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Release and Formation of Varietal Aroma Compounds during Alcoholic Fermentation from Nonfloral Grape Odorless Flavor Precursors Fractions

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An odorless flavor precursor fraction extracted from different nonfloral grape varietals has been added to a grape must and has been fermented by three different yeast strains. The wines obtained were analyzed by sensory descriptive analysis and by gas chromatography mass spectrometry to determine more than 90 aroma chemicals. The addition of the precursor fraction brought about a significant increase of the wine floral notes, irrespective of the yeast used. The levels of 51 wine aroma chemicals were found to depend on the precursor fraction addition and, in most cases, also on the yeast strain. Only β-damascenone, β-ionone, and vinylphenols were produced at concentrations well above threshold. However, the concerted addition of groups of compounds has shown that lactones, cinnamates, vanillins, and terpenes are together active contributors to the floral note. Different observations suggest that the formation of varietal aroma is an integral part of yeast metabolism and not a simple hydrolytical process.

KEYWORDS: Glycosides; flavor; yeast; saccharomyces cerevisiae; wine; alcoholic fermentation

INTRODUCTION

The discovery in the seventies of the existence of some wine aroma molecules in the form of glycosides (*1*) has encouraged the study of these aroma precursors. Such research has made it possible to identify more than 100 different aglycones broadly classified in the categories of shikimates, terpenoids, and norisoprenoids (*2*−*6*). The sugar moiety of the glycosides has also been elucidated and four sugars (β-D-glucopyranose; α-L-arabinofuranosyl-β-D-glucopyranose; α-L-rhamnopyranosyl-β-D-glucopyranose, and β-D-apiofuranosyl-β-D-glucopyranose) have been identified as the major components of the sugar part of the molecule (*7*−*9*). From the aromatic point of view, the most important glycosides are those of terpenols in Muscat varieties, which have been the object of intensive research (*4*, *10*−*14*). These glycosides are also the easiest to analyze and interpret, since the aroma molecules exist as aglycones. This implies that a simple hydrolysis of the O-glycosyl bond will release the aroma molecule, although there are also some odorless aglycones that can yield the aromatic terpenols by chemical rearrangement (*10*). The case of norisoprenoids is far more complicated, since the most important aroma chemicals do not exist as aglycones, but are formed by complex chemical rearrangements of the odorless aglycones (*15*−*20*). At least four different relevant wine aroma compounds belong to this class. These are β-damascenone, β-ionone, TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), and TPB (t-1-(2,3,6-trimethylphenyl)but-1,3-diene) (*20*, *21*). The two first compounds are important aroma compounds of most wines (*22*) and have a positive sensory effect, although their sensory contribution to wine aroma is not yet well understood. The latter two are slowly formed during wine aging in some wines, and their sensory contribution is mainly considered negative (*17*, *20*, *23*). Shikimates have received far less attention.

It spite of all the progress done, the role played by the different aroma molecules derived from grape precursors on nonfloral young wine sensory properties is not clearly understood. The importance on wine sensory properties of β-damascenone, β-ionone and the terpenols, and of course of the cysteinyl-derivatives has been clearly documented, but these compounds all together do not seem to be enough to explain the varietal odors and flavors noted in some nonfloral wines. Different studies have demonstrated that the precursor fractions have enough potential to explain some of these varietal odor nuances, since the mild acid hydrolysates (not the enzyme hydrolysates) retain some of the odor properties linked to the wines made with those varieties (*3*, *24*−*28*). Therefore, some other compounds coming from the precursor fractions should have some role, but this is not yet known.

There are also some gaps in our understanding of the process of wine aroma formation from the aroma precursors because-fermentation is one of the most obscure steps. While several works have given quite complete lists of the aroma compounds formed from precursor fractions extracted from non-floral

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varietals by acid or enzimatic hydrolysis (*3, 4, 6, 26−29*), most of the reports about the role of yeast have focused mainly on the ability of different yeast strains to release terpenols from precursors extracted from Muscat grapes (*12−14, 30−32*). Only one report from Delfini et al. (*33*) gives factual evidence about the involvement of yeasts in the development of aroma notes from precursor fractions, but they did not identify the aroma molecules responsible for those changes (*33*). The numbers and types of aroma compounds that the yeast is able to form or release from the precursor fractions of nonfloral grapes are, therefore, not completely known.

The main goals of the present paper are to determine which aroma molecules are released or formed from fractions of odorless precursors during fermentation by the action of yeasts and to assess the potential sensory role played by those molecules on wine aroma.

MATERIALS AND METHODS

Reagents and Standards. Dichloromethane and methanol (LiChrosolv quality) were purchased from Merck (Darmstadt, Germany), pentane from Fluka (Buchs, Switzerland), ethyl acetate, absolute ethanol, sodium hydroxide, sodium fluoride, L(+)-ascorbic acid, ammonium sulfate, sodium dihydrogenphosphate 1-hydrate, and disodium hydrogenphosphate 12-hydrate were supplied by Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, U.S.). LiChrolut EN resins were purchased from Merck. The chemical standards were suppied by Aldrich (Gillingham, UK), Sigma (St. Louis, MO), ChemService (West Chester, PA), PolyScience (Niles, IL), Firmenich (Geneva, Switzerland), Panreac, Merck, Fluka, and Lancaster (Strasbourg, France) as shown in **Table 1**.

Samples. Grapes from *Vitis vinifera* vars. Macabeo, Sauvignon blanc, Merlot, and Parraleta cultivated in different regions of Spain in 2005, were harvested by hand and were stored frozen at −30 °C in the laboratory. Juice from Macabeo grapes was used for the laboratory fermentations.

Preparation of the Precursor Extract. The precursors were extracted from four different nonfloral grape varieties (Macabeo, Sauvignon blanc, Merlot, and Parraleta) to obtain a complex "multivarietal" pool of precursors. The procedure is based on that described is ref *34*. Grapes were treated in batches of 500 g of a single variety, and they were destemmed by hand and homogenized with a mixer Ultra Turrax T25 Basic (Ika, Labortechnik) in the presence of 0.13 M NaF and 50 mg/L ascorbic acid. The triturate was centrifuged at 4500 rpm for 15 min at 5 °C to separate the must from the skins, followed by a filtration through filter paper. The mashes of skins obtained (around 80 g per batch) were suspended in 380 mL of a buffer solution (0.1 M Na_2HPO_4/NaH_2PO_4) at pH 7 and 13% ethanol and allowed to macerate in the dark (36 h, 20 °C, nitrogen atmosphere) to extract the precursors. This solution was centrifuged at 4500 rpm for 15 min at 20 °C, and the supernatant was filtered through filter paper. Ethanol was then removed at room temperature by vacuum distillation in a rotary evaporator. This solution (ca. 260 mL per batch) is the "macerate". The must (ca. 300 mL per batch) and the macerate were percolated through two LiChrolut EN (1300 mg) resin beds (previously preconditioned with 32 mL of dichloromethane, 32 mL of methanol, and 65 mL of water). In both cases the column was washed with 26 mL of water, and then with 40 mL of a pentane: dichloromethane (2:1 v/v) mixture. The retained precursors were finally eluted with 50 mL of an ethyl acetate:methanol (9:1 v/v) mixture (ethyl acetate extract). Three batches per variety were processed, and the corresponding ethyl acetate extracts were mixed and evaporated under vacuum to dryness. These dry extracts were reconstituted in 20 mL of a 50% ethanol solution (coming from 900 mL must or around 240 g of skins). Finally, the macerate and must extracts coming from the four varieties were mixed to form the multivarietal mix used to spike the musts.

Yeasts and Fermentation Conditions. Three commercial *Saccharomyces cerevisiae* yeasts were used in this study, strain AR2 (yeast strain 1) from DMS Food Specialties Oenology S.A.S. (France), strain NT 116 (yeast strain 2) from Anchor Bio-Technologies (South Africa), and strain QA23 (yeast strain 3) from Lallemand (France). Yeast cultures were grown from 0.5 g active dry yeast rehydrated in 30 mL of sterile water at 35 °C for 30 min. One mL of must was added to improve the yeast growth.

Laboratory fermentations were carried out in triplicate using 350 mL-bottles filled with 250 mL of sterile grape must. Grape juice was sterilized by filtration (0.45 μm Schleicher & Schull, Postfch, Germany). For each yeast strain, fermentations were carried out with and without precursor extract. The precursor extract was added to reach 2 times the concentration of precursors in must (30 mL of the precursor mix per L of must). The samples were inoculated at 20 °C with 2 mL of the activated yeast solution. The fermentation process was monitored by weight. All fermentations were completed after 24−28 days.

Immediately following the alcoholic fermentation, yeast lees were removed by centrifugation and samples for quantitative analysis were then taken and analyzed. The wines were then bottled in 250 mL vessels and kept for 2 days at 4 °C. After this time, samples were analyzed by sensory analysis.

Control. The control sample was composed of a synthetic wine (12% v/v ethanol, 5 g L^{-1} tartaric acid, pH 3.4, 200 mg L^{-1} $NaHSO_3$) supplemented with the precursor fraction at the same concentration level than the supplemented must samples (30 mL of precursor mix per L). The control was stored at 20 °C in the dark for 28 days before its analysis.

Extraction and Analysis of Minor Volatile Compounds (SPE and GC−Ion Trap−MS Analysis). This analysis was carried out using the method proposed and validated by Lopez et al. (*35*). The method was modified to use a smaller quantity of sample and also incorporates a new washing step to improve the chromatographic resolution. In accordance with this method, 15 mL of wine, containing 10 μL of a surrogate standards solution (isopropyl propanoate, 3-octanone, heptanoic acid, and β-damascone, 2000 μg/g in ethanol), was passed through a 50 mg LiChrolut EN cartridge at about 2 mL min^{-1}. The sorbent was washed with 5 mL of 40% methanol solution and dried by letting air pass through (−0.6 bar, 10 min). Analytes were recovered by elution with 600 μL of dichloromethane. An internal standard solution (4-methyl-4-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol, at a concentration of 350, 450, and 500 μg/g, respectively, in dichloromethane) was added to the eluted sample. The extract was then analyzed by GC with Ion Trap−MS detection under the conditions described below.

Extraction and Analysis of Aroma Precursors. The determination of the aroma precursors in the initial must and in the pool of precursors was carried out indirectly by the analysis of the volatiles liberated by harsh acid hydrolysis of the aroma precursors using the method proposed by Ibarz et al. (*34*), which is an improvement of the method originally proposed by Gunata et al. (*36*). The must (50 mL) was percolated through a 200 mg LiChrolut EN resin cartridge (previously conditioned with 5 mL dichloromethane, 5 mL methanol, and 10 mL of water). Then

Table 1. Identified Compounds, Chemical Standards Used in the Study and MS Fragments Used for Quantitative Analysis.

RI[a]	compounds	source, purity	*m/z*
C_6 compounds			
1390	Z-3-hexen-1-ol	Aldrich, 98%	67
1413	E-2-hexen-1-ol	Aldrich, 99%	57
lactones			
1970	E-whiskylactone[c]	Aldrich, 98%	99
1988	δ-octalactone	Lancaster, 98%	99
2068	γ-nonalactone	Aldrich, 97%	85
2141	γ-decalactone	Aldrich, 98%	85
2260	δ-decalactone	Lancaster, 98%	99
benzenes			
1520	benzaldehyde	Fluka, 99%	105
1659	phenylacetaldehyde	Aldrich, 90%	91
1908	ethyl dihydrocinnamate	Aldrich, 99%	104
2081	ethyl cinnamate	Aldrich, 99%	131
2219	2-phenoxyethanol	Fluka, 98%	94 + 138
volatile phenols			
1876	2-methoxyphenol	Aldrich, 98%	109 + 124
2030	o-cresol	Aldrich, 99%	108
2068	4-ethyl-2-methoxyphenol	Lancaster, 98%	137
2157	m-cresol	Aldrich, 99%	108
2237	4-allyl-2-methoxyphenol	Aldrich, 99%	164
2262	4-vinyl-2-methoxyphenol	Aldrich, 98%	135 + 150
2279	E-4-propenyl-2-methoxyphenol	Lancaster, 97%	164
2404	4-vinylphenol	Lancaster, 10% soln.	91 + 120
2563	4-allyl-2,6-dimethoxyphenol	Aldrich, 90%	194
2725	1,2-dimethoxy-4-propylbenzene	Tentatively identified	151
3090	ethyl 4-hydroxybenzoate	Aldrich, 99%	121
vanillins			
2592	vanillin	Panreac, 99%	151 + 152
2629	methyl vanillate	Aldrich, 99%	151 + 182
2654	ethyl vanillate	Lancaster, 97%	151 + 196
2664	acetovanillone	Aldrich, 98%	151 + 166
2829	zingerone[c]	Aldrich, 96%	137 + 168
2892	homovanillyl alcohol	Aldrich, 99%	137 + 168
3040	syringaldehyde[c]	Aldrich, 98%	181 + 182
3099	homovanillic acid	tentatively identified	137 + 182
3123	acetosyringone	Aldrich, 97%	181 + 196
norisoprenoids			
1526	vitispirane A[c]	tentatively identified	93 + 121+136
1529	vitispirane B[c]	tentatively identified	93 + 121+136
1637	Riesling acetal[c]	tentatively identified	138
1748	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	tentatively identified	157
1829	β-damascenone	Firmenich, 90%	121
1832	t-1-(2,3,6-trimethylphenyl)but-1,3-diene (TPB)	tentatively identified	157
1848	α-isomethyl-ionone	Fluka, 85%	135 + 150
1939	3-oxo-β-ionone	tentatively identified	163
1950	β-ionone	Sigma, 98%	177
1952	actinidols[c]	tentatively identified	163
2657	3-oxo-α-ionol	tentatively identified	108
2698	3-hydroxy-7,8-dihydro-β-ionol	tentatively identified	193
2730	3-oxo-7,8-dihydro-α-ionol	tentatively identified	135
terpenes			
1447	Z-linalool oxide (furan)	tentatively identified	59
1476	E-linalool oxide (furan)	tentatively identified	59
1556	linalool	Fluka, 98.5%	93 + 121+136
1565	linalool acetate	tentatively identified	93 + 121
1608	terpinen-4-ol	tentatively identified	93 + 111
1613	2,6-dimethyl-1,7-octadien-3,6-diol	tentatively identified	71
1688	ocimenol	tentatively identified	93
1705	α-terpineol	Fluka, 97%	121 + 136
1709	terpinyl acetate	tentatively identified	68
1775	β-citronellol	Fluka, 90–95%	RIC
1811	nerol	Fluka, 90–95%	69
1963	3,7-dimethyl-1,5-octadien-3,7-diol	tentatively identified	82
2244	terpin	tentatively identified	59 + 81
2391	farnesol (2E,6E)	Fluka, 98%	69
miscellaneous			
1426	furfural	ChemService, 99%	95 + 96
2077	pantolactone	Aldrich, 99%	71
major compounds			
692	acetaldehyde	Aldrich, 99.5%	GC–FID[b]
995	2,3-butadione	Aldrich, 99%	GC–FID[b]
1116	1-butanol	Sigma, 99%	GC–FID[b]
1891	benzyl alcohol	Aldrich, 99%	GC–FID[b]
1672	3-methylbutyric acid	Lancaster, 98%	60

Table 1 (Continued)

RI[a]	compounds	source, purity	*m/z*
major compounds			
1677	2-methylbutyric acid	Aldrich, 98%	74
1960	2-ethylhexanoic acid	Aldrich, 99%	73 + 88
1279	hexyl acetate	ChemService, 99%	GC–FID[b]
1828	phenylethyl acetate	Fluka, 99%	104
1644	ethyl decanoate	Fluka, 99%	157 + 201
1490	ethyl 3-hydroxybutyrate	Aldrich, 98%	GC–FID[b]
1627	butyric acid	PolyScience, 99.5%	GC–FID[b]

[a] RI, retention index calculated in a DBWAXetr column. [b] Compound determined by microextraction and GC-FID analysis (*52*). [c] Zingerone: Vanillin acetone; Riesling Acetal: 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0(1,6)]undec-4-ene; Vitispirane: 2,10,10-trimethyl-6-methylen-1-oxaspiro- (*4,5*)dec-7-ene; Syringaldehyde: 3,5-dimethoxy-4-hydrxybenzaldehyde; Actinidols: 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; E-whiskylactone: (E)-β-methyl-γ-octalactone.

the column was rinsed with 4 mL of water and 4 mL of a pentane:dichloromethane (2:1 v/v) mixture. The precursors were eluted with 10 mL of a ethyl acetate:methanol (9:1 v/v) mixture. The solvent was evaporated under vacuum in a rotary evaporator to 1 mL, and then taken to dryness under gentle nitrogen current. The dry extract was reconstituted in 10 mL of hydrolysis solution (0.2 M citric acid buffer solution at pH 2.5). Acid hydrolysis (100 °C, 1 h) and extraction of the volatiles released was carried out under the conditions described in ref *34*. The obtained extract was finally analyzed by GC with ion trap−MS detection under the conditions described below.

Gas Chromatography−Mass Spectrometry Conditions. Gas chromatographic analysis was performed with a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system from Varian (Sunnyvale, CA). A DB-WAXETR capillary column (J&W Scientific, Folsom, CA) (60 m × 0.25 mm I.D., film thickness 0.5 μm) preceded by a 3 m × 0.25 mm uncoated (deactivated, intermediate polarity) precolumn from Supelco (Bellefonte, PA) was used. Helium was the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature program was 3 min at 40 °C, 10 °C min^{-1} up to 90 °C, 2 °C min^{-1} up to 230 °C, and finally held at this temperature for 37 min. Initially the injector was kept at 35 °C during 0.3 min and a pressure pulse of 25 psi during 2.60 min was applied. The injector was then heated to 250 °C at rate of 200 °C min^{-1}. The splitless time was 2.60 min. Silanized glass wood was used as a packing material in the insert. The injection volume was 4 μL. The global run time was recorded in full scan mode (40−220 *m/z* mass range). The chromatographic data were analyzed by Varian Saturn GC−MS Version 6.3 software.

Wine Descriptive Analysis. The sensory panel was composed of six females and three males, 25−40 years of age, all of them belonging to the laboratory staff and with a long experience in sensory analysis. Eight aroma terms, as shown in **Figure 1**, were selected by the panel for the descriptive analysis of the wines following standardized practices (ISO 6564:1985 and 4121:1987). Standards for some of the terms were defined: sweet (5 mL of brine from canned peach to 40 mL of white wine), flowery (100 μg L^{-1} of linalool and 1 mg L^{-1} of phenylethyl acetate to white wine), oxidized (2 g of overripen melon soaked for 1 h in 50 mL white wine and 1 drop of honey), and sweet fruit (6 mL of apple jam added to 50 mL of white wine). Panelists scored the intensity of each attribute using a seven-point scale. In all cases, wines (20 mL at 20 °C) were presented in coded, black tulip-shaped wine glasses covered by glass Petri dishes. Samples were presented in a random order. Because of the small amount of sample available, each judge evaluated each of the three replicate samples once.

Evaluation of the Sensory Influence of the Identified Odorants. The potential sensory effect of the odorants identified was studied via triangular tests (ISO 4120: 1983). The odorants were added by groups either to a model wine (10% ethanol in water v/v containing 5 g/L tartaric acid and pH 3.2) or to a neutral white wine (12% ethanol v/v, pH 3.2) and confronted with the unspiked controls. The concentrations and the odorant groups added can be seen in **Table 2**. In case a significant difference was found, judges were then asked to retest the samples and note down the descriptors that had changed. In all cases, solutions (20 mL at 20 °C) were presented in coded, black tulip-shaped wine glasses covered by glass Petri dishes.

Statistical Analysis. The quantitative data were analyzed by two-way analysis of variance (ANOVA). The yeast strain and the precursor fraction addition were the factors. The analyses were carried out using SPSS (SPSS Inc., Chicago, IL) for Windows, version 11.5.

Descriptive analysis data were also analyzed by two-way ANOVA.

RESULTS

Sensory Analysis. The results of the sensory analysis can be seen in **Figure 1**. The addition of grape extracts containing diverse odorless flavor precursors to a must exerted a significant sensory effect on the aroma profiles of the wines obtained. As can be seen, wines from musts supplemented with the precursor fraction presented, in all cases, a higher score for the floral term. For the other descriptors, the results were variable depending on the yeast strain. For instance, the score for the sweet descriptor was higher for the wine obtained with precursor fraction addition for yeast strain 3; however, for yeast strain 2, this score was higher for the wine obtained without precursor fraction addition.

Quantitative Composition of the Obtained Wines. The addition of the precursor fraction to the must and the yeast strain used in the fermentation exerted an important influence on the aroma composition of the wines. **Table 3** gives the results of the two-way ANOVA for both factors. As is shown in the table, the levels of 56 and 51 compounds were found to depend on the yeast strain and on the precursor fraction addition, respectively. The interaction between both factors was significant in 37 cases. A remarkable observation is that, in most cases in which the addition of precursor fraction was significant, the effect of the yeast strain was also significant.

General Effect of the Yeast Strain on the Volatile Profile. The effect of the yeast strain was evident not only for those compounds of known fermentative origin such as fatty acids, fatty acid ethyl esters, and fusel alcohols (data not shown) but also for many other compounds with varietal or prefermentative origins, as can be seen in **Tables 3** and **4**. The effect of the yeast strain was particularly strong (*F* quotient higher than 100)

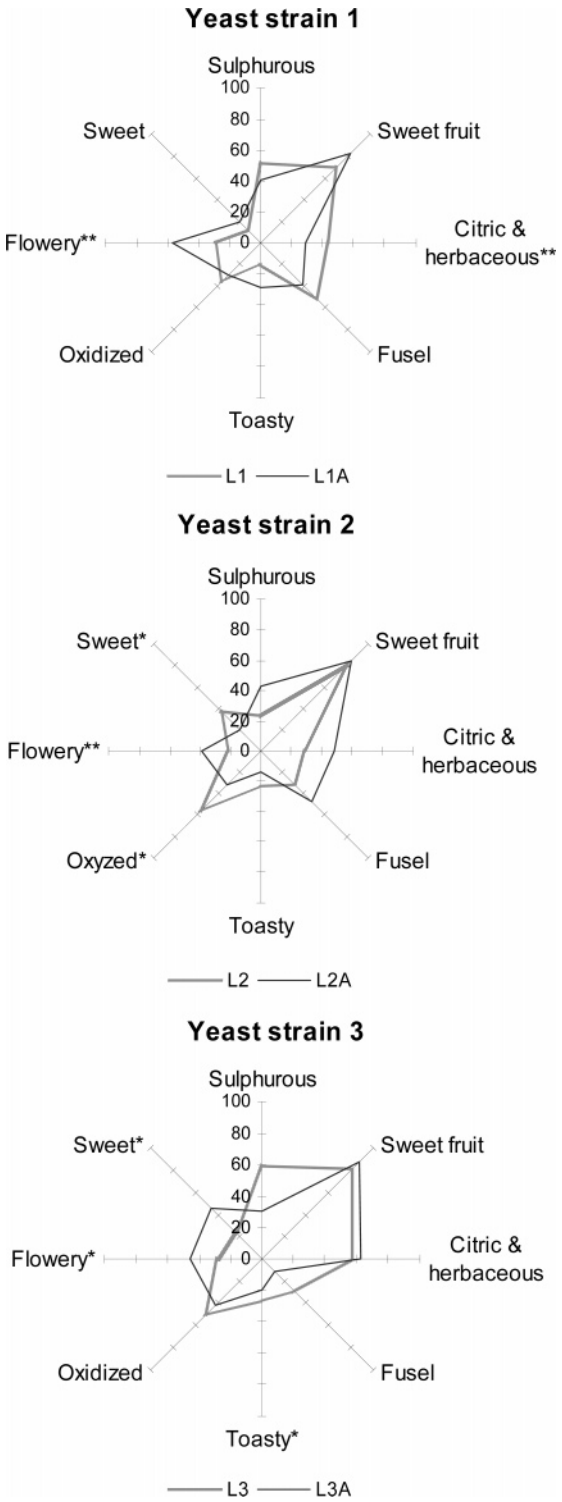

Figure 1. Spider webs showing the measured aromatic descriptors of the six different wines of the study (data are averages of three replicate samples). Codes: L1, L2, L3: wines made from nonsupplemented must with yeast strains 1, 2, or 3; L1A, L2A, and L3A, are the wines made from precursor-supplemented musts. *Difference significant at $P > 0.95$; **at $P > 0.99$.

for compounds, such as E-2-hexenol, E-whiskylactone, δ-octalactone, γ-nonalactone, o- and m-cresols, 4-ethyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, 4-vinylphenol, zingerone, actinidiols, 3,7-dimethyl-1,5-octadien-3,7-diol, and farnesol. This implies that for many of these compounds, the levels can vary in average factors between 2 and 4 depending on the yeast strain used. The strongest case is 4-vinylphenol for which the levels of the samples fermented with yeast 3 are up to 10 times higher than those fermented with yeast 2. This obviously indicates that yeast 3 has a powerful decarboxylase activity (*37*, *38*).

Table 2. Addition Experiments: Groups and Concentrations of Odorants Added to Synthetic or Neutral Wine.

	concentration added (μg/L)	odor threshold (μg/L)[a]
lactones		
δ-octalactone	9.5	400 (48)
γ-nonalactone	3.6	30 (49)
cinnamates		
ethyl dihydrocinnamate	0.47	1.6 (22)
ethyl cinnamate	1.3	1.1 (22)
phenols I		
2-methoxyphenol	7.5	9.5 (22)
o-cresol	0.39	31 (50)
m-cresol	1.3	68 (50)
phenols II		
4-ethyl-2-methoxyphenol	0.11	33 (22)
4-vinyl-2-methoxyphenol	128	40 (51)
4-vinylphenol	353	180 (50)
vanillins		
vanillin	2.2	200 (51)
methyl vanillate	23	3000 (35)
ethyl vanillate	21	990 (35)
acetovanillone	80	1000 (35)
zingerone	41	
syringaldehyde	16	50000 (48)
norisoprenoids		
β-damascenone	3.8	0.05 (51)
α-isomethyl-ionone	4.6	
β-ionone	3.3	0.09 (22)
terpenes		
linalool	7.8	25 (22)
α-terpineol	2.3	250 (22)
β-citronellol	7.7	100 (51))
farnesol	82	200 (48)

[a] The reference from the odor threshold is shown in parenthesis.

In spite of the high significance of the yeast strain factor, the effect of the yeast strain on the hydrolysis or formation of varietal compounds is extremely complex, so that it is not easy to assign a clear "champion" for the formation of most of the different families of compounds. So far, leaving aside the huge production of vinylphenols by yeast 3, the single clear facts are that wines made with yeast 1 contain lesser amounts of Z-3-hexenol, δ-lactones, and cinnamates, and maximal amounts of E-2-hexenol and of γ-lactones, while wines made with yeast 3 contain maximal levels of δ-lactones.

General Effect of the Addition of the Precursor Fraction. The addition of the precursor fraction affected to compounds from nearly all biochemical origins, including even several fermentative compounds such as acetaldehyde, 2,3-butadione -diacetyl-, ethyl decanoate, butyric, 3-methylbutyric, and 2-methylbutyric acids and phenylethyl acetate. In general, the addition brought about an increment of the levels of compounds, as can be seen in **Table 4**. However, the levels of Z-3-hexenol and of 3-methylbutyric and 2-methylbutyric acids in the supplemented samples were significantly lower than those found in nonsupplemented samples. The highest increments corresponded to some vanillins (factor 7 for methyl vanillate, factor 4 for ethyl vanillate, factor 5 for acetosyringone). Other important increments are those observed for ethyl cinnamate, 1,2-dimethoxy-4-propylbenzene -dihydromethyleugenol-, and 3-oxo-α-ionol (more than a factor 2 in average), ethyl dihydrocinnamate, 2-methoxyphenol -guaiacol-, acetovanillone, α-isomethylionone, and β-ionone (near factor 2) and m-cresol.

Table 3. Results of the ANOVA Study Carried Out on the Different Volatile Compounds Identified in the Wines[a]

	yeast strain		precursor fraction addition		interaction	
compounds	F	p	F	p	F	p
C_6 compounds						
Z-3-hexen-1-ol	35	8.9×10^{-6}[b]	85	8.1×10^{-7}[b]	6.8	0.011[b]
E-2-hexen-1-ol	144	1.3×10^{-8}[b]	11	0.006[b]	27	5.5×10^{-5}[b]
lactones						
E-whiskylactone	240	2.1×10^{-10}[b]	12	0.005[b]	1.6	0.238
δ-octalactone	229	2.8×10^{-10}[b]	2.6	0.132	45	2.7×10^{-6}[b]
γ-nonalactone	145	4.0×10^{-9}[b]	85	8.8×10^{-7}[b]	5.2	0.023[b]
γ-decalactone	61	1.1×10^{-6}[b]	3.0	0.110	1.9	0.194
δ-decalactone	30	3.4×10^{-5}[b]	0.49	0.498	1.3	0.304
benzenes						
benzaldehyde	23	0.0001[b]	2.0	0.186	13	0.001[b]
phenylacetaldehyde	6.8	0.012[b]	0.05	0.827	0.03	0.967
ethyl dihydrocinnamate	34	1.2×10^{-5}[b]	30	0.0001[b]	2.5	0.122
ethyl cinnamate	7.0	0.010[b]	115	1.6×10^{-7}[b]	4.7	0.031[b]
2-phenoxyethanol	5.8	0.019[b]	16	0.002[b]	12	0.002[b]
volatile phenols						
2-methoxyphenol	6.8	0.011[b]	81	1.1×10^{-6}[b]	0.06	0.941
o-cresol	1022	4.0×10^{-14}[b]	11	0.006[b]	11	0.002[b]
4-ethyl-2-methoxyphenol	193	1.0×10^{-8}[b]	10	0.011[b]	2.7	0.115
m-cresol	180	4.0×10^{-9}[b]	977	4.3×10^{-12}[b]	2.1	0.169
4-allyl-2-methoxyphenol	323	$3.7 \times 10^{-}11$[b]	1.6	0.226	8.5	0.005[b]
4-vinyl-2-methoxyphenol	46	$2.3 \times 10^{-}6$[b]	5.7	0.035[b]	1.3	0.314
E-4-propenyl-2-methoxyphenol	4.5	0.034[b]	0.004	0.951	6.2	0.014[b]
4-vinylphenol	634	2.9×10^{-11}[b]	25	0.0005[b]	35	3.0×10^{-5}[b]
4-allyl-2,6-dimethoxyphenol	0.56	0.587	8.3	0.015[b]	1.6	0.239
1,2-dimethoxy-4-propylbenzene	14	0.0007[b]	905	1.1×10^{-12}[b]	6.3	0.014[b]
ethyl 4-hydroxybenzoate	1.6	0.237	56	7.4×10^{-6}[b]	37	7.0×10^{-6}[b]
vanillins						
vanillin	24	0.0002[b]	31	0.0004[b]	2.5	0.135
methyl vanillate	1.9	0.189	1250	1.7×10^{-13}[b]	0.3	0.745
ethyl vanillate	1.3	0.307	978	7.2×10^{-13}[b]	4.0	0.048[b]
acetovanillone	2.9	0.091	351	3.0×10^{-10}[b]	0.3	0.752
zingerone	274	9.6×10^{-11}[b]	304	6.9×10^{-10}[b]	37	7.5×10^{-6}[b]
homovanillyl alcohol	24	0.0001[b]	3.2	0.103	13	0.002[b]
syringaldehyde	25	0.0001[b]	148	2.5×10^{-7}[b]	6.6	0.015[b]
homovanillic acid	14	0.0008[b]	54	9.3×10^{-6}[b]	5.2	0.024[b]
acetosyringone	7.1	0.012[b]	701	1.4×10^{-10}[b]	7.0	0.013[b]
norisoprenoids						
Riesling acetal	3.4	0.068	4.9	0.047[b]	0.79	0.478
β-damascenone	135	6.0×10^{-9}[b]	56	7.4×10^{-6}[b]	5.6	0.019[b]
α-isomethyl-ionone	52	2.5×10^{-5}[b]	55	7.6×10^{-5}[b]	50	2.9×10^{-5}[b]
β-ionone	28	5.1×10^{-5}[b]	30	0.0002[b]	27	6.2×10^{-5}[b]
actinidiols	122	1.1×10^{-8}[b]	718	4.5×10^{-12}[b]	35	1.0×10^{-5}[b]
3-oxo-α-ionol	22	9.4×10^{-5}[b]	461	6.0×10^{-11}[b]	6.6	0.012[b]
3-hydroxy-7,8-dihydro-β-ionol	6.3	0.013[b]	83	9.8×10^{-7}[b]	2.7	0.110
3-oxo-7,8-dihydro-α-ionol	20	0.0001[b]	12	0.005[b]	5.7	0.018[b]
terpenes						
E-linalool oxide (furan)	13	0.001[b]	2.0	0.184	0.63	0.548
linalool	42	6.9×10^{-6}[b]	21	0.0008[b]	18	0.0003[b]
linalool acetate	65	4.4×10^{-6}[b]	13	0.005[b]	22	0.0003[b]
2,6-dimethyl-1,7-octadien-3,6-diol	14	0.0008[b]	37	5.6×10^{-5}[b]	2.8	0.104
α-terpineol	29	4.2×10^{-5}[b]	47	2.7×10^{-5}[b]	18	0.0003[b]
terpinyl acetate	10	0.003[b]	4.0	0.068	5.8	0.017[b]
β-citronellol	56	1.7×10^{-6}[b]	49	2.3×10^{-5}[b]	35	1.7×10^{-5}[b]
nerol[a]	69	2.6×10^{-7} [b]	0.36	0.560	0.13	0.879
3,7-dimethyl-1,5-octadien-3,7-diol	129	7.9×10^{-9}[b]	17	0.002[b]	34	1.1×10^{-5}[b]
farnesol (2E,6E)	119	3.5×10^{-8}[b]	202	2.0×10^{-8}[b]	12	0.002[b]
miscellaneous						
furfural	71	1.3×10^{-6}[b]	13	0.005[b]	8.0	0.009[b]
pantolactone	24	0.0003[b]	0.3	0.596	0.3	0.753
major compounds						
acetaldehyde	10.3	0.006[b]	6.0	0.040[b]	0.36	0.711
2,3-butadione	95	8.8×10^{-7}[b]	9.7	0.013[b]	3.6	0.070
1-butanol	84	8.6×10^{-8}[b]	44	2.4×10^{-5}[b]	1.3	0.312
benzyl alcohol	1.9	0.214	20	0.002[b]	7.6	0.014[b]
3-methylbutyric acid	9.9	0.005[b]	85	7.0×10^{-6}[b]	13	0.002[b]
2-methylbutyric acid	0.23	0.802	19	0.001[b]	2.7	0.114
2-ethylhexanoic acid	74	4.3×10^{-7}[b]	158	7.2×10^{-8}[b]	29	4.5×10^{-5}[b]
hexyl acetate	146	1.9×10^{-6}[b]	0.001	0.970	0.001	0.999
phenylethyl acetate	83	2.2×10^{-7}[b]	23	0.001[b]	15	0.001[b]
ethyl decanoate	48	1.9×10^{-6}[b]	11	0.006[b]	1.6	0.237
ethyl 3-hydroxybutyrate	110	4.7×10^{-7}[b]	5.7	0.041[b]	1.7	0.240
butyric acid	16	0.0007[b]	10	0.009[b]	3.0	0.094

[a] Only compounds for which any of the factors (yeast strain and precursor fraction addition) was found to exert a significant influence are given. [b] Significance level 95%.

Table 4. Concentration (in μg L^{-1}, except Where Indicated[a,b]) of the Volatile Compounds Quantified in the Wines, in the Control, or in the Harsh Acid Hydrolysates from Odorless Precursor Fractions Extracted from the Supplemented or Nonsupplemented Musts of the Study (Data Are the Average of the Three Replicate Samples)

				yeast strain 1		yeast strain 2		yeast strain 3	
compounds	control	must[d]	must + A[d]	L1[e]	L1A[e]	L2[e]	L2A[e]	L3[e]	L3A[e]
C_6 compounds									
Z-3-hexen-1-ol	0.12	0.20	0.23	187 ± 13^{g}	145 ± 15^{a}	267 ± 17^{i}	191 ± 5^{g}	$236 \pm 19^{h,i}$	$203 \pm 18^{g,h}$
E-2-hexen-1-ol	nd[c]	3.2	2.9	2.9 ± 0.1^{h}	1.8 ± 0.2^{g}	0.8 ± 0.4^{f}	1.0 ± 0.02^{f}	1.1 ± 0.1^{f}	1.3 ± 0.2^{f}
lactones									
E-whiskylactone	nd	nd	nd	1.9 ± 0.1^{f}	1.47 ± 0.01^{f}	5.8 ± 0.4^{g}	5.3 ± 0.5^{g}	5.8 ± 0.1^{g}	6 ± 1^{g}
δ-octalactone	nd	nd	nd	10.7 ± 0.6^{f}	17 ± 1^{g}	22.5 ± 0.6^{h}	$15 \pm 2^{f,g}$	29 ± 1^{i}	39 ± 6^{j}
γ-nonalactone	1.0	0.29	0.54	9.1 ± 0.3^{j}	11.5 ± 0.1^{j}	$5.0 \pm 0.4^{f,g}$	$6.6 \pm 0.2^{g,h}$	4.3 ± 0.3^{f}	$8 \pm 1^{h,i}$
γ-decalactone	nd	0.56	0.49	1.6 ± 0.1^{f}	1.6 ± 0.1^{f}	0.653 ± 0.002^{g}	0.7 ± 0.1^{g}	0.48 ± 0.07^{g}	0.6 ± 0.2^{g}
δ-decalactone	nd	0.85	0.84	29 ± 2^{f}	29 ± 1^{f}	$45 \pm 6^{g,h}$	40 ± 4^{g}	$44 \pm 3^{g,h}$	$49 \pm 8^{h,i}$
benzenes									
benzaldehyde	5.0	5.8	4.9	8.1 ± 0.7^{f}	6.3 ± 0.6^{f}	6.1 ± 1.6^{f}	6.0 ± 0.7^{f}	6.1 ± 0.2^{f}	7.1 ± 1.0^{f}
phenylacetaldehyde	nd	3.5	2.7	$6.5 \pm 0.3^{f,g}$	$6.6 \pm 1.2^{f,g}$	8 ± 3^{g}	$6 \pm 2^{f,g}$	4.0 ± 0.1^{f}	3.6 ± 0.1^{f}
ethyl dihydrocinnamate	nd	nd	nd	nd[f]	0.5 ± 0.1^{g}	$0.67 \pm 0.03^{g,h}$	0.9 ± 0.2^{h}	0.37 ± 0.04^{g}	$0.7 \pm 0.1^{g,h}$
ethyl cinnamate	0.1	nd	nd	0.8 ± 0.1^{f}	1.33 ± 0.02^{g}	$1.0^{f,g}$	1.9 ± 0.2^{h}	0.6 ± 0.2^{f}	1.9 ± 0.5^{h}
2-phenoxyethanol	5.9	1.5	1.5	6.1 ± 0.2^{g}	5.5 ± 0.6^{g}	3.7 ± 0.1^{f}	8 ± 1^{h}	$6 \pm 1^{g,h}$	8 ± 1^{h}
volatile phenols									
2-methoxyphenol	0.3	0.43	0.46	7.6 ± 0.3^{f}	$13.2 \pm 0.2^{g,h}$	8.7 ± 1.3^{f}	$16 \pm 2^{g,h}$	$10.1 \pm 0.8^{f,g}$	18 ± 4^{h}
o-cresol	nd	nd	nd	1.6 ± 0.1^{g}	1.96 ± 0.04^{h}	nd[f]	nd[f]	nd[f]	nd[f]
4-ethyl-2-methoxyphenol	nd	nd	nd	nd[f]	nd[f]	$0.17 \pm 0.05^{g,h}$	$0.20 \pm 0.02^{g,h}$	0.13 ± 0.05^{g}	0.2 ± 0.1^{h}
m-cresol	nd	nd	nd	nd[f]	1.0 ± 0.1^{g}	nd[f]	1.3 ± 0.1^{g}	0.9 ± 0.3^{g}	2.0 ± 0.4^{h}
4-allyl-2-methoxyphenol	0.4	0.28	0.28	nd[a]	nd[f]	1.2 ± 0.3^{g}	1.15 ± 0.06^{g}	0.8 ± 0.1^{g}	1.3 ± 0.3^{g}
4-vinyl-2-methoxyphenol	6	27	25	290 ± 31^{f}	$417 \pm 20^{f,g}$	441 ± 79^{g}	$538 \pm 93^{g,h}$	$671 \pm 24^{h,i}$	760 ± 135^{i}
E-4-propenyl-2-methoxyphenol	nd	0.94	0.73	4.5 ± 0.2^{f}	5.3 ± 0.4^{f}	6.0 ± 0.3^{f}	5.4 ± 0.7^{f}	4.7 ± 0.5^{f}	6 ± 2^{a}
4-vinylphenol	12	28	25	661 ± 45^{f}	960 ± 104^{f}	555 ± 97^{f}	461 ± 48^{f}	6060 ± 403^{h}	4476 ± 717^{g}
4-allyl-2,6-dimethoxyphenol	nd	1.1	1.7	0.9 ± 0.3^{a}	0.61 ± 0.08^{f}	0.66 ± 0.07^{f}	0.68 ± 0.03^{f}	0.9 ± 0.2^{f}	0.63 ± 0.08^{f}
1,2-dimethoxy-4-propylbenzene[a]	nd	1.7	1.6	11.1 ± 0.6^{f}	26 ± 2^{g}	14.1 ± 0.5^{f}	28 ± 2^{g}	12.6 ± 0.3^{f}	32 ± 5^{g}
ethyl 4-hydroxybenzoate	nd	1.1	4.8	171 ± 12^{f}	318 ± 17^{h}	235 ± 5^{g}	218 ± 29^{g}	178 ± 4^{f}	288 ± 38^{h}
vanillins									
vanillin	6	3.2	3.6	9.2 ± 0.2^{g}	10.0 ± 0.3^{g}	7.0 ± 0.3^{f}	9.22 ± 0.003^{g}	9.4 ± 0.5^{g}	10.7 ± 0.5^{h}
methyl vanillate	1.2	0.40	0.64	3.7 ± 0.5^{f}	24.7 ± 0.5^{g}	3.9 ± 0.3^{f}	27 ± 3^{g}	4.5 ± 0.4^{f}	29 ± 4^{g}
ethyl vanillate	2	nd	nd	6.3 ± 0.2^{f}	26 ± 1^{b}	9 ± 1^{f}	$27 \pm 2^{g,h}$	6.6 ± 0.5^{f}	30 ± 4^{h}
acetovanillone	3	2.4	2.7	95 ± 7^{f}	167 ± 3^{g}	104 ± 1^{f}	184 ± 13^{g}	97 ± 3^{f}	185 ± 26^{g}
zingerone	1	4.1	3.9	70 ± 3^{g}	110 ± 5^{h}	41 ± 2^{f}	$54 \pm 5^{f,g}$	65 ± 2^{g}	105 ± 16^{h}
homovanillyl alcohol	nd	7.7	14	12 ± 2^{f}	15.9 ± 0.8^{f}	26 ± 6^{h}	$18 \pm 2^{f,g}$	$25 \pm 6^{g,h}$	47 ± 3^{i}
syringaldehyde	5	4.1	6.0	$12 \pm 2^{g,h}$	17 ± 1^{i}	nd[f]	14 ± 1^{h}	10.0 ± 0.5^{g}	$15 \pm 3^{h,i}$
homovanillic acid[a]	33	50	63	$53 \pm 2^{f,g}$	77 ± 3^{i}	46 ± 6^{f}	$58 \pm 6^{g,h}$	$54.3 \pm 0.8^{f,g}$	$68 \pm 2^{h,i}$
acetosyringone	nd	1.9	4.2	$17 \pm 2^{f,g}$	66 ± 2^{h}	9 ± 3^{f}	63 ± 2^{h}	24 ± 0.3^{g}	66 ± 14^{h}
norisoprenoids									
vitispirane A[a]	nd	49	44	nd	nd	nd	nd	nd	nd
vitispirane B[a]	nd	48	43	nd	nd	nd	nd	nd	nd
Riesling acetal[a]	nd	16	11	0.6 ± 0.1^{f}	0.79 ± 0.06^{f}	0.58 ± 0.02^{f}	0.7 ± 0.2^{f}	0.54 ± 0.02^{f}	0.6 ± 0.1^{f}
1,1,6-trimethyl-1,2-dihydronaphthalene[a]	nd	51	61	nd	nd	nd	nd	nd	nd
β-damascenone	nd	2.5	3.2	3.5 ± 0.3^{f}	$4.6^{f,g}$	8.0 ± 0.1^{h}	10.0 ± 0.5^{i}	5.5 ± 0.7^{g}	$9 \pm 2^{h,i}$
t-1-(2,3,6-trimethylphenyl) but-1,3-diene[a]	nd	1.7	2.8	nd	nd	nd	nd	nd	nd
α-isomethyl-ionone	nd	nd	nd	5.9 ± 0.4^{g}	5.3 ± 0.1^{g}	1.8 ± 0.2^{f}	6 ± 2^{g}	$4.4 \pm 0.4^{f,g}$	6 ± 1^{g}
3-oxo-β-ionone[a]	nd	15	28	nd	nd	nd	nd	nd	nd
β-ionone	0.08	0.11	0.24	5.2 ± 0.6^{h}	$4.3 \pm 0.4^{g,h}$	1.2 ± 0.2^{f}	4.5 ± 0.7^{h}	3.5 ± 0.4^{g}	5.0 ± 0.8^{h}
actinidiols[a]	nd	21	36	nd[f]	0.71 ± 0.06^{h}	0.5 ± 0.1^{g}	0.93 ± 0.03^{i}	nd[f]	$0.8 \pm 0.2^{h,i}$
3-oxo-α-ionol[a]	0.7	8.4	10.9	16.5 ± 0.2^{f}	34 ± 1^{h}	13.2 ± 0.5^{f}	27 ± 2^{g}	15.2 ± 0.9^{f}	$32 \pm 6^{g,h}$
3-hydroxy-7,8-dihydro-β-ionol[a]	nd	nd	nd	1.2 ± 0.2^{f}	$1.7^{g,h}$	1.2 ± 0.1^{f}	$1.\pm 0.2^{h}$	$1.2 \pm 0.1^{f,g}$	2.3 ± 0.3^{i}
3-oxo-7,8-dihydro-α-ionol[a]	nd	nd	nd	$1.5 \pm 0.2^{f,g}$	1.92 ± 0.05^{g}	1.22 ± 0.06^{f}	1.3 ± 0.2^{f}	1.38 ± 0.07^{f}	$1.7 \pm 0.4^{f,g}$
terpenes									
Z-linalool oxide (furan)[a]	nd	11	10	nd	nd	nd	nd	nd	nd
E-linalool oxide (furan)[a]	nd	nd	nd	$1.0 \pm 0.1^{i,j}$	$1.0 \pm 0.1^{h,j}$	$0.75 \pm 0.05^{f,g}$	0.69 ± 0.05^{f}	$0.8 \pm 0.1^{g,h}$	$0.9 \pm 0.1^{h,i}$
linalool	0.59	0.50	0.64	17 ± 1^{h}	15 ± 2^{h}	8 ± 1^{f}	16 ± 2^{h}	9.0 ± 0.4^{f}	12 ± 2^{g}
linalool acetate[a]	0.6	0.45	0.30	12 ± 1^{g}	9 ± 2^{g}	3.1 ± 0.2^{f}	9 ± 1^{b}	4.8 ± 0.6^{f}	5.3 ± 0.2^{f}
terpinen-4-ol[a]	nd	0.41	0.65	nd	nd	nd	nd	nd	nd
2,6-dimethyl-1,7-octadien-3,6-diol[a]	0.5	5.5	5.0	0.5 ± 0.1^{g}	0.63 ± 0.07^{g}	$0.42 \pm 0.02^{f,g}$	0.60 ± 0.04^{g}	0.29 ± 0.06^{f}	0.6 ± 0.2^{g}
ocimenol[a]	0.3	5	8	nd	nd	nd	nd	nd	nd
α-terpineol	0.35	3.1	5.3	4.9 ± 0.4^{g}	4.8 ± 0.6^{g}	2.4 ± 0.2^{f}	4.7 ± 0.3^{g}	3.5 ± 0.1^{f}	5 ± 1^{g}
terpinyl acetate[a]	nd	3.8	3.2	$0.75 \pm 0.05^{f,g}$	$0.86 \pm 0.06^{f,g}$	1.2 ± 0.2^{g}	$1.1 \pm 0.1^{f,g}$	0.46 ± 0.04^{f}	$1.1 \pm 0.5^{f,g}$
β-citronellol	nd	1.7	1.2	5.8 ± 0.4^{f}	5.4 ± 0.1^{f}	5.9 ± 0.2^{f}	8 ± 1^{f}	6.4 ± 0.3^{f}	14 ± 4^{g}
nerol	nd	nd	0.27	nd[f]	nd[f]	2.4 ± 0.5^{g}	3 ± 1^{g}	3 ± 1^{g}	3.6 ± 0.3^{g}
3,7-dimethyl-1,5-octadien-3,7-diol[a]	0.5	2.2	1.3	3.8 ± 0.2^{f}	$4.7 \pm 0.4^{f,g}$	7.5 ± 0.5^{h}	7.5 ± 0.2^{h}	5.7 ± 0.5^{g}	9 ± 1^{c}
terpin[a]	nd	2.0	2.0	nd	nd	nd	nd	nd	nd
farnesol (2E,6E)	nd	nd	4.2	74 ± 5^{f}	$99 \pm 6^{f,g}$	111 ± 14^{g}	193 ± 15^{h}	110 ± 7^{g}	181 ± 34^{h}

Table 4 (Continued)

compounds	control	must[d]	must + A[d]	yeast strain 1 L1[e]	yeast strain 1 L1A[e]	yeast strain 2 L2[e]	yeast strain 2 L2A[e]	yeast strain 3 L3*
miscellaneous								
furfural	1.7	nd	nd	1.7 ± 0.2^{h}	$1.4 \pm 0.3^{g,h}$	0.5 ± 01^{f}	1.0 ± 0.1^{g}	$1.3 \pm 0.1^{g,h}$
pantolactone	1.8	18	16	2.7 ± 0.2^{f}	2.7 ± 0.1^{f}	4.6 ± 0.5^{g}	4.8 ± 0.9^{g}	$3.7 \pm 0.6^{f,g}$
major compounds								
acetaldehyde[b]	nd	nd	nd	8 ± 2^{f}	$11.5 \pm 3.2^{f,g}$	10.5 ± 0.4^{f}	13 ± 1^{g}	14 ± 2^{g}
2,3-butadione[b]	nd	nd	nd	nd[f]	nd[f]	$1.3 \pm 0.2^{h,i}$	2.0 ± 0.4^{h}	0.9 ± 0.3^{g}
1-butanol[b]	nd	nd	nd	$<1.2^{f}$	$<1.2^{f,g}$	$<1.2^{h,i}$	1.25 ± 0.05^{j}	$< 1.2^{g,h}$
benzyl alcohol[b]	nd	nd	13	nd[f]	$<0.02^{g}$	$<0.02^{f,g}$	$<0.02^{f,g}$	$<0.02^{f,g}$
3-methylbutyric acid	1.4	2.3	1.6	65 ± 8^{g}	52 ± 3^{g}	80 ± 10^{h}	$36 \pm 3f$	85 ± 6^{h}
2-methylbutyric acid	1.0	3.1	1.7	$23 \pm 7^{f,g}$	$17 \pm 5^{f,g}$	29 ± 5^{f}	$11 \pm 1f$	$21 \pm 4^{f,g}$
2-ethylhexanoic acid	nd	nd	2.6	$10.8 \pm 0.5^{f,g}$	$11.2 \pm 0.6^{f,g}$	8.3 ± 0.8^{f}	13.1 ± 0.3^{g}	11.5 ± 0.4^{g}
hexyl acetate[b]	nd	nd	nd	nd[f]	nd[f]	nd[f]	nd[f]	$<0.03^{g}$
phenylethyl acetate[b]	nd	nd	nd	0.86 ± 0.08^{g}	1.21 ± 0.03^{h}	0.56 ± 0.04^{f}	0.59 ± 0.07^{f}	0.67 ± 0.01^{f}
ethyl decanoate[b]	0.1	nd	nd	0.58 ± 0.08^{f}	0.78 ± 0.04^{f}	1.08 ± 0.06^{f}	1.7 ± 0.5^{g}	$1.7 \pm 0.1^{g,h}$
ethyl 3-hydroxybutyrate[b]	nd	nd	nd	$< 0.04^{g}$	0.05 ± 0.01^{g}	nd[f]	nd[f]	0.07 ± 0.01^{g}
butyric acid[b]	nd	nd	nd	$0.41 \pm 0.03^{f,g}$	$0.47 \pm 0.03^{f,g}$	nd[a]	$0.67 \pm 0.02^{f,g}$	0.8 ± 0.1^{g}

[a] Relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000) of the volatile compounds for which pure references were not available. [b] Data in mg/L. [c] nd, not detected. [d] Must, must + A: volatile compounds resulting from the harsh acid hydrolysis of the precursor fraction extracted from non-supplemented and supplemened must, respectively. [e] L, wine obtained without precursor extract addition, LA = wine obtained with precursor extract addition. [f–j] Different letters indicate significant differences (significant level 95%).

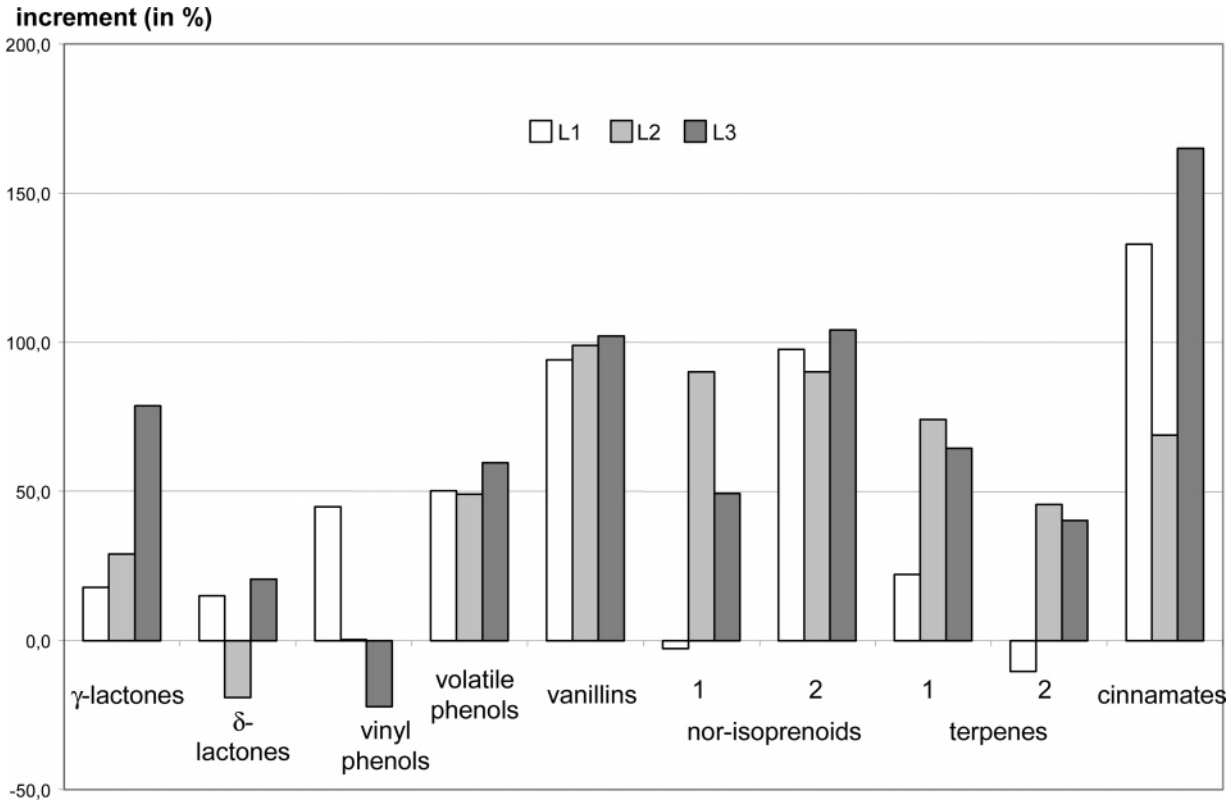

Figure 2. Increments observed in the supplemented wines normalized to the amount of compound formed in the nonsupplemented samples. Categories correspond to those shown in Table 3 with the following remarks: volatile phenols do not include data from vinyl phenols; nor-isoprenoids and terpenols have been split into two groups, the first one includes the calibrated compounds (data in concentration units), the second one includes the compounds measured only as relative area; the two cinnamates form an independent group.

It should be also noted the absence in the wines of some compounds that can be observed in the acid-hydrolysates of the precursor fraction (shown in the third and fourth columns of **Table 4**), such as γ-octalactone, vitispiranes, TDN, TPB, 3-oxo-β-ionone, Z and E linalool oxides, terpinen-4-ol, ocimenol, or terpin.

Role of Yeast Strain on the Hydrolysis/Formation of Varietal Aroma Compounds. The interdependence between factors yeast strain and precursor fraction addition is very complex and can be more easily understood with the help of **Figure 2**. The figure shows for each family of compounds the increment caused by the supplementation (expressed as percent of the amount found in the nonsupplemented sample).

As can be seen, different patterns can be identified:

(1) Aroma compounds for which the normalized increments are equal for the three yeast strains (volatile phenols, vanillins, and the second family of norisoprenoids)

(2) Aroma compounds for which the normalized increments are always positive, but that differ between strains (γ-lactones, terpenes -first group-, and cinnamates)

(3) Aroma compounds for which one of the strains fails in forming the compound from the precursor fraction (δ-lactones, vinylphenols, norisoprenoids -first group-, and terpenes, second group)

A remarkable observation is that in some of the compounds following the third pattern, the yeast which failed in the release or production of the aroma compound from the precursor fraction was the most efficient at forming that compound in the nonsupplemented sample. Examples of this are β-ionone or linalool (yeast 1 failed), δ-decalactone (yeast 2 failed), or 4-vinyl-2-methoxyphenol (yeast 3 failed). In all these cases the addition of the precursor fraction seems to level up the differences observed between yeasts in the nonsupplemented samples

Fermentative Induced or Acid Hydrolysis?. The second column of **Table 4** gives the aroma composition of the nonfermented control sample after the 28 days of the experiment. As can be seen, most of the aroma compounds were at concentrations below the method detection limits, which indicates that the natural acid hydrolysis from the precursor fraction is very slow, by comparison with both the fast acid hydrolysis carried out at high temperatures (shown in the third and fourth columns of **Table 4**) and the hydrolysis caused by fermentation. Exceptions are benzaldehyde, 2-phenoxyethanol, vanillin, homovanillic acid, and furfural. These compounds are found in the control at concentrations similar or even higher than those observed in the fermented supplemented samples. Leaving aside these compounds, data in the table demonstrate that the roles of yeast and of fermentation are decisive in the formation of aroma compounds from the precursor fraction.

Sensory Effects Linked to the Addition of the Precursor Fraction. None of the increments in the levels of aroma compounds linked to the addition of the precursor fraction is "per se" relevant enough to cause any important sensory effect. In fact, and in terms of aroma units, only in four cases (β-damascenone, β-ionone, 4-vinylphenol, and 4-vinyl-2-methoxyphenol) the increments were well above the corresponding odor thresholds, and in two other cases (ethyl cinnamate and 2-methoxyphenol), the increments were close to the corresponding thresholds. Nevertheless, there are a relatively large number of aroma compounds whose concentration increases with the addition of the precursor fraction and such numbers of compounds may exert a concerted action on wine aroma. This was checked by grouping the most relevant aroma compounds into seven different categories attending to chemical structure and/or biochemical origin and by studying the effect of the joined addition of the compounds in one, two, or more categories to a model wine. The composition of such groups is presented in **Table 2** and the results of the sensory tests are presented in **Table 5**. When the addition of individual groups was carried out, only in three cases (norisoprenoids and the two fractions with volatile phenols) was a significant sensory effect noted. The smell of the phenolic fractions was, in both cases, not very pleasant, as expected, particularly in the case of vinylphenols. The combined addition of two families of the four remaining families was then tried. Only in the case of the joined addition of cinnamates and terpenes a clear sensory effect was noted which evidence the existence of a synergic or additive effect between both groups of odorants. When three of the families were added together, the smell of the mixture could be significantly perceived in all possible combinations. The aromas of all these mixtures were flowery and sweet. The sensory effect was clearer still when the four categories: cinnamates, vanillins, terpenes, and lactones were added together. In this case, the aroma of the mixture reminded more of peach in a synthetic wine, but was mainly floral when the addition was carried out in a neutral wine. Finally, the aroma of the complete mixture was easily detected even when added to a neutral white wine. The aroma of this mixture was defined as flowery and sweet, quite close to those found in the mixtures of cinnamates, vanillins, terpenes, and lactones.

Table 5. Results of the Triangular Tests and Sensory Description of the Effects Caused by the Addition of One or Several Groups of Odorants to Synthetic or Neutral Wine

compounds added[a]	significance (α)	sensory effects
addition of a single group		
norisoprenoids	<0.05	fruity, blackberry
phenols I	<0.01	phenol
phenols II	<0.001	dirty, unpleasant, medicinal
cinnamates	ns	
vanillins	ns	
terpenes	ns	
lactones	ns	
addition of two groups		
cinnamates + terpenes	<0.01	floral
cinnamates + vanillins	ns	
cinnamates + lactones	ns	
vanillins + terpenes	ns	
vanillins + lactones	ns	
terpenes + lactones	ns	
addition of three groups		
cinnamates + vanillins + terpenes	<0.05	floral
cinnamates + vanillins + lactones	<0.05	floral
cinnamates + terpenes + lactones	<0.001	floral, terpenic
vanillins + terpenes + lactones	<0.01	sweet
addition of four groups		
cinnamates + vanillins + terpenes + lactones	<0.001	sweet fruit, peach
idem to neutral wine	<0.01	floral, sweet
addition of the seven groups		
all compounds	<0.001	floral, sweet, fruity, citric
all compounds in neutral wine	<0.001	floral, sweet, perfume

[a] Except where indicated, the additions were carried out in synthetic wine.

DISCUSSION

The experiments presented in this paper confirm the key role played by fermentation and by yeast on the formation of wine varietal aroma. As results in **Figure 1** indicate, the addition of an odorless aroma precursor extract from nonfloral grapes to a must has an effect on the aroma of the fermented wine, increasing its flowery nuances. The sensory effect of the addition is not extreme, in accordance with the nonfloral character of the grapes from which the extract was prepared, but it is strong enough to be significantly detected in all the experiments.

Similarly, results in **Tables 3** and **4** confirm that the presence of odorless aroma precursor fractions in the must brings about some increase in the levels of many wine volatiles, some of which bear important aroma properties. Twenty-three out of the 40 aroma compounds whose level increases with the addition of the precursor fraction have been identified in different experiments as normal constituents of the aroma of young wines (*22*, *39*−*41*). The other 17 compounds are also important aroma compounds but, so far and leaving aside 1,1,6-trimethyl-1,2-dihydronaphthalene -TDN-, they never have been clearly detected as wine-aroma-active compounds in the different experiments carried out using gas chromatography−olfactometry. Most of the compounds in this last group are, however, normal constituents of the different hydrolysates obtained from grape precursor fractions (*3*, *4*, *27*, *36*). Interestingly, most of

the 23 compounds in the first group have been related to the grape variety in different studies: The levels of some of them, such as β-damascenone, γ-nonalactone, 2-methoxyphenol, 4-vinyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, linalool, and α-terpineol have been found to be significantly related to the grape variety (*22*, *42*), most of the compounds in that list were also found in the mild acid hydrolysates obtained from precursor fractions (*28*), ethyl cinnamate and ethyl dihydrocinnamate have been related to the specific aroma of Pinot noir wines (*43*−*45*).

The sensory experiment presented in **Tables 2** and **5** demonstrates that the 23 compounds in this group exert a concerted action on wine aroma and, furthermore, the final sensory effect is an increase of the floral notes of wine. This finding suggests that those 23 aroma compounds are directly responsible for the floral notes linked to the addition of the precursor fraction to the must. It is particularly noteworthy that the floral notes seem to be mostly due to compounds, such as cinnamates, vanillins, terpenes, and lactones, present at subthreshold and perithreshold levels. In any case, these findings make it possible to state that the varietal aroma of nonfloral varieties, leaving aside the cysteinyl-related mercaptans, should be attributed to the presence of a large number of compounds belonging to different chemical classes: linear aliphatic lactones, ethyl cinnamates, vanillin-derived compounds, volatile phenols, terpenes, and norisoprenoids. In most cases, none of the compounds reaches concentrations high enough to be clearly identified in the mixture, so that it is not possible to talk about impact compounds. This is in accordance with the complex and subtle aroma nuances of most nonfloral wines.

It may be thought that the production of those varietal aroma compounds is the result of a simple hydrolytical process caused by the yeast glycosydases released during fermentation. However, some of the results presented in this paper suggest that the formation of varietal aroma, or at least of some of the compounds forming it, is a much more complex process than a simple enzyme-driven hydrolytical process. Such "simple" hydrolytical process should be consistent with the first pattern of behavior identified in **Figure 2**. However, the second and third patterns of behaviors, particularly the last one, seem to indicate that varietal aroma formation is part of yeast metabolism and has a complex regulation. Similar phenomena have been reported in the production of lactones by the yeast *Sporobolomyces odorus* (*46*). Some other indicators of a deep involvement of the precursor fraction on yeast metabolism are seen in the production of some esters (ethyl decanoate, phenylethyl acetate, ethyl hydrocinnamate, ethyl cinnamate, linalyl, and terpinyl acetates) which obviously cannot be present in the precursor fraction and in the decrement in the levels of 3-methylbutyric and 2-methylbutyric acids.

Finally, it should be noted that the precursor molecules for some of the odorants formed have not been described. This is the case of γ and δ-lactones. Small amounts of these compounds can be found in the acid hydrolysates of precursor fractions (*28*), which suggests that more or less specific precursors should exist. Recent research in whisky has shown that *Saccharomyces* yeasts are able to form lactones from the hydroxyacids formed by previous oxidation of different fatty acids by lactic bacteria (*47*). The precursor molecules could be free of hydroxyacids, but could also be glycerol esters.

In conclusion, this research has demonstrated that a part of varietal aroma in nonfloral grape varieties is due to the concerted action of more than 20 aroma chemicals present at relatively low concentrations and odor activities. These compounds are formed or released during fermentation by the action of yeasts from not well-known nonvolatile molecules and through processes which should be further studied.

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