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Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry

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Abstract

The odorants released by mild acid hydrolysis from an Amberlite XAD-2 odourless fraction of flavour precursors isolated from Tempranillo and Grenache grapes were studied in an aroma extract concentration analysis (AECA) experiment. Ninety-eight odour-active regions were detected in the AECA. The most important odorants released were unsaturated fatty acid derivatives, such as hexanal, octanol, 1-octen-3-one, E-2-heptenal, E,E-2,4-decadienal, fatty acids and several aliphatic lactones: γ-nona, -deca- and undecalactones, δ-decalactone and ε-dodecalactone (tentative identification). Some shikimic acid derivatives, such as guaiacol, 2-phenylethanol, ethyl dihydrocinnamate, ethyl cinnamate, 2,6-dimethoxyphenol, 4-vinylphenol, isoeugenol, phenyl acetic acid, and vanillin, were also important. By contrast, β-damascenone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and an unknown were the only impact odorants derived from carotenoids, and farnesol and 3,7-dimethyl-1-ene-3,7-diol the only terpenols.

Keywords: GC-olfactometry; AECA; Grapes; Tempranillo; Grenache; Flavour precursor; Glycosides; Aroma

1. Introduction

Vitis vinifera cv. Tempranillo and Grenache are two of the most important Spanish red grape cultivars, and they are, with minor quantities of Graciano grapes, the base of Rioja wines. Rioja is the most emblematic Spanish area in the production of high quality red wines. As neutral grape cultivars, the aroma and flavour of Tempranillo and Grenache grapes are subtle; however, the well matured wines made with Tempranillo develop intense aroma and flavour nuances reminiscent of fruits (blackberry, plum, fig, raisin), spices and wood. Wines made with Grenache can develop kirsch, chocolate, peach and even flowery notes. Some of these nuances are due to compounds directly released from the oak wood in which the wines are traditionally matured; however, there is an important but unknown part of wine aroma that comes from odourless flavour precursors present in the grape.

The presence of these precursors in grapes and other fruits is well documented (Francis, Kassara, Noble, & Williams, 1999; Takeoka et al., 1992; Williams, Sefton, & Francis, 1992; Williams, Sefton, & Wilson, 1989; Winterhalter, 1992; Winterhalter, Knapp, & Straubinger, 1999). Some authors were able to demonstrate that terpenols released from grape precursors play an actual or potential role in the aroma of Muscat wines (Cordonnier & Bayonove, 1974; Ribéreau-Gayon, Boidron, & Terrier, 1975; Williams, Strauss, & Wilson, 1980; Williams, Strauss, Wilson, & Massy Westropp, 1982) or in the formation of some characteristic off-flavours in Riesling wines (Simpson & Miller, 1983; Winterhalter, 1991; Winterhalter, Sefton, & Williams, 1990). In the case of non-floral grape cultivars, a series of investigations have established a connection between the aroma attributes of hydrolyzed flavour precursors from the

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grapes and aroma attributes of their wines (Abbott, Coombe, & Williams, 1991; Francis, Sefton, & Williams, 1992). The work presented by Francis et al. (1999) relating chemical composition of hydrolysates to sensory descriptive analysis of Cabernet Sauvignon and Merlot wines went a step further, and showed that important sensory attributes were correlated with the concentrations of specific components of the hydrolysates. Yet, these relationships did not prove that those chemical compounds were really responsible for the observed sensory attributes, since a previous study of the nature and number of odour molecules present in those hydrolysates was not carried out. The work presented in this paper intends to bridge (in part) this gap by using a gas chromatography-olfactometric (GC-O) technique known as aroma extract concentration analysis (AECA) (Kerscher & Grosch, 1997). The purpose of this paper is to identify and classify the main odour-active chemicals released by mild acid hydrolysis from a fraction of odourless flavour precursors extracted from Tempranillo and Grenache grapes, according to their potential aroma importance and possible biochemical origin.

2. Material and methods

2.1. Harvesting of grapes

Tempranillo and Grenache grapes were hand-harvested at approximately 24° Brix from different vineyards of Cariñena (Zaragoza, Spain) during the 1999 vintage. Grapes were chilled to 10 °C during transportation and stored at −30 °C. Four kg of berries were taken at random from frozen clusters. Berries were crushed in a roller press, maintaining low temperature; juice and skins were separated by means of centrifugation at 100*g* for 30 min.

To simulate glycoside extraction during wine making, the grape skins were suspended in 2 l of a solution of 13% v:v ethanol, pH 7 buffer (NaH_2PO_4/Na_2HPO_4, 0.1 M) for 24 h at 20 °C in the dark. Afterwards the solution was filtered and centrifuged at 100*g* for 10 min.

2.2. Isolation and extraction of hydrolyzed aroma compounds

Glycosidically-bound compounds in the clarified juice or in the solutions containing the extracts from the skins were isolated on Amberlite XAD-2 resin (Supelco, Bellefonte, PA, USA), according to the technique of Günata, Bayonove, Baumes, and Cordonnier (1985). Acid hydrolysis of the bound fraction was performed in a model wine (13% v:v ethanol, 6 g/l tartaric acid, pH 3.2) maintained for 4 weeks at 50 °C. After this period, the solutions were stored at −30 °C prior to analysis.

Aroma compounds from the hydrolysates were extracted with dichloromethane. Aliquots of the hydrolysates were salted out with 0.3 g $(NH_4)_2SO_4$ per ml and extracted with two volumes of dichloromethane (organic-aqueous phase ratio 1:10). The dichloromethane extracts were mixed and concentrated in a micro Kuderna-Danish concentrator (Supelco) fitted to a 3-ball Snyder column, first to a volume of about 1 ml and later, under a N_2 stream, to a final volume equivalent to 500, 1000 or 2000-fold, of the original concentration of the compounds in the grapes.

2.3. HPLC fractionation

The extracts were too complex for unequivocal MS identification of odorants; therefore, they were fractionated by HPLC. An aliquot of the hydrolysate, equivalent to 0.5 l of the initial sample, was injected in a Kromasil-C18 column (25 cm length, 1 cm i.d., 5 μm particle size) according to the method of Ferreira, Hernandez Orte, Escudero, Lopez, and Cacho (1999). Thirty fractions were collected at the column exit. The fractions were diluted with water to adjust their ethanol content to 13% v/v and were then re-extracted with two volumes of dichloromethane (phase ratio 1/10). The extracts were concentrated under a stream of nitrogen to 100 μl and then analyzed by GC-MS and GC-O (only odour-active fractions) in the systems described below.

2.4. Gas chromatography-mass spectrometry conditions

A star 3400CX (Varian, Walnut Creek, CA, USA) gas chromatograph fitted with a Saturn 4 electronic impact MS detector (Varian) was used. The column was a DBWAX 20M type from J&W (Folsom, CA, USA) 60 m × 0.25 mm i.d. and 0.25 μm film thickness. The carrier gas was He at 1.5 ml/min. The injector used was a 1077 split/splitless injector from Varian kept at 250 °C. One μl of the extract (from the samples or from one of the HPLC fractions) was injected in split mode using a 1/10 split ratio. The column was initially at 40 °C and, after 5 min, it was raised to 220 °C at 3 °C/min. A mass range of 35–250 m/z was recorded at 1 scan per s.

The identity of the odorants was determined by comparison of the mass spectra, chromatographic and odour properties of the unknowns with those of pure reference compounds if available.

2.5. Sensory analysis and gas chromatography-olfactometry

Before the AECA, the samples were tested to verify that the aromas released from precursors could change wine sensory properties. A neutral red wine was used as a reference. One litre of this wine was spiked with hydrolysate from the must extracted from 1 kg of grapes

(experiment A) or with hydrolysate from the skins contained in 1 kg of grapes (experiment B). The sensory evaluation was conducted via a duo-trio test to discriminate between the neutral wine and the spiked. The panel was composed of 6 panellists, who were given two coded samples containing the neutral wine and the spiked wine and a reference which, alternately, was the spiked or the neutral wine. The total number of judgments was 12 per experiment.

The gas chromatograph was an 8360 Carlo Erba (Fisons Instruments, Milan, Italy) fitted with a FID detector and an ODO-1 Olfactory Detector Outlet Kit from SGE (Ringwood, Australia) that allowed for simultaneous sniffing of GC effluents. The split ratio between the FID and the olfactometer was 1:1. The column was a DBWAX 20M from J&W 30 m × 0.32 mm i.d. and 0.5 μm film thickness. The carrier gas was H_2 at 3 ml/min. A 1 μl sample was introduced in splitless mode into a split/splitless injector kept at 250 °C. The splitless time was 60 s, and the subsequent split ratio was 1/10. The column was initially at 40 °C and, after 5 min, this was raised to 220 °C at 3 °C/min. Two different extracts were used for the olfactometric analysis of each cultivar. The first one contained the volatiles released from precursors extracted from the skins and the second one the volatiles released from precursors extracted from the must. Three different dilutions of each extract were studied (see above), and each dilution of each extract was olfactometrically analyzed by four trained judges. The odorants, which could be perceived in the most diluted extracts (those concentrated 500 times), were arbitrarily assigned a 4-flavour dilution factor (FD); the odorants that could be detected in the 1000-fold-concentrated extracts were assigned a 2-FD value, and the odorants that could only be detected in the 2000-fold-concentrated extracts received a 1-FD value.

3. Results and discussion

3.1. General

The hydrolysates of Tempranillo grape flavour precursor fractions had intense aromas, reminiscent of dry fruits, such as fig, grape or quince, and of herbs. In the case of Grenache hydrolysates, aroma was reminiscent of ripe fruit, flowery, phenolic and balsamic. The addition of these hydrolysates to a neutral wine exerted a significant effect on the aroma of the wine, as Table 1 reveals. This result agrees with other authors' (Abbott et al., 1991; Francis et al., 1992) findings of the contributions of aroma compounds released by mild acid hydrolysis of grape flavour precursors to the wine bouquet and justifies the subsequent olfactometric analysis.

Results of the AECA are presented in Table 2. The table shows the 98 odorants that were detected by the sniffers in the olfactometric experiments, ordered first according to their biochemical origin and second by their retention index in the chromatographic column. Thirty-seven odorants were found to have maximum flavour dilution (FD) factors in at least one of the extracts, and 30 out of these 37 could be identified. Overall, 42 aroma compounds were identified. Odorants released from grape flavour precursors can be classified according to their biochemical origin in the following categories: unsaturated fatty acid derivatives, shikimic acid derivatives, nor-isoprenoids, terpenes, thiols, and compounds of miscellaneous origin.

Table 1
Significance of duo–trio tests results for must and skin hydrolysates ($n = 12$)

	Must	Skin
Tempranillo	$p = 0.005$	$p = 0.001$
Grenache	$p = 0.001$	$p = 0.001$

Table 3 shows the identity of a further 24 volatile compounds identified in the extract but which were not detected in the AECA experiment. Apart from these compounds and those presented in Table 2, the extracts contained large amounts of odourless high molecular weight alcohols that were not identified. Therefore, from a quantitative point of view, the extracts were composed of alcohols, ethyl acetate, acetic acid, benzyl alcohol, 1-hexanol, syringaldehyde and hexadecanoic acid and its ethyl ester. The presence of benzyl alcohol as a major compound was in agreement with the results of Lopez Tamames, Carro Marino, Gunata, Sapis, Baumes, and Bayonove (1997). However, the major contribution to the odour potential of these hydrolysates was not due to compounds present in highest concentration but to minor although powerful odorants. The precise molecules from which compounds in Tables 2 and 3 are derived are not known because no analysis of the precursor fraction was carried out before the hydrolysis. Some molecules could be aglycones in the grapes, but some others could be the result of different hydrolytic and oxidation processes taking place during the hydrolysis. These problems will have to be addressed in further research. The present research focusses on the odorants released by a technique of hydrolysis that resembles the process of wine maturation (Francis et al., 1992).

3.2. Unsaturated fatty acids derivatives

At least 19 compounds listed in Table 2 belong to this class. In the first place, there were several aliphatic compounds with 6 or 8 carbon atoms, such as hexanal, octanol and hexanoic and octanoic acids. Hexanal and octanol were present in wines at very low concentrations but were not detected in wine olfactometry. Hexanal was reported in an AECA list of aroma compounds

Table 2
Main odorants found in juice and skin hydrolysates

RI	Odour description	Identity	Tempranillo juice	Tempranillo skins	Grenache juice	Grenache skins	Abundance[f]
	Lipid derivatives						
1094	Fruity, crushed grapes	Hexanal[a]	4	2	4	4	++
1221	Green	E-2-Hexenal[a]			1		+
899	Fruity, sweet	Ethyl acetate[a]	4	4	1		++
1248	Flowery, fruity	Ethyl hexanoate[a]			2		++
1309	Damp, humid	1-Octen-3-one[d]	4	4	4	1	Trace
1336	Beer	E-2-Heptenal[b]		2	1	1	+
1382	Green, cypress	Z-3-Hexen-1-ol[a]	1	1	1		++
1573	Pleasant, sweet	1-Octanol[a]	2	2	4	1	++
1646	Green	E-2-Decenal[b]			1		+
1743	Fatty, sweet	Pentanoic acid[a]		4			+
1825	Fatty	E,E-2,4-Decadienal[a]	1		4	2	++
1860	Fatty,cheese	Hexanoic acid[a]	4	4	4	4	+
2056	Lactone-like, peach	γ-Nonalactone[a]	1		1		+
2075	Fatty	Octanoic acid[a]		4			+
2177	Coconut	γ-Decalactone[a]	1	4	1		Trace
2220	Coconut, lactone-like	δ-Decalactone[d]	4	4		2	Trace
2247	Spice, lactone-like	γ-Undecalactone[a]	1	1	4	1	Trace
2264	Lactone-like, coconut	ε-Dodecalactone[b]	4	4	4	4	Trace
2276	Aromatic herbs	Ethyl hexadecanoate[a]		1			+++
2371	Lactone-like	γ-Dodecalactone[d]				1	Trace
2398	Dry wood, oak	(Z)-6-Dodecene-7-lactone[d]	2	2	1		Trace
	Shikimic derivatives						
1550	Dry plastic, synthetic	Benzaldehyde[a]	2	2			+
1879	Phenolic, wood	Guaiacol[a]	4	2	4	4	+
1900	Pleasant, faint	Ethyl dihydrocinnamate[a]	4	1			+
1932	Flowery, dry fruits	2-Phenylethanol[a]			4	2	++
1969	Phenolic	Phenol[a]			1		+
2112	Unpleasant, machine	m-Cresol[a]			1	1	+
2156	White flowers	Ethyl cinnamate[a]	1	1	4	2	Trace
2183	Alcoholic	2-Phenoxyethanol[a]	1				++
2192	Phenolic, flowery	Eugenol[a]	2				+
2209	Shoe polish	4-Ethylphenol[a]		1	1		Trace
2213	Phenolic, synthetic	4-Vinylguaiacol[a]	1	1	2	2	+++
2303	Phenolic, synthetic	2,6-Dimethoxyphenol[a]	4	4	4	4	+
2376	Tangerine, wood	Isoeugenol[a]			4	1	Trace
2425	Almond shell	4-Vinylphenol[a]	4	2	1	1	+
2457	Aromatic, flowery	Benzoic acid[a]			2	1	+
2575	Honey, flowery	Phenylacetic acid[a]	4	4	1	1	+
2589	Vanilla	Vanillin[a]	4	4	4	4	++
2653	Dry herbs	Methyl vanillate[a]				1	+
2668	Flowery	Ethyl vanillate[a]			1	1	++
2685	Tangerine, flowery	Acetovanillone[a]				1	+
	Norisoprenoids						
1751	Pleasant, flowery	TDN[b]		4			+
1834	Dry plum	β-Damascenone[a]	4	1	4	4	++
1891	Flowery	Unknown norisoprenoid[d,e]	1	1	4	2	Trace
	Terpenes						
1660	Sweet, cookie, bun	Citronellyl acetate[b]	1		1		+
1721	Pleasant	α-Terpineol[a]			1		++
1983	Aromatic, flowery	3,7-Dimethyloct-1-ene-3,7-diol[c]	2	2	4	4	+
2382	Flowery	Farnesol[a]	2	4	1		++
	Thiols						
1741	Mango, anise	3-Mercaptohexyl acetate[d]			1		Trace
1866	Lemon, green	3-Mercaptohexanol[a]	1	1		1	Trace

Table 2 (*continued*)

RI	Odour description	Identity	Tempranillo juice	Tempranillo skins	Grenache juice	Grenache skins	Abundance[f]
	Miscellaneous						
1074	Fruity	Ethyl 2-methylbutyrate[a]			4		+
1466	Vinegar	Acetic acid[a]	4	4	4	4	++
1499	Pleasant, soap	2-Ethyl-1-hexanol[a]	1	4			++
1535	Sweet, fruity	2,5-Dimethyl-3(2H)-furanone[d]			2	2	Trace
1556	Blue cheese	Propanoic acid[a]	1				+
1629	Toasty, burnt	2-Acetylpyrazine[d]	2		2	2	Trace
2225	Liquorice, celery	Sotolon[d]	2	2	4	4	Trace
2257	Sweet, honey	Methyl antranilate[d]	2	2	2		Trace
	Unknown odorants						
977	Lactic	n.i.		4			
981	Butter, strawberry	n.i.	2	2			
983	Orange, sweet	n.i.	1				
1060	Fruity	n.i.	4		2		
1086	Fruity, ester	n.i.				1	
1113	Fruity	n.i.			4		
1116	Rancid	n.i.	1				
1164	Apple	n.i.		1			
1241	Gas	n.i.		2			
1298	Perfume	n.i.		1			
1388	Pleasant, sweet	n.i.			1	1	
1390	Synthetic, metallic	n.i.	1				
1395	Flowery	n.i.			1		
1407	Dust, pollen, jasmine	n.i.			1		
1432	Flowery, pleasant	n.i.	1				
1443	Toast bread, ashen	n.i.	1	1			
1517	Chlorine, damp	n.i.	4	2	4	4	
1600	Green, flowery	n.i.			1	1	
1625	Chlorine	n.i.	4	2	4		
1694	Rubber	n.i.	2	2			
1734	Strange	n.i.	4				
1783	Rubber	n.i.	1				
1872	Rubber	n.i.	2				
1896	Sour, green tomatoes	n.i.		1			
2015	Citric	n.i.			1		
2026	Dry fruit, violets	n.i.		4	4	4	
2064	Vanilla	n.i.			1		
2086	Dry fruit	n.i.			1		
2137	Phenolic	n.i.		1			
2240	Synthetic, chemical,	n.i.		1			
2315	Wine, fruity	n.i.			1		
2334	Cherry candy, liquor	n.i.	1		1		
2423	Fatty, rancid	n.i.				1	
2517	Fresh	n.i.			1	1	
2527	Machine, unpleasant	n.i.				1	
2531	Clove	n.i.		1			
2556	Toasty	n.i.		1			
2607	Phenolic	n.i.				1	
2765	Dry herbs	n.i.				1	
2810	Flowery	n.i.				1	

Gas chromatographic retention data, olfactory description, chemical identity and flavour dilution values obtained in AECA experiments.

RI: Gas chromatographic retention data (as Retention Index in a DBWAX 20M column).

[a] Reliability: Identification based on coincidence of gas chromatographic retention and mass spectrometric data with those of the pure compound available in the lab.

[b] Reliability: The pure compound was not available, but gas chromatographic retention and mass spectrometric data were coincident with those reported in literature.

[c] Reliability: Identification based solely on coincidence of mass spectrometric data.

[d] Reliability: Identification based in gas chromatographic retention data and odour quality. The compound did not produce any clear signal in the mass spectrometer because of its low concentration; n.i.: not identified.

[e] Reliability: Mass spectrum available in Ferreira, Lopez, Escudero, and Cacho (1998).

[f] Average abundance: Trace: (concentration below 0.1 μg/kg); +: small peak (concentration between 0.1 and 1 μg/kg); ++: peak of intermediate size (concentration between 1 and 20 μg/kg); +++: large peak (concentration above 20 μg/kg).

Table 3
Other volatile compounds found in the extracts from the hydrolyzed precursors but not detected in the AECA experiment

RI	Compound	Reliability	Abundance[c]
1138	1-Butanol	[a]	+++
1230	Isoamyl alcohol	[a]	++
1398	1-Hexanol	[a]	+++
1428	Linalool oxide	[b]	Trace
1446	Ethyl octanoate	[a]	+
1470	Furfural	[a]	+
1523	Theaspirane	[b]	Trace
1527	Vitispirane	[a]	+
1565	Linalool	[a]	+
1648	Ethyl decanoate	[a]	+
1710	γ-Hexalactone	[a]	+
1827	Phenylethyl acetate	[a]	Trace
1843	Ethyl dodecanoate	[a]	+
1857	Geraniol	[a]	Trace
1860	α-Ionone	[a]	Trace
1905	Benzyl alcohol	[a]	+++
1952	β-Ionone	[a]	Trace
2048	4-Ethylguaiacol	[a]	Trace
2470	Benzophenone	[a]	++
2993	Hexadecanoic acid	[a]	+++
3008	Zingerone	[a]	+
3408	Syringaldehyde	[a]	+++
3415	4-Hydroxymethyl benzoate	[a]	++
3447	4-Hydroxybenzaldehyde	[a]	++

RI: Gas chromatographic retention data (as Retention Index in a DBWAX 20M column).
[a] Reliability: Identification based on coincidence of gas chromatographic retention and mass spectrometric data with those of the pure compound available in the lab.
[b] Reliability: The pure compound was not available, but gas chromatographic retention and mass spectrometric data were coincident with those reported in literature.
[c] Abundance: Trace: weak, albeit distinguishable, ms signal (corresponding to concentration below 0.1 μg/kg); +: small peak (concentration between 0.1 and 1 μg/kg); ++: peak of intermediate size (concentration between 1 and 20 μg/kg); +++: large peak (concentration above 20 μg/kg).

from must (Kotseridis & Baumes, 2000). Two homologues of hexanal, octanal and decanal, were reported by different researchers as wine odour-active compounds (Guth, 1997; Lopez, Ortin, Perez-Trujillo, Cacho, & Ferreira, 2003; Kotseridis & Baumes, 2000). Hexanoic and octanoic acids are important wine odorants, but they are mostly formed as by-products of yeast metabolism and the contribution of grape precursors is residual from a quantitative point of view. In second place was 1-octen-3-one, which is a powerful odorant with very low perception thresholds. It has been previously tentatively identified by Baek, Cadwallader, Marroquin, and Silva (1997) and by Lopez et al. (2003) as a free volatile in Muscadine grape juice and in white wines, but it has not been previously reported as component of the grape precursor fraction. Its high FD suggests that it could play a role in wine aroma. A third group consisted of four unsaturated aldehydes, E-2-hexenal, E-2-heptenal, E-2-decenal and E,E-2,4-decadienal. Only E-2-hexenal has been previously detected in wine by olfactometry (Chisholm, Guiher, & Zaczkiewicz, 1995). In the fourth group there were several aliphatic lactones, such as γ-nona-, -deca-, -undeca-, and dodecalactones, δ-decalactone and ε-dodecalactone (the identification of the latter is merely tentative, as shown in Table 2). γ-Nonalactone was identified by Miranda-Lopez, Libbey, Watson, and McDaniel (1992) as an important odorant of Pinot noir wines. This last compound, and γ and δ decalactones, have also been detected in Spanish wines (Aznar, Lopez, Cacho, & Ferreira, 2001; Ferreira et al., 1998; Ferreira, Ortin, Escudero, Lopez, & Cacho, 2002; Lopez, Ferreira, Hernandez, & Cacho, 1999; Lopez et al., 2003). γ-Undecalactone has been reported in aged Champagne (Escudero, Charpentier, & Etiévant, 1999), and it has recently been unequivocally identified and quantified in different wines (Ferreira, Jarauta, Ortega, & Cacho, 2004).

As stated above, the precise genesis of all these compounds is not clear. Some could be genuine aglycones synthesized by the grapes from fatty acids (Albrecht, Heidlas, Schwarz, & Tressl, 1992) and further released by simple hydrolysis. Some others could be formed, however, during sample treatment by the degradation of residual fatty acids present in the flavour precursor fraction. Unsaturated fatty acid derivatives have not been previously reported as important grape

flavour precursors, although some researchers have included some of them under the headings "alcohols" or "volatile acids" (Cabaroglu, Canbas, Baumes, Bayonove, Lepoutre, & Gunata, 1997) or "aliphatics" (Francis et al., 1999). Results presented here, however, suggested that these compounds could be precursors, directly or indirectly, of important aroma-active compounds from wine. It should be noted, in addition, that the levels in wine of several compounds belonging to this group, such as γ-nona and γ-hexalactone, depend on the variety of grape from which the wine has been made (Ferreira, Lopez, & Cacho, 2000).

3.3. Shikimic acid derivatives

3.3.1. General

This was the second most important group of odorants in hydrolysates. At least three different subgroups can be found: volatile phenols, vanillin and related compounds and the rest.

3.3.2. Volatile phenols

There were 9 volatile phenols among the identified odorants, and three of them, guaiacol, 2,6-dimethoxyphenol, 4-vinylphenol and isoeugenol, had maximum FD values. All of them are important odorants of red wines (Ferreira, Aznar, Lopez, & Cacho, 2001; Ferreira et al., 2000; Kotseridis & Baumes, 2000). Guaiacol has been previously reported in flavour precursor fractions of Syrah grapes (Bureau, Baumes, & Razungles, 2000), 2,6-dimethoxyphenol in flavour precursor fractions of Merlot and Cabernet Sauvignon grapes (Francis et al., 1999) and 4-vinylphenol in precursors from Chardonnay juices (Sefton, Francis, & Williams, 1993). Isoeugenol has not been previously reported as an aglycone. Other phenols, such as eugenol, phenol, *m*-cresol, 4-vinylguaiacol and 4-ethylphenol, showed less intense FD values, although the similarities of their odours may make them important odour contributors. In addition, several authors (Ferreira et al., 2001; Ferreira et al., 2000) have reported that they are important odorants in both young and wood-aged red wines, and, probably, the flavour precursor fraction is the main source of volatile phenols in young wines. The odorant 4-ethylphenol has not been previously reported as component of a hydrolysate, and the presence of this compound in wine is usually associated with microbial spoilage (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). However, results presented here suggest that some part derives directly from precursors present in the grape.

3.3.3. Vanillin and related odorants

Vanillin reached a maximum FD factor in our AECA experiment, both in juice and skin samples. It is a well known constituent of flavour precursor fractions (Williams et al., 1989). Its odour threshold is quite high, due to its low volatility, but its characteristic flavour could influence aroma by retronasal perception (Larsson & Larsson, 1997). Methyl and ethyl vanillate and acetovanillone showed less intense FD, and were only detected in Grenache hydrolysates. Recently, acetovanillone has been related to the *honey* attribute in red wines by sensory descriptive analysis (Francis et al., 1999).

3.3.4. Other shikimic acid-derivatives

Other shikimic acid-derivatives detected in the olfactometric experiment were 2-phenylethanol, 2-phenoxyethanol, benzaldehyde, benzoic acid, phenyl acetic acid, ethyl dihydrocinnamate, and ethyl cinnamate. Among these, 2-phenylethanol, phenylacetic acid and ethyl dihydrocinnamate reached maximum FD factors. Ethyl dihydrocinnamate has been detected in wine by GCO (Lopez et al., 1999; Moio & Etievant, 1995) and several authors have shown that it can be present in wine at concentrations above its perception threshold (Aubry, Etievant, Ginies, & Henry, 1997; De Freitas, Ramalho, Azevedo, & Macedo, 1999; Ferreira et al., 2000). In this case, the contribution of the flavour precursor fraction could be important. 2-Phenylethanol and phenyl acetic acid have been detected in wine by GCO (Kotseridis & Baumes, 2000; Aznar et al., 2001) but, although their presence in grapes and in precursor hydrolysates has been previously reported (Schreier, Drawert, & Junker, 1976; Sefton et al., 1993), most of both compounds is produced during fermentation.

3.4. Terpenes

Farnesol shows a maximum FD factor in Tempranillo skins; it has not been reported in any olfactometric study of wines. 3,7-Dimethyloct-1-ene-3,7-diol reaches maximum FD in Grenache grapes. This compound has been previously reported as a component of grape hydrolysates (Williams et al., 1982) but it has not been described previously as an odour-active molecule in Tempranillo or Grenache red wine. Only two other monoterpenes, citronellyl acetate and α-terpineol, were identified in the AECA. The small number of terpenes is in agreement with the non-floral character of these grapes.

3.5. Norisoprenoids

Only three nor-isoprenoids, β-damascenone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and an unknown at RI1891 (mass spectra similar to vitispirane) have been found in the olfactometric experiment. β-Damascenone is a well-known component of many grape glycosidic fractions (Braell, Acree, Butts, & Zhou, 1986; Francis et al., 1999; Marais, Van Wyk, & Rapp, 1992; Sefton et al., 1993). The norisoprenoid TDN is known as a contributor to the kerosene-like character of some Riesling wines (Simpson & Miller, 1983), and it

has recently been related to the *honey* attribute in wines (Francis et al., 1999). In addition, both β-damascenone and the unknown at RI1891 have been reported as odour active components of red young wines (Ferreira et al., 1998; Lopez et al., 1999). Remarkably, two potent norisoprenoid odorants namely α-and β-ionones, have not been found in the olfactometric experiment, in agreement with the results presented in the aforementioned work by Francis et al. (1999).

3.6. Sulfur-containing compounds

The presence of 3-mercaptohexanol and 3-mercaptohexyl acetate (tentative identification) in the AECA list is in agreement with the outstanding role that the former has been found to play in the aroma of Grenache rosé wines (Ferreira et al., 2002). Both compounds originate from S-cysteine or S-gluthationyl conjugates presented in must (Tominaga, Peyrot des Gachons, & Dubourdieu, 1998), and are normal components of matured wines made with Tempranillo and Grenache grapes (Aznar et al., 2001).

3.7. Miscellaneous compounds

Noteworthy is the presence of sotolon (tentative identification) in Table 2, since this is the first time that it has been reported as a component of healthy grapes. Another interesting compound that has been tentatively identified is methyl anthranilate, important component of some non-*Vitis vinifera* grapes (Rapp & Versini, 1996).

4. Conclusions

This study presents results from the first olfactometric experiment performed on the flavour precursor hydrolysates of a grape. The study reveals the presence of a large number of odorants, some of which are well known wine flavour components. Notably, most of these odorants are minor compounds, present at very low concentrations. The most relevant contributions of Tempranillo and Grenache flavour precursors to the aroma of wine were from volatile phenols and unsaturated fatty acid derivatives, together with β-damascenone, vanillin, and ethyl dihydrocinnamate. The absence of some nor-isoprenoids and of monoterpenes was remarkable. The actual ability of some of these odorants to influence wine aroma will have to be confirmed by further experiments.

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