ROLE OF GLYCOSIDIC AROMA PRECURSORS ON THE ODORANT PROFILES OF GRENACHE NOIR AND SYRAH WINES FROM THE RHONE VALLEY. PART 2: CHARACTERISATION OF DERIVED COMPOUNDS

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Abstract

Aims: Grenache noir and Syrah are the predominant grape varieties in the French Rhone valley vineyard. This study aimed at identifying the odorants generated from glycoconjugates extracted from wines made with Grenache noir and Syrah.

Methods and results: Synthetic model wines enriched with glycoconjugates, treated or not with enzymes, were stored at 45 °C for 3 weeks, or at 13 °C for 18 months. Aromas generated were extracted and analyzed by GC-Olfactometry (only samples from accelerated aging) and were further quantitatively determined by GC-MS. Analysis of the extracts allowed to identify 49 odorants, including 27 that could be aglycons, or related compounds, of glycoconjugates from the grapes. In addition, the active compounds were quantified in similar experiments led in conditions of natural aging for 18 months.

Conclusion: The two varieties, Grenache noir and Syrah, were distinguishable by 14 odorant zones. Multivariate analyses (PCA) performed with the amounts of aroma compounds formed during both model and natural aging confirmed the effect of the glycosidase treatment on the acceleration of the aroma compounds formation and on the increase of the varietal differences of the wines.

Significance and impact of study: GC-Olfactometry coupled with GC-MS were good techniques to indentify and apreciate the odorants generated from glycoconjugates in the wines of Syrah and Grenache Noir, but in the context of a blend of odors, these techniques showed their limits and did not permit to determine the real impact of a molecule in the global aroma of the wine perceived by the taster. Other methods as additive techniques should be used to complete this study.

Keywords: Aging, aroma precursors, glycoconjugates, Grenache noir, olfactometry, Syrah, wine

Résumé

Objectif : Le Grenache noir et la Syrah sont les cépages les plus utilisés en vallée du Rhône. L'objectif de cette étude était de caractériser les composés odorants, issus des glycosides du raisin, susceptibles d'intervenir dans le profil olfactif des vins de ces deux variétés. Pour y parvenir, des solutions modèles de vins enrichies en précurseurs glycosylés, provenant de raisins de Grenache et de Syrah, ont été utilisées.

Méthodes et résultats : Les solutions modèles de vin enrichies ont été soumises à un vieillissement artificiel accéléré. Les aglycones formées ont été analysées par CPG-olfactométrie. L'analyse des extraits par CPG-SM a permis d'identifier 49 composés odorants, dont 27 pourraient être des aglycones ou des composés dérivant d'aglycones provenant des glycosides du raisin. De plus, ces composés odorants ont été quantifiés sur ces solutions modèles vieillies de façon accélérée, et sur les mêmes solutions soumises à un élevage naturel de 18 mois.

Conclusions : Les deux variétés, Grenache noir et Syrah, se sont différenciées par 14 zones odorantes. Des analyses multivariées (ACP), réalisées en utilisant les teneurs en composés obtenues par les deux vieillissements artificiel et naturel, ont confirmé l'influence du traitement par des glycosidases sur l'accélération de la formation des composés odorants issus des glycosides et sur l'amplification de la différence variétale entre les vins.

Signification et impact de l'étude : La CPG couplée à l'olfactométrie et à la spectrométrie de masse permet d'apprécier le caractère odorant d'un composé volatil dans l'extrait analysé et son implication possible dans l'arôme du vin. Cette étude a permis de déterminer les principaux composés odorants provenant des glycosides des baies de Grenache noir et de Syrah. Cependant dans le contexte d'un mélange d'odeurs, ces techniques ont montré leurs limites et n'ont pas permis de démontrer l'effet d'un composé sur l'arôme global du vin, tel que perçu par le dégustateur. D'autre méthodes comme les techniques additives pourraient être utilisées pour compléter cette étude.

Mots clés : élevage, précurseurs d'arômes, glycosides, Grenache noir, olfactométrie, Syrah, vin

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INTRODUCTION

Except for a few aromatic varieties, the berries from most *Vitis vinifera* varieties have no specific odors. Yet, some of these so called neutral varieties, such as Grenache noir and Syrah yield wines with an aroma specific of the variety. Such specific olfactive notes were attributed mainly to odorants generated from non odorous precursors occurring in grape berries. Among them are the glycoconjugates which make up an important reserve of aroma, and contributes to the aroma complexity acquired by the wines during aging. Grape glycoconjugates are composed of a sugar moiety, including always a glucose bound to a volatile aglycone by β-glucosidic bond. Glycosides can be hydrolysed enzymatically and/or chemically (Marais, 1983; Winterhalter, 1993) to release the volatile aglycons which can eventually undergo structural modification. The mild conditions used generally in enzymatic hydrolysis do not alter the structure of the aglycons (Gunata *et al.*, 1985, Winterhalter and Skouroumounis, 1997) which can be analyzed by GC-MS. On the contrary, acid catalyzed hydrolysis favours structural transformations of the aglycons such as those observed during wine aging (Sefton *et al.*, 1993). The GC-Olfactometry (GC-O) was used to identify odorant zones and the compounds potentially responsible for the Grenache wine aroma (Ferreira *et al.*, 2002; Lopez *et al.*, 2004).

Several GC-O methods were suited for the identification and ranking attending to potential importance of odorants from complex products. They were grouped in two categories:

- Methods of successive dilutions, AEDA (Aroma Extract Dilution Analysis; (Acree *et al.*, 1984)) and CHARM (Combined Hedonic Response Measurement; (Ullrich and Grosch, 1987)).

- Methods based on the analysis of an extract with one single concentration as the detection frequency method. In this method developed more recently (Van Ruth *et al.*, 1996, Pollien *et al.*, 1997), the measured variable was the detection frequency of odours by the whole panel. In this case, there was a good correlation between the intensity of the odour and the detection frequency of this same odour.

Strickly speaking, these methods include two different types of strategies, the detection frequency based methods and the posterior intensity measurement methods. The comparison of the advantages and drawbacks of these different methods (Van Ruth, 2001) revealed that methods based on the analysis of an extract with one single concentration are the most reproducible and best adapted to measuring the olfactory intensity because they take into account the Stevens law which relates the odour intensity to the concentration of the odorant. Moreover, contrary to the dilution techniques, they offered the possibility of validate statistically the results, and need less analyses. This method was used in this study.

In a previous paper (Ségurel *et al.*, 2009), we demonstrated that the olfactory profiles of Syrah and Grenache noir wines were different and that the odorants resulting from glycosidic precursors played a significant role in this difference. These results confirmed those previously obtained with wines of the two varieties Grenache and Syrah (Abbot *et al.*, 1991; Ormieres *et al.*, 1997; Ferreira *et al.*, 1998; Lopez *et al.*, 1999, Ferreira *et al.*, 2002; Lopez *et al.*, 2004). In this paper, the aim was to identify the aroma compounds resulting from grape glycosides implicated in the aroma expression of these varietal wines. The addition of glycosidic extracts enabled us to determine the impact of these precursors on wine's odours (Segurel *et al.*, 2010). The same experimental approach was applied in the context of comparisons by GC-olfactometry (GC-O) to reveal the odour variations between the samples enriched with glycoside precursors.

MATERIALS AND METHODS

Grenache noir and Syrah wines from vineyards situated in the Southern Cotes du Rhone area, vintage 2001, were selected for this study. Two experimental vineyards were selected, one planted with Grenache noir (GB), and the other with Syrah (SL). Grapes were harvested at technological maturity. For each variety, about 80 kg of grapes were sampled at random.

The grapes of each experimental vineyard were vinified according to the Inter-Rhone standard winemaking process: grapes were manually harvested, mechanically destemmed and crushed, then put in 1 hL (100 L) stainless steel tanks. Musts were added with SO_2 at 4 g/hL, and inoculated with 10 g/hL of L2056 commercial yeast strain (Lallemand, France). Fermentation and maceration were carried out during 7 days at 25 °C-30 °C. After pressing, malolactic fermentation was performed with addition of Vitilactic bacteria (Martin Vialatte, France). The two wines obtained (Grenache noir GB-01 and Syrah SL-01) were filtered, bottled, and stored at 13 °C until extraction of their glycosidic precursors. Each glycosidic extract was used as described below.

1. Synthetic model wines enriched with glycosidic aroma precursors

a) Synthetic model base wine

To obtain the synthetic model wine, 3.5 g of tartaric acid were added to 120 mL of ethanol and 800 mL of water. The pH of the mixture was adjusted to 3.5 with

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potassium carbonate and the volume was then adjusted to 1 L with water.

Two synthetic wines were respectively enriched with pools of glycosidic precursors from wines of Grenache and Syrah, coming from the two experimental plots of Grenache (GB-01) and Syrah (SL-01), in 2001. The preparation of these pools of glycosidic precursors was described in the previous paper (Segurel *et al.*, 2010). They were obtained from 5 L of wine and the synthetic model wines enriched by a factor 5 with glycosidic extract were analysed by GC-O and Gas Chromatography-Mass Spectrometry (GC-MS). This enrichment permited to increase the detection of the odorants generated from glycosides.

Half of the volume of precursor enriched synthetic wine was added with a commercial enzyme preparation (50 mg/L) (AR2000, DSM, Delf, Netherlands) containing the following glycosidase activities: β-D-apiofuranosidase (564.6 nkat/g), α-L-rhamnopyranosidase (236.7 nkat/g), α–L-arabinofuranosidase (13,500 nkat/g), α-L-arabinopyranosidase (576 nkat/g), and β-D-glucopyranosidase (4,380 nkat/g). The samples were closed with screw caps under CO_2 atmosphere, homogenized, then stored at room temperature for one week.

These synthetic model wines underwent an accelerated or natural aging, to generate the odorant compounds from these precursors according to the following methods:

Synthetic model wines / Accelerated aging by heating at 45 °C for three weeks :

- T-*Mod:* heated synthetic model wine without glycosides addition (control wine),
- G-D-*Mod:* Enriched with Grenache glycosides / Heated,
- G-DE-*Mod:* Enriched with Grenache glycosides / Enzyme added/ Heated,
- S-D-*Mod*: Enriched with Syrah glycosides / Heated,
- S-DE-*Mod*: Enriched with Syrah glycosides / Enzyme added / Heated.

Synthetic model wines / Natural aging by storage at 13 °C for eighteen months :

- T-*Nat:* Synthetic model wine / Natural aging,
- G-D-*Nat*: Enriched with Grenache glycosides / Natural aging,
- G-DE-*Nat*: Enriched with Grenache glycosides /Enzyme added / Natural aging,
- S-D-*Nat*: Enriched with Syrah glycosides / Natural aging,
- S-DE-*Nat*: Enriched with Syrah glycosides / Enzyme added / Natural aging.

2. Sensory analysis

The difference between the synthetic model wines enriched with Grenache noir and Syrah glycosides was determined by carrying out a triangular difference test. Two panels of 17 and 20 judges were respectively used to compare samples submitted to accelerated aging (17 judges) and to natural aging (20 judges). The olfactory assessment of the wines was performed by direct (orthonasal) perception.

3. GC-MS and GC-O analyses of the volatile compounds

The volatiles generated by controlled heating with or without enzyme addition were analyzed by GC-O, identified and quantified by GC/MS. At the same time, the synthetic model wines were submitted to natural aging (13 °C for 18 months) and analysed as above.

a) Extraction

The volatile compounds were extracted from the samples issued from the two types of aging according to the following method:

80 µg of 3,5-di-tert-butyl-4-hydroxytoluene (BHT), used as internal standard and antioxidant (Escudero and Etiévant, 1999), was added to 125 mL of a sample. The samples were extracted by 25 mL of dichloromethane by liquid-liquid extraction, at 4 °C under nitrogen during 15 min. The emulsion formed was demixed by centrifugation at 4 °C (20 min; 8,000 g). The organic phase was separated, then the extraction was repeated one time more. The two organic phases were gathered, dried on anhydrous Na_2SO_4 and filtered. The extract was concentrated at 47 °C in a Vigreux column, then in a Dufton column up to 500 µL volume. Extracts were stored under nitrogen at -80 °C.

b) GC-Olfactometry analyses

These analyses were performed to focus on the odorants generated from the glycosidic precursors, likely to contribute to the aroma expression of this glycosylated potential, and to the differentiation of both varieties. They were performed in the INRA Laboratory for Aromas in Dijon (UMR Flavic). For each variety, analyses were carried out on extracts from the 5 samples of synthetic model wines submitted to accelerated aging: T-*Mod*, G-D-*Mod*, G-DE-*Mod*, S-D-*Mod* et S-DE-*Mod*). The GC-O was carried out using the detection frequency method which consists to register the frequency of perception of an odour by the panelists during the chromatography separation (Van Ruth *et al.*, 1996; Pollien *et al.*, 1997). For each sample, the total duration of analysis was 70 min. To avoid fatigue, each panelist carried out half of the analysis, then the second half was carried

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out by an other panelist. During another session with the same sample injected, the roles were inverted.

Thirteen panelists were selected among 40 candidates to perform the sample analyses. The selection of panelists was carried out using a test of odor recognition (Thomas-Danguin *et al.*, 2003), and the training was performed with synthetic solutions containing twenty different odorants. The 13 panelists performed the GC-O analysis of 5 samples. For each sample, the individual olfactograms of each panelist, prompting yes/no responses for an odour (associated to a description of this odour) were juxtaposed, then an aromagram giving the detection frequency, in percentage of subjects having perceived an odour, was obtained by cumulating the individual olfactograms. The description of odours by the panelists was used to facilitate the overlapping of the olfactograms from the different panelists.

For each aging type, natural and accelerated, two analysis on the olfactory difference between Grenache or Syrah synthetic model wines enriched with glycosides were performed, one on enzyme added synthetic model wines (DE), the other on synthetic model wines without enzyme addition (D).

c) Gas chromatography conditions

A gas chromatograph (Hewlett-Packard 5890 Series II) was fitted with a DB-WAX fused silica capillary column (J&W SCIENTIFIC; 30 m x 0.25 mm i.d.; 0.5 µm film thickness). Operating conditions were as follows: the injector temperature program was set at 20 °C to 280 °C at 180 °C min^{-1}, then isothermal for 80 min. The oven temperature program was set from 40 °C to 75 °C (70°C min^{-1}) then to 245 °C at 3 °C min^{-1}, then isothermal for 20 min. Hydrogen carrier gas flow-rate was 1.5 mL min^{-1}. The column outlet was fitted with a fixed divider to an olfactometric detector (2/3 of the flow) and to a flame ion detector (1/3 of the flow). The injector and the detector were kept at 280 °C. The injected volume was 1.5 µL.

d) Detection by GC-O

The transfer column carrying the chromatographic effluent was kept at 280 °C, connected to a glass sniffing port adjusted to the human nose. The effluent reaching the sniffing port was carried by a humidified air make up (100 mL min^{-1}). Each panellist indicated each odour he perceived by a click of the computer's mouse. Simultaneously, the recording of every perceived odour was made on a tape recorder. The yes/no response to an odour expressed by the click of the mouse according to elution time was obtained by Fingerclick, a specifically designed software (INRA-UMR Flavic, Dijon, France).

e) Treatment of olfactometric data

The Fingerclick software was used to perform the following successive operations for each extract : the 13 individual olfactograms were cumulated to establish the aromagram giving the detection frequencies in percentage, (number of citations of a detected odour at the same linear retention time/number of panellists x 100).

f) Gas chromatography / Mass Spectrometry

The gas chromatography separation was carried out as previously described. For the mass spectrometry, a Hewlett-Packard 5889 A mass spectrometer equipped with a quadrupole detector was used for electron impact (EI) mode spectra. The transfer line from GC to MS was heated to 250 °C. The source temperature was kept at 250 °C. EI was recorded at 70 eV in the mass range of m/e 29-350 at 1 s intervals (fullscan mode). Identifications were carried out by comparing the linear retention index, EI mass spectra, and the odour descriptors of the compounds eluted, with published data or with data from authentic compounds; 1 µL was injected. The compounds were quantified by correlating the peak areas to 4-nonanol used as internal standard (16.3 µL/100 mL of sample). Results (means of four replications) were expressed as 4-nonanol equivalent.

RESULTS AND DISCUSSION

The sensory analyses of wines enriched with glycosides, described in the previous paper (Ségurel *et al.*, 2009), have demonstrated the implication of the grape glycosidic potential in the aroma of Grenache noir and Syrah wines, particularly in their aroma differentiation. In this paper, synthetic model wines enriched with Grenache or Syrah glycosides extracts, with or without enzyme addition, were submitted to aging treatments similar to those used in the sensory analyses (Ségurel *et al.*, 2009). However, in this case, the glycoside extracts were not added to wine but to synthetic model wines in order to make easier the GC-O and GC-MS analysis of the volatiles arising from glycoside, the only source of volatiles in such samples.

1. Preliminary difference test

Before performing such extensive analysis, the inter-varietal difference of the aromas generated from the different preparations was checked by comparative sensory analysis using the triangular test as previously described (Ségurel *et al.*, 2009). For each aging type, natural and accelerated, the olfactory difference between Grenache or Syrah synthetic model wines enriched with glycosides was significant in each case: ($p = 0.01$) for the D solutions (without enzyme addition) and for the DE solutions (with enzyme addition) ($p = 0.001$), indicating that the aromatic

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expression of glycosidic precursors in the model solution differentiated significantly the two varieties for the two aging modes. However, the difference seemed to be more difficult to perceive in the case of natural aging without enzyme addition. This could be explained by the relatively short time of natural aging (18 months at 13 °C), whereas model aging and enzyme addition increased the differences between aromas of Grenache and Syrah synthetic wines.

2. GC-O analyses

a) Odorant compounds profiles generated by accelerated aging of the model solutions

Four areas, perceived in the T-Mod sample of the non enriched heated synthetic model wines, were eliminated from the other aromagrams as they were generated from the matrix and not from glycosides. Among the 4 aromagrams, corresponding to the G-D-*Mod*, S-D-*Mod*, G-DE-*Mod* et S-DE-*Mod* samples, 186 odorant areas were perceived at least by one judge, giving evidence of the aroma complexity of the extracts, but 86 odours were perceived by only one panelist. Only 70 odours, perceived by at least 30% of the panel (4 panelists out of 13), were retained as significant as previously reported, regardless of the size of the jury (Pollien *et al.*, 1997, Aubry, 1999, Bernet, 2000). These odorous areas were often perceived in the 4 samples with different detection frequencies (figures 1 and 2).

The total average of the detection frequencies of all the odours perceived for each aromagram did not differ more than 5% between the varieties, for the same preparations (with or without enzyme addition): 47% for G-D-*Mod* against 50 % for S-D-*Mod* (figure 1), and 63% for G-DE-*Mod* against 57% for S-DE-*Mod* (figure 2). However, the enzyme addition increased this average intensity from 50% to 57% for Syrah and from 47% to 63% for Grenache. Thus, enzyming enhanced the aromatic intensity more significantly for Grenache synthetic model wines.

Similarities and differences appeared between the varieties concerning the detection frequencies of different odorous areas. To compare these detection frequencies, the least significant difference (LSD) of 30%, reported in the literature, was generally admitted. However, as this threshold is liable to variations depending of the odor, we decided to choose a less restrictive LSD of 3 panelists, corresponding to a frequency of 23% (3 panelists out of 13), to prevent removing any potentially discriminating areas.

b) Odorous areas common to the both varieties

The odour areas with detection frequency difference between varieties less than 23% are shown in table 1.

Some of the compounds identified in these odours had already been identified as aglycones in the bound fraction of the aroma of Grenache and Syrah berries (Ségurel, 2005). Among them were non terpenic alcohols (butan-1-ol, 2- and 3-methyl butanols, heptan-2-ol, 1-octen-3-ol, 2-phenylethanol), C_6-compounds (hexan-1-ol, (*Z*)-hexen-3-ol), volatile phenols (methyl salicylate,

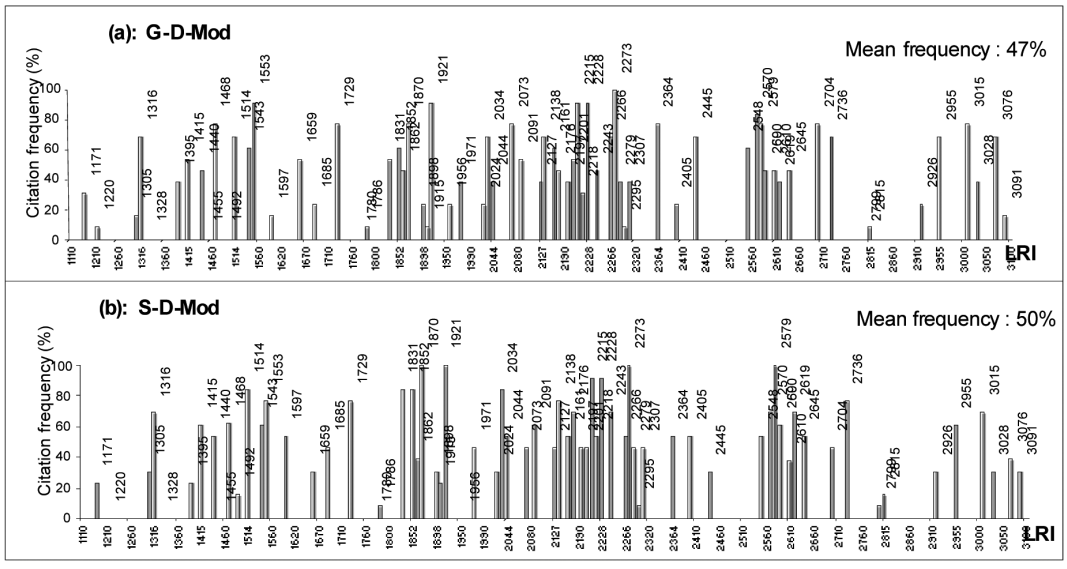


Figure 1 - Aromagrams of two volatile extracts, from model wine solutions added with glycoconjugates of (a): Grenache noir wine (G-D-*Mod* sample), (b): Syrah wine (S-D-*Mod* sample) and submitted to model aging (controlled heating, 45 °C for three weeks).
Citation frequency (% of panelists perceiving an odor)
as a function of LRI: Linear Retention Index on polar type column (DBWAX)

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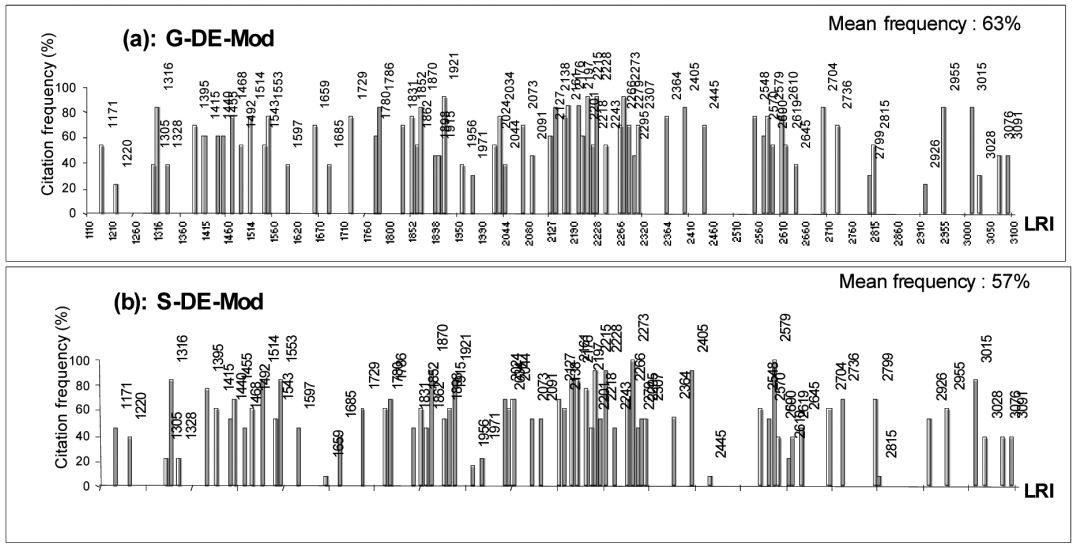


Figure 2 - Aromagrams of two volatile extracts, from model wine solutions added with glycoconjugates of (a): Grenache noir wine (G-DE-*Mod* sample), (b): Syrah wine (S-DE-*Mod* sample), enzymed with glycosidasic preparation, and submitted to model aging (controlled heating, 45 °C for 3 weeks).
Citation frequency (% of panelists perceiving an odor) as a function of LRI: Linear Retention Index during the GC analysis performed on polar type column (DBWAX)

Table 1 - Odorant compounds generated by glycoconjugates after model aging: qualitative and quantitative analysis by GC-Olfactometry. Odors common to Grenache and Syrah, i.e. for which citation frequency in Grenache and Syrah samples do not differ more than 23 % among one modality (enzymed or not).

RI[a]	Odor description [b]	Identification[c]	Odor citation frequency (%) [d]				Concentration (µg/L) [e]				Olfactive perception threshold[f]
			G-D	S-D	G-DE	S-DE	G-D	S-D	G-DE	S-DE	
1171	Herbaceous	Butan-1-ol	31	23	54	46	nq	nq	2.7	2.5	150000 (v)
1220	Gas, Alcohol	2- and 3-méthylbutanols	8	0	23	38	10.0	11.4	50.1	66.0	180000 (v)
1305	Lemon	NI	15	31	38	23	/	/	/	/	
1316	Mushroom	Heptan-2-ol	69	69	85	85	nq	nq	3.2	4.1	2500 (v)
1328	Cooking	Hexan-1-ol	0	0	38	23	nq	nq	289.4	153.7	5000 (v)
1395	Fruity / herbaceous	Cis-hexen-3-ol	38	23	69	77	nq	nq	26.2	12.9	400 (s)
1415	Mushroom	NI	54	62	62	62	/	/	/	/	
1440	Fruity	Ethyl octanoate	46	54	62	54	23.7	16.2	69.1	45.9	580 (v)
1455	Mushroom	1-octen-3-ol	0	0	62	69	nq	nq	8.0	3.2	200 (b)
1492	Earthy / spicy	NI	0	15	54	62	/	/	/	/	
1514	Carrot	2-Ethyl-1-hexanol	69	85	77	85	1.2	1.4	2.8	1.6	
1543	Vegetable	Vitispiranes	62	62	54	54	7.6	3.3	20.7	5.5	800 (v)
1553	Floral	Linalol	92	77	77	85	2.5	3.7	12.9	22.1	25 (s)
1729	Floral / toothpaste	α-Terpineol	77	77	77	62	5.5	3.4	32.9	16.7	250 (s)
1780	Floral, citrus fruit	citronellol	0	0	62	62	nq	nq	0.5	0,1	100 (s)
1786	Eucalyptus	Methyl salicylate	8	8	85	69	nq	nq	118.4	90.9	
1862	Animal	Hexanoic acid	46	38	54	46	0.2	0.4	1.4	0.2	8000 (v)
1898	Fruity, citrus	Ethyl Dihydrocinnamate *	23	31	46	54	nq	nq	nq	nq	1,6 (s)
1915	Mushroom	NI	8	23	46	62	/	/	/	/	
1921	Floral	2-phenyl-ethanol	92	100	92	85	778.4	47.7	978.9	486.7	100000 (v)
1956	Spicy / chemical	NI	23	0	38	15	/	/	/	/	
1971	Vegetable/floral	NI	38	46	31	23	/	/	/	/	
1987	Rubber	Benzothiazole	62	62	92	69	nq	nq	nq	nq	
2024	Mushroom, woody	*O*-cresol + phenol	23	31	54	69	0.1	nq	0.5	0.3	30 (v)
2034	Fruity / anis / coco	Pantolactone +/- nonalactone	69	85	77	62	0.2	0.3	0.3	0.4	30 (s)
2091	Floral / eucalyptus / coco	*m*- and *p*-cresols	54	62	46	54	nq	nq	0.4	0.2	68 (s)
2127	Leather, spicy	*p*-cymen-7-ol	38	46	62	69	nq	nq	0.3	1.2	
2161	Minty	NI	62	54	77	85	/	/	/	/	
2197	Animal	NI	38	46	85	77	/	/	/	/	
2201	Sweet, coco	δ-decalactone	54	46	62	46	0.1	0.4	0.6	0.8	
2215	Spicy, coffee	Hydroxyméthylacetophenone	92	92	92	92	nq	nq	0.3	4.7	
2218	Plastic / Animal	4-vinyl-gaiacol	31	54	54	54	nq	nq	0.5	nq	10 (e)
2228	Floral	*o*-amino-acetophenone *	92	92	92	92	nq	nq	nq	nq	
2243	Coco	Methyl anthranilate *	46	62	54	46	nq	nq	nq	nq	11 (s)
2266	Animal	2,6-dimethoxyphenol	69	54	69	69	0.2	1.1	0.6	0.7	570 (s)
2273	Dentist, minty	NI	100	100	92	100	/	/	/	/	
2295	Floral	NI	8	8	46	54	/	/	/	/	
2307	Minty	NI	38	46	69	54	/	/	/	/	
2548	Spicy, fruity	Methoxyeugenol	62	54	77	62	0.1	0.4	0.7	2.9	1200 (s)
2570	Floral	Phenylacetic acid	85	69	62	54	0.5	0.5	0.6	0.2	1000 (s)
2590	Spicy	NI	46	62	54	38	/	/	/	/	
2645	Spicy / Floral / vanilla	Ethyl Vanillate	46	54	38	46	0.8	5.7	1.4	6.7	1000 (s)
2736	Wax/herbaceous	NI	69	77	69	69	1.6	3.9	3.0	3.2	
3015	Candy, berry fruit	4-(*p*-hydroxy-phenyl)-butan-2-one	77	69	85	85	0.3	0.1	1.4	0.2	
3028	Medical	NI	38	31	31	38	/	/	/	/	
3091	Floral, berry fruit	4-(p-hydroxy-phényl)-butan-2-ol	15	31	46	38	0.4	0.2	0.8	0.2	

[a] RI: Retention Index on DBWAX column. [b]Descriptors given by the panel during GC-O analysis. [c]Identification based on mass spectra and analysis of reference compounds; NI: Non identified; * Tentative identification based only on RI and odor description. In boldscript: compounds previously identified in the glycosylated fraction of the corresponding grapes. [d]The four samples correspond to extracts of model wines added with glycoconjugates extracted from Grenache noir (G) or Syrah (S), enzymed with glycosidasic preparation or not (DE or D), and submitted to model aging treatment (controlled heating at 45 °C for 3 weeks. [e]Estimated concentration, in equivalents of BHT, used as external standard, per model wine liter; nq: non quantifiable. [f]Olfactive perception threshold (µg/L) in water (e), wine (v) or model wine (s), according to literature (Simpson, 1978; Etiévant, 1991; Culléré *et al.*, 2004).

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methoxyeugenol, acetovanillone), and monoterpenols (linalol, α-terpineol, citronellol). The vitispiranes, identified at the index retention IR=1543, were generated from a glycosylated norisoprenoidic triol (3,6,9-trihydroxy-7,8-dihydro-β-ionol) occurring in grape berry (Winterhalter, 1991; 1992).

GC/MS of the areas around the retention index 2210 revealed numerous compounds, including an isomer of the methyl-hydroxy-acetophenone, already identified in the glycosidic fraction of berries (Ségurel, 2005). This compound had only been quantified in the samples with enzyme addition (table 1). As in the bound fraction of the berries, it seemed to be more abundant in the Syrah than in the Grenache samples (Ségurel, 2005), but the observed GC-O frequencies did not confirm this difference.

Other compounds had not been identified in the glycosylated fraction of the berries. However, several phenolic compounds were previously found in the bound fraction of the aroma of Syrah berries or leaves, particularly phenol, cresols, 2,6-dimethoxyphenol, 4-(4-hydroxyphenyl)-butan-2-one (frambinone) and its corresponding alcohol 4-(4-hydroxyphenyl)-butan-2-ol (Wirth *et al.*, 2001).

It was interesting to note the presence of lactones (pantolactone, γ-nonalactone, δ-decalactone). Some lactones have been also previously found in acid hydrolysates of precursors extracts from Grenache noir grapes (Lopez *et al.*, 2004). However, such lactones, specially δ-decalactone, have been already found in the hydrolysates enzymatically obtained from glycoconjugates of several fruits like raspberry, pineapple and several varieties of peaches and nectarines (Pabst *et al.*, 1991, Wu *et al.*, 1991; Aubert *et al.*, 2003). They could arise from glycosides of their open form (5-hydroxydecanoic acid in the case of δ-decalactone), by hydrolysis followed by cyclization; this mechanism has already been demonstrated for whisky-lactone (Masson *et al.*, 2000) and marmelolactone (Winterhalter *et al.*, 1991).

Finally, ethyl octanoate and ethyl dihydrocinnamate could be generated by the glycoside hydrolysis of the corresponding acids, followed by the esterification of

Table 2 - Odorant compounds generated by glycoconjugates. Odors discriminating Grenache and Syrah samples.

RI[a]	Odor description[b]	Identification[c]	Odor Citation frequency (%)[d]				Odors predominant in Grenache*	Odors predominant in Syrah *	Concentration (µg/L) [e]				Olfactive perception threshold[f]
			G-D	S-D	G-DE	S-DE			G-D	S-D	G-DE	S-DE	
Odors discriminant at the 30% level of citation frequency													
1468	Cooking / potatoe	3-methyl-thio -propanal	77	62	77	46	DE^{30}		Nq	Nq	Nq	Nq	0.15 (s)
1597	Vegetable / cucumber	(*E,Z*)-Nona-2,6-dienal	15	54	38	46		D^{30}	Nq	Nq	Nq	Nq	0.02 (s)
1659	Floral	Riesling acetal	54	31	69	8	D^{23}, DE^{30}		1.1	0.5	2.7	0.5	
1831	Fruity / cooked apple	β-damascenone	54	85	69	46	DE^{23}	D^{30}	0.2	0.4	0.3	0.3	0.05 (s)
2044	Caramel / strawberry	Furaneol	38	54	38	69		DE^{30}	Nq	Nq	Nq	Nq	5 (s)
2405	Floral / chemical	4-vinyl-phenol	23	54	85	92		D^{30}	Nq	Nq	4.3	2.1	180 (s)
2445	Minty / spiced	NI	69	31	69	8	D^{30}, DE^{30}		/	/	/	/	
2610	Spiced	Methyl vanillate	46	38	77	23	D^{23}, DE^{30}		0.1	0.1	0.6	0.3	3000 (s)
2704	Pungent / hospital	3,4-dihydro-3-hydoxyactinidol	77	46	85	62	D^{30}, DE^{23}		4.9	0.8	5.8	0.9	
2799	Floral	NI	0	8	31	69		DE^{30}	/	/	/	/	
2815	Spicy / fresh	Zingerone	8	15	54	8	DE^{30}		0.5	0.3	15.7	5.1	
2926	Undescribed	Zingerol	23	31	23	54		DE^{30}	1.4	0.9	3.8	1.4	
3076	charcoal/Euca-lyptus/Orange/coco	NI	69	38	46	38	D^{30}		/	/	/	/	
Odors discriminant at the 23% level of citation frequency													
1685	Cheese / transpiration	3-methyl-butanoïc acid	23	46	38	38		D^{23}	0.1	0.2	0.1	0.2	8 (s)
1852	Floral	Geraniol	62	85	77	62		D^{23}	0.1	0.2	1.4	2.9	
1870	Minty / medical	Gaiacol	77	100	85	92		D^{23}	Nq	0.2	0.2	1.2	9.5 (s)
2073	Cheese / dusty	Octanoic acid	77	46	69	54	D^{23}		128.9	96.9	141.1	76.9	500 (s)
2138	Candy / berry fruit	Ethyl cinnamate	69	77	85	62	DE^{23}		0.1	Nq	0.7	0.4	1.1 (s)
2176	Dentist / clove	Eugenol	46	69	85	85		D^{23}	Nq	Nq	0.2	0.9	6 (s)
2279	Burnt / spicy / rubber	Decanoic acid	38	46	69	46	DE^{23}		30.5	23.3	32.7	16.2	1000 (s)
2364	Spicy / floral	NI	77	54	77	54	D^{23}, DE^{23}		0.1	0.1	0.1	0.2	6 (s)
2579	Vanilla	Vanilline	100	100	92	100		D^{23}, DE^{23}	1.6	2.5	4.1	2.5	60 (s)
2955	Sweet / chocolate	Syringaldéhyde	69	62	85	62	DE^{23}		1.3	2.8	3.2	2.6	50000 (e)

a, b, c, d, e, f see table 1. * Odors for which there is a difference in citation frequency between Grenache and Syrah of 23% or 30% at least, while comparing among non enzymed modalities (D) or enzymed (DE).

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these acids, under the conditions of the accelerated model aging.

c) Odorant areas potentially differentiating the two varieties

We could distinguish 23 odour areas which differentiate the two varieties with a detection frequency difference higher than 23% (table 2): 13 odour areas predominated in the Grenache samples and 11 predominated in the Syrah. Differences of detection frequency between the enzyme added samples (G-DE-Mod vs S-DE-*Mod*) was above 50% for 4 odours characterizing Grenache, whereas it reached exactly 30% for all the areas characterizing Syrah. That demonstrated the more important effect of enzyme addition on the Grenache variety, in accordance with the sensory analyses of wines enriched with glycosides (Ségurel *et al.*, 2009).

The analysis by GC-MS allowed the identification of 20 possible compounds for these odours, however 4 areas were unknown (table 2).

Among the compounds differentiating both varieties, three C13-norisoprenoids were identified: Riesling acetal (RI=1659), contributing to the typicity of old Riesling wines (Strauss *et al.*, 1987; Winterhalter *et al.*, 1990); β-damascenone (RI=1831) a powerful odorant in wines (Ferreira *et al.*, 2002; Lopez *et al.*, 2004); and 3,4-dihydro-3-hydroxyactinidol (RI=2704). These three compounds were generated from glycosylated precursors occurring in grape berry as reported previously (Strauss *et al.*, 1987; Winterhalter *et al.*, 1990; 1991). Riesling acetal and 3-hydroxy-3,4-dihydroactinidol odours were more intense in Grenache samples with or without enzyme treatment. β-damascenone predominated in Syrah sample without enzyme addition (S-D), but its odour was more intense in enzyme added Grenache sample (G-DE). That could be explained by the different reactivities of its precursors, free aglycones or glycosides (Winterhalter and Skouroumounis, 1997).

Nine volatile phenols differentiated both varieties: Methyl vanillate, zingerone, zingerol and 4-vinyl-phenol displayed the most significant differences in olfactory intensities (threshold ≥ 30%), the first two odorants characterizing Grenache, and the latter ones Syrah. That was consistent with the higher levels in zingerone and zingerol aglycones in the glycosidic fraction from the Grenache berries, but the other phenolic aglycones did not show significant differences in the berries of both varieties (Ségurel, 2005). With a lower detection frequency difference (≥ 23%), gaiacol, eugenol and vanillin could contribute to the aromatic typicity of Syrah wines. The odour area perceived at the retention index IR= 2044, more intense in the Syrah samples, was attributed to furaneol, based on its odour descriptors of caramel/strawberry jam only as it could not be detected using GC-MS. This compound was also found in the GC-O analysis of several old red wines (Kotseridis and Baumes, 2000, Aznar *et al.*, 2001; Cullere *et al.*, 2004). The furaneol contents in these samples, and in the corresponding glycosidic extracts, were probably underestimated due to the high polarity of the glucosides, making them difficult to extract from juices or wines (Kotseridis, 1999). Moreover, this compound could act in synergy with other keto-enols odorants such as homofuraneol and sotolon (Ferreira *et al.*, 2002).

Lastly, ethyl cinnamate, 3-methyl-butanoic acid, nona-2,6-dienal and 3-methylthiopropanal (methional) were detected in the samples of both varieties. The two aldehydes were only identified using their retention index and their characteristic odour descriptor (respectively cucumber and boiled potato). They were frequently reported in GC-O studies due to their formation in oxidized wines and their very low perception thresholds (Escudero *et al.*, 2000).

d) Odorant compound profiles generated by natural aging of model solutions of wines enriched with glycosides

The previously identified aroma compounds were quantified in the natural aged samples (table 3). Several compounds could not be quantified in the two samples without enzyme treatment (G-D-*Nat* et S-D-*Nat*), many more than in the corresponding accelerated model aging (G-D-*Mod* et S-D-*Mod*) (tables 1 and 2). Thus, the natural aging conditions used 18 months of storage at 13 °C was less efficient in generating odorants from glycosides than model aging conditions, which was consistent with the sensory analysis of wines enriched with glycosides (Ségurel *et al.*, 2009).

A principal component analysis (PCA) was carried out on the detection frequencies of the odorants identified by GC/O, in the 8 samples of both varieties submitted to model and natural aging, but odorants differentiating both varieties only were used (figure 3). In the PCA first plane, most variables were located in the Eastern part of the correlation circle, thus characterized by the abundance of compounds. The samples were separated according to the variety and the enzyme treatment, whereas the two aging modes Mod and Nat were grouped, which validated the thermal aging as a good model for natural aging. The first axis (49.7% of variability) separated the samples without enzyme addition in the Western zone, from the enzyme added samples, in the Eastern zone. The second axis (30.3% of variability) separated the Grenache from the Syrah samples. Both varieties were clearly separated when enzyme added samples were considered. Similarly, enzyme treatment differentiated more easily the Grenache than the Syrah samples, as already observed in the sensory analysis (Ségurel *et al.*, 2009) and the model aged samples.

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Table 3 - Amounts of odorant compounds in model wines added with glycoconjugates extracted from Grenache noir (G) or Syrah (S), and submitted to natural aging (storage at 13 °C for 18 months).

RI[a]	Odorant compound[a]	Concentration (µg/L) [b]			
		G-DE-Nat	S-DE-Nat	G-D-Nat	S-D-Nat
Compounds attributed to odors discriminant at the 30% level of citation frequency in GC-O					
1468	3-methyl-thio-propanal	Nd	Nd	Nd	Nd
1597	(*E,Z*)-Nona-2,6-dienal	Nd	Nd	Nd	Nd
1659	Riesling acetal	0.20	0.01	0.40	0.07
1831	β-damascenone	0.05	0.05	0.05	0.08
2044	Furaneol	Nd	Nd	Nd	Nd
2405	4-vinyl-phenol	Nd	Nd	2.09	2.05
2610	Methyl Vanillate	1.53	4.82	4.55	6.72
2704	3,4-dihydro-3-hydroxyactinidol	Nd	Nd	Nd	Nd
2704	3-[(2-hydroxyethyl)-thio]-hexan-1-ol	4.07	0.59	0.5	Nd
2815	Zingerone	0.41	Nd	8.06	3.74
2926	Zingerol	1.25	0.49	2.01	1.13
Compounds attributed to odors discriminant at the 23% level of citation frequency in GC-O					
1685	3-methyl-butanoic acid	Nd	0.02	1.43	0.14
1852	Geraniol	Nd	Nd	1.60	4.35
1870	Gaiacol	Nd	Nd	0.07	1.12
2073	Octanoic acid	84.07	44.9	69.75	38.83
2138	Ethyl cinnamate	0.22	Nd	0.24	0.31
2176	Eugenol	Nd	Nd	0.11	0.85
2279	Decanoic acid	17.20	10.67	9.26	6.24
2579	Vanillin	0.87	0.87	1.27	1.46
2955	Syringaldehyde	0.82	0.73	0.98	0.94
Compounds attributed to odor common to Grenache and Syrah					
1171	Butan-1-ol	Nd	Nd	1.28	1.77
1220	2- and 3-methylbutanols	4.15	4.50	21.97	30.23
1316	Heptan-2-ol	Nd	Nd	1.61	2.53
1328	Hexan-1-ol	Nd	Nd	116.84	75.06
1395	(*Z*)-hex-1-en-3-ol	Nd	Nd	10.60	6.82
1440	Ethyl octanoate	11.19	6,25	59.23	36.96
1455	Oct-1-en-3-ol	Nd	Nd	2.92	1.59
1514	2-ethyl-hexan-1-ol	0.46	0.48	1.31	0.58
1543	vitispiranes	Nd	Nd	0.07	Nd
1553	Linalol	0.45	0.31	5.36	10.12
1729	α-terpineol	Nd	Nd	10.34	3.66
1780	Citronellol	Nd	Nd	0.2	Nd
1786	Methyl salicylate	Nd	Nd	58.50	77.84
1862	Hexanoic acid	0.59	0.51	1.29	0.84
1921	2-phenylethanol	403.02	185.51	522.36	255.78
2024	*o*-cresol + phenol	Nd	Nd	0.21	0.16
2034	Pantolactone + γ-nonalactone	0.05	0.07	0.14	0.08
2091	*m*- and *p*-cresols	Nd	Nd	0.12	0.17
2127	*p*-cymen-7-ol	Nd	Nd	0.15	0.06
2201	δ-decalactone	Nd	Nd	0.10	0.11
2215	hydroxymethylacetophenone	Nd	Nd	0.15	5.98
2218	4-vinyl-gaiacol	Nd	Nd	0.95	Nd
2266	2,6-dimethoxyphenol	Nd	Nd	0.06	0.08
2548	Methoxyeugenol	0.01	0.08	0.29	1.86
2570	Phenylacetic acid	0.55	0.36	0.95	0.71
2645	Ethyl vanillate	0.62	2.63	1.00	5.78
3015	4-(*p*-hydroxy-phenyl)-butan-2-one	0.38	0.11	0.81	0.25
3091	4-(p-hydroxy-phenyl)-butan-2-ol	0.28	0.08	0.79	0.36

[a] RI: Retention Index on DBWAX column; compounds are those listed in table 1. [b] Estimated concentration, in equivalents of 4-nonanol, used as external standard, per liter of model wine; Nd: non detected. The four samples correspond to extracts of model wines added with glycoconjugates, enzymed with glycosidases or not (DE or D).

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e) Correlations with the data of the sensory analysis

The correlation between the data of the sensory analysis (Ségurel *et al.*, 2009) and that obtained by GC-O was difficult to establish because the CG-O resolutive approach did not take into account complex interactions between odorant compounds and between these compounds and their matrix (Aznar *et al.*, 2001; Ferreira *et al.*, 2002; Escudero *et al.*, 2004). Compounds of very different aroma classes can be involved in the expression of one single odour and conversely, one single component can contribute to the expression of different sensory notes, with no obvious link between these notes and the odour of the pure compound. These interactions are particularly important in complex liquids such as wine.

Furthermore the comparison with the wines tasted in the previous work (Ségurel *et al.*, 2009) was difficult to establish because the fermentation-derived esters and other fermentation-derived odorants were present only in the wines and some of them (ethyl esters of branched-chains acids) increased during aging.

CONCLUSION

The olfactometric analysis of synthetic model wines, enriched with glycosides and aged using model and natural aging, revealed several odorants contributing to their aroma. Moreover, varietal differences in the relative proportions of certain odorants generated from glycosides of both varieties were observed. Although several compounds eluted at the same retention time could be at the origin of some odorant areas, odors perceived by GC-O were tentatively linked to compounds identified by GC-MS. However it would be interesting to perform a bi-dimensional chromatography to confirm this hypothesis.

Correlations with the data of the sensory analysis (Segurel *et al.*, 2010) were difficult to establish, however the differences observed in GC-O of synthetic model wines enriched with glycosides and artificially aged were consistent with those detected by the sensory analysis of the corresponding wines. In both cases, enzyme treatment modified more significantly the aroma profiles of Grenache wines than those of Syrah.

Moreover, for the glycosidic fraction, the model aging, as proposed by Francis *et al.* (1994), appeared to be a good technic to reproduce a natural aging of several years. The compounds arising during this artificial aging were similar to those naturally obtain after several years.

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