

Glycosidic aroma compounds of some Portuguese grape cultivars

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Abstract: In order to evaluate the potential aroma of some Portuguese grapes, several glycosidically bound aroma compounds of ten grape varieties grown in Évora, Alentejo, Portugal, were released, identified and quantified using gas chromatography–mass spectrophotometry. White varieties were Arinto, Perrum, Rabo-de-Ovelha, Roupeiro and Antão Vaz. Red varieties were Trincadeira, Aragonez, Tinta Caiada, Moreto and Castelão. Grapes of Roupeiro and Trincadeira from five other vines in the Alentejo region were also studied. For each variety pulp and skin were analyzed separately. The compounds produced by enzymatic hydrolysis of glycoside precursors extracted from pulps and skins of these grapes were similar to the ones appearing in the so-called ‘aromatic’ varieties; however, the amounts found were significantly lower. These results seem to show the neutral characteristic of these grapes to indicate that each variety has a distinctive profile regarding compounds from the linalool class. The ratios of *trans*:*cis* forms of some linalool compounds remain the same in all samples of Roupeiro and Trincadeira, regardless their origin. In Trincadeira grapes no linalool was detected. Skins are always richer than pulps. The acid hydrolysis of norisoprenoid aglycons produced highly odorant compounds previously described in grape varieties. Although some differences between varieties could be detected, the red grapes Aragonez and Castelão are richer in vitispiranes while Moreto, Trincadeira and Tinta Caiada are richer in actinidols; in white grapes, only Roupeiro and Perrum show detectable amounts of vitispiranes. These results need further investigation and analysis to be considered as a statement and should be confirmed in an extended study.

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INTRODUCTION

All cultivars of *Vitis vinifera* studied up until now show differences in the composition of secondary metabolites such as terpene alcohols, norisoprenoids and benzenoids sufficiently great to justify their use in varietal characterization.¹

The typical flavour of wines is mainly due to volatile compounds that proceed from the grapes where they are in free volatile form or in bound form, usually as glycosides. In most cases these glycosides accumulate in the fruit and can be found in higher amounts than in the free forms.²

Chemical analyses of volatile secondary metabolites of wine grapes are increasingly employed to understand the influence of grape variety on wine flavour. While volatile metabolites are found in low concentrations in grapes, they usually accumulate in much higher concentrations as bound forms, mainly as flavourless glycoconjugates, which can be an additional reservoir of flavour, releasing further volatile compounds via mild acid hydrolysis that takes place naturally during vinification and storage of wines.³ The analysis of volatiles released from flavourless glycoconjugates is a valuable strategy for flavour studies

on fruits that contain low concentrations of free aroma compounds.⁴

According to the quantities of free and bound forms of varietal compounds, grapes can be classified as neutral or aromatic, the last having higher levels of terpenic compounds. Neutral grapes also have terpenic compounds and, like the aromatic ones, we can split them into classes of the same biosynthetic activities. Aroma compounds identified in grapes have been found to differ in quantity with the variety and to pass more or less unchanged into the wine aroma, thus being considered for variety classification and/or characterization. The growing area only marginally influences the characteristic varietal composition of the monoterpene compounds in the various grape varieties.⁵ Agronomic and climatic aspects have greater influence on the amounts rather than in the type of the aroma compounds present in a grape variety.

Terpenic compounds found in grape cultivars may be grouped into classes having structures similar to linalool and geraniol. Cyclic compounds are related to nerol. Compounds of the linalool class include isomers of furan and pyran oxides of

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linalool, *E*-3,7-dimethyl-3,7-dihydroxi-1,5-octadiene 3,7-dimethyl-3,7-dihydroxi-1-octene, and *E*- and *Z*-2,6-dimethyl-2,7-octadien-1,6-diol, while compounds of the geraniol class include geraniol, citronellol, geranic acid, 3,7-dimethyl-1,7-octadiol, and *Z*-3,7-dimethyl-2-en-1,7-octandiol *E*-3,7-dimethyl-2-en-1,7-octandiol. Nerol, α -terpineol, *Z*-3,7-dimethyl-2-en-1,7-octandiol and *p*-menthene-7,8-diol may be grouped into a nerol class.¹ All these compounds are present in grapes in free and glycosidically conjugated forms.^{6,7} The bound fraction of the terpenic compounds is usually higher than the free fraction,⁸ even though the glycosidically conjugated forms of the monoterpenes make no direct contribution to the aroma of grapes.⁹

Although the origin of norisoprenoids is yet to be clarified, recent results¹⁰ support the hypothesis that C₁₃ glycosylated norisoprenoids in grape berries derive exclusively from carotenoid. The relation of the norisoprenoid content to xanthophyll degradation was also considered since xanthophylls decrease during ripening while the norisoprenoid content increases.¹ C₁₃-Norisoprenoids are considered as those that contribute mostly to the aroma of wines made with non-aromatic grapes.

There are many compounds already identified upon enzymatic hydrolysis of glycoside compounds extracted from berries; most show a megastigma ring: 3-hydroxymegastigma-5,8-dien-7-one, 9-hydroxymegastigma-4,7-dien-3-one and 6,9-dihydroxymegastigma-4,7-dien-3-one. By chemical hydrolysis at pH 3 and 100 °C norisoprenoids produce volatile compounds with a small odour threshold, namely damascenone, Riesling acetal, vitispirane isomers, actinidol isomers and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene).¹¹

Knowledge of the distribution of glycosidically bound compounds between skins and pulp in grapes used for winemaking offers a valuable guide in applying skin contact, press conditions and pomace contact time to optimize wine aroma.¹² In fact it is known that although monoterpenes are the compounds responsible for part of the aroma in wine, the so-called PVT (potentially volatile monoterpenes) constitute the major fraction of monoterpenes in wines and as such they are odourless. When released, or if released, however, their aroma thresholds vary from 100–130 $\mu\text{g L}^{-1}$ to 400–500 $\mu\text{g mL}^{-1}$ or even 3–5 mg mL^{-1} depending on the monoterpene considered. Besides the terpene alcohols, known as C₁₃-norisoprenoids, frequently have attractive sensory qualities combined with low flavour thresholds (the lowest concentrations at which they can be smelt) and have been found to be important aroma constituents of wine; thus their release can be beneficial to the final wine aroma.¹³ The beneficial effect of these practices may, however, be overridden by the deleterious

effects on wine flavour and colour resulting from the simultaneous extraction of bitter and astringent phenolic compounds, which are located primarily in grape skins.

Studies in varietal characterization have been carried out for some white varieties from the north of Portugal^{14,15} as well as for a few Portuguese red varieties.^{16,17}

The present study is a first step in the varietal characterization of the most important grapes of the Alentejo region in the south of Portugal, based on the composition of their glycosidic flavour precursors. Ten varieties – Roupeiro, Arinto, Rabo de Ovelha, Antão Vaz and Perrum for white varieties and Trincadeira, Moreto, Castelão Aragonez and Tinta Caiada for red varieties – all from vines in Évora, were followed during a one-year harvest. Trincadeira and Roupeiro from the other five sub-regions of Alentejo, namely Reguengos, Redondo, Vidigueira, Borba and Portalegre, were also studied in order to evaluate the possible impact of different agonomic and climatic factors on the composition of glycosidic flavour precursors. Because they are usually known as non-floral varieties (neutral) a low concentration of free aroma compounds would be expected. Flavour precursors analysis has been used as a strategy to determine the real flavour potential of these Portuguese varieties, thus giving hints for future technological changes/improvements related to skin and/or pomace contact time as well as the use of enzymes during winemaking to release the bound aroma.

EXPERIMENTAL

Grapes

The grapes used for this work were picked during the 1998 harvest from vines in Évora, Alentejo, Portugal – five white varieties: Roupeiro, Antão Vaz, Perrum, Rabo de Ovelha and Arinto; and five red varieties: Trincadeira, Aragonez, Tinta Caiada, Moreto and Castelão. All these varieties of *Vitis vinifera* L. are recommended in the Alentejo region, and were picked at commercial maturation. Grapes of Roupeiro and Trincadeira varieties were also picked from vines in Portalegre, Borba, Redondo, Reguengos and Vidigueira, the most important sub-regions in Alentejo besides Évora. From each variety only one sample (ca 1000 g) was collected in all cases; grapes within a sample were randomly picked within the whole vine according to a previously proposed scheme.¹⁸

Sample preparation

Aroma compounds were extracted following a previously reported method^{19,20} with modifications. The aroma content in pulps and skins was separately analysed. A sample of 100 berries was peeled. Skins were placed in 20 mL of methanol and frozen until analysis. Pulps were collected in a beaker, 100 mg

Na₂SO₃ was added and the mixture was frozen until further analysis. After being defrozen, skins were homogenized in an Omni-Mixer (Sorvall, Labequip, Ontario, Canada), centrifuged for 15 min at 8000 × g; the liquid phase was recovered. The pellet was washed with water and centrifuged again. This last operation was repeated twice. The liquid phases were assembled until 250 mL of 'skin extract' was obtained. To remove phenolic compounds 1 g of polyvinylpyrrolidone (Sigma, St Louis, MO, USA) was added to the skin extract. After defreezing pulps were also homogenized and centrifuged for 15 min at 8000 × g. The juice was recovered, while the pellet was washed with water and centrifuged twice. The liquids were assembled

until 250 mL extract was obtained. 100 mg of a commercial pectolitic enzyme (Vinozym, Novo Nordisk Ferment Ltd, Dittingen), without glycosidase activity was added, and after 4 h centrifuged. 200 mL of these extracts was eluted through a Sep Pack® Cartridge C18 5 g (Waters, Milford, MA, USA) previously activated with 25 mL of methanol and 50 mL of water in sequence. After elution the cartridge was washed with 50 mL of water followed by 30 mL of dichloromethane. The bound compounds were eluted with 30 mL of methanol. Methanol was eliminated under vacuum and the residue solubilized in 5 mL of a phosphate–citrate buffer pH 5. The glycosidically bound fraction of skins or juice, isolated on

Table 1. Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from red grapes

Compounds (µg kg ⁻¹)	T (E)		M (E)		TC (E)		Az (E)		C (E)	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
C6 compounds										
Hexanol	122.0	113.3	87.9	40.7	106.5	75.6	77.1	60.7	61.8	37.5
<i>trans</i> -3-Hexenol	6.9	9.5	nd	2.2	12.6	2.8	1.7	2.5	nd	1.4
<i>cis</i> -3-Hexenol	21.6	20.2	50.4	30.3	52.1	44.7	28.0	25.6	20.9	16.8
<i>trans</i> -2-Hexenol	33.9	21.1	32.5	8.2	33.3	13.5	9.9	7.0	16.7	10.1
Total	184.4	164.1	170.8	81.4	204.5	136.6	116.7	95.8	99.4	65.8
Terpenes										
<i>trans</i> -Furan linalool oxide	16.1	6.5	14.6	2.6	4.9	6.6	2.2	2.2	15.4	9.7
<i>cis</i> -Furan linalool oxide	12.8	4.7	18.7	3.7	3.6	5.6	6.0	2.6	13.2	9.4
<i>trans</i> -Pyran linalool oxide	33.5	9.5	23.6	7.5	nd	nd	3.4	3.3	12.1	8.8
<i>cis</i> -Pyran linalool oxide	20.2	9.5	34.2	8.2	nd	nd	4.3	3.3	10.9	10.9
Nerol	nd	2.6	nd	3.7	nd	9.0	nd	2.9	nd	2.9
Geraniol	13.3	15.9	46.4	12.0	27.9	29.4	29.3	9.5	39.2	16.3
<i>Z</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	14.9	14.2	38.7	12.1	7.8	nd	10.2	7.8	28.0	7.9
<i>E</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	33.2	21.7	64.2	14.6	18.0	12.4	25.2	17.5	13.2	14.1
<i>E</i> -3,7-Dimethyl-2-en-1,7-octandiol	10.0	11.8	23.6	8.5	9.0	4.4	13.4	9.1	37.6	4.9
Geranic acid	10.0	6.8	16.0	4.2	14.4	4.3	30.5	3.9	52.1	4.0
<i>p</i> -Menthene-7,8-diol	10.8	nd	21.7	23.1	72.2	nd	6.4	nd	9.7	Nd
Total	174.8	103.2	301.7	100.2	157.8	71.7	130.9	62.1	231.4	88.9
Benzenoids										
Benzaldehyde	4.6	4.7	13.0	5.2	36.8	26.4	3.4	2.7	6.7	3.2
Methyl salicylate	13.3	28.8	10.6	18.7	11.7	14.9	2.6	2.2	36.7	71.3
α-Methyl-benzenemethanol	nd	2.1	nd	1.9	nd	5.7	nd	1.9	nd	1.6
Eugenol	nd	7.4	9.4	9.1	6.0	4.2	4.8	6.7	3.5	nd
Benzyl alcohol	378.8	323.3	428.4	238.0	218.4	238.8	230.0	184.9	390.2	203.0
2-Phenylethanol	221.5	160.3	245.3	118.0	149.4	114.7	242.1	116.8	55.9	82.5
4-Hydroxy-3-methoxyphenethanol	33.2	60.8	43.4	40.7	71.3	64.3	44.0	51.8	39.9	8.0
Dihydroconiferyl alcohol	22.4	58.0	18.9	81.4	15.0	17.4	8.0	23.7	8.4	5.9
Total	673.8	645.4	769	513	508.6	486.4	534.9	390.7	541.3	375.5
Phenols										
4-Vinylphenol	16.6	9.3	18.9	75.3	114.9	33.6	100.3	68.8	222.1	28.1
4-Vinylguaicol	18.3	22.3	21.7	113.9	2.4	44.7	5.9	31.1	10.9	41.3
Total	34.9	31.6	40.6	189.2	117.3	78.3	106.2	99.9	233	69.4
Norisoprenoids										
3-Hydroxy-β-damascone ^a	14.3	11.1	55.7	21.8	78.4	35.1	5.0	41.8	22.5	21.2
3-Oxo-α-ionol ^b	18.1	23.5	81.2	27.3	69.0	29.3	46.7	71.4	35.4	7.1
3,9-Dihydroxy-mega-5-ene	nd	35.9	33.0	14.6	34.5	8.9	45.4	53.9	12.9	7.7
Vomifolol ^c	192.6	60.8	219.7	113.9	262.0	133.3	136.1	155.3	282.4	77.8
Total	225	131.3	389.6	177.6	443.9	206.6	233.2	322.4	353.2	113.8

T, Trincadeira; M, Moreto; TC, Tinta Caiada; Az, Aragonez; C, Castelão; (E), Évora.

^a 3-Hydroxymegastigma-5,8-diene-7-one.

^b 9-Hydroxymegastigma-4,7-diene-3-one.

^c 6,9-Dihydroxymegastigma-4,7-diene-3-one.

Table 1A. Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from red grapes

Compounds ($\mu\text{g kg}^{-1}$)	T (P)		T (B)		T (Rd)		T (Rg)		T (V)	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
C6 compounds										
Hexanol	287.6	172.9	67.7	72.5	94.9	99.9	105.2	76.9	277.3	395.8
<i>trans</i> -3-Hexenol	22.2	14.2	1.9	5.1	4.6	8.8	3.9	4.6	5.3	11.3
<i>cis</i> -3-Hexenol	73.7	52.3	23.2	19.6	15.8	10.8	22.4	11.7	27.9	50.1
<i>trans</i> -2-Hexenol	62.0	24.0	22.6	14.4	30.2	9.7	27.5	11.7	21.1	32.8
Total	445.5	263.4	115.4	111.6	145.5	129.2	159.0	104.9	331.6	490.0
Terpenes										
<i>trans</i> -Furan linalool oxide	24.9	25.5	9.7	5.1	7.0	6.2	14.1	8.8	4.9	15.5
<i>cis</i> -Furan linalool oxide	19.9	17.2	5.2	4.0	4.9	4.1	11.4	7.3	6.8	13.1
<i>trans</i> -Pyran linalool oxide	24.9	15.1	5.2	4.5	7.0	6.4	16.5	8.6	6.4	11.2
<i>cis</i> -Pyran linalool oxide	16.4	20.9	4.2	4.6	4.9	6.6	15.7	11.4	9.8	25.1
Nerol	nd	4.3	nd	1.5	nd	1.6	nd	1.1	nd	4.5
Geraniol	39.8	20.0	19.3	8.5	7.0	8.3	9.4	7.7	7.5	30.1
Z-2,6-Dimethyl-2,7-octadiene-1,6-diol	42.1	9.2	25.5	9.0	11.5	6.8	12.3	6.7	21.0	21.6
E-2,6-Dimethyl-2,7-octadiene-1,6-diol	70.2	13.9	50.6	17.7	14.5	9.2	13.1	7.6	37.2	31.7
E-3,7-Dimethyl-2-en-1,7-octandiol	16.4	13.9	18.5	10.9	6.4	8.8	8.5	9.1	24.6	30.7
Geranic acid	70.2	5.5	20.7	3.9	6.4	4.0	7.7	4.6	17.5	10.1
<i>p</i> -Menthene-7-8-diol	39.8	nd	8.8	nd	10.7	nd	12.3	nd	12.6	nd
Total	364.6	145.5	167.7	69.7	80.3	62.0	121.0	72.9	148.3	193.6
Benzenoids										
Benzaldehyde	18.7	6.8	5.5	4.2	12.7	6.0	7.1	4.0	9.8	8.5
Methyl salicylate	39.8	54.2	6.5	16.5	61.2	98.3	11.0	16.0	15.1	109.6
α -Methyl-benzenemethanol	nd	9.9	nd	0.9	nd	1.3	nd	1.6	nd	5.6
Eugenol	12.9	16.6	3.1	5.4	4.3	8.3	2.7	4.8	5.6	18.4
Benzyl alcohol	877.4	366.2	214.1	215.7	309.0	398.1	264.3	215.1	231.5	622.9
2-phenylethanol	596.7	230.8	134.8	104.5	158.2	153.0	213.0	118.0	164.0	330.9
O-movanillic alcohol	238.7	80.0	35.2	25.3	51.2	28.8	54.7	54.2	56.5	165.6
Dihydroconiferyl alcohol	28.1	29.2	7.0	12.2	12.8	12.2	23.1	18.2	14.0	48.8
Total	1812.3	793.7	406.2	384.7	609.4	706.0	575.9	431.9	496.5	1310.3
Phenols										
4-Vinylphenol	233.9	172.3	82.6	19.2	43.9	28.1	310.1	37.6	71.1	77.9
4-Vinylguaicol	11.7	97.9	2.2	20.8	4.3	27.3	6.9	24.2	6.3	177.6
Total	245.6	270.2	84.4	40.0	48.2	55.4	317.0	61.8	77.4	255.5
Norisoprenoids										
3-Hydroxy- β -damascone ^a	49.1	34.8	14.1	17.9	17.9	13.2	21.6	13.8	9.8	65.9
3-Oxo- α -ionol ^b	107.6	53.2	31.2	24.7	23.5	16.8	25.4	17.3	38.6	42.9
3,9-Dihydroxy-mega-5-ene	49.1	39.1	11.4	13.0	21.4	24.3	13.1	17.4	36.5	92.5
Vomifolol ^c	400.1	131.7	151.7	72.8	129.0	71.8	132.1	99.6	164.0	292.0
Total	605.9	258.8	208.4	128.4	191.8	126.1	192.2	148.1	248.9	493.3

T, Trincadeira; (P), Portalegre; (B), Borba; (Rd), Redondo; (Rg), Reguengos; (V), Vidigueira.

^a 3-Hydroxymegastigma-5,8-dien-7-one.^b 9-Hydroxymegastigma-4,7-dien-3-one.^c 6,9-Hihydroxymegastigma-4,7-dien-3-one.

C18 cartridges, was hydrolysed with 200 μL of a commercial glycosidase rich enzyme (pectinol, Genencor International, Roncq, France) at 40 °C for 24 h; 1-heptanol was added as internal standard (40 mg L^{-1}). This mixture, containing the aglycons released by enzymatic hydrolysis, was eluted through a Sep Pack[®] Cartridge C18 1 g previously activated with 5 mL of methanol and 10 mL of water, and the free compounds recovered with 6 mL of dichloromethane. The dichloromethane extract was split in two. One volume, after drying under anhydrous sodium sulfate, was evaporated at ambient temperature and pressure. After concentration the sample was ready for GC/GC-MS analysis.

To the second volume of the dichloromethane extract 10 mL of tartaric buffer at pH 3.0 and 1 g of sodium chloride were added. The mixture was kept in a water bath at 100 °C for 1 h. After cooling the sample (containing the product of chemical hydrolysis of varietal aromatic aglycones) was eluted through a Sep Pack[®] Cartridge C18 (300 mg), previously activated with 2 mL of methanol and 3 mL of water. After washing with 5 mL of water, the volatile compounds were eluted with 5 mL of dichloromethane, dried under anhydrous sodium sulfate and concentrated at ambient temperature and pressure prior to injection on GC/GC-MS system.

Standards chemicals and solvents

All solvents/chemicals and standards were GC or pa grade from Sigma–Aldrich Co. (St Louis, MO, USA).

Chromatographic conditions

The capillary column was an Innovax (J&W Scientific, Palo Alto, CA, USA) 30 m × 0.25 mm d.i. and 0.25 µm d.f. The gas chromatograph was a Hewlett Packard 5890 series II linked online with a selective detector MSD 5970 (Hewlett Packard, Wilmington, DE, USA); injector: 250 °C; interface 230 °C. The oven was programmed from 30 °C for 2 min, then linearly increased at a rate of 30 °C min⁻¹ to 60 °C and then at 2 °C min⁻¹ to 160 °C and 3 °C min⁻¹ to 230 °C, and held for 13 min. Carrier gas was helium: 70 kPa. The injection mode was splitless for 2 min. Acquisition mass range was from 28 to 300 u.m.a.; ionization energy 70 eV. Compounds were identified by comparing retention times and mass spectra with standards, when available, or comparing the retention time and mass spectra with those reported in the literature.^{21–23} Semi-quantitative data were obtained by the ratio of peak height of individual compounds vs. internal standard peak height.

RESULTS

Red varieties

The volatile compounds released by enzymatic hydrolysis from the glycoside precursors in the Portuguese red grape varieties are shown in Table 1. Data show that, for all varieties, skins are richer than pulps.

Geraniol is the most abundant monooxygenated terpenic alcohol. Linalool and α -terpineol were found in amounts lower than 1 µg L⁻¹. Nerol was only quantified in pulps. Di-oxygenated compounds derived from linalool and geraniol are well represented,

the most abundant being the furanic and pyranic oxides of linalool and the two isomers of 8-hydroxylinalool. The *p*-ment-1-ene-7,8-diol, a very important aroma compound that derives from α -terpineol, was found in important amounts in pulps from Tinta Caiada. Moreto variety seems to be the richer in terpenic compounds, followed by Castelão and Trincadeira. In Tinta Caiada the pyranic oxides of linalool were not found in detectable amounts.

The content of norisoprenoid compounds is higher in the Tinta Caiada variety, but all other varieties, except Trincadeira, have amounts greater than 400 µg kg⁻¹.

The compounds produced by chemical hydrolysis of the aglycons obtained by enzymatic hydrolysis of the glycosylated precursors are presented in Table 2. The compounds obtained were easily identified by GC–MS. When these compounds are considered Moreto and Aragonez are the only varieties where pulps are richer than skins. Vitispirane isomers are the most representative compounds in Aragonez, and actinidol isomers in Moreto and Tinta Caiada. Moreto and Aragonez are also the richest varieties in Riesling acetal and TDN. Moreto is the richest variety in β -damascenone.

As far as soil is concerned we studied the same variety (Trincadeira) in eight different sub-regions. These results are presented in Table 1A for enzymatic hydrolysis from the glycosylated precursors from red grapes and in Table 2 for the compounds released by chemical hydrolysis.

For all samples skins are richer than pulps in terpenic and norisoprenoid compounds, once again with the exception of Vidigueira (Table 1A). Although there are some important differences in the amounts of compounds found in these grapes, the aromatic profile is very similar. As for the compounds produced by chemical hydrolysis of the aglycons generated by

Table 2. Compounds obtained by chemical hydrolysis from glycosylated precursors extracted from red grapes

Compounds (µg kg ⁻¹)	T (E)		M (E)		TC (E)		Az (E)		C (E)	
	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin
Vitispiranes	nd	8.7	43.0	51.2	9.6	9.1	59.9	58.8	17.2	25.0
Riesling acetal	0.9	13.5	87.9	20.2	1.5	6.5	28.9	21.7	10.6	15.1
TDN	0.9	23.2	24.7	34.8	4.9	4.8	26.4	7.7	8.1	5.3
β -Damascenone	4.3	6.3	16.5	27.5	9.3	4.3	11.5	3.6	8.6	4.5
Actinidols	9.6	15.9	93.4	82.4	45.4	25.8	19.0	10.9	12.0	7.11

Compounds (µg kg ⁻¹)	T(P)		T (B)		T (Rd)		T (Rg)		T (V)	
	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin
Vitispiranes	10.9	27.1	6.9	6.6	8.7	nd	8.9	nd	8.4	9.9
Riesling acetal	27.4	10.2	7.1	7.2	14.9	2.3	8.9	7.1	6.9	5.3
TDN	5.5	25.9	7.7	4.4	7.5	nd	1.6	2.9	6.1	8.0
β -Damascenone	18.3	16.6	5.3	3.7	4.2	4.1	4.1	3.3	7.6	15.2
Actinidols	21.9	21.5	12.4	11.1	15.4	9.9	6.8	12.3	4.6	22.4

T, Trincadeira; M, Moreto; TC, Tinta Caiada; Az, Aragonez; C, Castelão; E, Évora; P, Portalegre; B, Borba; Rd, Redondo; Rg, Reguengos; V, Vidigueira.

enzymatic hydrolysis (Table 2), Trincadeira seems to be poor in precursors of vitispirane isomers, Riesling acetal, TDN, damascenone and actinidol isomers, but the ratios between these compounds, however, remain the same in different environments.

White varieties

As can be observed in Table 3, skins are richer than pulps, as expected. Arinto has highest values of terpenes. In all these varieties we found more compounds from the linalool class, with

cis-8-hydroxy-linalool being the most important. Arinto variety, however, shows a high level of compounds from the geraniol class. Nevertheless all grape varieties have an important terpenic component that can be expressed in aromatic notes depending on the ratio linalool:geraniol.

As far as content of norisoprenoids is concerned, they are poorly represented, with vomifoliol being the most important. Arinto is richer in norisoprenoid compounds and Roupeiro is poorest. From the results in Table 4 we see that several differences

Table 3. Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from white grapes

Compounds ($\mu\text{g kg}^{-1}$)	R		Av		RO		Pr		A	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
C6 compounds										
Hexanol	57.4	49.2	43.2	31.9	29.2	38.1	15.7	12.2	79.1	19.0
<i>cis</i> -3-Hexenol	9.8	7.9	17.2	9.2	9.1	13.6	9.6	5.4	6.4	1.8
<i>Trans</i> -2-Hexenol	5.9	6.7	11.6	6.6	3.2	4.2	5.1	1.6	27.5	1.5
Total	73.1	63.8	72	47.7	41.5	55.9	30.4	19.2	113	22.3
Terpenes										
<i>trans</i> -Furan linalool oxide	11.9	10.5	9.4	7.2	12.3	9.7	11.2	7.3	19.4	3.7
<i>cis</i> -Furan linalool oxide	9.5	11.1	8.9	5.8	7.8	8.1	9.3	10.0	6.4	2.3
Linalool	58.2	26.8	13.9	4.1	70.7	31.4	6.1	1.4	75.1	4.9
α -Terpineol	11.9	3.8	nd	nd	4.5	1.9	nd	nd	4.4	2.1
<i>trans</i> -Pyran linalool oxide	8.9	7.5	8.3	5.9	10.4	9.7	1.9	1.9	6.4	1.6
<i>cis</i> -Pyran linalool oxide	10.7	9.8	10.5	6.9	13.6	13.6	16.6	12.0	12.1	2.3
Nerol	1.8	2.7	26.7	6.3	8.4	5.3	4.5	3.1	9.7	1.1
Geraniol	14.9	10.1	49.9	17.3	34.4	40.4	22.4	23.9	64.6	5.0
<i>E</i> -3,7-Dimethyl-3,7-dihydroxi-1,5-octadiene	10.1	25.9	10.5	15.1	13.7	25.2	8.6	17.6	25.0	11.6
3,7-Dimethyl-3,7-dihydroxi-1-octene	4.2	7.0	4.4	4.3	6.9	3.4	3.2	6.0	14.5	7.0
3,7-Dimethyl-1,7-octadiol	9.9	0.9	9.3	1.9	22.7	7.5	9.0	1.3	158.4	9.3
2,6-Dimethyl-7-octane-1,6-diol	7.3	6.2	10.2	8.4	21.3	20.5	7.6	2.1	47.5	8.6
<i>Z</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	36.0	21.6	63.0	16.9	59.7	53.9	22.2	8.1	82.4	12.1
<i>E</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	64.8	81.1	143.4	47.6	259.9	81.2	142.0	37.6	285.0	60.2
<i>E</i> -3,7-Dimethyl-2-en-1,7-octandiol	23.9	14.2	nd	9.1	47.7	13.0	nd	12.4	161.1	19.6
Geranic acid	59.3	6.3	107.5	2.8	37.8	11.6	37.4	5.9	158.4	3.9
<i>p</i> -Menthene-7,8-diol	26.4	9.0	16.1	3.9	9.6	nd	25.6	7.3	53.7	9.3
Total	369.7	254.5	492	163.5	641.4	336.4	327.6	157.9	1184	164.6
Benzenoids										
Benzaldehyde	3.3	2.8	8.3	2.0	4.5	13.1	4.5	1.4	7.3	1.5
Methyl salicylate	27.9	81.1	11.1	11.3	3.2	15.3	2.2	1.4	8.1	3.0
α -Methyl-benzenemethanol	1.5	2.4	3.3	2.1	1.9	2.5	1.6	1.5	4.0	1.2
Benzyl alcohol	200.7	209.4	143.4	122.5	294.4	262.5	164.0	103.7	279.9	92.5
2-Phenylethanol	191.2	152.7	128.5	86.2	175.0	172.8	143.3	91.7	364.3	88.2
Omanillic alcohol	31.6	45.9	70.4	60.1	98.1	nd	118.8	101.0	31.7	18.2
Dihydroconiferyl alcohol	6.6	14.8	24.7	7.8	nd	121.2	9.7	16.6	9.6	8.7
Total	462.8	509.1	389.7	292	577.1	587.4	444.1	317.3	704.9	213.3
Phenols										
4-vinylguaicol	2.2	43.5	6.7	39.3	13.7	69.6	10.4	103.8	14.3	32.2
4-vinylphenol	12.8	16.4	11.7	8.1	9.6	9.5	5.5	7.1	23.8	36.2
Total	15	59.9	18.4	47.4	23.3	79.1	15.9	110.9	38.1	68.4
Norisoprenoids										
3-Hydroxy- β -damascone ^a	18.5	15.8	43.3	19.9	6.9	nd	31.2	17.4	82.5	21.2
3-Oxo- α -ionol ^b	16.5	10.1	95.0	26.2	58.3	31.4	27.0	15.7	31.7	12.0
3,9-Dihydroxy-mega-5-ene	20.2	12.9	30.5	41.3	3.4	nd	46.4	29.9	88.7	17.4
Vomifoliol ^c	101.1	52.6	128.5	76.5	169.7	85.3	93.0	66.6	322.1	62.9
Total	156.3	91.4	297.3	163.9	238.3	116.7	197.6	129.6	525	113.5

R, Roupeiro; AV, Antão Vaz; RO, Rabo de Ovelha; Pr, Perrum; A, Arinto; (E), Évora.

^a 3-Hydroxymegastigma-5,8-dien-7-one.

^b 9-Hydroxymegastigma-4,7-dien-3-one.

^c 6,9-Dihydroxymegastigma-4,7-dien-3-one.

Table 3A. Compounds released by enzymatic hydrolysis from the glycosylated precursors extracted from white grapes

Compounds ($\mu\text{g kg}^{-1}$)	R (P)		R (B)		R (Rd)		R (Rg)		R (V)	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
C6 compounds										
Hexanol	55.0	49.7	36.2	23.8	56.3	49.4	67.2	38.0	97.6	61.6
<i>cis</i> -3-Hexenol	15.4	12.0	7.3	3.6	10.5	9.6	17.7	7.1	19.2	11.5
<i>Trans</i> -2-Hexenol	9.5	8.6	12.3	11.7	4.3	5.5	7.2	5.0	9.2	4.4
Total	79.9	70.3	55.8	39.1	71.1	64.5	92.1	50.1	126.0	77.5
Terpenes										
<i>trans</i> -Furan linalool oxide	12.8	14.3	7.8	4.2	12.1	11.8	15.2	5.9	17.0	8.4
<i>cis</i> -Furan linalool oxide	12.5	14.9	5.3	2.9	11.8	12.8	15.9	6.4	16.3	10.0
Linalool	86.6	42.0	13.9	3.2	57.3	26.3	27.4	8.4	43.3	14.0
α -Terpineol	5.9	4.1	1.7	1.3	16.6	3.8	7.5	2.2	11.4	3.3
<i>trans</i> -Pyran linalool oxide	10.3	8.6	6.7	2.6	9.0	8.0	13.0	5.5	15.3	7.0
<i>cis</i> -Pyran linalool oxide	13.2	13.4	7.3	2.9	12.5	9.9	18.1	6.9	23.1	14.8
Nerol	nd	3.3	nd	0.3	2.0	5.2	nd	1.4	5.7	2.8
Geraniol	30.8	9.2	9.5	2.9	30.2	9.6	14.4	5.5	19.2	8.4
<i>E</i> -3,7-Dimethyl-3,7-dihydroxi-1,5-octadiene	13.9	25.8	5.0	3.6	10.9	23.4	4.3	5.5	9.9	12.2
3,7-Dimethyl-3,7-dihydroxi-1-octene	7.3	3.5	1.7	2.1	1.1	2.9	2.9	5.0	3.6	4.2
3,7-Dimethyl-1,7-octadiol	8.8	2.0	4.0	0.8	8.3	1.0	5.8	1.3	6.9	1.6
2,6-Dimethyl-7-octane-1,6-diol	10.4	6.8	8.1	1.8	8.0	7.2	8.7	4.6	13.0	7.2
<i>Z</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	41.1	22.7	26.3	8.3	32.0	18.3	4.1	16.3	51.5	18.6
<i>E</i> -2,6-Dimethyl-2,7-octadiene-1,6-diol	83.7	82.6	28.2	17.9	34.8	63.6	12.0	79.1	90.5	87.5
<i>E</i> -3,7-Dimethyl-2-en-1,7-octandiol	71.1	11.1	14.1	6.6	24.2	13.0	24.8	18.2	31.1	17.9
Geranic acid	85.3	1.6	36.3	3.7	47.3	2.7	62.3	5.3	81.6	5.3
<i>p</i> -Menthene-7,8-diol	32.5	14.0	23.0	6.1	23.0	10.7	22.5	11.2	35.6	16.7
Total	526.2	279.9	198.9	71.2	341.1	230.2	258.9	188.7	475.0	239.9
Benzenoids										
Benzaldehyde	1.5	3.7	1.4	2.2	4.9	4.8	2.2	3.2	2.1	3.9
Methyl salicylate	6.6	22.3	15.6	30.5	32.7	59.6	9.4	20.1	7.1	14.8
α -Methyl-benzenemethanol	3.7	5.9	2.2	1.7	2.2	4.3	2.2	1.4	1.4	2.1
Benzyl alcohol	202.1	243.7	230.6	205.0	200.3	209.5	204.5	178.0	227.1	237.9
2-Phenylethanol	176.9	134.8	163.4	95.4	147.3	141.4	164.6	111.1	211.2	158.6
O-movanillic alcohol	66.3	33.3	45.4	17.8	30.8	40.7	55.9	53.4	67.4	70.8
Dihydroconiferyl alcohol	33.2	10.4	14.1	9.5	5.0	4.6	9.1	9.6	10.8	12.4
Total	490.3	454.1	472.7	362.1	423.2	464.9	447.9	376.8	527.1	500.5
Phenols										
4-Vinylguaicol	4.0	44.9	3.6	24.9	1.9	31.8	1.7	53.5	4.6	53.2
4-Vinylphenol	17.5	12.0	14.5	14.3	9.4	6.8	19.0	11.5	16.6	18.1
Total	21.5	56.9	18.1	39.2	11.3	38.6	20.7	65.0	21.2	71.3
Norisoprenoids										
3-Hydroxy- β -damascone ^a	14.4	28.7	13.7	10.9	17.7	16.6	14.5	22.7	18.6	26.8
3-Oxo- α -ionol ^b	30.0	17.8	45.6	10.1	25.3	10.8	20.7	12.8	24.0	11.4
3,9-Dihydroxy-mega-5-ene	8.0	12.4	8.1	6.7	19.3	14.6	15.3	12.0	24.8	14.7
Vomifolol ^c	140.5	61.2	143.5	46.1	97.3	33.9	135.8	47.8	142.0	57.8
Total	129.9	120.1	210.9	73.8	159.6	75.9	186.3	95.3	209.4	110.7

R, Roupeiro; (P), Portalegre; (B), Borba; (Rd), Redondo; (Rg), Reguengos; (V), Vidigueira.

^a 3-Hydroxymegastigma-5,8-dien-7-one.^b 9-Hydroxymegastigma-4,7-dien-3-one.^c 6,9-Dihydroxymegastigma-4,7-dien-3-one.

can be observed among the varieties but, as noticed before, the greatest differences are between pulp and skin amounts rather than variety and/or region.

When soil is considered (Table 3A) the ratios found between the isomers remain the same in all sub-regions and as observed before skins are richer than pulps. Differences among the different sub-regions can be observed when the amounts of individuals is considered. The differences, however, lie in the intervals attributed before for the varieties.

The same is true when the norisoprenoid compounds are considered.

Similar results are obtained when the compounds generated by acid hydrolysis of the aglycons produced by enzymatic hydrolysis of the bound aromatic compounds (Table 4) are evaluated.

DISCUSSION AND CONCLUSIONS

Bound aroma compounds, namely terpenes and norisoprenoids, of ten Portuguese varieties of grapes

Table 4. Compounds obtained by chemical hydrolysis from glycosylated precursors extracted from white grapes

Compounds ($\mu\text{g kg}^{-1}$)	R (E)		AV (E)		RO (E)		Pr (E)		A (E)	
	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin
Vitispiranes	31.8	29.5	40.9	16.0	10.0	11.1	41.3	56.3	26.2	67.4
Riesling acetal	27.4	25.9	15.6	35.1	20.5	5.2	15.6	66.3	4.3	44.9
TDN	21.9	11.8	15.3	8.2	4.1	4.7	15.4	11.9	3.1	15.0
β -Damascenone	5.4	1.8	5.5	7.8	nd	7.0	7.5	10.4	9.0	13.5
Actinidols	12.4	10.0	34.6	75.3	51.5	79.9	14.5	25.2	57.8	107.8

Compounds ($\mu\text{g kg}^{-1}$)	R (P)		R (B)		R (Rd)		R (Rg)		R (V)	
	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin
Vitispiranes	9.6	20.9	17.0	11.3	20.2	20.1	49.5	25.7	52.4	35.9
Riesling acetal	10.3	6.8	9.5	13.7	28.3	18.0	30.4	18.2	31.5	22.2
TDN	2.1	5.2	5.0	12.1	10.8	11.3	24.7	23.6	23.2	20.4
β -Damascenone	2.7	8.5	1.9	6.0	3.2	6.4	3.2	6.4	3.0	7.2
Actinidols	11.0	19.8	7.6	18.2	10.1	15.2	12.7	15.5	9.7	19.5

R, Roupeiro; AV, Antão Vaz; RO, Rabo de Ovelha; Pr, Perrum; A, Arinto; E, Évora; P, Portalegre; B, Borba; Rd, Redondo; Rg, Reguengos; V, Vidigueira.

were studied. The same aroma compounds as in aromatic grapes were found, namely terpenes, benzenoids and norisoprenoids, but the amounts were rather small. Geraniol, linalool and α -terpineol, the dominant terpenes in Muscat varieties (aromatic grapes),⁹ did not show relevant amounts in all cases. In fact they were all present in the white varieties but not in the red ones. Chemical hydrolysis of norisoprenoid aglycons released by enzymatic hydrolyses produced vitispirane isomers, actinidol isomers, Riesling acetal, TDN and β -damascenone. In some varieties skins are richer than pulps, which is not usually stated.⁷ In some cases amounts of C13 norisoprenoid aglycones

are higher than the usual amounts, which rarely reach $100 \mu\text{g L}^{-1}$.

If the aroma impact of these results was to be estimated, however, we could more or less consider that the amounts released still remain far below the usually detected amounts in aromatic grapes, where monoterpene concentrations can reach 14 mg L^{-1} .

Results obtained indicate some differences in syntheses of varietal glycosylated aroma compounds among the Portuguese red and white grape varieties studied. Differences seem to be higher in white varieties. The differences concern terpenic,

Table 5. Ratios between aroma compounds of white grapes pulps and skins

	RE	RRd	RP	RB	RV	RRg	AV	A	Pr	RO
Pulps										
OxA/OxB	<1	<1	<1	<1	<1	<1			<1	
OxC/OxD	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
T8OHlin/C8OHlin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
3OH β dam/3oxo α ion							<1			
alc.Omo./diidrocon										
(T8OH + C8OH)/pment										
Lin/ α terp										
Lin/ger							<1	<1	<1	<1
Skins										
OxA/OxB						<1				
OxC/OxD	<1	<1	<1	<1	<1	<1	<1	<1		<1
T8OHlin/C8OHlin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
3OH β dam/3oxo α ion		<1	<1	<1	<1	<1	<1		<1	<1
alc.Omo./diidrocon										
(T8OH + C8OH)/pment						<1				
Lin/ α terp										
Lin/ger							<1		<1	

RV, Roupeiro (Vidigueira); RRd, Roupeiro (Redondo); RRg, Roupeiro (Reguengos); RB, Roupeiro (Borba); RP, Roupeiro (Portalegre); RE, Roupeiro (Évora); AV, Antão Vaz; RO, Rabo de Ovelha; Pr, Perrum; A, Arinto.

OxA, *trans* isomer of the furanic oxide of linalool; OxB, *cis* isomer of the furanic oxide of linalool; lin, linalol; α terp, α -terpineol OxC, *trans* isomer of the pyranic oxide of linalool; OxD, *cis* isomer of the pyranic oxide of linalool; ger, geraniol; T8OHlin, *Z* isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; C8OHlin, *E* isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; pment, *p*-menthene-7,8-diol; alc.Omo., 4-hydroxy-3-methoxyphenethanol; diidrocon, dihydroconiferilic alcohol; 3OH β dam, 3-hydroxy- β -damascone; 3oxo α ion, 3-oxo- α -ionol (one of the compounds was not detected).

Table 6. Ratios between aroma compounds of red grapes pulps and skins

	TV	TRd	TRg	TB	TP	TE	TC	Az	C	M
Pulps										
OxA/OxB								<1		<1
OxC/OxD	<1	<1	<1	<1	<1			<1	<1	<1
T8OHlin/C8OHlin	<1	<1	<1	<1	<1	<1		<1	<1	<1
3OH β dam/3oxo α ion		<1	<1	<1	<1	<1		<1		<1
alc.Omo./diidrocon										<1
(T8OH + C8OH)/pment										
Skins										
OxA/OxB	<1							<1		<1
OxC/OxD	<1							<1		<1
T8OHlin/C8OHlin	<1	<1	<1	<1	<1	<1	<1	<1		<1
3OH β dam/3oxo α ion	<1	<1	<1	<1	<1	<1		<1	<1	<1
alc.Omo./diidrocon										
(T8OH + C8OH)/pment							<1			

TV, Trincadeira (Vidigueira); TRd, Trincadeira (Redondo); TRg, Trincadeira (Reguengos); TB, Trincadeira (Borba); TP, Trincadeira (Portalegre); TE, Trincadeira (Évora); M, Moreto; TC, Tinta Caiada; Az, Aragonez; C, Castelão.

OxA, *trans* isomer of the furanic oxide of linalool; OxB, *cis* isomer of the furanic oxide of linalool; OxC, *trans* isomer of the pyranic oxide of linalool; OxD, *cis* isomer of the pyranic oxide of linalool; T8OHlin, *Z* isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; C8OHlin, *E* isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; pment, *p*-menthene-7,8-diol; alc.Omo., homovanillic alcohol; diidrocon, dihydroconiferilic alcohol; one of the compounds was not detected.

norisoprenoid and benzenoid compound amounts as well as ratios between isomeric compounds. The ratios studied are those usually used to classify the varieties according to their potential flavour.¹ When these specific ratios are evaluated (Tables 5 and 6) it can be concluded that all the grapes studied can be considered neutral.

Another volatile compound also found in all varieties was *p*-menthene-7,8-diol. This result is in agreement with similar data that consider this compound the one that usually prevails in grapes.²⁴

An influence of different environments in the two varieties studied can probably be outlined, although study has to be extended over several years to be conclusive. Qualitative profile and ratios between isomeric compounds remain more or less the same regardless of the sub-region (Tables 5 and 6). This fact should be explored to search for a possible varietal discrimination. When quantitative data are considered differences are noticed (Tables 1A, 2, 3B, and 4), which is in agreement with previous results.^{5,9,11}

The technological impact of this study is yet far from being concluded; as a first approach we can consider that the enzymatic and acidic hydrolysis followed by a higher skin contact (especially for the white varieties) can improve/enhance wine aroma. By increasing the concentrations of free monoterpenes in wine as well as other potent odorants such as norisoprenoids, the varietal aroma and hence perceived quality can also be increased. The impact of the early release of these compounds on wine ageing has to be evaluated as well as contact time to avoid deleterious effect of phenols and/or rearrangement of the monoterpenes liberated under the mild acidic conditions of wine¹³.

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