievant and Bayonove (1983)
Ethyl <i>N,N</i> -dimethylcarbamate	a	6		tr	Joe <i>et al</i> (1977)
Betaine	c	2	0	tr	Dubois and Simand (1976)
<i>Hydrocarbons</i>					
Bencene	a	1	0	tr	Etievant and Bayonove (1983)
Toluene	a	1	0	tr	Schreier <i>et al</i> (1976)
<i>p</i> -Dimethyl bencene	a	1	0	1	
Xylene	a	4	0	1	Etievant and Bayonove (1983)
Estyrene	a	3	0	tr	Etievant and Bayonove (1983)
<i>trans</i> -Vitispirane	b	0, 2	tr	2	Simpson and Miller (1984)
<i>cis</i> -Vitispirane	b	2	0	1	Simpson and Miller (1984)
Naphthalene	a	8	0	1	Simpson and Miller (1984)
1,1,6-Trimethyl	b	2	0	1	Simpson and Miller (1984)
Trihydronaphthalene					
2-Methyl naphthalene	a	9	0	tr	Simpson and Miller (1984)
<i>Acetals</i>					
2,4,5-Trimetil-1,3-dioxolane isomer	c	10	0	2	Simpson and Miller (1984)
2,4-Dimethyl-5-ethyl-1,3-dioxolane	c	10	0	tr	Schreier <i>et al</i> (1976)
1,1-Diethoxybutane	c	5	0	1	Etievant and Bayonove (1983)
1,1-Diethoxypentane	b	5	0	2	Etievant and Bayonove (1983)
Unknown 1,3-dioxolane		9	0	tr	
Unknown 1,3-dioxolane		9	0	tr	



TABLE 1—Continued

Compound	Reliability <sup>a</sup>	Fraction	Total abundance <sup>b</sup>	Abundance in the fraction <sup>c</sup>	Reference
Isobutyl oxirane isomer		9	0	1	
1-Ethoxy-1-hexoxy ethane	b	9	0	1	Simpson and Miller (1984)
<i>Miscellaneous</i>					
2-Butylthiophene	c	10	0	tr	
3-Methyl-thio-1-propanol	a	0	tr		Maarse and Visscher (1989)
Unknown thiazole		8		tr	
Benzothiazol	c	9		2	Schreier <i>et al</i> (1976)
Ethyl vainillin ether	c	7	0	tr	
Escopoletin isomer	c	6	0	1	

<sup>a</sup> Reliability: a—Identification based on coincidence of gas chromatographic retention and mass spectrometric data with those of the pure compound available in the lab. b—The pure compound was not available, but gas chromatographic retention and mass spectrometric data were coincident with those reported in literature. c—Identification based solely on coincidence of mass spectrometric data.

<sup>b</sup> Total abundance is abundance in the main extract: tr, peak height smaller than  $15 \times$  background noise (sub-ppb range); 1, peak height between 15 and 100 background noise (ppb range); 2, peak height between 100 and 1000 background noise (10–100 ppb range); 3, peak height between 1000 and 10000 background noise (100–1000 ppb range); 4, peak height bigger than 10000 background noise (1–20 ppm range); 5, overload peak (more than 20 ppm); o, occluded.

<sup>c</sup> Abundance in the fraction (when the compound was present in more than one fraction, only data referent to the fraction with the greatest amount are given): tr, peak height smaller than  $15 \times$  background noise (sub-0.1 ppb range); 1, peak height between 15 and 100 background noise (0.1–1 ppb range); 2, peak height between 100 and 1000 background noise (1–10 ppb range); 3, peak height between 1000 and 10000 background noise (10–100 ppb range); 4, peak height bigger than 10000 background noise (more than 100 ppb); 5, overload peak (ppm range).

merely serve as examples. It should be noted that some of them (Liebich *et al* 1970; Laub and Woller 1974; Ter Heide *et al* 1978; Tress *et al* 1978) do not refer to wine, but to beer, rum or other alcoholic beverages.

The data show the complexity of the wine flavour and the numerous metabolic pathways that are undergone in its formation. Fusel alcohols, the major compounds are formed in the amino acid metabolism; the aliphatic higher alcohols may be formed from related aldehydes by reduction during the yeast fermentation (Nykänen 1986); fatty acids and their esters are formed by the yeast lipid metabolism. Of the aldehydes and ketones found in the wine, the two vicinal diketones and acetoin arise from the amino acid catabolism, and the aliphatic members from the anabolic metabolism (Nykänen 1986). The furfural derivatives come from carbohydrate dehydration and cyclation (Tressl 1979), while the aromatic ketones and alcohols are mainly derivatives of the phenylpropanoid metabolism in the grapes or are originated by the yeast from several grape precursors (Flanzy *et al* 1987). Terpenes are products linked to the carotenoid anabolism in the grape, while some hydrocarbons such as TDN and vitispirane are considered to be carotenoid degradation products, also from the grape (Strauss *et al* 1987). Although some lactones have been found in grapes stored in anaerobic

conditions under CO<sub>2</sub> (Dourtoglou *et al* 1994), they are mainly formed by the yeasts along their amino acid and keto acid metabolism (Tressl and Albrecht 1986).

Several points should be remarked. Although several terpenes have been identified, among them two sesquiterpenes, their quantity is very small and they probably do not exert any influence over the aroma in accordance with the neutral character of the grape variety. Therefore, this grape variety can be considered as non-terpene dependent, although the quantification of these compounds may be useful for classification purposes (Schreier 1979).

By contrast, the amount of products derived from the phenylpropanoid metabolism is very important, both from a qualitative and a quantitative point of view. In fact, numerous compounds were identified which used to be considered oak derived products, particularly acetovanillone, propiovanillone, escopoletin and other aromatic ketones. Since the wine had no contact with wood these compounds must be formed along different stages of the fermentation. The technology used in the wine making prevented an excessive grape crushing, and a significant quantity of the grapes passed to the fermentation tanks uncrushed. Thus, it is likely that carbonic maceration took place to some extent, conditions in which it has been postulated (Dourtoglou *et al* 1994)

that the phenylpropanoid metabolism is reactivated. It is not clear if these compounds arise from the hydrolysis of some glycosidic precursors already present in the grape (Williams *et al* 1989), if they are generated in the first stages of the wine making, or if they are the consequence of the cinnamoyl esterase activity of the yeast (Thurston and Tubbs 1980), bacteria (Dubois 1983) or some other exogenous enzymes (Dugelay *et al* 1993).

Another compound generally considered as a wood product is the *cis*-oak lactone that has been identified although in a very small amount. The same compound was found by Schreier and Drawert (1974) also in non-wood-aged German wines, and then it is clear that a biosynthetic pathway should exist in yeast. The lactone composition is an important question as well. It has been claimed that lactones could be formed as artefacts from the chromatographic injection of their corresponding  $\gamma$ - or  $\delta$ -hydroxy acids (Maga 1976), or even by its silica catalysed cyclisation. Even though this may happen to some extent, there are several reasons to think that those phenomena are not the main source of the detected lactones but most of them are natural products of this wine. First, the most concentrated lactones can be easily detected in the non fractionated extract; second, the flavour extract of the wine showed a clear lactone odour which was predominant in one of the fractions; and third, in the fractionation lactones are well separated from their precursors and if they were originated in the chromatographic injection, they should appear with the acidic compounds in the ether fraction. All of the homologous series of the  $\gamma$ -lactones from  $\gamma$ -butirolactone to  $\gamma$ -decalactone are reported, plus the cited oak lactone and several  $\delta$ -lactones.

Finally, it should be note that none of the aromatic ketones arising from the carotenoid degradation pathway,  $\beta$ -damascenone and  $\beta$ -ionone, could be found in the flavour fractions. This is an important fact, as these compounds are largely responsible for the charac-

teristic flavour and aroma of some red wines grown in the same area, showing typical red-fruit and violet-like odour (Ferreira *et al* 1993).

### Sensory analysis

As can be seen in Table 1, the elution of compounds from the silica gel column follows a typical compound class separation pattern. Ethyl esters are eluted in fraction 3 and are well separated from fusel alcohol acetates that are eluted in fraction 4. Lactones are mainly eluted in fraction 6, aromatic ketones in fractions 6 and 7, hydroxy esters elute in the last fractions with some phenols and dioxolanes, while alcohols are distributed in fractions 6–10. In the conditions chosen, even terpenols are eluted together despite dissimilarities between their structures. As compounds with the same functional group, differing only in the lengths of their carbonylic chains, very often show similar odours this allows us to have not only a compound class separation but an odour-class separation. The extracts, therefore represent one or several families of compounds and one or several families of odours. After a mild evaporation of the solvent (see methods), the fractions can be reconstituted in a matrix that can be added to the original product. In this way it is possible to screen the relative importance of the families of flavourants even though those compounds could not be identified.

Results concerning the sensory analysis performed with the different fractions are presented in Table 2. None of the fractions could be identified in the wine when added as 100-fold dilution. That is to say, if we define the aroma values of the fractions as the quotient between their original concentration and the maximum dilution in which they can be perceived, there is no one fraction with an aroma value over 100. This means that there are no clear impact compounds in this wine, and that its aroma and flavour must be considered as an

TABLE 2  
Sensory data of the different flavour fractions<sup>a</sup>

Fraction	Odour	Dil 1/100	Dil 1/10	Dil 1/3	Dil 1/1	Odourous substances present
1	None	NA	NA	NA	NA	Hydrocarbons
2	Oily	NS	NS	NS	NS	Hydrocarbons
3	Fruity, apple, sweet	NS	NS	**	****	Ethyl esters
4	Fruity, banana, sweet	NS	*	***	****	Fusel alcohol acetates
5	Fruity, blackberry, exotic	NS	NS	**	***	Phenones
6	Cocoa, peach	NS	***	****	****	Lactones, vanillin
7	Flowery, creamy	NS	NS	***	****	Vanillin, <i>g</i> -butyro-lactone, alcohols
8	Flowery, phenolic	NS	NS	**	***	Hydroxy esters, phenyl ethanol
9	Tea, liquorice	NS	NS	*	**	Dioxolanes
10	Liquorice, medicine	NS	*	***	****	Phenols, dioxolanes, unknowns

<sup>a</sup> NA, not analysed; NS not significant; \*\*\*\* Significant ( $P < 0.001$ ), \*\*\* Significant ( $P < 0.01$ ); \*\* Significant ( $P < 0.05$ ); \* Significant ( $P < 0.1$ )

overall contribution of a large number of compounds, this result is in agreement with the conclusion of Schreier (1980). However, there are three fractions whose estimated aroma values are greater than 10, thus contributing significantly to the observed flavour and aroma nuances of the wine. These fractions are the 4th, 6th and 10th.

Fraction 4 is mainly composed of the fusel alcohol acetates and may play some role in the fruity aroma of this wine. Although from a statistical point of view we were not able to find correlations between these compounds and the fruity notes of some young Spanish red wines (Ferreira *et al* 1995), some other authors have demonstrated that isoamyl acetate, the major fusel alcohol acetate, plays a key role in the aroma of some red grape varieties (Van Wyk *et al* 1979).

Fraction 6 is a very complex fraction from a chemical point of view. It is composed of terpene alcohols, fusel alcohols, aromatic aldehydes and  $\delta$ - and  $\gamma$ -lactones. However, from a sensory point of view, the lactones seem to dominate, as their aromatic descriptors are the same as the ones of the fraction. These compounds have low sensory detection thresholds and Nakamura *et al* (1988) stated that they may play a role in some red wines. On the other hand, Etievant *et al* (1983), found that their aroma thresholds in a sweet white wine were too high to consider that they may play some important role in its aroma, but this could be due to the effect that sugar can play on aromatic perception. Anyway, results in Table 2 show that this fraction is very important in the aroma of the vidadillo wine. We postulate that lactones in this fraction are responsible for the peach note observed in the wine. The role played by the aromatic aldehydes found in this fraction and in the following one is not clear, but they could be responsible for the vanilla-like note in the wine. These hypothesis will have to be confirmed through descriptive analysis.

Fraction 10 is composed of several dioxolanes, 5-hydroxy-methyl furfural, and several phenols. In this case the descriptors are very difficult to assign to only one compound or to a class of compounds, but phenols are usually described as medicinal. 5-Hydroxy-methyl-furfural is unlikely to be an important contributor to the wine aroma due to its high flavour threshold reported by Meilgaard *et al* (1970). The flavour properties of dioxolanes are still not very clear, but they do not seem to be important flavourings, according to data presented by Etievant (1979). Several important flavourings in this fraction remain unidentified and further research must be performed, particularly with regard to the liquorice note displayed in this fraction, and with the sensory note that this fraction engenders in the wine.

## CONCLUSIONS

The flavour fractions dilution analysis reported in this work seems to be a very promising tool for character-

ising the flavour of the wines. The fractionation of the aroma extract in an adsorption chromatographic column is attractive since it leads to a class compounds separation. Consequently, compounds with similar chemical structures and a high likelihood of similar aromatic properties are eluted in the same fraction, allowing the study of their additive effects.

The flavour of the young wines made with *Vitis vinifera* var vidadillo is the balance of a large number of compounds. However, fusel alcohols acetates,  $\delta$ - and  $\gamma$ -lactones and some unidentified compounds may play an outstanding role, contributing to some of the characteristic nuances of this wine.

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