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Determination of Volatile Compounds in Grenache Wines in Relation with Different Terroirs in the Rhone Valley

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This paper describes the study of 19 wines of the Grenache Noir cultivar obtained from representative soils of the Rhone Valley according to their geographical site, climatic conditions, hydrological regulation, and soil profile. Among the volatile compounds analyzed by GC/MS/FID, the concentrations of the varietal compounds (i.e., β-damascenone, β-ionone, and geraniol) and those of the compounds without direct influence on the wine aroma (i.e., hexenols and methanol) indicated the existence of two groups of wines. These concentrations were correlated with grape maturity due to the ecosystem and particularly the soil.

KEYWORDS: Wine; Grenache; volatile compounds; β-damascenone; β-ionone; α-ionone

INTRODUCTION

Grenache Noir is the principal type of vine in the Appellation Origine Contrôlée Côtes du Rhone and in most of the Rhone Valley appellations. There are few reference data concerning the composition of Grenache wines in terms of volatile compounds (*1*−*3*). However, some work has been done on flavor precursors of a glycosidic nature such as the carotenoids in Grenache grapes (*4*−*6*). Ormieres (*7*) showed that the aromatic potential of Grenache is dependent on molecules that might be released during wine-making. In the climate−soil−vine ecosystem, it is difficult to determine their influence on the constitution and the quality of grapes and wines (*8*). The relationships between precursor contents and the nature of the soil are currently being studied (*9*). Although the varietal compounds result directly from the grape, they are also closely related to the soil and the conditions of production.

Among grape varietal aromatic compounds, the terpenols are interesting for their flowery odors. For example, Baumes et al. (*1*) showed that Grenache is a neutral type of vine and has low free terpenol contents (i.e., a few micrograms per liter) compared to other types of vines, in particular Muscat types, the contents of which are ∼1 mg/L. β-Ionone (violet note) and damascenone (floral and honey notes) result directly from the grape but have different origins. β-Ionone is regarded as a degradation product of the carotenoids, the content of which decreases 8-fold during Grenache maturation (*6*). However, for β-damascenone, several other precursors have been found (*10*) such as 3,5,9-trihydroxymegastigma-6,7-diene (allenic triol) and 3,9-dihydroxymegastigm-5-en-7-yne (acetylenic diol). The hexenols and hexanol result from the transformation of aldehydes due to the enzymatic oxidation of grape fatty acids during alcoholic fermentation (*4*). These compounds have vegetal (herbaceous) notes; their thresholds being >2 mg/L.

As in other neutral types of wines, the flavor of Grenache wines is the result of the interaction of ∼40 odorous compounds, the levels or which vary from <1 μg/L (ionones) to >100 mg/L (isoamylic alcohol) (*2*). According to the latter authors, the most important odorous compounds are the ethyl esters, the volatile

Table 1. Classification of the 19 Wines in Relation to Soil Characteristics and Water-Holding Capacity

Deep Soils without Gravel

water-holding capacity				
>200 mm			100–140 mm	
deep sands	loess	old terrace without gravel	sands on marl	sands on sandstone
wines				
652	235	562	638	840
205				
789				

Gravelly Soils

water-holding capacity				
>200 mm	90–120 mm		50–80 mm	<50 mm
gravelly soils on marl	old Villafranchien or Mindelian terrace	gravelly colluviums	old Riss terrace on sandy gravel	gravelly soils on calcareous rock
wines				
867	383	941	412	986
736	162	422	103	
		456	597	
			318	

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Table 2. Levels (Milligrams per Liter) of Fermentative Volatile Compounds in the 19 Grenache Wines

	compounds[a]														
wine	VAC	FFA	Acetat	FAEE	EBut	ELa	DSuc	PhE	Actoi	Bd	Pd	But	Ade	EA	Alc
103	6.3	3.5	0.63	0.55	0.21	55	2.10	62	7.8	1025	72	31.6	5.0	69	392
162	5.7	4.1	1.28	0.76	0.33	33	0.87	42	18.5	1067	73	15.2	8.4	59	357
205	8.8	3.9	0.81	0.78	0.12	32	1.17	46	12.8	889	65	16.2	8.3	51	363
235	6.3	4.1	0.69	0.79	0.44	44	1.63	56	16.6	702	57	21.9	12.1	64	384
318	5.3	2.8	0.38	0.37	0.35	27	1.30	61	2.8	761	66	14.3	2.8	44	334
383	4.2	2.3	0.61	0.45	0.32	28	0.76	38	10.4	1046	70	19.0	7.7	51	285
412	6.0	3.1	0.69	0.62	0.19	26	1.24	66	8.3	831	64	19.9	18.7	60	360
422	5.3	3.8	1.60	0.79	0.46	53	0.99	34	13.7	1483	97	24.0	8.4	80	332
456	5.4	5.2	0.51	0.90	0.37	27	1.66	49	21.7	995	81	12.8	12.8	70	287
562	5.4	4.9	1.54	0.88	0.39	64	1.96	47	8.1	739	61	16.1	6.7	66	310
597	4.8	1.7	0.59	0.34	0.40	19	0.46	66	13.6	1039	69	18.6	8.7	53	322
638	6.1	5.3	0.78	0.94	0.23	29	1.48	60	12.6	962	71	16.4	6.1	59	352
652	4.2	3.2	0.63	0.59	0.40	53	1.45	20	17.7	1368	77	22.7	7.2	63	222
736	5.9	3.8	1.56	0.75	0.33	29	0.89	51	19.2	998	68	17.5	10.6	59	353
789	7.7	3.6	0.94	0.64	0.32	36	0.64	59	43.2	763	59	16.2	14.9	60	402
840	6.5	3.9	0.47	0.84	0.20	56	3.61	53	6.3	920	64	34.9	9.9	59	341
867	6.2	3.9	0.66	0.73	0.39	36	1.84	53	10.0	909	70	17.8	11.7	59	333
941	5.2	4.3	0.68	0.96	0.42	62	1.44	33	7.9	1152	83	20.2	5.7	69	308
986	4.7	2.3	0.26	0.50	0.33	23	1.13	47	4.7	1239	92	16.6	5.6	59	294
min	4.2	1.7	0.26	0.34	0.12	19	0.46	20	2.8	702	57	13	2.8	44	222
max	8.8	5.3	1.60	0.96	0.46	64	3.61	66	43.2	1483	97	35	18.7	80	402
mean	5.8	3.7	0.81	0.69	0.33	38	1.40	50	13	994	72	20	9.0	61	333
SD	1.1	0.9	0.39	0.18	0.09	14	0.68	12	9	203	10	5	3.7	8	42
variation (%)	19	24	48	26	28	36	48	24	64	20	14	28	41	13	13

[a] VAC, 2-methylpropionic acid + butyric acid + 3-methylpropionic acid; FFA, hexanoic acid + octanoic acid + decanoic acid + dodecanoic acid; Acetat, isoamyl acetate + hexyl acetate + phenylethyl acetate; FAEE, ethyl hexanoate + ethyl octanoate + ethyl decanoate; EBut, ethyl butyrate; ELa, ethyl lactate; DSuc, diethyl succinate; PhE, 2-phenylethanol; Actoi, acetoin; Bd, butane-2,3-diol D-(−) and meso; Pd, propane-1,2-diol; But, γ-butyrolactone; Ade, acetaldehyde; EA, ethyl acetate; Alc, propan-1-ol + 2-methylpropan-1-ol + butan-1-ol + 2-methylbutan-1-ol + 3-methylbutan-1-ol.

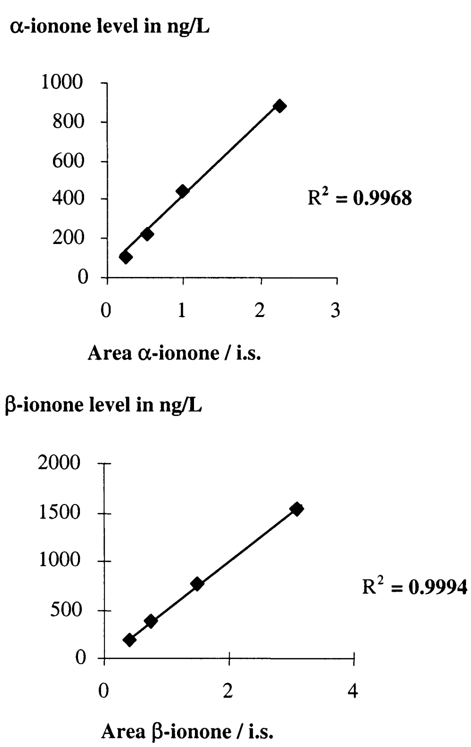

Figure 1. Linearity of α-ionone and β-ionone detection in Grenache red wine.

Table 3. Levels of Varietal Compounds in the 19 Grenache Wines

wine	hexenols[a] (μg/L)	terpene alcohols[b] (μg/L)	noriso-prenoids[c] (μg/L)	benz-aldehyde (mg/L)	benzyl alcohol (mg/L)
103	1150	41	1.77	nd[d]	nd
162	670	53	2.07	0.29	0.23
205	960	42	2.12	0.04	0.67
235	1260	96	1.81	nd	0.33
318	1150	63	4.17	nd	nd
383	800	39	2.36	0.08	0.50
412	960	20	2.25	nd	nd
422	870	50	2.43	0.11	0.52
456	1180	57	2.37	nd	nd
562	800	20	2.09	nd	nd
597	860	50	2.82	0.09	0.58
638	1010	47	2.47	nd	nd
652	870	55	2.39	0.14	0.40
736	650	61	2.19	0.09	0.72
789	1020	29	1.84	0.06	0.50
840	980	34	1.35	nd	nd
867	1210	38	2.98	0.07	0.73
941	1180	51	2.61	0.04	0.59
986	870	53	3.14	0.22	0.39
min	650	20	1.35	0	0
max	1260	96	4.17	0.29	0.73
mean	40	47	2.38	0.06	0.32
SD	15	17	0.67	0.08	0.28
variation (%)	36	35	28	133	87

[a] Hexanol + *cis*-hex-2-enol + *trans*-hex-3-enol. [b] Linalol + α-terpineol + citronelol + nerol + geraniol. [c] α-Ionone + β-ionone + β-damascenone. [d] Not detected.

phenols, the terpenols, lactones, and some norisoprenoidic derivatives. The objective of the present study is to show the influence of Rhone Valley soils (i.e., water reserve, stony ground or not, earliness, different geographical sites) on the aromatic composition (grape variety volatile compounds and fermentation) of Grenache wines.

MATERIALS AND METHODS

Selection of Experimental Area. Nineteen homogeneous parcels in terms of vine crops and cultivation techniques were selected to constitute the Grenache variety observatory (**Table 1**) described in ref *11*. The parcels representative of the Rhone Valley were selected

Table 4. In-Wine Levels of Compounds Characteristic of the Very Early Maturing Areas and Total Sugar Levels at Harvest

wine	methanol (mg/L)	damascenone (μg/L)	geraniol (μg/L)	β-ionone (μg/L)	*cis*-hex-2-enol (μg/L)	TA[a] (g/L)	sugars in must (g/L)
162	116	1.89	19.0	0.187	51	3.17	240.5
318	120	4.01	24.0	0.137	41	4.07	236.3
383	155	2.27	14.5	0.095	41	4.36	246.7
597	176	2.67	15.0	0.159	33	4.30	264.0
652	115	2.22	16.7	0.178	30	5.22	235.3
736	116	2.05	9.0	0.153	59	4.63	248.4
986	136	3.01	25.8	0.137	28	3.90	243.9
min	115	1.89	9.0	0.095	28	3.17	235.3
max	176	4.01	25.8	0.187	59	5.22	248.4
mean	133.4	2.59	17.7	0.149	40	4.24	264.0
SD	23.8	0.73	6	0.031	11	0.64	9.7
variation (%)	17.8	28	33	21	28	15	3.9

[a] TA expressed in g/L of tartaric acid.

Table 5. In-Wine Levels of Compounds Characteristic of the Relatively Late or Normally Maturing Areas and Total Sugar Levels at Harvest

wine	methanol (mg/L)	damascenone (μg/L)	geraniol (μg/L)	β-ionone (μg/L)	*cis*-hex-2-enol (μg/L)	TA[a] (g/L)	sugars in must (g/L)
103	116	1.561	nd[b]	0.215	101	6.55	240.0
205	135	1.869	9.8	0.253	70	4.53	232.8
235	122	1.593	nd	0.219	73	6.13	223.0
412	119	2.106	nd	0.146	66	5.87	236.3
422	100	2.185	7.6	0.251	52	5.41	248.4
456	83	2.038	1.4	0.290	69	5.49	219.0
562	108	1.816	nd	0.283	52	7.04	219.0
638	116	2.270	8.6	0.205	98	5.08	243.9
789	132	1.631	6.5	0.213	57	5.14	223.2
840	112	1.175	nd	0.177	113	7.04	245.6
867	106	2.786	8	0.194	59	4.76	231.0
941	96	2.365	7.5	0.254	97	5.64	235.0
min	83	1.175	1.4	0.146	52	4.53	219.0
max	135	2.786	9.8	0.290	113	7.03	248.4
mean	112	1.950	7.1	0.225	76	3.74	233.1
SD	15	0.433	3	0.043	20	0.83	10.3
variation (%)	13	22	38	19	28	15	4.4

[a] TA expressed in g/L of tartaric acid. [b] Not detected.

according to two principal soil criteria (*8*): the presence or not of stones and the water mode (dryness or favorable) in this southern region of France. The single rootstock used was the 110 R. The planting density was 3600−4000 vines/ha. The vines aged between 15 and 20 years were trellised in "cordon de Royat double" (except for Châteauneuf du Pape, where only the "gobelet" is authorized for Grenache). Inside each parcel, a zone of 100 vines generally distributed in four rows was identified, and 15 groups of three successive vines were selected for testing.

Wine-making. The 1995 harvest of each parcel was performed manually. The various batches of grapes were destemmed and crushed separately and then put in 1 hL stainless steel tanks. Must was added by SO_2 at 4−6 g/hL depending on the acidity and the health status of the grapes. Ten grams per hectaliter of the L2056 yeast (Institut Rhodanien) was then added. Fermentation and maceration lasted 7 days at ∼25 °C. Musts were inoculated with lactic acid bacteria Viniflora Oenos (Chr Hansen). The wines were bottled in January.

Acidity and Sugar Determination. Titratable acidity (TA) and reducing sugars were determined according to the method recommended by the OIV.

Chromatographic Analyses. *Varietal Compounds.* The method used to determine the free terpenols and alcohols with six carbon atoms was developed by Bertrand (*12*) and was modified by Guedes de Pinho (*13*). Fifty milliliters of wine adjusted to pH 8 was added with 50 μL of decan-4-ol (internal standard) at a concentration of 670 mg/L. This mixture was extracted with 4 mL, 2, mL, and again 2 mL of ether/hexane (1:1, v/v) by stirring each extraction for 5 min. The organic phases were collected, mixed, and concentrated 10 times under a nitrogen stream. Two microliters of the extract was injected into a gas chromatograph (Carlo Erba 4130) coupled to a flame ionization detector (FID). The column was an FFAP type (BP 21, 50 m × 0.25 mm × 0.2 μm, SGE, Courtaboeuf, France). The injector (split/splitless type) was heated to 250 °C with a splitless time of 0.3 min. The pressure of the carrier gas (hydrogen) was 180 kPa. The temperature of the oven was maintained at 60 °C for 5 min after the injection, was increased at a rate of 2 °C/min up to 200 °C, and was then maintained for 20 min. The method described in ref *14* was used to determine the norisoprenoids: β-ionone, β-damascenone, and α-ionone. The chromatograms were obtained by analyzing the extract of a wine by ether/hexane; this extract was then concentrated 10-fold by a nitrogen stream and injected (2 μL) into a GC coupled to an MSD in SIM mode, monitored with the ions 121, 190, and 177. The column was a Carbowax 20 M (Biochrom, 50 m × 0.25 mm × 0.25 μm, Champniers, France). Linearity was satisfactory for β-damascenone and for α-ionone and β-ionone (**Figure 1**).

Fermentative Compounds. Chromatographic methods (GC-FID) developed in the laboratory enabled us to determine the principal secondary compounds of the fermentation, that is, volatile fatty acids with short and long chains, acetates, ethyl esters, diethyl succinate, and phenylethanol (*12*); acetaldehyde, methanol, and higher alcohols (*15*); and acetoin and γ-butyrolactone (*16*). Briefly, esters and acids were determined after the addition of internal standard [i.s. (octan-3-ol, 20 mg/L)] and extraction with ether/hexane. Two microliters of extract was injected into the Carlo Erba 5300 GC (column BP 21, 50 m × 0.25 mm × 0.2 μm, SGE). Acetaldehyde and alcohols were determined by direct injection of 0.4 μL of wine after the addition of

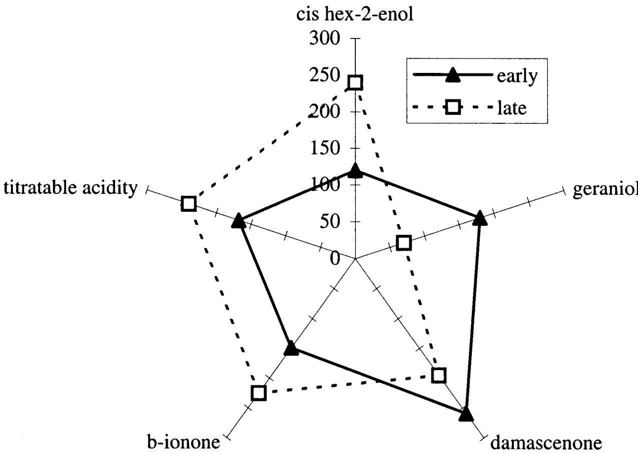

Figure 2. Compound distribution profile of Grenache wines from different areas of the Rhone Valley and at different stages of maturity (early and late parcels).

i.s. (4-methylpentan-2-ol, 100 mg/L) into the HP 5890 GC (column CPWax 57CB, 50 m × 0.25 mm × 0.2 μm, Varian, Les Ulis, France). Acetoin and γ-butyrolactone were determined by direct injection of 1 μL of wine after the addition of i.s. (octan-3-ol, 100 mg/L) into the GC HP 5890 (column Carbowax 20 M, 50 m × 0.25 mm × 0.25 μm, Biochrom).

RESULTS AND DISCUSSION

The use of the same yeast during alcoholic fermentation and strict respect for the conditions of wine-making explain the low coefficient of variation found (13%) for higher alcohols and to a lesser extent for ethyl esters of fatty acids (**Table 2**). In addition, there was a good correlation between the acidity of the wines and the ethyl lactate and diethyl succinate contents. The levels of all these fermentative compounds were close to those obtained in Grenache wines by Baumes et al. (*1*) and generally in young red wines (*3*).

With regard to the terpenols (**Table 3**), Grenache is a neutral type of vine like Cinsault, Carignan, Syrah, and Cabernet Sauvignon. Terpenol contents were ~47 μg/L and, similar to those found in other types of vines by Baumes et al. (*1*), were much lower than the sensory thresholds in white wine of 130 μg/L for geraniol, 100 μg/L for linalol, and 400 μg/L for α-terpineol (*17*).

With regard to norisoprenoids, β-ionone, β-damascenone, and α-ionone levels were strongly linked to the variety of Grenache. Baumes et al. (*1*) already noted higher concentrations for these substances in Grenache compared to Cinsault and Carignan, but curiously Ferreira et al. (*3*) did not find any β-damascenone in the characteristic grape variety odorants. In fact, the wines richest in β-damascenone were also the richest in geraniol (**Tables 4** and **5**). The average β-ionone levels (197 ng/L) are similar to those found in Merlot by Koseridis et al. (*14*) in 1998 (153 ng/L). On the other hand, Grenache wines were less rich in α-ionone (average of 4 ng/L in Grenache and 154 ng/L in Merlot) but were richer in β-damascenone (2185 ng/L in Grenache and 552 ng/L in Merlot). The β-damascenone contents varied from 1000 g/L to 4000 ng/L. Because the olfactory threshold in wine is 4000 ng/L, β-damascenone could be an important compound in the flavor of Grenache wines. Despite their low levels, the three aromatic ketones (α-ionone, β-ionone, and β-damascenone) could play a role in flavor due to their high volatility (*2*).

These findings suggest the existence of two major groups in the wines studied. The first consists of wines produced in the most southerern zone and on the warmest soils, where maturation occurs early (**Table 4**). They generally have the highest concentrations in β-damascenone and in geraniol. They also have a low total acidity due to their high degree of maturity as confirmed by their high potential alcohol in must. The average acidity for these seven wines was 4.24 g/L of tartaric acid, whereas for all the wines taken together it was 4.99 g/L. However, the geraniol concentrations were much lower than the sensory threshold, and the hexenol contents were low. The seven wines had higher methanol contents, a finding possibly due to the high pectin content resulting from slight over-ripening. Geraniol, hexenol, and methanol contents had no direct influence on flavor but seem to be important markers for differentiating soils. The other group consists of wines obtained from soils giving grapes that mature later. These wines had a higher total acidity and a lower total sugar levels and were poor in β-damascenone but richer in β-ionone (**Table 5**). **Figure 2** shows both groups of wines according to a compound distribution profile involving β-damascenone, geraniol, β-ionone, *cis*-hex-2-enol, and titratable acidity.

In conclusion, this study used chromatographic methods to measure fermentative and some varietal compounds occurring in Grenache in various ecosystems of the Rhone Valley, France. The volatile compound profile would seem to indicate that the origin of these wines may be established according to the degree of grape maturity. Many factors were found to influence the levels of varietal volatile compounds in wine such as cultivar, region, vintage, and technological process. These findings based on β-damascenone, geraniol, β-ionone, and *cis*-hex-2-enol levels therefore represent an initial approach to understanding the influence of the ecosystem on the constitution of Grenache wines.

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