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Investigating the Aroma of Syrah Wines from the Northern Rhone Valley Using Supercritical CO₂-Dearomatized Wine as a Matrix for Reconstitution Studies

Olivier Geffroy,* Marie Morère, Ricardo Lopez, Grégory Pasquier, and Jean-Stéphane Condoret

ABSTRACT: This study aimed to investigate the key compounds involved in the aroma of French Syrah wines from the northern Rhone valley from two vintages characterized by distinct climatic conditions. The volatile composition of the wines was assessed through the determination of 76 molecules. After identifying the best matrix and best model for aroma reconstitution studies, omission tests were conducted using the Pivot profile method. For both vintages, 35 molecules with odor activity values (OAVs) above 0.5 were identified. While remarkably high levels of 2-furfurylthiol (FFT) were reported in both wines, rotundone and 3-sulfanylhexanol (3SH) enabled the strongest discrimination between the two wines. Wine dearomatized using supercritical carbon dioxide (sCO₂) was identified as the best matrix. The best models built using this matrix were composed of molecules with OAV > 5 and OAV > 10 highlighting that this dearomatization approach can be valuable to reconstitute the aroma of wine using a small number of molecules. For the cool vintage wine, the omission of rotundone and FFT had the greatest impact on the olfactive profile for nonanosmic and anosmic respondents to rotundone, respectively. 3SH, whose omission decreased the rating of the “fruity” attribute, was identified as the main contributor to the aroma of Syrah wine produced in the warm vintage.

KEYWORDS: *Syrah, wine, aroma reconstitution, omission tests, supercritical CO₂, Pivot profile*

■ INTRODUCTION

With 190,000 ha under vines mainly in France (64,000 ha) and Australia (40,000 ha), *Vitis vinifera* L. Syrah was the eighth most planted cultivar in the world in 2015.¹ Several hypotheses on its origin have been proposed, including those that place it in Iran, in the ancient viticultural area of “Shiraz/Chiraz”, or in Sicily in Syracuse.² However, Syrah is more likely to originate from the French Alps area as genetic research studies have shown that it was obtained from a spontaneous cross between Mondeuse blanche and Dureza, two almost extinct grape varieties from this area.² Syrah remains a historic variety in the Rhone Valley in France where it is usually blended with Grenache and Mourvèdre in the southern vineyards. Red wines from the northern part of the valley such as those made within the protected designations of origin (PDOs) Côte-Rotie, Saint-Joseph, Crozes-Hermitage, Hermitage, and Cornas are made from pure Syrah.

Despite the importance of Syrah for these PDOs, little research has been undertaken to investigate the volatiles involved in the aroma of these wines. To the best of our knowledge, there is only one such study, conducted by a Japanese research group,³ which showed that wines from Côte-Rotie, Saint-Joseph, and Crozes-Hermitage could contain substantial levels (above 100 ng/L) of rotundone, a sesquiterpene responsible for black pepper aroma.⁴ Apart from rotundone, research studies undertaken in other vineyards indicate that Syrah red wines contain negligible concentrations of methoxypyrazines⁵ and that hydrolyzed glycosidic precursors together with dimethyl sulfide are responsible for the fruity, black olive and earthy, and

tobacco/cigar note characteristics of this cultivar.⁶ When vinified in rosé, Syrah wines can also exhibit significant levels of 3-sulfanylhexanol (3SH) imparting grapefruit and tropical aromas.⁷ The most complete and consistent work characterized the key aroma compounds, through reconstitution studies and omission tests, of ultrapremium Australian Syrah wines sourced in the Barossa Valley and in the Margaret River wine regions.⁸ Using a synthetic wine made by mixing 44 aroma compounds in concentrations measured in Syrah wines, this study showed that compounds such as β -damascenone, linalool, and fatty acids had a small impact on aroma. It also highlighted that the greatest impact was observed when omitting nonvolatile compounds such as organic acids, sugars, minerals, and glycerol, which emphasizes the importance of the matrix when conducting such aroma reconstitution studies.

Most of the research studies using this latter approach, popularized by Ferreira et al.⁹ and inspired by Grosch and Schieberle,¹⁰ have been carried out using hydroalcoholic solutions with a relatively simple composition.^{8,9} However, because of the large impact of the nonvolatile fraction on the aroma perception,^{8,11,12} the use of a native wine that has undergone a dearomatization could be a valuable technique to

increase the quality of the aroma reconstitution. This dearomatization could be achieved through rotary evaporation followed by a resin treatment, as proposed by Lytra et al.,¹³ or using supercritical carbon dioxide (sCO₂), a promising physical extraction technique that has proved its efficiency for the recovery of aroma from wine.¹⁴ sCO₂ is a fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure, which can adopt properties midway between a gas and a liquid.

The aim of the present work was to study the key volatiles involved in the aroma of Syrah wines from the Northern Rhone valley from two vintages with distinct climatic conditions using sCO₂ dearomatized wine as a matrix for reconstitution studies.

■ MATERIALS AND METHODS

Wine. Two commercial unwooded Syrah wines from the Crozes-Hermitage PDO were selected for the study in February 2018. These wines, produced by the same winery and from the same vineyard blocks located in the south part of the PDO, were from two vintages with distinct climatic conditions (cool for 2013 and warm for 2015). To characterize accurately these two vintages from a climatic point of view, daily rainfall and air temperature (minima, maxima, and mean values) from 1994 to 2017 were provided by Météo France (Toulouse, France) for the Mercuriol weather station. This station is located less than 10 km from the sites where the grapes were sourced. The data were used to calculate the Huglin index¹⁵ and the cumulative rainfall between 1 April and 30 September and between 1 January and 31 December. An informal olfactory evaluation (orthonasal and retronasal) organized with a small group of tasters confirmed that the two wines had distinct sensory features (spicy/peppery for 2013 and fruity for 2015) and were representative of the diversity of vintage styles found within the PDO. To avoid any modification in the composition or sensory profile over the different phases of the study that ended in May 2019, the wines were stored at 4 °C. All the classical analyses performed on the wines from the identification of the best matrix experiment, either before or after the dearomatization processes, were carried out using a Winescan FT-120 (Foss France SAS, Nanterre, France). These analyses included the alcohol content (% v/v), titratable acidity (g/L tartaric acid), pH, tartaric acid (g/L), malic acid (g/L), lactic acid (g/L), volatile acidity (g/L acetic acid), and modified color intensity (MCI) calculated as the sum of absorbance at 420, 520, and 620 nm, total phenolic index (TPI), and carbon dioxide (mg/L). All determinations were carried out once, but the Winescan produced duplicate spectra and provided mean values for each parameter.

Chemicals. All chemicals used for the reconstitution studies were of analytical reagent grade (purity >95%). Dilutions, when needed, were prepared in dipropylene glycol (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Rotundone was supplied by Firmenich (Geneva, Switzerland), and *cis*-oak-lactone together with 2,3-butanedione by Diffusions Aromatiques (Saint-Cézaire-sur-Siagne, France). Because of the difficulties of handling gaseous molecules, hydrogen sulfide (H₂S) and methanethiol were used in the nanoencapsulated form from mother solutions prepared just before use (Cara Technology Limited, Surrey, United Kingdom). 3SH and 3-sulfanylhexyl acetate were supplied by Cluzeau Info Labo (Sainte-foy-La-Grande, France). The rest of the molecules were supplied by Sigma-Aldrich (Saint-Quentin-Fallavier, France). These include ethyl hexanoate, ethyl lactate, ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate, ethyl acetate, isoamyl acetate, acetic acid, butyric acid, isobutyric acid, isovaleric acid, hexanoic acid, octanoic acid, isobutanol, isoamyl alcohol, *cis*-3-hexenol, methionol, 2-phenylethanol, acetaldehyde, β -damascenone, β -ionone, dimethyl sulfide, 2-furfurylthiol (FFT), benzyl mercaptan (BM), guaiacol, eugenol, 4-ethylphenol (4-EP), 4-vinylguaiacol (4-VG), *trans*-isoeugenol, and γ -butyrolactone.

Quantitative Analysis of Aroma Compounds. Seventy-six molecules belonging to several chemical families were analyzed in the

wine using all the analytical methods routinely implemented in our laboratory, apart from 3-isobutyl-2-methoxypyrazine (IBMP) and 2-isopropyl-3-methoxypyrazine (IPMP), which were not analyzed. Indeed, previous research showed that IBMP and IPMP did not contribute to the aroma of Syrah wines with odor activity value (OAV) < 0.5, including those from cool climate wine regions,^{5,8} some weather conditions well known to be favorable to obtaining substantial levels of methoxypyrazines.

The quantitative analysis of major compounds was carried out using a validated published method.¹⁶ In accordance with this method, 3 mL of wine containing the internal standards (IS) (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, ethyl heptanoate, heptanoic acid, and 2-octanol) and 7 mL of water were salted with 4.5 g of ammonium sulfate and extracted with 0.2 mL of dichloromethane. The extract was then analyzed by gas chromatography (GC) with flame ionization detection. The area of each analyte was normalized by that of its corresponding IS and then interpolated in the corresponding calibration plot. The plots were built by applying exactly the same analytical method as that applied to synthetic wines containing known amounts of the analytes, covering the natural range of occurrence of these compounds. Typical R² varied between 0.9938 and 0.9998.

The analysis of minor compounds was carried out using the method proposed by López et al.¹⁷ In accordance with this method, 50 mL of wine, containing 25 μ L of the BHA solution and 75 μ L of a surrogate standard solution (3-octanone, β -damascone, heptanoic acid, and isopropyl propanoate), were passed through a LiChrolut EN (Merck, Darmstadt, Germany) 200 mg cartridge at a rate of about 2 mL/min. The sorbent was dried under nitrogen stream (purity 99.999%). Analytes were recovered by elution with 1.3 mL of dichloromethane. A volume of 25 μ L of an IS solution (4-hydroxy-4-methyl-2-pentanone, 3,4-dimethylphenol and 2-octanol, both at 300 mg per g of dichloromethane) were added to the eluted sample. The extract was then analyzed by GC with ion trap mass spectrometry (MS) detection under the conditions described in the reference. This method has a satisfactory linearity with R² higher than 0.99 in all cases.

The analysis of highly volatile sulfur compounds was carried out using the method proposed by Ontañón et al.¹⁸ based on automated headspace solid-phase microextraction and GC-pulsed flame photometric detection. A volume of 4.9 mL of saturated NaCl brine was placed in a 20 mL standard headspace vial and sealed. After this, the vial was purged with a nitrogen stream of 2 bar for 1 min. Immediately after this operation, 100 μ L of the wine sample, 5 μ L of the ethanediol solution, and 20 μ L of the IS solution (isopropyl disulfide, 1-propanethiol, 2-propanethiol, cyclopentanethiol, 1-hexanethiol, and [²H₆]dimethyl sulfide at 200 μ g/L) were injected through the septum with a syringe. Samples were incubated for 5 min at 35 °C and then extracted for 20 min at the same temperature. The extraction was performed with agitation at 250 rpm in cycles of 8 s on and 2 s off. Desorption took place in the injection port at 300 °C for 7 min, and analysis was carried out under the conditions listed in the reference. In all cases, the linearity of the method was satisfactory with R² ranging from 0.9823 (H₂S) to 0.9980 (methanethiol).

The analysis of polyfunctional mercaptans was carried out using the method proposed by Mateo-Vivaracho, et al.¹⁹ First, 0.2 g of ethylenediaminetetracetic acid and 0.6 g of L-cysteine chlorohydrate were added to 25 mL of wine. This sample mixture was then transferred to a 20 mL volumetric flask where it was spiked with 15 μ L of an ethanolic solution containing 1400 μ g/L of [²H₂]-2-furfurylthiol, [²H₃]-benzyl mercaptan, and [²H₃]-3-mercaptohexyl acetate as IS. The complete volume was then transferred into a 24 mL screw-capped vial together with 0.2 g of O-methylhydroxylamine, shaken for 15 s, purged with pure nitrogen (99.999%), sealed, and incubated in a water bath at 55 °C for 45 min. Six milliliters of this incubated sample was then loaded into a 50 mg Bond Elut-ENV solid-phase extraction (SPE) cartridge (Varian, Walnut Creek, USA). Major wine volatiles were removed by rinsing with 4 mL of a 40% methanol–water solution (0.2 M) in phosphate buffer at pH 7.7. A second IS was also loaded into the cartridge by passing it through 220

Table 1. Odor Threshold, Concentration, OAV, Accumulated Uncertainty of the Method As Expressed in RSD, %, and Inter-Vintage RSD_{vintage}, % of Aroma Compounds, Grouped by Chemical Family, for the Two Crozes-Hermitage Syrah Wines

chemical family	aroma compound	odor threshold ^a (μg/L)	2013		2015		uncertainty as expressed in RSD (%)	RSD _{vintage} (%)	
			concentration (μg/L)	OAV ^b	concentration (μg/L)	OAV			
ethyl esters	ethyl propanoate	5500 ⁴¹	137	<0.5	240	<0.5	15	39	
	ethyl butyrate	125 ⁴¹	<30	^c	<30	—	9	—	
	ethyl hexanoate	62 ⁴¹	586	9.5	744	12.0	7	17	
	ethyl octanoate	580 ⁴¹	191	<0.5	277	<0.5	7	26	
	ethyl decanoate	200 ⁴¹	<20	—	<20	—	4	—	
	ethyl lactate	154,000 ⁴²	195,585	1.3	160,072	1.0	3	14	
	diethyl succinate	200,000 ⁴²	16740	<0.5	17949	<0.5	4	5	
	ethyl isobutyrate	15 ⁴¹	100	6.7	51	3.4	2	46	
	ethyl 2-methylbutyrate	18 ⁴¹	27	1.5	12	0.7	5	53	
	ethyl isovalerate	3 ⁴¹	42	14.1	22	7.4	3	44	
Acetates	ethyl acetate	12,300 ⁴²	64,955	5.3	76,925	6.3	7	12	
	isoamyl acetate	30 ⁴³	111	3.7	190	6.3	6	37	
	hexyl acetate	1500 ⁴²	<10	—	<10	—	6	—	
	isobutyl acetate	1605 ²²	8.7	<0.5	5.8	<0.5	3	29	
	butyl acetate	1800 ⁴²	1.9	<0.5	1.8	<0.5	4	4	
Acids	2-phenylethyl acetate	250 ⁴³	4.1	<0.5	5.6	<0.5	7	22	
	acetic acid	300,000 ⁴³	429,857	1.4	442,911	1.5	10	2	
	butyric acid	173 ⁴¹	186	1.1	186	1.1	4	0	
	isobutyric acid	2300 ⁴¹	3139	1.4	4726	2.1	3	29	
	isovaleric acid	33 ⁴¹	1719	52.1	943	28.6	3	41	
	hexanoic acid	420 ⁴¹	1382	3.3	1684	4.0	10	14	
	octanoic acid	500 ⁴¹	1666	3.3	1915	3.8	5	10	
	decanoic acid	1000 ⁴¹	247	<0.5	433	<0.5	9	39	
	Alcohols	isobutanol	40,000 ⁴³	63,758	1.6	25,701	0.6	2	60
		1-butanol	150,000 ⁴²	781	<0.5	921	<0.5	4	12
isoamyl alcohol		30,000 ⁴³	334,668	11.2	219,489	7.3	3	29	
1-hexanol		8000 ⁴³	1849	<0.5	1864	<0.5	4	1	
<i>cis</i> -3-hexenol		400 ⁴³	252	0.6	274	0.7	4	6	
methionol		1000 ⁴¹	2414	2.4	1064	1.1	4	55	
benzyl alcohol		200,000 ⁴⁴	294	<0.5	220	<0.5	11	20	
2-phenylethanol		14,000 ⁴¹	39,602	2.8	24,696	1.8	10	33	
carbonyl compounds	acetaldehyde	500 ⁴³	816	1.6	333	0.7	11	59	
	2,3-butanedione	100 ⁴³	417	4.2	658	6.6	8	32	
	acetoin	150,000 ⁴²	11,052	<0.5	22,410	<0.5	5	48	
	benzaldehyde	1500 ⁴²	11	<0.5	2.0	<0.5	3	99	
terpenes and norisoprenoids	linalool	25 ⁴¹	6.7	<0.5	7.9	<0.5	9	12	
	α -terpineol	250 ⁴¹	11	<0.5	8.1	<0.5	3	20	
	citronellol	100 ⁴²	1.2	<0.5	2.1	<0.5	4	39	
	geraniol	20 ⁴³	1.3	<0.5	1.5	<0.5	9	12	
	rotundone	0.008 ⁴	0.054	6.7	0.007	0.9	10	109	
	β -damascenone	0.05 ⁴³	1.0	19.3	1.1	22.1	4	10	
	α -ionone	2.6 ⁴²	0.47	<0.5	<0.02	—	4	—	
	β -ionone	0.09 ⁴¹	0.20	2.2	0.19	2.1	6	1	
highly volatile mercaptans	hydrogen sulfide	10 ⁴⁵	14	1.4	11	1.1	3	17	
	methanethiol	2.2 ⁴⁵	3.7	1.7	3.6	1.7	2	2	
	ethanethiol	0.008 ⁴⁵	<1.3	—	<1.3	—	3	—	
	dimethyl sulfide	10 ⁴⁵	43	4.3	23	2.3	3	42	
	dimethyl disulfide	30 ⁴⁵	<0.20	—	<0.20	—	3	—	
polyfunctional mercaptans	FFT	0.0004 ⁴⁶	0.016	40.5	0.017	42.8	23	4	
	4-methyl-4-sulfanylpentan-2-one	0.0008 ⁴⁶	<0.0035	—	<0.0035	—	18	—	
	3-SH	0.06 ⁴⁶	<0.023	—	0.933	15.6	9	141	
	benzyl mercaptan	0.0003 ⁴⁷	0.004	14.3	0.004	12.8	20	8	
	3-sulfanylhexyl acetate	0.004 ⁴⁶	0.009	2.3	0.008	2.1	22	5	
Phenols	guaiaicol	9.5 ⁴³	27	2.7	22	2.3	5	13	

Table 1. continued

chemical family	aroma compound	odor threshold ^a ($\mu\text{g/L}$)	2013		2015		uncertainty as expressed in RSD (%)	RSD _{vintage} (%)
			concentration ($\mu\text{g/L}$)	OAV ^b	concentration ($\mu\text{g/L}$)	OAV		
	<i>o</i> -cresol	31 ¹⁷	<0.01	–	<0.01	–	3	–
	4-ethylguaiaicol	33 ⁴¹	5.5	<0.5	1.0	<0.5	3	97
	<i>m</i> -cresol	68 ⁴¹	0.60	<0.5	0.41	<0.5	3	28
	4-propylguaiaicol	10 ⁴⁴	0.27	<0.5	<0.01	–	4	–
	eugenol	6 ⁴¹	12	1.9	6.4	1.1	2	41
	4-EP	35 ⁴¹	77	2.2	17	<0.5	4	91
	4-VG	40 ⁴³	42	1.1	95	2.4	3	54
	<i>trans</i> -isoeugenol	6 ⁴⁴	2.6	<0.5	3.0	0.5	4	11
	2,6-dimethoxyphenol	570 ¹⁷	69	<0.5	58	<0.5	3	12
	4-vinylphenol	180 ⁴⁸	51	<0.5	40	<0.5	2	17
	4-allyl-2,6-dimethoxyphenol	1200 ⁴⁹	13	<0.5	7	<0.5	5	42
vanillin derivatives	vanillin	60 ⁴⁴	19	<0.5	11	<0.5	3	34
	methyl vanillate	3000 ¹⁷	44	<0.5	36	<0.5	3	13
	ethyl vanillate	990 ¹⁷	282	<0.5	123	<0.5	4	56
	acetovanillone	1000 ⁴⁴	51	<0.5	42	<0.5	6	14
Lactones	γ -butyrolactone	35,000 ⁴⁴	18,190	0.5	16,120	<0.5	4	9
	<i>trans</i> -oak-lactone	790 ⁴²	64	<0.5	54	<0.5	3	12
	<i>cis</i> -oak-lactone	67 ⁴²	102	1.5	72	1.1	2	24
	γ -nonalactone	31 ⁵⁰	6.7	<0.5	6.9	<0.5	3	1
	γ -decalactone	10 ⁴²	<0.27	–	<0.27	–	4	–
cinnamates	ethyl dihydrocinnamate	1.6 ⁴¹	0.45	<0.5	0.42	<0.5	3	4
	ethyl cinnamate	1.1 ⁴¹	0.38	<0.5	0.41	<0.5	3	5

^aOdor threshold; reference given. All odor thresholds were determined in hydroalcoholic solutions or synthetic wines except for refs 4 41, and 45, which were given in water. ^bOdor activity value. ^cNot determined.

μL of solution (20 μL of 4-methoxy- α -toluenethiol, 150 $\mu\text{g/L}$ in ethanol, and 200 μL water). Mercaptans retained in the cartridge were directly derivatized by passing 1 mL of an aqueous solution of DBU (6.7%) and 50 μL of a 2000 mg/L solution of PFBBR in hexane and letting the cartridge imbibe with the reagent for 20 min at room temperature (25 °C). The cartridge was then rinsed with 4 mL of a 40% methanol/water solution 0.2 M in H_3PO_4 and with 1 mL of water. Derivatized analytes were finally eluted with 600 μL of a solvent mixture (hexane 25% in diethyl ether), and then 10 μL of the chromatographic IS solution (octafluoronaphthalene 22.5 $\mu\text{g/L}$ in hexane) was added to the extract. The eluate was finally washed with five 1 mL volumes of brine (200 g/L NaCl water solution), transferred to a 2 mL vial, and spiked with a small amount of anhydrous sodium sulfate. Four microliters of this sample was directly injected in cold splitless mode into the GC-negative chemical ionization MS system, which was operated as described in the published method. Linearity of the method ($0.978 < R^2 < 0.999$) was satisfactory.

Rotundone was quantitated in wine using the method developed by Culleré et al.²⁰ A volume of 30 μL of the IS solution (benzyl benzoate $\approx 100 \text{ mg L}^{-1}$ in ethanol) was added to 50 mL of wine, and then this volume was loaded into a 200 mg bond ELUT PPL cartridge. The cartridges were previously conditioned with 4 mL of dichloromethane, 4 mL of methanol, and 4 mL of a hydroalcoholic solution containing 12% (v/v) ethanol. After loading the wine, the bed was washed with 5 mL of water, 20 mL of an aqueous solution containing methanol [70% (v/v)], and 1% NaHCO_3 . After the cartridges were dried, the elution was carried out with 2 mL of hexane containing 25% (v/v) of ethyl ether. Then, this extract was concentrated to 200 μL by a nitrogen stream. The area of the peak corresponding to the m/z fragment chosen for the rotundone was normalized to that of the IS. These relative areas were interpolated in the calibration curve, prepared by the SPE-GC-MS analysis of synthetic wine samples containing known amounts of rotundone and the IS. The methodology used for the analysis of synthetic wine samples was the same as

the proposed method for the real wine samples. This method showed satisfactory linearity ($R^2 = 0.9974$) in red wine.

All determinations were carried out once, except for highly volatile sulfur compounds, which were determined in duplicate and for which mean values were considered. Such duplicates enable to detect occasional errors in the analysis that can occur because of the high volatility of these compounds. The response factors for the aroma compounds quantitated in the two Crozes-Hermitage wines are shown in Table S1.

Accumulated uncertainty of the analytical methods, expressed as relative standard deviation (RSD), is shown in Table 1 for each quantitated aroma compound. For each aroma compound, the RSD between the two vintages ($\text{RSD}_{\text{vintage}}$) was calculated and the OAV was determined by dividing the concentration of the compound by its odor threshold in water or in the hydroalcoholic solution. When the concentration of an analyte was below its limit of quantitation, its OAV was not determined.

Identification of the Best Matrix. A first experiment was carried out to identify the best matrix for aroma reconstitution. Two synthetic wines with two levels of alcohol content (SW 10% and SW 12.5%) were prepared by mixing tartaric acid (2 g/L), lactic acid (1.5 g/L), glycerol (8 g/L), and absolute ethanol to get a final concentration of 10% and 12.5% v/v. The pH was adjusted to 3.50 with 1 M NaOH. These reduced concentrations of ethanol were used as preliminary studies showed that the synthetic wines with the ethanol content of the original wines were smelling alcohol despite the use of glycerol to limit the volatility of ethanol. For producing dearomatized red wine through supercritical carbon dioxide (sCO_2), an extraction pilot SFE200 from Separex (Champigneulle, France) was used. This comprises a 200 mL stainless steel extractor, which was loaded with 130 mL of native red wine (Crozes-Hermitage PDO). The system was operated at a constant temperature of 40 °C and a pressure of 100 bar, optimal conditions to remove the aroma.¹⁴ Pure CO_2 was passed into the cell for 30 min with the flow rate kept constant at 20 g/min. Several runs were carried out under the same conditions to obtain enough dearomatized red wine for each vintage. Dearomatized wines

obtained from these runs were blended and used in the classical analysis and reconstitution studies. Containers hosting the dearomatized wine were maintained opened for enough time to allow the elimination of the excess of carbon dioxide. After such treatment, the wine was completely odorless with no aroma perceived at tasting. For each vintage, dearomatized red wine was also prepared using a method adapted from Lytra et al.¹³ Native red wines (Crozes-Hermitage PDO) were treated using a Rotavapor EL 131 (Büchi, Rungis, France) with a 20 °C bath temperature to obtain two-thirds of their original volume. Then, the liquid was supplemented with 5 g/L LiChrolut resin (40–120 μm) and was stirred for 12 h. The solution was centrifuged (11, 200 rpm for 20 min), and classical analyses were performed. The dearomatized wine obtained after such treatment (Rotavapor) was not completely odorless and had a very low aroma intensity at tasting.

For each vintage, reconstitution studies were conducted by mixing aroma compounds, whose OAV was strictly above 0.5, in concentrations measured in Syrah wines. Indeed, a previously published wine reconstitution study demonstrated that such models showed the highest qualitative similarity with the aroma of the native wine.⁹

As in other reconstitution studies,^{8,9,11} differences were expected between the native wines and the reconstituted wines. Therefore, to assess the quality of the reconstitutions, the degree of difference (DOD) test²¹ whose procedure can be summarized as follows was preferred over triangular tests. The panel was composed of 15 panelists (8 males and 7 females, ages 22–53) who had prior experience in wine tasting. They were asked to proceed only with an olfactory assessment. For each vintage, their task was to rate the DOD between pairs of samples on a discontinuous scale ranging from “0” (similar) to “10” (extremely different). Pairs of samples consisted of the native wine (identified as the reference) and the four reconstituted wines (SW 10%, SW 12.5%, sCO₂, and Rotavapor) coded with three-digit codes. The native wine served blind was also compared to itself to get a baseline DOD score. A constant volume of 15 mL of each sample was poured into black wine-tasting glasses at 18 °C. Pairs were presented according to a balanced design (Williams Latin square). The panelists received a new pair of samples every 5 min. The questionnaire also contained ample space for free comments. The panelists had first to assess the DOD for the five pairs of samples from the 2013 series and after a 5 min rest those from the 2015 series. Sensory analysis was organized at 22 °C, under daylight lighting, in an air-conditioned professional tasting room.

Optimization of the Model. To optimize the model, a second experiment was conducted on the matrix that received the lowest DOD score during the identification of the best matrix experiment. For each vintage, several models were built by mixing aroma compounds whose OAVs were strictly above 0.5 (OAV > 0.5), above 1 (OAV > 1), above 2 (OAV > 2), above 5 (OAV > 5), and above 10 (OAV > 10), in concentrations measured in Syrah wines. For each vintage, the reference sample was the native wine, which was also compared to itself served blind. Sensory evaluation was conducted under the same conditions as during the previous experiment. The panelists had first to assess the DOD for the 6 pairs of samples from the 2013 series and after a 5 min rest those from the 2015 series.

Omission Tests. Omission experiments were carried out using the best matrix and the best model identified in the two previous experiments. For each vintage, aroma compounds to be omitted were selected on the basis of belonging to the best model and having a grape-derived origin. Nineteen panelists (11 females, 8 males, ages 28–55) who had extensive experience in wine tasting were recruited from a local winemakers' association and a technical institute. Samples were assessed using Pivot Profile,²² a relatively new descriptive method based on free description, which can be summarized here briefly. The panelists were provided with pairs of samples consisting of a single reference product called the pivot and the sample product following a balanced design (Williams Latin square). They were then asked to freely describe the differences between the two samples. For each vintage, the pivot was built as an average sample of all the test products (fully optimized reconstitution, optimized reconstitutions

with omission of some aroma compounds). The panelists were only asked to smell the samples and to write down each attribute that the samples had in smaller or larger amounts than the pivot (e.g., less green, more animal). They were instructed to use only descriptive words without providing any sentences and were not allowed to use the negative form (e.g., closed for nonexpressive). For each pair of samples, the pivot was regenerated. A constant volume of 15 mL of each sample was poured into black wine-tasting glasses at 18 °C, and the panelists received a new pair of samples every 5 min. The panelists had first to evaluate the pairs of samples from the 2013 series and after a 5 min rest those from the 2015 series. As specific anosmia has been previously reported for rotundone,⁶ a triangular test was performed at the end of the session to detect anosmic panelists. One of the three coded samples contained either a 200 ng/L rotundone solution or water alone. The position and the nature of the different samples within each test were randomized. Those who were not able to identify the different sample after the olfactory assessment were considered as anosmic respondents. Omission experiments were organized at 22 °C, under daylight lighting, in an air-conditioned professional tasting room.

Statistical Treatments. For the identification of the best matrix and the best model optimization experiments, statistical analyses were conducted with XLSTAT software (Addinsoft, Paris, France). DOD scores were subjected to a three-way analysis of variance (ANOVA) treatment (vintage × treatment × panelist) with first-order interactions. Fisher's least significant difference test was used as a post-hoc comparison of means at $P < 0.05$. For the omission experiments, data were treated with Tastel software version 2019 (ABT Informatique, Rouvroy sur Marne, France). For each series of data, terms were regrouped by categories and only the categories which were mentioned at least five times for each series were maintained. Then, negative and positive frequencies were calculated for each category and each sample. The negative frequency was subtracted from the positive one, and the resulting scores were translated to obtain positive scores. The final matrix was treated through correspondence analysis (CA) to obtain sensory maps of the samples. For the 2013 spicy vintage, data were treated separately for anosmic and nonanosmic respondents to rotundone.

■ RESULTS AND DISCUSSION

Aroma Compound Composition. Among the 76 volatile compounds analyzed, 35 molecules were found with an OAV > 0.5 in the 2013 and 2015 Syrah wines (Table 1). For both vintages, the three compounds with the greatest OAV were isovaleric acid (52.1 in 2013 and 28.6 in 2015), FFT (40.5 in 2013 and 42.8 in 2015), and β-damascenone (19.3 in 2013 and 22.1 in 2015). The contribution of isovaleric acid, a fatty acid produced by yeasts imparting a cheesy character to the aroma of red wines, has been previously emphasized notably in Syrah.⁸ In the same way, β-damascenone, a C₁₃-norisoprenoid formed by oxidative cleavage of neoxanthin, has been widely detected in wines and appears to be almost ubiquitous.²³

FFT has been previously identified as one of the main aroma compounds involved in the aging bouquet of Bordeaux wooded red wines with FFT concentrations, up to 150 ng/L, increasing with the aging time in the bottle.²⁴ However, to the best of our knowledge, this is the first time that such levels of FFT have been reported in relatively young unwooded red wines. Indeed, FFT is mainly formed in wine by the reaction of H₂S with furfural released from the barrels.²⁵ However, furfural can also be produced by Maillard reactions, which naturally occur in red wine.²⁶ Syrah is considered as a “reductive” cultivar, and we can assume that the expected large amount of H₂S formed by the yeast during fermentation might have promoted the formation of FFT. Together with other furan-derived compounds previously identified in Syrah wines,⁸ FFT,

which has a strong roast coffee aroma, could contribute to the burnt rubber notes characteristic of some wines made from this cultivar.

Despite the large difference between the olfactive profiles of the two wines, their aroma compound composition did not greatly differ. As reflected by their intervintage coefficients of variation, the compounds that accounted for the strongest discrimination between the two vintages were 3SH (141%), rotundone (109%), 4-EP (91%), isobutanol (60%), acetaldehyde (59%), methionol (55%), 4-VG (54%), and ethyl 2-methylbutyrate (53%).

For most of these compounds, the observed intervintage differences might be directly (for grape-derived aroma compounds) or indirectly (for fermentation-derived aroma compounds) related to variations in the climate conditions.²⁷ The data shown in Figure 1 enable a comparison of the two

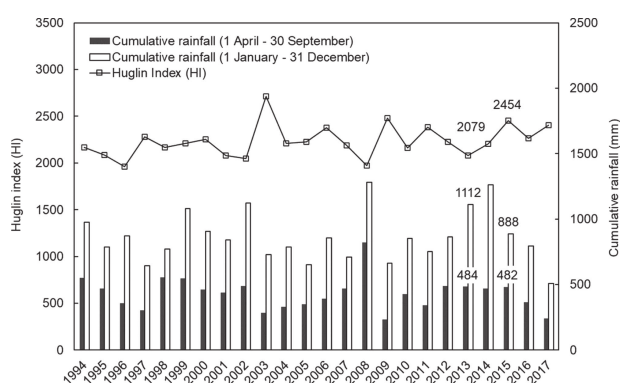


Figure 1. Huglin index, cumulative rainfall between 1 April and 30 September, and between 1 January and 31 December from 1994 to 2017 for the Mercuriol weather station.

studied vintages from a climatic standpoint. As reflected by the Huglin index, 2013 and 2015 were, respectively, the coolest and warmest vintages among the five vintages available on the market at the launch of the study. While 2013 was the fifth coolest vintage since 1994 and the coolest since 2008, 2015 was the third warmest vintage since 1994 and the warmest since 1999. If cumulative rainfall between 1 April and 30 September did not enable the two vintages to be discriminated, 2013 can be seen to have been rainier when considering the period between 1 January and 31 December. However, it should be mentioned that 199 mm of rainfall was recorded in 2015 between 12 September and 17 September. As 2015 was an early-ripening vintage with the harvest generally occurring before mid-September in the Crozes-Hermitage area, the climatic conditions during the vine vegetative growth and the fruit-ripening period are likely to have been dryer for this vintage than those reflected by the cumulative rainfall between 1 April and 30 September. In the context of climate change, it is worth mentioning that cool vintages such as 2013 are likely to become less and less frequent, and the olfactive profile of Crozes-Hermitage wines will probably look more like that of 2015 in the future. This trend toward warmer vintages can be clearly observed in the historical data shown in Figure 1.

The greater rotundone concentration in wine from the 2013 vintage is consistent with previous research work highlighting that wines made from vintages with cool and wet climatic conditions usually exhibit high levels of rotundone.²⁸ The warmer conditions observed in 2015 might have contributed to

enhance nitrogen assimilation through a greater mineralization of organic matter in the soil. For this vintage characterized by a remarkably high level of 3SH, the likely larger nitrogen content in the berries could have positively affected the concentration of varietal thiol precursors.²⁹ Furthermore, it might have limited the production of fermentation-derived higher alcohols such as isobutanol and methionol whose formation is promoted under low yeast assimilable nitrogen conditions.³⁰

If rotundone is known to be very stable in bottled wine,³¹ the 2-year extended period of storage might also explain the lower concentration of 3SH found in the 2013 wine. Indeed, thiols are chemically unstable because they are easily oxidizable in disulfide under mild oxidative conditions.³⁸

Similarly, the higher levels for the 2013 wine of acetaldehyde formed by oxidation of ethanol and ethyl 2-methylbutyrate, whose esterification is enhanced during wine aging to reach the acid-ester equilibrium,³² might be the consequence of this extra period of storage.

As neither wines were aged in oak barrels, 4-EP and 4-VG are likely to have originated from the metabolism of hydroxycinnamic acids (HAs), suggesting a *Brettanomyces* activity. 4-Vinylphenol and 4-VG are first produced by *Brettanomyces* through enzymatic decarboxylation from HA, before yielding 4-EP and 4-ethylguaiacol through reduction of the vinyl group.³³ As concentrations in 4-VG and 4-EP are, respectively, higher and lower in 2015, we can assume that the *Brettanomyces* contamination and the related reaction leading to the production of 4-EP from 4-VG were controlled in an earlier stage during this vintage.

Without performing an aroma extract dilution analysis (AEDA), a quantitative GC olfactometry procedure, we cannot completely exclude the possibility that some key aroma contributors were not quantitated. However, a negligible impact would be expected on the results as among the 48 key molecules identified in a AEDA work aiming at investigating the compounds involved in the aroma of two Syrah wines,⁸ only nine were not analyzed in our study. It is also worth mentioning that among these nine aroma compounds, 2-methylbutyric acid and ethyl 2-methylpropanoate were the only potential key odorants showing an OAV > 1 in this former study. As the Syrah wines from this past study were both aged in oak barrels, the aroma compound composition of the two unwooded Crozes-Hermitage wines is likely to be simpler, which must also have limited the possibility of missing a key compound.

However, the AEDA procedure also has some limitations and does not always allow the identification of the main odorants because of the coelution of the compounds, notably those present at a trace level, which can result in the perception of odor cluster. For this reason, additional compounds not detected in the AEDA are also frequently analyzed and included in models for reconstitution studies.^{8,9} This limitation can be illustrated by FFT and BM, two molecules detected in the ng/L range showing an OAV > 10 in the Crozes-Hermitage wines. Indeed, these two compounds were not identified as potential key contributors to the aroma of Syrah wines in a previous study based on AEDA.⁸ It is also worth mentioning that some key compounds identified by AEDA, including those with AEDA values of 1000, can be found in wines at very low concentrations and have no sensory contribution.⁸

Identification of the Best Matrix and Best Model. The impact of the dearomatization processes on the classical

laboratory analysis is shown in Table 2. By removing one-third of the sample volume mainly composed of volatile components, the Rotavapor treatment had a great impact on the alcohol content, volatile acidity, and dissolved carbon dioxide. In most cases, it also tended to increase the content in nonvolatile molecules through concentration. Before carrying out the reconstitution studies, absolute ethanol was added to increase the ethanol content of 8% v/v to reproduce the concentration in the native wine. In comparison, the sCO₂ treatment had a weak impact on most of the measured parameters apart from dissolved CO₂, which was slightly increased. Depending on the settings, sCO₂ has been previously applied to wine either to remove volatiles,³⁴ alcohol,^{35,36} or for both applications.¹⁴ The impact of such a treatment on the raffinate has already been characterized for ethanol and aroma but never for other basic parameters.

For these initial reconstitution studies, 35 molecules were included in the model for both wines. The matrix, designed as a “treatment” factor, had a significant impact on the DOD score (Table 3). For both vintages, the lowest score was observed for the sCO₂ treatment (Figure 2). In comparison with SW 10% and SW 12.5%, the superiority of sCO₂ could be related to the greater complexity of the matrix and notably its composition in nonvolatile fractions, which was very similar to the native wine.^{8,12} The difference with Rotavapor could be explained by the absence of odor perceived at tasting for the sCO₂ sample with the former treatment being slightly marked by oxidative notes. Despite the relative neutrality of absolute ethanol, the addition of a large amount of exogenous ethanol for Rotavapor might also have played a role by enhancing the overall alcohol perception.

Following these results, the sCO₂ matrix was selected for the rest of the study. It can be seen that the DOD score remained high in comparison with the native wine served blind (Figure 2). In the space available for free comments on the tasting sheet, several panelists highlighted that the test samples exhibited intense reductive notes. To improve the quality of the reconstitution, H₂S and methanethiol, which might have been responsible for these notes, were excluded from the model optimization experiment.

This exclusion had a positive effect on the quality of the model built by mixing aroma compounds with OAV > 0.5 as the average DOD score was decreased by 0.6 ± 1.5 and 1.0 ± 2.5 for the 2013 and 2015 vintages, respectively (Figure 3). The number of molecules included in the models was 33 for OAV > 0.5 in both vintages, 31 in 2013 and 27 in 2015 for OAV > 1.0, 21 in 2013 and 20 in 2015 for OAV > 2.0, 10 in 2013 and 11 in 2015 for OAV > 5, and 6 for OAV > 10 in both vintages. This number had a significant impact on the quality of the reconstitution (Table 3). For both vintages, the best reconstitution was observed for the OAV > 5 and OAV > 10 models (Figure 3). These findings are consistent with the recent work highlighting that multiple esters can be substituted by simpler reconstitutions using very few compounds.³⁷ However, they contradict other research emphasizing that the most qualitative reconstitutions using synthetic wines were obtained using complex models.⁹ The aroma of simple models containing only molecules with a high OAV were often very different from that of the wine.⁹ Even though wine dearomatized through sCO₂ was odorless, we can assume that this matrix contained reduced amounts of aroma compounds in comparison to the original wine. Indeed, previous results obtained using similar operating conditions

Table 2. Impact of the Dearomatization Treatments on the Classical Laboratory Analysis

vintage	treatment	alcohol content (% v/v)	titratable acidity (g/L tartaric acid)	pH	tartaric acid (g/L)	malic acid (g/L)	lactic acid (g/L)	volatile acidity (g/L acetic acid)	glucose + fructose (g/L)	MCI ^c	TPI ^d	CO ₂ (mg/L)
2013	native wine	12.9 ± 0.1	4.6 ± 0.1	3.78 ± 0.05	1.4 ± 0.3	0.0 ± 0.3	1.6 ± 0.3	0.42 ± 0.05	0.9 ± 0.5	11.5 ± 0.2	65 ± 5	363 ± 45
	sCO ₂ ^a	12.7 ± 0.1	4.7 ± 0.1	3.77 ± 0.05	1.4 ± 0.3	0.0 ± 0.3	1.6 ± 0.3	0.48 ± 0.05	0.9 ± 0.5	11.3 ± 0.2	65 ± 5	429 ± 45
	rotavapor ^b	4.1 ± 0.1	6.9 ± 0.1	3.75 ± 0.05	1.2 ± 0.3	0.0 ± 0.3	1.3 ± 0.3	0.09 ± 0.05	1.2 ± 0.5	12.2 ± 0.2	87 ± 5	<60
2015	native wine	13.5 ± 0.1	5.3 ± 0.1	3.74 ± 0.05	1.2 ± 0.3	0.0 ± 0.3	1.3 ± 0.3	0.45 ± 0.05	1.1 ± 0.5	15.0 ± 0.2	81 ± 5	419 ± 45
	sCO ₂	12.9 ± 0.1	5.4 ± 0.1	3.73 ± 0.05	1.2 ± 0.3	0.0 ± 0.3	1.3 ± 0.3	0.36 ± 0.05	1.0 ± 0.5	16.8 ± 0.2	86 ± 5	468 ± 45
	rotavapor	4.3 ± 0.1	7.8 ± 0.1	3.75 ± 0.05	1.0 ± 0.3	0.0 ± 0.3	0.9 ± 0.3	0.01 ± 0.05	1.4 ± 0.5	23.5 ± 0.2	99 ± 5	<60

^aWine dearomatized through supercritical carbon dioxide. ^bWine dearomatized through rotary evaporation followed by a resin treatment. ^cModified color intensity. ^dTotal Phenolic Index. The uncertainty of measurement is expressed as 95% confidence interval.

Table 3. Results of Three-Way Analysis of Variance of DOD Data for the Identification of the Best Matrix and Optimization of the Model Experiments

experiment	vintage (V)	treatment (T)	panelist (P)	V × T	V × P	T × P
identification of the best matrix	0.110	<0.0001	<0.0001	0.145	0.014	0.051
optimization of the model	0.046	<0.0001	<0.0001	0.457	0.429	0.114

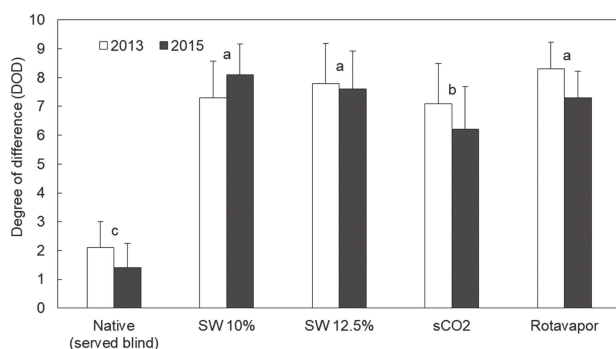


Figure 2. Mean scores of the DOD between the native wine, and the native wine served blind and the wines reconstituted using several matrices for the 2013 and 2015 vintages. Abbreviations: SW 10% and SW 12%, synthetic wines with ethanol concentrations of 10 and 12% v/v, respectively; sCO₂, wine dearomatized through supercritical carbon dioxide; Rotavapor, wine dearomatized through rotary evaporation followed by a resin treatment. Different letters indicate means significantly different at $P < 0.05$ by the Fisher test. Error bars represent 95% confidence interval.

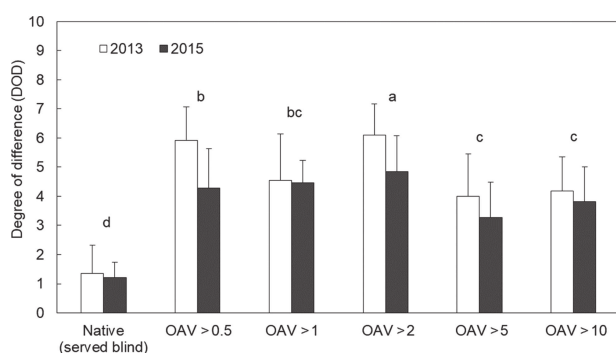


Figure 3. Mean scores of the DOD between the native wine, and the native wine served blind, and the wines reconstituted by mixing aroma compounds with OAV strictly above 0.5 (OAV > 0.5), above 1 (OAV > 1), above 2 (OAV > 2), above 5 (OAV > 5), and above 10 (OAV > 10) for the 2013 and 2015 vintages. Different letters indicate means significantly different at $P < 0.05$ by the Fisher test. Error bars represent 95% confidence interval.

highlighted that peaks were either reduced or suppressed in the chromatogram, in most cases.¹⁴ For example, in this former study, 2-furfural, acetic acid, and 2,3-butanediol were not removed after the sCO₂ treatment and could be found in the raffinate. Under our conditions, the presence of acetic acid, which is the main contributor to volatile acidity, can be clearly observed after dearomatization as the volatile acidity was not decreased for the sCO₂ treatment (Table 2). Therefore, reconstitutions might have enhanced the overall contribution of some molecules included in the models. For the more complex models composed of compounds with low OAVs, this effect might have been greater than for the simpler model. The fact that the efficiency of sCO₂ for removing aroma compounds might be selective and dependent notably on the

volatility of the molecule could also be of importance.¹⁴ As with H₂S and methanethiol, we cannot discard the possibility that some specific aroma compounds included in the OAV > 0.5, OAV > 1, and OAV > 2 models were overexpressed and contributed to decrease the quality of the reconstitution. The fact that the OAV > 2 model had the highest DOD score suggests that complex interactions occurred.

As no differences were observed between the OAV > 5 and OAV > 10 models for both vintages, we can suppose that the molecules from the first model, which were not included in the second one (rotundone, ethyl acetate, ethyl isobutyrate, and ethyl hexanoate for 2013; ethyl isovalerate, ethyl acetate, isoamyl acetate, isoamyl alcohol, and 2,3-butanedione for 2015), made a small contribution to the aroma of Syrah wines. However, for the 2013 vintage, the presence of rotundone for which specific anosmia has been documented could have brought some noise into the data.⁴ As the panel was not tested for anosmia in this phase of the study, it remains difficult to draw firm conclusions for this aroma compound. In order to have a sufficient choice of grape-derived molecules for the last phase of the study, the OAV > 5 model was retained.

Omission Tests. Because of the low availability of native wine, a bulk Syrah wine from the 2018 vintage produced in a neighboring wine region was chosen to replace the Crozes-Hermitage wine. This wine had the following features: alcohol 14.1% v/v, titratable acidity 4.1 g/L expressed as tartaric acid, pH 4.02, tartaric acid 2.2 g/L, malic acid 0.0 g/L, lactic acid 1.1 g/L, volatile acidity 0.40 g/L expressed as acetic acid, glucose + fructose 1.1 g/L, MCI 17.8, TPI 89, and dissolved CO₂ 655 mg/L. Semi-industrial SCO₂ equipment from Separex (Champigneulle, France) with a 25 L capacity was used to treat the large volumes necessary for omission studies. It was loaded with 13 L of bulk Syrah wine and operated under the same conditions as previously reported (40 °C and 100 bar). Because of the change of scale, the CO₂ flow was increased to 500 g/min and the treatment maintained for 120 min. In the meantime, the SFE200 equipment was used to produce some 2013 and 2015 dearomatized Crozes-Hermitage wine using the procedure previously described. For each vintage, models built by mixing aroma compounds with OAV > 5 in the Crozes-Hermitage and bulk Syrah wines dearomatized through sCO₂ using the pilot scale and semi-industrial equipment, respectively, were compared using triangular tests. For both vintages, no significant differences were observed between the two reconstitution models at $P < 0.05$ (six out of 15 correct responses for 2013 and seven out of 15 correct responses for 2015). Therefore, the remaining amount of dearomatized bulk Syrah wine was used to perform omission tests.

The grape-derived aroma compounds chosen for the omission studies were rotundone for 2013, 3SH for 2015, and FFT, BM, and β-damascenone for both vintages. The aroma composition of the samples presented to the panelists during the omission tests is shown in Table 4. Of the 19 panelists, eight rotundone anosmic respondents representing 42% of the whole panel were identified. This was a greater percentage of specific anosmia than expected^{6,38} enabling a

Table 4. Aroma Composition of the Samples Presented to Panelists during Omission Tests

aroma compound ^a	2013 series						2015 series					
	concentration (μg/L)						concentration (μg/L)					
	pivot	fully optimized reconstitution	-rotundone	-2-furfurylthiol	-benzyl mercaptan	-β-damascenone	pivot	fully optimized reconstitution	-3-SH	-2-furfurylthiol	-benzyl mercaptan	-β-damascenone
ethyl hexanoate (fruity)	586	586	586	586	586	586	744	744	744	744	744	744
ethyl isobutyrate (fruity)	100	100	100	100	100	100	–	–	–	–	–	–
ethyl isovalerate (fruity)	42	42	42	42	42	42	22	22	22	22	22	22
ethyl acetate (varnish, fruity)	64,955	64,955	64,955	64,955	64,955	64,955	76,925	76,925	76,925	76,925	76,925	76,925
isoamyl acetate (banana)	– ^b	–	–	–	–	–	190	190	190	190	190	190
isovaleric acid (cheese, lactic)	1719	1719	1719	1719	1719	1719	943	943	943	943	943	943
isoamyl alcohol (fusel)	334,668	334,668	334,668	334,668	334,668	334,668	219,489	219,489	219,489	219,489	219,489	219,489
2,3-butanedione (butter, lactic)	–	–	–	–	–	–	658	658	658	658	658	658
rotundone (spicy, peppery)	0.043	0.054	–	0.054	0.054	0.054	–	–	–	–	–	–
β-damascenone (dried plums)	0.8	1.0	1.0	1.0	1.0	–	0.9	1.1	1.1	1.1	1.1	–
FFT (roasted coffee)	0.013	0.016	0.016	–	0.016	0.016	0.014	0.017	0.017	–	0.017	0.017
3-SH (grapefruit, tropical fruit)	–	–	–	–	–	–	0.746	0.933	–	0.933	0.933	0.933
benzyl mercaptan (garlic)	0.003	0.004	0.004	0.004	–	0.004	0.003	0.004	0.004	0.004	–	0.004

^aAroma compounds; sensory attributes are given in parentheses. ^bNot included in the model.

relatively balanced distribution of anosmic and nonanosmic respondents to rotundone.

In the case of the 2013 series (Figure 4A), for the respondents anosmic to rotundone, the fully optimized reconstitution and the sample in which rotundone was omitted were very close on the CA maps. This indicates that the omission of rotundone did not provoke any changes in the aroma perception for this population. Although this conclusion was not unexpected, it has also been suggested that rotundone could induce molecular mechanisms of flavor reduction for anosmic respondents.³⁸ However, as this mechanism is concentration-dependent,³⁹ we cannot completely discard the possibility that the relatively small amount of rotundone spiked in the fully optimized reconstitution sample (54 ng/L) was not sufficient to suppress the olfactory signal transduction. These two samples were perceived to be higher in the “green” attribute and surprisingly lower in the “spicy/peppery” aroma, which is difficult to explain. The compound whose omission induced the greatest effect on the olfactory profile, as reflected by its distance from the fully optimized reconstitution on the CA map, was FFT. Although its omission had no effect on the “toasted/roasted” character, it decreased the overall “aromatic” intensity. The omission of BM and β -damascenone increased the rating for the “lactic” attribute, which suggests that these two compounds might interact with isovaleric acid through a masking effect. The omission of β -damascenone also decreased the “fruity” character, which is relevant in view of previous research studies highlighting the enhancing effect of this molecule on the fruity character of red wine.⁴⁰

For the nonanosmic respondents testing the 2013 series, the omission of rotundone had the greatest impact on the aroma, while the contribution of BM and FFT was weak (Figure 4B). The sample in which rotundone was omitted was logically perceived as less “spicy/peppery” and more “fruity”. The reconstitution without β -damascenone was rated higher for “aromatic” and lower in the “green” and “animal” attributes.

For the 2015 series, most of the omissions had a large impact on the olfactory profile. However, the sample in which FFT was removed showed the most similarity with the fully optimized reconstitution, which was perceived as “aromatic”. The omission of FFT increased the rating of “fruity” and “floral” and decreased the perception of the “toasted/roasted” attribute characteristic of this aroma compound. The greatest impact was observed when 3SH was omitted. Its omission decreased the “fruity” and “floral” characters and increased the “animal” and “toasted/roasted” attributes, which suggest that 3SH can interact with FFT. The reconstitution model without β -damascenone was perceived as less “fruity” and “floral”, in accordance with previous studies.⁴⁰ The omission of BM increased the rating for the “green” attribute.

This study investigated the key compounds involved in the aroma of Syrah wines from the northern Rhone valley. For the first time, FFT was found at remarkably high concentration levels in relatively young wines, which were not aged in barrels. Rotundone and 3SH were the two grape-derived aroma compounds that enabled the best discrimination between the two studied vintages. For the nonanosmic respondents, rotundone appeared to be the most impacting compound in wine from the cool 2013 vintage. For anosmic respondents, the removal of FFT had the greatest impact, while the omission of rotundone did not induce any changes in the aroma perception. 3SH was identified as the key contributor to the “fruity” aroma for the warm 2015 vintage. Furthermore, this

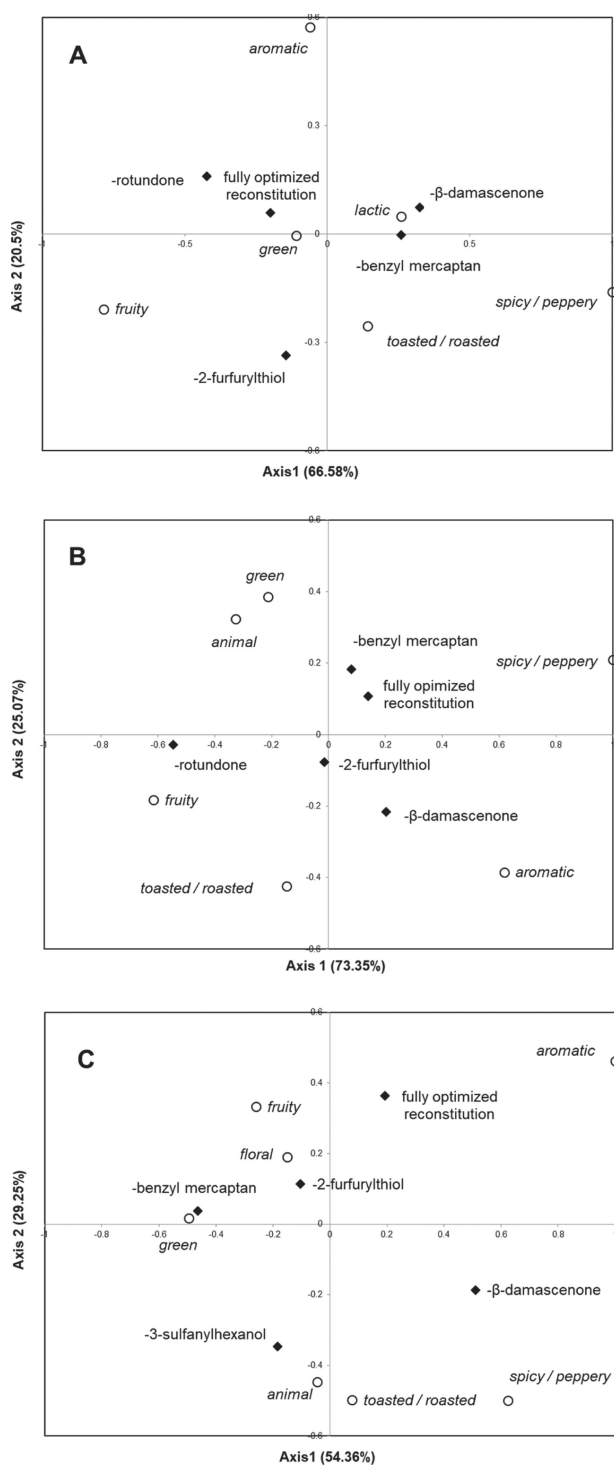


Figure 4. Projection of the samples presented during omission tests in the CA map (subspace 1–2) (A) for anosmic panelists to rotundone for the 2013 series, (B) for nonanosmic panelists to rotundone for the 2013 series, and (C) for the whole panel for the 2015 series. Abbreviations: fully optimized without rotundone (-rotundone), β -damascenone (β -damascenone), benzyl mercaptan (-benzyl mercaptan); 2-furfurylthiol (-2-furfurylthiol), and 3-SH (-3-sulfanylhexanol).

study has found that wine dearomatized through supercritical CO_2 could be valuable for aroma reconstitution studies. Using this matrix, the best models were observed when mixing a

small number of molecules with OAV > 5 or OAV > 10. The Pivot Profile also proved to be a very convenient and easy-to-use sensory method for omission tests.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.0c04328>.

Response factors for the aroma compounds quantitated in the two Crozes-Hermitage wines (PDF)

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■ ABBREVIATIONS

PDO, protected designation of the origin; 3SH, 3-sulfanylhexanol; sCO₂, supercritical carbon dioxide; OAV, odor activity value; MIC, modified color intensity; TPI, total phenolic index; H₂S, hydrogen sulfide; FFT, 2-furfurylthiol; BM, benzyl mercaptan; 4-EP, 4-ethylphenol; 4-VG, 4-vinylguaiacol; RSD, relative standard deviation; RSD_{vintage}, relative standard deviation between the two vintages; SW, synthetic wine; DOD, degree of difference; ANOVA, analysis of variance; CA,

correspondence analysis; HA, hydroxycinnamic acids; AEDA, aroma extract dilution analysis

■ REFERENCES

- (1) OIV. Distribution of the World's Grapevine Varieties in 2015. *Focus* 2017, 2014, p 54.
- (2) Meredith, C.; Boursiquot, J.-M. Origins and Importance of Syrah Around the World. *International Syrah Symposium; Lyon, France, 2008*; pp 17–20.
- (3) Takase, H.; Sasaki, K.; Shinmori, H.; Shinohara, A.; Mochizuki, C.; Kobayashi, H.; Saito, H.; Matsuo, H.; Suzuki, S.; Takata, R. Analysis of Rotundone in Japanese Syrah Grapes and Wines using Stir Bar Sorptive Extraction (SBSE) with Heart-Cutting Two-Dimensional GC-MS. *Am. J. Enol. Vitic.* 2015, 66, 398–402.
- (4) Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vössing, T.; Widder, S.; Krammer, G.; Sefton, M. A.; Herderich, M. J. From Wine to Pepper: Rotundone, an Obscure Sesquiterpene, is a Potent Spicy Aroma Compound. *J. Agric. Food Chem.* 2008, 56, 3738–3744.
- (5) Koch, A.; Doyle, C. L.; Matthews, M. A.; Williams, L. E.; Ebeler, S. E. 2-Methoxy-3-isobutylpyrazine in Grape Berries and its Dependence on Genotype. *Phytochemistry* 2010, 71, 2190–2198.
- (6) Segurel, M. *Contribution des Précurseurs Glycosidiques et du Sulfure de Diméthyle des Baies de Vitis vinifera L. Cv Grenache Noir et Syrah à l'Arôme des Vins de la Vallée du Rhône*; École Nationale Supérieure Agronomique: Montpellier, 2005.
- (7) Masson, G.; Schneider, R. Key Compounds of Provence Rosé Wine Flavor. *Am. J. Enol. Vitic.* 2009, 60, 116–122.
- (8) Mayr, C. M.; Geue, J. P.; Holt, H. E.; Pearson, W. P.; Jeffery, D. W.; Francis, I. L. Characterization of the Key Aroma Compounds in Shiraz Wine by Quantitation, Aroma Reconstitution, and Omission Studies. *J. Agric. Food Chem.* 2014, 62, 4528–4536.
- (9) Ferreira, V.; Ortín, N.; Escudero, A.; López, R.; Cacho, J. Chemical Characterization of the Aroma of Grenache Rosé Wines: Aroma Extract Dilution Analysis, Quantitative Determination, and Sensory Reconstitution Studies. *J. Agric. Food Chem.* 2002, 50, 4048–4054.
- (10) Grosch, W. Evaluation of the Key Odorants of Foods by Dilution Experiments, Aroma Models and Omission. *Chem. Senses* 2001, 26, 533–545.
- (11) Frank, S.; Wollmann, N.; Schieberle, P.; Hofmann, T. Reconstitution of the Flavor Signature of Dornfelder Red Wine on the Basis of the Natural Concentrations of Its Key Aroma and Taste Compounds. *J. Agric. Food Chem.* 2011, 59, 8866–8874.
- (12) Sáenz-Navajas, M.-P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the Nonvolatile Matrix on the Aroma Perception of Wine. *J. Agric. Food Chem.* 2010, 58, 5574–5585.
- (13) Lytra, G.; Tempere, S.; de Revel, G.; Barbe, J.-C. Distribution and Organoleptic Impact of Ethyl 2-Hydroxy-4-methylpentanoate Enantiomers in Wine. *J. Agric. Food Chem.* 2012, 60, 1503–1509.
- (14) Ruiz-Rodríguez, A.; Fornari, T.; Jaime, L.; Vázquez, E.; Amador, B.; Nieto, J. A.; Yuste, M.; Mercader, M.; Reglero, G. Supercritical CO₂ Extraction Applied Toward the Production of a Functional Beverage from Wine. *J. Supercrit. Fluids* 2012, 61, 92–100.
- (15) Tonietto, J.; Carbonneau, A. Régime Thermique en Période de Maturation du Raisin dans le Géoclimat Viticole: Indice de Fraîcheur des Nuits-IF et Amplitude Thermique. *Proceedings of the Symposium International sur le Zonage Vitivinicole, Avignon, France, 2002*; pp 279–289.
- (16) Ortega, C.; López, R.; Cacho, J.; Ferreira, V. Fast Analysis of Important Wine Volatile Compounds: Development and Validation of a New Method Based on Gas Chromatographic–Flame Ionisation Detection Analysis of Dichloromethane Microextracts. *J. Chromatogr. A* 2001, 923, 205–214.
- (17) López, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of Minor and Trace Volatile Compounds in Wine by Solid-Phase Extraction and Gas Chromatography with Mass Spectrometric Detection. *J. Chromatogr. A* 2002, 966, 167–177.

- (18) Ontañón, I.; Vela, E.; Hernández-Orte, P.; Ferreira, V. Gas Chromatographic-Sulfur Chemiluminescent Detector Procedures for the Simultaneous Determination of Free Forms of Volatile Sulfur Compounds Including Sulfur Dioxide and for the determination of their metal-complexed forms. *J. Chromatogr. A* **2019**, *1596*, 152–160.
- (19) Mateo-Vivaracho, L.; Zapata, J.; Cacho, J.; Ferreira, V. Analysis, Occurrence, and Potential Sensory Significance of Five Polyfunctional Mercaptans in White Wines. *J. Agric. Food Chem.* **2010**, *58*, 10184–10194.
- (20) Culleré, L.; Ontañón, I.; Escudero, A.; Ferreira, V. Straightforward Strategy for Quantifying Rotundone in Wine at ngL(-1) Level Using Solid-Phase Extraction and Gas Chromatography-Quadrupole Mass Spectrometry. Occurrence in Different Varieties of Spicy Wines. *Food Chem.* **2016**, *206*, 267–273.
- (21) Aust, L. B.; Gacula, M. C., Jr.; Beard, S. A.; Washam, R. W., II Degree of Difference Test Method in Sensory Evaluation of Heterogeneous Product Types. *J. Food Sci.* **1985**, *50*, 511–513.
- (22) Thuillier, B.; Valentin, D.; Marchal, R.; Dacremont, C. Pivot profile: A New Descriptive Method Based on Free Description. *Food Qual. Prefer.* **2015**, *42*, 66–77.
- (23) Sefton, M. A.; Skouroumounis, G. K.; Elsey, G. M.; Taylor, D. K. Occurrence, Sensory Impact, Formation, and Fate of Damascenone in Grapes, Wines, and Other Foods and Beverages. *J. Agric. Food Chem.* **2011**, *59*, 9717–9746.
- (24) Picard, M.; Thibon, C.; Redon, P.; Darriet, P.; de Revel, G.; Marchand, S.p. Involvement of Dimethyl Sulfide and Several Polyfunctional Thiols in the Aromatic Expression of the Aging Bouquet of Red Bordeaux Wines. *J. Agric. Food Chem.* **2015**, *63*, 8879–8889.
- (25) Blanchard, L.; Tominaga, T.; Dubourdieu, D. Formation of Furfurylthiol Exhibiting a Strong Coffee Aroma During Oak Barrel Fermentation from Furfural Released by Toasted Staves. *J. Agric. Food Chem.* **2001**, *49*, 4833–4835.
- (26) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the Formation Mechanisms of Some Volatile Compounds During the Aging of Sweet Fortified Wines. *J. Agric. Food Chem.* **1999**, *47*, 2837–2846.
- (27) Robinson, A. L.; Boss, P. K.; Solomon, P. S.; Trengove, R. D.; Heymann, H.; Ebeler, S. E. Origins of Grape and Wine Aroma. Part 1. Chemical Components and Viticultural Impacts. *Am. J. Enol. Vitic.* **2014**, *65*, 1–24.
- (28) Caputi, L.; Carlin, S.; Ghiglieno, I.; Stefanini, M.; Valenti, L.; Vrhovsek, U.; Mattivi, F. Changes in Rotundone Content During Grape Ripening and winemaking to manipulation of the 'peppery' character of wine. *J. Agric. Food Chem.* **2011**, *59*, 5565–5571.
- (29) Roland, A.; Schneider, R.; Razungles, A.; Cavelier, F. Varietal Thiols in Wine: Discovery, Analysis and Applications. *Chem. Rev.* **2011**, *111*, 7355–7376.
- (30) Bell, S.-J.; Henschke, P. A. Implications of Nitrogen Nutrition for Grapes, Fermentation and Wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295.
- (31) Herderich, M. J.; Siebert, T. E.; Parker, M.; Capone, D. L.; Jeffery, D. W.; Osidacz, P.; Francis, I. L. Spice up your life: analysis of key aroma compounds in Shiraz. *Flavor Chemistry of Wine and Other Alcoholic Beverages*; American Chemical Society, 2012; Vol. 1104, pp 3–13.
- (32) Díaz-Maroto, M. C.; Schneider, R.; Baumes, R. Formation Pathways of Ethyl Esters of Branched Short-Chain Fatty Acids during Wine Aging. *J. Agric. Food Chem.* **2005**, *53*, 3503–3509.
- (33) Chatonnet, P.; Dubourdieu, D.; Boidron, J.-n.; Pons, M. The Origin of Ethylphenols in Wines. *J. Agric. Food Chem.* **1992**, *60*, 165–178.
- (34) Macedo, S.; Fernandes, S.; Lopes, J. A.; de Sousa, H. C.; Pereira, P. J.; Carmelo, P. J.; Mendiña, C.; Simões, P. C.; Nunes da Ponte, M. Recovery of Wine-Must Aroma Compounds by Supercritical CO₂. *Food Bioprocess Technol.* **2008**, *1*, 74–81.
- (35) Gamse, T.; Rogler, I.; Marr, R. Supercritical CO₂ Extraction for Utilisation of Excess Wine of Poor Quality. *J. Supercrit. Fluids* **1999**, *14*, 123–128.
- (36) Ruiz-Rodriguez, A.; Fornari, T.; Hernández, E. J.; Señorans, F. J.; Reglero, G. Thermodynamic modeling of dealcoholization of beverages using supercritical CO₂: application to wine samples. *J. Supercrit. Fluids* **2010**, *52*, 183.
- (37) de-la-Fuente-Blanco, A.; Sáenz-Navajas, M.-P.; Valentin, D.; Ferreira, V. Fourteen Ethyl Esters of Wine Can Be Replaced by Simpler Ester Vectors Without Compromising Quality but at the Expense of Increasing Aroma Concentration. *Food Chem.* **2020**, *307*, 125553.
- (38) Geffroy, O.; Descôtes, J.; Serrano, E.; Li Calzi, M.; Dagan, L.; Schneider, R. Can a Certain Concentration of Rotundone be Undesirable in Duras Red Wine? A Study to Estimate a Consumer Rejection Threshold for the Pepper Aroma Compound. *Aust. J. Grape Wine Res.* **2018**, *24*, 88–95.
- (39) Takeuchi, H.; Kato, H.; Kurahashi, T. 2,4,6-Trichloroanisole is a Potent Suppressor of Olfactory Signal Transduction. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 16235–16240.
- (40) Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D. Which Impact for β -Damascenone on Red Wines Aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103–4108.
- (41) Ferreira, V.; López, R.; Cacho, J. F. Quantitative Determination of the Odorants of Young Red Wines from Different Grape Varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
- (42) Etievant, P. *Volatile Compounds in Food and Beverages*; Marse: New York, 1991.
- (43) Guth, H. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
- (44) Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas Chromatography– Olfactometry and Chemical Quantitative Study of the Aroma of Six premium Quality Spanish Aged Red Wines. *J. Agric. Food Chem.* **2004**, *52*, 1653–1660.
- (45) Mestres, M.; Busto, O.; Guasch, J. Analysis of Organic Sulfur Compounds in Wine Aroma. *J. Chromatogr. A* **2000**, *881*, 569–581.
- (46) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of New Volatile Thiols in the Aroma of *Vitis vinifera* L. var. Sauvignon Blanc Wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.
- (47) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution of Benzenemethanethiol to Smoky Aroma of Certain *Vitis vinifera* L. Wines. *J. Agric. Food Chem.* **2003**, *51*, 1373–1376.
- (48) Boidron, J.-N.; Chatonnet, P.; Pons, M. Influence du Bois sur Certaines Substances Odorantes des Vins. *OENO One* **1988**, *22*, 275–294.
- (49) Gemert, L.; Nettenbreijer, A. *Compilation of Odour Threshold Values in Air and Water*; National Institute Water Supply & Central Institute for Nutrition and Food Research, 1977.
- (50) Nakamura, S.; Crowell, E. A.; Ough, C. S.; Totsuka, A. Quantitative Analysis of γ -Nonalactone in Wines and Its Threshold Determination. *J. Food Sci.* **1988**, *53*, 1243–1244.