

## Fate of Grape Flavor Precursors during Storage on Yeast Lees

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The effect of the addition of a grape flavor precursor extract to a grape juice, before or after fermentation with three different *Saccharomyces cerevisiae* yeast strains, on the evolution of the wine aroma composition during a 9-month aging period on yeast lees has been studied. Wine aroma compounds were determined by gas chromatography–mass spectrometry after alcoholic fermentation and after 3 and 9 months of storage. The aging of wine on lees caused important changes in the aroma profiles of wines, making the concentrations of three terpenes, norisoprenoids (except  $\beta$ -damascenone and  $\beta$ -ionone), 4-allyl-2,6-dimethoxyphenol, ethyl vanillate, syringaldehyde, and ethyl cinnamate increase, whereas the concentrations of most of the rest of compounds tended to decrease. Lees are responsible for the observed increasing trends, except for linalool and  $\alpha$ -terpineol, and also for a large part of the observed decrements. As expected, the addition of precursors brings about an increment in the levels of most terpenes, norisoprenoids, vanillins, and ethyl cinnamate, and it is after an aging time when differences linked to the level of precursors in the must become more evident. The timing of the addition of precursors has a minor influence, except for  $\beta$ -damascenone, vanillin, and syringaldehyde, for which supplementation after fermentation is more effective. It has also been observed that the precursor fraction makes the levels of vinylphenols decrease. Finally, it has also been found that lees from different yeast strains may have a slightly different abilities to release volatile compounds derived from precursors.

**KEYWORDS:** Glycosides; flavor; aroma; yeast lees; wine; storage; aging on lees

### INTRODUCTION

Since the discovery of the presence of some wine aroma compounds in the form of glycosides (1), important research has been done to try to exploit technologically this aroma potential of grapes. Some authors have considered the use of selected or genetically modified yeast strains, as well as the enzymes produced by them, to enhance the aroma released from flavor precursors (2–4). Others studied the use of exogenous glycosidase enzymes to achieve an increase in wine flavor (5–7). Acid high-temperature conditions were also tested to hydrolyze glycosidic precursors; however, they can cause a deterioration of the quality of wine, and molecular rearrangements have also been described (8). The hydrolysis of flavor precursors by lactic acid bacteria during malolactic fermentation was also reported (9–12). Nevertheless, little information is available about the possibility to enhance wine aroma during wine aging on yeast lees.

Traditionally, only some white wines mainly from Burgundy and sparkling wines produced by the *champanoise* method are left in contact with lees. Nevertheless, wine aging on lees is now a more widely extended practice (13) to improve the organoleptic characteristics of wine (14, 15). After yeast death, an autolysis process takes place, and the intracellular constituents, such as hydrolytic enzymes, polysaccharides, and mannoproteins, are

released to the medium. Many works have been carried out to study the effect of lees autolysis on phenolic compounds (16, 17). It has been demonstrated that the hydrolytic enzymes released can modify the phenolic fraction (18) and mannoproteins can act on the tartaric stabilization (19) and interact with phenolic compounds, improving color stability and reducing the astringency of wine (20, 21). Several works have been carried out to study the evolution of the volatile fraction of wines during aging on lees (19, 22–24), but most of these works have focused on major or fermentative compounds such as esters, fatty acids, or alcohols. Bautista et al. (23) observed an increase of the concentrations of most volatile compounds with storage time, but in most cases this increase was also observed in control wine without lees. Bueno et al. (22) found a different behavior in Airen and Macabeo wines. Whereas in Airen wines most of the compounds increased their concentration, the opposite tendency was observed in Macabeo wines. The decrease in the volatile concentration could be due to the fact that yeast cell walls can adsorb volatile compounds (25, 26). This phenomenon is influenced by the degree of yeast autolysis, ethanol content, temperature, pH, and the presence of other wine constituents (27). This capacity of yeast lees to adsorb organic compounds has been investigated to reduce the content of some toxic compounds such as mycotoxins and pesticides (28, 29) and some off-flavor such as 4-ethylphenol and 4-ethylguaiaicol (27). Some oak volatile compounds can also be adsorbed by yeast lees during wine aging in barrels (30, 31).

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Up to now, only a few studies have been carried out to investigate the effect of aging on lees on grape flavor glycosides. Dubourdieu et al. (32) reported that  $\beta$ -glucosidases are excreted into the media as a result of yeast autolysis. Thus, an enhancement of the aroma concentration would be expected after wine aging on its lees by hydrolysis of flavor glycosides. However, flavor glycosides, as well as the released volatile compounds, can be adsorbed on the yeast cell wall (33). Zoecklein et al. (34, 35) found a reduction in the glycosyl glucose and potential volatile terpene content after storing wine on the yeast lees compared to newly fermented wine. However, the reduction, although lower, was also observed in wine without yeast lees. What is more, no statistic studies were carried out to prove the significant effect of the yeast lees. Furthermore, glycosyl glucose methods do not give information about the evolution of the individual compounds during the storage time and could be influenced by other glycosylated compounds in the wine.

The aims of this work are to study the evolution of the concentration of the volatile compounds from grape flavor precursors during storage on yeast lees and to evaluate the influence of the addition of grape flavor precursors before and after alcoholic fermentation on wine aroma composition.

## MATERIALS AND METHODS

**Reagents and Standards.** Dichloromethane and methanol (LiChrosolv quality) were purchased from Merck (Darmstadt, Germany); pentane was from Fluka (Buchs, Switzerland) and citric acid from Aldrich (Gillingham, U.K.). Ethyl acetate, absolute ethanol, sodium hydroxide, sodium fluoride, L-(+)-ascorbic acid, ammonium sulfate, sodium dihydrogenphosphate 1-hydrate, disodium hydrogenphosphate 12-hydrate, sodium bisulfite, and L-(+)-tartaric acid were supplied by Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). LiChrolut EN resins were purchased from Merck. The chemical standards (with purities between 90 and 99%) were supplied by Aldrich, Sigma (St. Louis, MO), Chemservice (West Chester, PA), Polyscience (Niles, IL), Firmenich (Geneva, Switzerland), Panreac, Merck, Fluka, and Lancaster (Strasbourg, France).

**Grapes.** Grapes from *Vitis vinifera* vars. Macabeo, Sauvignon Blanc, Merlot, and Parraleta cultivated in different regions of Spain in 2005 were harvested by hand and stored frozen at  $-30\text{ }^{\circ}\text{C}$  in the laboratory. Juice from Macabeo grapes was used for the laboratory fermentations.

**Preparation of the Precursor Extract.** Flavor precursors were extracted from four different nonfloral grape varieties (Macabeo, Sauvignon Blanc, Merlot, and Parraleta) following the procedure described by Loscos et al. (36). Grapes from each variety (1.5 kg grapes per variety) were destemmed and then blended in a mixer. Must and skins were separated by centrifugation, followed by filtration through filter paper. The mashes of skins obtained were suspended in buffer solution (0.1 M  $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ ) at pH 7 and 13% (v/v) ethanol and allowed to macerate in the dark (36 h,  $20\text{ }^{\circ}\text{C}$ , and nitrogen atmosphere). The flavor precursors from both macerate and must were extracted using LiChrolut EN resins. In both cases, after the resins had been washed consecutively with water and with a pentane/dichloromethane (2:1 v/v) mixture, the retained precursors were eluted with an ethyl acetate/methanol (9:1 v/v) mixture. These extracts were evaporated under vacuum to dryness and reconstituted in 20 mL of a 50% ethanol solution (from 900 mL of must or around 240 g of skins). Finally, the macerate and must extracts from the four varieties were mixed to form a multivarietal mixture 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 SAS (France), strain NT 116 (yeast strain 2) from Anchor Bio-Technologies (South Africa), and strain QA23 (yeast strain 3) from Lallemant (France). Yeast cultures were grown from 2.5 g of active dry yeast rehydrated in 30 mL of sterile water at  $35\text{ }^{\circ}\text{C}$  for 30 min.

Laboratory fermentations were carried out using 350 mL bottles filled with 300 mL of sterile grape juice from Macabeo variety and were capped with a synthetic cork pierced with a small (100  $\mu\text{L}$ ) micropipet tip containing

**Table 1.** Winemaking Variables of the Wines at the End of the Alcoholic Fermentation

	yeast strain 1		yeast strain 2		yeast strain 3	
	T <sup>a</sup>	A <sup>a</sup>	T	A	T	A
ethanol (% v/v)	13.3	13.8	13.3	13.8	14.0	15.1
residual sugars (g L <sup>-1</sup> )	2.6	3.7	2.9	4.0	2.4	2.1
pH	3.36	3.25	3.28	3.29	3.26	3.24
volatile acidity (g L <sup>-1</sup> of acetic)	0.34	0.36	0.45	0.43	0.48	0.45

<sup>a</sup>T, wines obtained with unsupplemented must; A, wines obtained with supplemented must.

a plug of cotton. Grape juice was sterilized by filtration (0.45  $\mu\text{m}$ , Schleicher & Schull, Postfch, Germany). For each yeast strain, three different wines were prepared in duplicate: wine obtained from the must unsupplemented with the flavor precursor extract (T), wine obtained from the must supplemented with the flavor precursor extract (A), and wine (obtained from the unsupplemented must) spiked with the flavor precursor extract after fermentation (TC). In each case, the precursor extract was added to reach 2 times the concentration of precursors in must (30 mL of the precursor mix per liter of must or wine). Samples were inoculated at  $20\text{ }^{\circ}\text{C}$  with 2 mL of the activated yeast solution. The fermentation process was monitored by weight. Bottles were stirred once a day during fermentation. All fermentations were completed after 25 days. Winemaking variables at the end of fermentation are shown in Table 1.

**Storage in Contact with Yeast Lees.** Immediately following the alcoholic fermentation, a concentration of 50 mg L<sup>-1</sup> sodium bisulfite was used to avoid the development of lactic acid bacteria. A control wine was prepared to study the contribution of slow acid hydrolysis at wine pH to the aroma release. This control consisted of a model wine [5 g L<sup>-1</sup> of tartaric acid, 13% (v/v) of ethanol, 5 g L<sup>-1</sup> of glycerol, and 50 mg L<sup>-1</sup> sodium bisulfite at pH 3.4] supplemented with the flavor precursor extract at the same concentration noted above. In all cases, headspace oxygen was replaced by nitrogen gas flushing, and the bottles were sealed with synthetic corks. All wines were kept in contact with their yeast lees for 9 months at  $20\text{ }^{\circ}\text{C}$ , with regular agitation of the yeast lees. The control wine was also kept for the same time in the same conditions. Minor aroma compounds were analyzed after alcoholic fermentation, after 3 and 9 months of storage following the procedure described below.

**Study of the Adsorption of the Volatile Compounds on Yeast Lees.** To study the possible adsorption of the volatile compounds on the yeast lees, a model wine supplemented with several aroma compounds and added with yeast lees was kept for 17 weeks at  $20\text{ }^{\circ}\text{C}$ , simulating the wine aging on lees. The model wine consisted of 12% (v/v) ethanol, 5 g L<sup>-1</sup> tartaric acid, 5 g L<sup>-1</sup> glycerol, and 50 mg L<sup>-1</sup> sodium bisulfite with pH adjusted to 3.4. Aroma compounds consisted of major aromas (20 mg L<sup>-1</sup> ethyl acetate, 100 mg L<sup>-1</sup> isoamyl alcohol, 20 mg L<sup>-1</sup>  $\beta$ -phenylethanol, 4 mg L<sup>-1</sup> hexanoic acid, 3 mg L<sup>-1</sup> octanoic acid, 1 mg L<sup>-1</sup> ethyl hexanoate, 1 mg L<sup>-1</sup> ethyl octanoate, and 0.2 mg L<sup>-1</sup> phenylethyl acetate) and minor aromas from the harsh acid hydrolysis of flavor precursors. To obtain these minor aromas, 60 mL of a flavor precursor extract obtained following the procedure described above was submitted to harsh acid hydrolysis (240 mL of a 0.2 M citric acid solution, pH 2.5, 1 h,  $100\text{ }^{\circ}\text{C}$ ). The released aroma compounds were extracted using LiChrolut EN resins [two cartridges of 500 mg, previously conditioned with 15 mL of dichloromethane, 15 mL of methanol, and 20 mL of a hydroalcoholic solution 10% (v/v)]. After the resins had been washed with 10 mL of water, volatile compounds were eluted with 10 mL of ethanol. Both extracts were mixed and added to the model wine (20 mL of extract per 1200 mL of model wine).

In this case, and with the purpose of having maximum control of the volatiles taken by the lees, these came from the fermentation of a model medium with Stellevin NT 116 yeast. The yeast lees were washed twice with a 0.9% NaCl solution, and they were finally separated by centrifugation (4500 rpm, 30 min,  $12\text{ }^{\circ}\text{C}$ ). The yeast lees were added without further treatment. The lees obtained in the fermentation of 2 L of synthetic must were added to 1 L of synthetic wine, which corresponded, approximately, to 37 g L<sup>-1</sup>, wet weight. Three different trials were prepared in triplicate: model wine supplemented with aroma compounds and yeast lees (WLA), model wine supplemented with aroma compounds without yeast lees (WA), and model wine supplemented with yeast lees without aromas

(WL). Wines were kept in 350 mL bottles filled with 200 mL of wine. After the headspace oxygen had been replaced by nitrogen gas flushing, the bottles were sealed with synthetic corks. Yeast lees were regularly stirred. Minor aroma compounds were analyzed initially, after 2, 6, and 17 weeks of storage following the procedure described below.

**Extraction and Analysis of Minor Volatile Compounds (SPE and GC–Ion Trap–MS Analysis).** Minor volatile compounds were extracted and determined following the method proposed and validated by Lopez et al. (35) with the modifications proposed by Loscos et al. (36). In accordance with this method, 15 mL of wine was passed through a 50 mg LiChrolut EN cartridge at about 2 mL min<sup>-1</sup>. 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.

**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<sup>-1</sup>. The oven temperature program was 3 min at 40 °C, 10 °C min<sup>-1</sup> to 90 °C, 2 °C min<sup>-1</sup> 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<sup>-1</sup>. The splitless time was 2.60 min. CarboFrit plugs (Restek, Bellefonte, PA) were used as packing material in the insert. The injection volume was 4 µL. The global run time was recorded in full scan mode (*m/z* 40–220 mass range). The chromatographic data were analyzed by Varian Saturn GC–MS version 6.3 software.

**Statistical Analysis.** Data were analyzed by three-way analysis of variance (ANOVA), storage time, yeast strain, and the addition of flavor precursors being the factors. Data from the study of the adsorption of the yeast lees were also analyzed by two-way analysis of variance, the time of storage and the different kinds of wine being the factors. The analyses were carried out using SPSS (SPSS Inc., Chicago, IL) for Windows, version 11.5. Principal component analysis (PCA) was carried out with Unscrambler 11.5 from Camo (Oslo, Norway).

## RESULTS AND DISCUSSION

In this work wines (supplemented or not with precursors) obtained by fermentation of a natural grape juice (also supplemented or not with precursors) were stored on their lees for 9 months and the concentration of the minor aroma compounds from grape flavor precursors were determined at the end of fermentation, after 3 and 9 months of storage. A total of 47 volatile compounds (Table 2), classified into 7 categories (terpenes, norisoprenoids, volatile phenols, vanillin derivatives, benzenes, lactones, and miscellaneous) were determined. Results of the analysis are shown in Tables 3 (yeast strain 1), 4 (yeast strain 2), and 5 (yeast strain 3). Data were studied by three-way ANOVA, the factors being the storage time, the addition of flavor precursors, and the yeast strain. Results (data not shown) indicate that all factors exert a significant effect in nearly all cases, except in the cases of β-ionone and 4-allyl-2,6-dimethoxyphenol (addition factor not significant); 4-ethylguaiacol, methyl vanillate, and 1,2-dimethoxy-4-propylbenzene (yeast strain factor not significant); phenylacetaldehyde, 2-phenoxyethanol, and (*E*)-2-hexen-1-ol (addition and yeast strain factors not significant); and eugenol (none of the factors significant). Such a high level of significance is the consequence of the high reproducibility of the assays. For dealing with such a complex set of data, the discussion will focus on the factors with highest weight in the data set. A first idea of

**Table 2.** Retention Indices and Chemical Standards Used for Identification and Quantitation of Volatile Compounds

	RI <sup>b</sup>	source, purity	
Terpenes			
1	1478	tentatively identified	nerol oxide
2	1556	Fluka, 98.5%	linalool
3	1613	tentatively identified	2,6-dimethyl-1,7-octadiene-2,6-diol
4	1705	Fluka, 97%	α-terpineol
5	1775	Fluka, 90–95%	β-citronellol
6	1811	Fluka, 90–95%	nerol
7	2366	tentatively identified	neric acid
Norisoprenoids			
8	1526	tentatively identified	vitispirane A <sup>a</sup>
9	1529	tentatively identified	vitispirane B <sup>a</sup>
10	1637	tentatively identified	Riesling acetal <sup>a</sup>
11	1748	tentatively identified	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)
12	1832	tentatively identified	tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB)
13	1829	Firmenich, 90%	β-damascenone
14	1939	tentatively identified	3-oxo-β-ionone
15	1950	Sigma, 98%	β-ionone
16	1952	tentatively identified	actinidols <sup>a</sup>
Volatile Phenols			
17	1876	Aldrich, 98%	guaiacol
18	2068	Lancaster, 98%	4-ethylguaiacol
19	2237	Aldrich, 99%	eugenol
20	2244	Aldrich, 99%	4-ethylphenol
21	2262	Aldrich, 98%	4-vinylguaiacol
22	2404	Lancaster, 10% solution	4-vinylphenol
23	2563	Aldrich 90%	4-allyl-2,6-dimethoxyphenol
Vanillin Derivatives			
24	2592	Panreac, 99%	vanillin
25	2629	Aldrich, 99%	methyl vanillate
26	2654	Lancaster, 97%	ethyl vanillate
27	2664	Aldrich, 98%	acetovanillone
28	2829	Aldrich, 96%	zingeronol
29	2892	Aldrich, 99%	homovanillyl alcohol
30	3040	Aldrich, 98%	syringaldehyde
31	3099	tentatively identified	homovanillic acid
32	3123	Aldrich, 97%	acetosyringone
Benzenes			
33	1520	Fluka, 99%	benzaldehyde
34	1659	Aldrich, 90%	phenylacetaldehyde
35	1908	Aldrich, 99%	ethyl dihydrocinnamate
36	2081	Aldrich, 99%	ethyl cinnamate
37	2219	Fluka, 98%	2-phenoxyethanol
38	2725	tentatively identified	1,2-dimethoxy-4-propylbenzene
Lactones			
39	1988	Lancaster, 98%	δ-octalactone
40	2068	Aldrich, 97%	γ-nonalactone
41	2154	Aldrich, 98%	δ-nonalactone
42	2141	Aldrich, 98%	γ-decalactone
43	2260	Lancaster, 98%	δ-decalactone
Miscellaneous			
44	1390	Aldrich, 98%	( <i>Z</i> )-3-hexen-1-ol
45	1413	Aldrich, 98%	( <i>E</i> )-2-hexen-1-ol
46	1672	Lancaster, 98%	3-methylbutyric acid
47	1677	Aldrich, 98%	2-methylbutyric acid

<sup>a</sup> Actinidols, 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; 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. <sup>b</sup> Retention index calculated in a DB-WAXetr column.

**Table 3.** Evolution of the Concentration (in Micrograms per Liter, Except Where Indicated<sup>a</sup>) of Volatile Compounds during the Storage of the Wines Obtained with Yeast Strain 1 on Their Lees (Data Are the Average of Two Replicate Samples)

		T <sub>EF</sub>	A <sub>EF</sub>	T <sub>3m</sub>	TC <sub>3m</sub>	A <sub>3m</sub>	T <sub>9m</sub>	TC <sub>9m</sub>	A <sub>9m</sub>
Terpenes									
1	nerol oxide <sup>a</sup>	1.2 ± 0.2h	0.88 ± 0.21fg	1.0 ± 0.1gh	0.71 ± 0.07f	0.69 ± 0.06fg	0.68 ± 0.03f	0.73 ± 0.21fg	0.57 ± 0.07f
2	linalool	2.4 ± 0.4f	3.6 ± 0.1gh	3.0 ± 0.3fg	4.0 ± 0.2gh	4.2 ± 0.1h	4.3 ± 0.3h	4.4 ± 1.0h	4.6 ± 0.3h
3	2,6-dimethyl-1,7-octadiene-3,6-diol <sup>a</sup>	1.9 ± 0.1f	2.2 ± 0.9f	2.0 ± 0.2f	1.8 ± 0.1f	2.2 ± 0.1f	1.7 ± 0.2f	1.7 ± 0.1f	1.7 ± 0.2f
4	α-terpineol	1.2 ± 0.2f	1.4 ± 0.2fg	1.2 ± 0.2f	1.7 ± 0.1g	1.8 ± 0.1g	2.5 ± 0.2h	3.4 ± 0.3i	3.3 ± 0.1i
5	β-citronellol	8.3 ± 0.6h	4.1 ± 0.3fg	7.1 ± 0.4h	4.5 ± 1.1g	3.6 ± 0.8fg	4.4 ± 0.7g	2.8 ± 0.2f	2.9 ± 0.3f
6	nerol	3.5 ± 0.2h	3.2 ± 0.1h	4.9 ± 0.7i	1.3 ± 0.3g	1.7 ± 0.1g	0.44 ± 0.11f	nd f	nd f
7	neric acid <sup>a</sup>	3.8 ± 0.4f	4.6 ± 1.5fg	6.2 ± 1.3gh	8.7 ± 0.3i	9.1 ± 0.9i	3.3 ± 0.8f	6.6 ± 0.7h	gh6.1 ± 0.3
Norisoprenoids									
8	vitispirane A <sup>a</sup>	0.99 ± 0.06f	1.2 ± 0.3fg	2.0 ± 0.1hi	1.9 ± 0.3hi	2.6 ± 0.3jk	1.6 ± 0.1gh	2.9 ± 0.2k	2.2 ± 0.3ij
9	vitispirane B <sup>a</sup>	0.80 ± 0.03f	1.2 ± 0.3fg	1.4 ± 0.3gh	1.6 ± 0.1hi	2.4 ± 0.2j	1.6 ± 0.1gi	2.3 ± 0.1j	1.9 ± 0.2i
10	Riesling acetal <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	5.7 ± 0.2g	9.3 ± 0.3i	7.9 ± 0.5h
11	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) <sup>a</sup>	0.59 ± 0.01f	0.57 ± 0.1f	0.65 ± 0.03fg	0.65 ± 0.08fg	0.70 ± 0.04fg	0.88 ± 0.14g	1.5 ± 0.2h	1.4 ± 0.2h
12	tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	nd f	0.22 ± 0.02h	0.14 ± 0.01g
13	β-damascenone	3.7 ± 0.2g	4.5 ± 0.9h	3.1 ± 0.2g	3.4 ± 0.2g	3.1 ± 0.3g	1.7 ± 0.1f	2.0 ± 0.1f	1.7 ± 0.4f
14	3-oxo-β-ionone <sup>a</sup>	0.48 ± 0.15f	1.4 ± 0.1gh	0.91 ± 0.10fg	2.0 ± 0.2i	3.1 ± 0.5j	1.7 ± 0.1hi	6.8 ± 0.1k	6.6 ± 0.3k
15	β-ionone	0.28 ± 0.02g	0.33 ± 0.13gh	<DL f	0.25 ± 0.04g	0.28 ± 0.03g	0.50 ± 0.07h	0.37 ± 0.16gh	0.31 ± 0.07gh
16	actinidols <sup>a</sup>	0.70 ± 0.01g	2.4 ± 0.1g	1.3 ± 0.1f	3.2 ± 0.4h	4.5 ± 0.1i	3.5 ± 0.2h	12 ± 0.4j	11 ± 0.4j
Volatile Phenols									
17	guaiacol	2.5 ± 0.1h	1.9 ± 0.3g	1.5 ± 0.1f	1.3 ± 0.1f	1.3 ± 0.1f	f1.5 ± 0.1	1.3 ± 0.1f	1.4 ± 0.1f
18	4-ethylguaiacol	0.30 ± 0.02g	0.21 ± 0.06fg	0.23 ± 0.01g	0.13 ± 0.02f	0.11 ± 0.02f	0.57 ± 0.04h	0.50 ± 0.04h	0.55 ± 0.10h
19	eugenol	1.8 ± 0.1g	1.3 ± 0.6fg	1.8 ± 0.1g	1.4 ± 0.2fg	1.4 ± 0.1fg	1.5 ± 0.1f	1.3 ± 0.2f	1.3 ± 0.1fg
20	4-ethylphenol	0.60 ± 0.08i	0.09 ± 0.01fg	0.25 ± 0.13h	<DL f	<DL f	0.20 ± 0.01gh	0.15 ± 0.05gh	0.10 ± 0.03fg
21	4-vinylguaiacol	658 ± 64j	341 ± 41i	286 ± 48hi	57 ± 1f	82 ± 2fg	148 ± 17g	130 ± 19g	144 ± 13g
22	4-vinylphenol	2757 ± 41j	679 ± 117h	1249 ± 52i	260 ± 23f	296 ± 1fg	399 ± 36g	253 ± 58f	207 ± 3f
23	4-allyl-2,6-dimethoxyphenol	4.0 ± 0.1gh	3.1 ± 0.7f	4.6 ± 0.4h	3.4 ± 0.1fg	4.0 ± 0.6fgh	3.5 ± 0.4fg	4.6 ± 0.3h	4.6 ± 0.5h
Vanillin Derivatives									
24	vanillin	14 ± 2h	7.8 ± 0.5f	10 ± 0.7g	7.8 ± 0.2f	7.7 ± 0.2f	12 ± 1h	9.5 ± 0.3g	8.9 ± 0.4fg
25	methyl vanillate	4.3 ± 0.2f	24 ± 3j	3.8 ± 0.1f	8.6 ± 0.2g	19 ± 1i	3.2 ± 0.3f	11 ± 0.4h	20 ± 0.2i
26	ethyl vanillate	16 ± 1f	48 ± 5h	18 ± 1f	46 ± 4h	66 ± 4i	34 ± 3g	99 ± 3j	135 ± 1k
27	acetovanillone	99 ± 2j	117 ± 11k	57 ± 1fg	54 ± 2f	78 ± 4i	63 ± 3gh	66 ± 2h	91 ± 3j
28	zingerone	57 ± 4j	48 ± 8i	34 ± 2g	26 ± 1f	37 ± 3gh	34 ± 4g	32 ± 1g	42 ± 1hi
29	homovanillyl alcohol	nd f	8.2 ± 1.5hi	3.8 ± 0.9g	5.5 ± 0.3gh	4.5 ± 0.4g	5.6 ± 0.9gh	9.1 ± 2.8i	11 ± 1i
30	syringaldehyde	nd f	9.3 ± 1.5g	nd f	9.5 ± 0.2g	8.3 ± 0.1g	9.3 ± 0.5g	13 ± 1h	13 ± 1h
31	homovanillic acid <sup>a</sup>	126 ± 16h	127 ± 13h	104 ± 2g	108 ± 2g	125 ± 3h	56 ± 10f	69 ± 3f	69 ± 3f
32	acetosyringone	20 ± 1h	34 ± 5j	13 ± 1f	15 ± 1fg	26 ± 3i	12 ± 1f	17 ± 1gh	30 ± 1ij
Benzenes									
33	benzaldehyde	11 ± 0.1i	6.5 ± 0.8h	11 ± 0.5i	4.6 ± 0.3g	4.7 ± 0.2g	13 ± 1j	2.8 ± 0.3f	2.3 ± 0.2f
34	phenylacetaldehyde	2.6 ± 0.5fg	5.0 ± 1.7h	5.1 ± 0.6h	2.4 ± 0.3f	3.2 ± 0.8fg	4.0 ± 0.3gh	3.3 ± 0.2fg	3.0 ± 0.2fg
35	ethyl dihydrocinnamate	0.20 ± 0.04g	0.20 ± 0.03g	0.16 ± 0.03fg	0.15 ± 0.01fg	0.11 ± 0.01f	0.15 ± 0.02fg	0.12 ± 0.02f	0.11 ± 0.03f
36	ethyl cinnamate	0.52 ± 0.03f	0.96 ± 0.05g	0.58 ± 0.02f	1.2 ± 0.1h	1.1 ± 0.2gh	0.68 ± 0.04f	1.0 ± 0.1g	0.94 ± 0.10g
37	2-phenoxyethanol	4.4 ± 0.8g	4.1 ± 1.0g	2.8 ± 0.6f	3.2 ± 0.5fg	2.8 ± 0.3f	2.8 ± 0.1f	2.4 ± 0.4f	2.8 ± 0.2f
38	1,2-dimethoxy-4-propylbenzene <sup>a</sup>	20 ± 0.1h	30 ± 3j	10 ± 1f	15 ± 2g	25 ± 1i	12 ± 1f	19 ± 1h	30 ± 1j
Lactones									
39	δ-octalactone	3.2 ± 0.2h	3.0 ± 0.1h	1.8 ± 0.1fg	2.1 ± 0.2g	1.5 ± 0.1f	1.5 ± 0.1f	1.5 ± 0.3f	1.6 ± 0.2f
40	γ-nonalactone	9.6 ± 0.5j	5.8 ± 0.2h	6.7 ± 0.5i	3.8 ± 0.1f	4.4 ± 0.2g	6.5 ± 0.5hi	4.0 ± 0.2fg	4.5 ± 0.1g
41	δ-nonalactone	1.2 ± 0.1i	0.70 ± 0.12gh	0.46 ± 0.06fg	0.29 ± 0.07f	0.34 ± 0.01fg	0.96 ± 0.04hi	0.72 ± 0.01fg	0.51 ± 0.06fh
42	γ-decalactone	10 ± 1i	4.1 ± 0.7g	7.0 ± 0.7h	2.8 ± 0.1f	3.5 ± 0.2fg	6.3 ± 0.8h	2.8 ± 0.1f	3.1 ± 0.1fg
43	δ-decalactone	60 ± 6h	59 ± 1h	30 ± 2f	34 ± 1fg	31 ± 2f	33 ± 2fg	38 ± 3g	32 ± 2f
Miscellaneous									
44	(Z)-3-hexen-1-ol	143 ± 4i	127 ± 5h	65 ± 7g	66 ± 2g	69 ± 2g	50 ± 1f	53 ± 7f	60 ± 5fg
45	(E)-2-hexen-1-ol	8.9 ± 3.4j	9.7 ± 5.2g	nd f	nd f	nd f	0.46 ± 0.20f	1.0 ± 0.1f	1.2 ± 0.2f
46	3-methylbutyric acid	58 ± 5i	29 ± 1h	24 ± 1gh	16 ± 2f	18 ± 4fg	20 ± 3fg	14 ± 5f	16 ± 2fg
47	2-methylbutyric acid	43 ± 4i	19 ± 1h	16 ± 1gh	11 ± 2f	11 ± 3fg	14 ± 3fgh	10 ± 4f	10 ± 1f

<sup>a</sup>Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). Different letters indicate significant differences (significant level 95%). <sup>b</sup>T, wines obtained with unsupplemented must; TC, wines supplemented after fermentation; A, wines obtained with supplemented must. <sup>c</sup>EF, end of fermentation; 3m, 3 months of storage on lees; 9m, 9 months of storage on lees. <sup>d</sup>nd, not detected. <sup>e</sup><DL, under the detection limit.

**Table 4.** Evolution of the Concentration (in Micrograms per Liter, Except Where Indicated<sup>a</sup>) of Volatile Compounds during the Storage of the Wines Obtained with Yeast Strain 2 on Their Lees (Data Are the Average of the Two Replicate Samples)

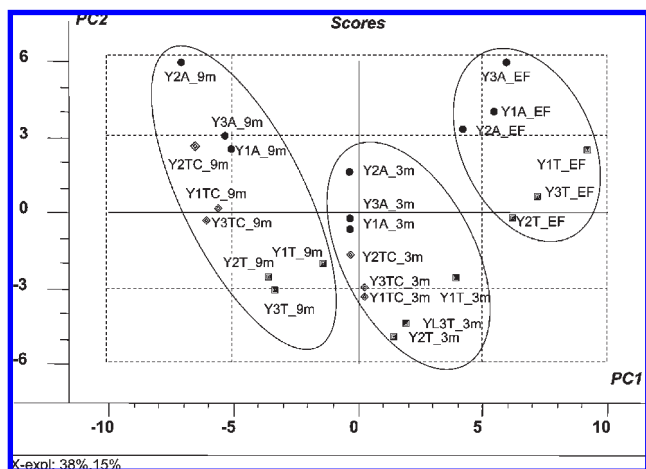
		T <sub>EF</sub>	A <sub>EF</sub>	T <sub>3m</sub>	TC <sub>3m</sub>	A <sub>3m</sub>	T <sub>9m</sub>	TC <sub>9m</sub>	A <sub>9m</sub>
Terpenes									
1	nerol oxide <sup>a</sup>	0.74 ± 0.04i	0.66 ± 0.09gi	0.51 ± 0.18fgh	0.41 ± 0.02f	0.47 ± 0.07fg	0.57 ± 0.04fi	0.61 ± 0.12gi	0.68 ± 0.04hi
2	linalool	3.4 ± 0.1f	5.0 ± 0.1hi	3.3 ± 0.3f	4.2 ± 0.5g	5.4 ± 0.2i	4.5 ± 0.2gh	5.4 ± 0.1i	6.1 ± 0.2j
3	2,6-dimethyl-1,7-octadiene-3,6-diol <sup>a</sup>	2.0 ± 0.1gh	2.4 ± 0.2ij	2.1 ± 0.1hi	2.6 ± 0.1jk	2.7 ± 0.1k	1.3 ± 0.1f	1.8 ± 0.1g	1.9 ± 0.1gh
4	α-terpineol	1.3 ± 0.3f	1.5 ± 0.2fg	1.3 ± 0.1f	1.8 ± 0.1gh	2.1 ± 0.1h	2.8 ± 0.2i	3.9 ± 0.2j	4.2 ± 0.2j
5	β-citronellol	5.9 ± 0.1gh	6.6 ± 0.2h	5.3 ± 0.6g	5.3 ± 0.6g	5.9 ± 0.2gh	4.2 ± 0.1f	4.1 ± 0.2f	4.2 ± 0.2f
6	nerol	1.9 ± 0.2h	2.7 ± 0.2i	1.2 ± 0.2g	2.0 ± 0.2h	1.2 ± 0.2g	0.35 ± 0.13f	1.1 ± 0.1g	1.1 ± 0.3g
7	neric acid <sup>a</sup>	2.1 ± 0.1f	3.5 ± 0.8g	2.8 ± 0.3fg	8.4 ± 0.2ij	8.8 ± 0.5j	3.4 ± 0.2g	7.8 ± 0.3hi	7.2 ± 0.1h
Norisoprenoids									
8	vitispirane A <sup>a</sup>	1.0 ± 0.03fg	0.81 ± 0.40f	1.7 ± 0.1gh	2.8 ± 0.7ij	2.7 ± 0.2i	2.3 ± 0.1hi	3.4 ± 0.1j	3.5 ± 0.1j
9	vitispirane B <sup>a</sup>	0.83 ± 0.06f	0.83 ± 0.27f	1.3 ± 0.1fg	2.3 ± 0.6h	2.2 ± 0.2h	1.8 ± 0.1gh	2.8 ± 0.1i	3.0 ± 0.2i
10	Riesling acetal <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	6.7 ± 0.1g	9.8 ± 0.4i	9.4 ± 0.2h
11	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) <sup>a</sup>	0.58 ± 0.13fg	0.46 ± 0.23f	0.70 ± 0.07fh	0.80 ± 0.11gh	0.90 ± 0.09h	1.3 ± 0.1i	1.7 ± 0.2j	1.6 ± 0.2ij
12	tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	nd f	0.23 ± 0.05g	0.25 ± 0.09g
13	β-damascenone	1.8 ± 0.2g	2.6 ± 0.2i	2.0 ± 0.4gh	3.2 ± 0.2j	2.4 ± 0.2hi	1.3 ± 0.1f	2.1 ± 0.1gh	1.9 ± 0.1g
14	3-oxo-β-ionone <sup>a</sup>	0.43 ± 0.05f	1.5 ± 0.1h	0.90 ± 0.10g	2.1 ± 0.2i	2.9 ± 0.2j	2.3 ± 0.1i	7.0 ± 0.3k	7.6 ± 0.2l
15	β-ionone	0.26 ± 0.05f	0.41 ± 0.13g	0.23 ± 0.08f	0.32 ± 0.06fg	0.25 ± 0.03f	0.59 ± 0.04h	0.63 ± 0.05h	0.66 ± 0.03h
16	actinidols <sup>a</sup>	0.88 ± 0.01f	2.5 ± 0.3gh	1.6 ± 0.2fg	3.6 ± 0.3hi	5.2 ± 0.1j	4.1 ± 0.6ij	12 ± 1k	14 ± 0.5k
Volatile Phenols									
17	guaiacol	2.0 ± 0.2h	2.0 ± 0.2h	1.4 ± 0.1fg	1.3 ± 0.1f	1.5 ± 0.1fg	1.5 ± 0.1fg	1.5 ± 0.1fg	1.6 ± 0.1g
18	4-ethylguaiacol	0.21 ± 0.05f	0.17 ± 0.01f	0.16 ± 0.04f	0.22 ± 0.03f	0.26 ± 0.12f	0.58 ± 0.03g	0.51 ± 0.07g	0.50 ± 0.08g
19	eugenol	1.6 ± 0.3f	1.4 ± 0.7f	1.4 ± 0.2f	1.4 ± 0.1f	1.6 ± 0.3f	1.6 ± 0.1f	1.8 ± 0.1g	1.9 ± 0.1f
20	4-ethylphenol	0.17 ± 0.03g	0.19 ± 0.07h	<DL f	0.16 ± 0.05g	0.04 ± 0.03f	0.24 ± 0.02g	0.21 ± 0.04g	0.23 ± 0.02g
21	4-vinylguaiacol	221 ± 26h	200 ± 55h	62 ± 4f	50 ± 5f	81 ± 3f	149 ± 7g	131 ± 21g	200 ± 7h
22	4-vinylphenol	186 ± 31gh	202 ± 58h	74 ± 7f	89 ± 11f	109 ± 3fg	252 ± 16hi	327 ± 72i	330 ± 8i
23	4-allyl-2,6-dimethoxyphenol	3.8 ± 0.3g	2.5 ± 0.6f	4.4 ± 0.3gh	4.2 ± 0.2gh	4.8 ± 0.2hi	4.4 ± 0.1gh	5.5 ± 0.2ij	6.1 ± 0.6j
Vanillin Derivatives									
24	vanillin	8.8 ± 2.0fg	7.7 ± 1.0fg	7.5 ± 0.1fg	9.9 ± 1.6g	8.9 ± 0.8fg	7.5 ± 0.1f	9.4 ± 1.0fg	9.8 ± 0.7fg
25	methyl vanillate	3.8 ± 0.6f	20 ± 8h	3.2 ± 0.4f	10 ± 1g	20 ± 1h	3.4 ± 0.1f	12 ± 1g	20 ± 1h
26	ethyl vanillate	13 ± 0.1f	40 ± 13hi	18 ± 1fg	50 ± 3i	68 ± 5j	33 ± 1gh	119 ± 16k	150 ± 3l
27	acetovanillone	71 ± 0.2h	95 ± 14j	47 ± 2f	61 ± 5g	86 ± 5ij	52 ± 1fg	76 ± 3hi	92 ± 1j
28	zingerone	28 ± 1f	44 ± 18g	19 ± 2f	25 ± 3f	46 ± 1g	18 ± 3f	29 ± 2f	44 ± 7g
29	homovanillyl alcohol	7.6 ± 0.5fg	11 ± 4hi	5.8 ± 0.2fg	6.5 ± 0.4fg	4.5 ± 0.4f	6.9 ± 0.8fg	8.4 ± 1.0gh	13 ± 2i
30	syringaldehyde	8.8 ± 0.5fg	8.1 ± 1.0f	6.5 ± 0.1f	11 ± 1gh	8.9 ± 2fg	7.5 ± 0.4f	13 ± 2i	12 ± 2hi
31	homovanillic acid <sup>a</sup>	82 ± 6g	73 ± 36g	83 ± 5g	122 ± 12h	146 ± 12i	53 ± 3f	73 ± 5fg	84 ± 1g
32	acetosyringone	16 ± 0.4fh	36 ± 8i	11 ± 0.3f	19 ± 2gh	32 ± 2i	11 ± 1f	19 ± 1h	32 ± 2i
Benzenes									
33	benzaldehyde	6.9 ± 0.1h	5.8 ± 0.7h	2.7 ± 0.3f	3.5 ± 0.7fg	3.7 ± 0.8fg	3.1 ± 0.4fg	4.2 ± 1.1g	8.8 ± 0.2i
34	phenylacetaldehyde	4.9 ± 1.1h	2.6 ± 0.7fg	2.0 ± 0.2f	3.6 ± 1.2gh	2.1 ± 0.5f	2.6 ± 0.3fg	3.4 ± 0.4fg	3.1 ± 0.1fg
35	ethyl dihydrocinnamate	0.26 ± 0.01gh	0.36 ± 0.01i	0.14 ± 0.04f	0.21 ± 0.01g	0.29 ± 0.05h	0.25 ± 0.03gh	0.24 ± 0.03gh	0.37 ± 0.02i
36	ethyl cinnamate	0.72 ± 0.03f	1.1 ± 0.2g	0.59 ± 0.11f	1.5 ± 0.1hi	1.4 ± 0.1h	0.63 ± 0.02f	1.7 ± 0.1j	1.7 ± 0.1ij
37	2-phenoxyethanol	4.0 ± 0.4g	4.5 ± 1.1g	2.9 ± 0.2f	3.1 ± 0.2f	2.7 ± 0.2f	2.8 ± 0.2f	3.0 ± 0.1f	2.8 ± 0.2f
38	1,2-dimethoxy-4-propylbenzene <sup>a</sup>	17 ± 0.1fg	28 ± 12h	12 ± 1f	20 ± 2fg	30 ± 1h	15 ± 1fg	23 ± 1gh	30 ± 1h
Lactones									
39	δ-octalactone	2.6 ± 0.2i	1.2 ± 0.4gh	1.5 ± 0.2h	1.4 ± 0.2h	0.85 ± 0.2fg	1.2 ± 0.1gh	1.0 ± 0.1fg	0.73 ± 0.08f
40	γ-nonalactone	2.5 ± 0.2gh	2.8 ± 0.7h	1.8 ± 0.2f	2.3 ± 0.2fg	2.6 ± 0.1h	1.9 ± 0.1fg	2.5 ± 0.1h	2.8 ± 0.1h
41	δ-nonalactone	nd f	nd f	nd f	nd f	nd f	nd f	nd f	nd f
42	γ-decalactone	1.7 ± 0.1fg	1.8 ± 0.5fg	2.2 ± 0.5gh	2.5 ± 0.3h	2.9 ± 0.5h	1.2 ± 0.1f	1.4 ± 0.2f	1.8 ± 0.1fg
43	δ-decalactone	42 ± 0.3i	30 ± 3h	25 ± 1fg	25 ± 1fg	20 ± 1f	29 ± 2gh	26 ± 2gh	24 ± 6fg
Miscellaneous									
44	(Z)-3-hexen-1-ol	200 ± 18j	167 ± 14i	98 ± 3h	75 ± 9g	81 ± 1gh	68 ± 1fg	64 ± 5fg	57 ± 6f
45	(E)-2-hexen-1-ol	10 ± 0.2i	8.6 ± 0.5h	nd f	nd f	nd f	0.57 ± 0.12g	0.64 ± 0.09g	0.58 ± 0.02g
46	3-methylbutyric acid	65 ± 5i	50 ± 9h	33 ± 5g	26 ± 5fg	21 ± 3f	22 ± 3f	19 ± 2f	17 ± 3f
47	2-methylbutyric acid	64 ± 8i	49 ± 11h	31 ± 5g	25 ± 4fg	20 ± 3fg	20 ± 3fg	18 ± 2f	15 ± 4f

<sup>a</sup>Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). Different letters indicate significant differences (significant level 95%). <sup>b</sup>T, wines obtained with unsupplemented must; TC, wines supplemented after fermentation; A, wines obtained with supplemented must. <sup>c</sup>EF, end of fermentation; 3m, 3 months of storage on lees; 9m, 9 months of storage on lees. <sup>d</sup>nd, not detected. <sup>e</sup><DL, under the detection limit.

**Table 5.** Evolution of the Concentration (in Micrograms per Liter, Except Where Indicated<sup>a</sup>) of Volatile Compounds during the Storage of the Wines Obtained with Yeast Strain 3 on Their Lees (Data Are the Average of the Two Replicate Samples)

		T <sub>EF</sub>	A <sub>EF</sub>	T <sub>3m</sub>	TC <sub>3m</sub>	A <sub>3m</sub>	T <sub>9m</sub>	TC <sub>9m</sub>	A <sub>9m</sub>
Terpenes									
1	nerol oxide <sup>a</sup>	0.86 ± 0.06i	0.78 ± 0.06i	0.82 ± 0.01i	0.53 ± 0.06g	0.43 ± 0.01f	0.63 ± 0.05h	0.56 ± 0.03gh	0.55 ± 0.04gh
2	linalool	2.9 ± 0.1f	3.8 ± 0.3gh	3.1 ± 0.5fg	3.7 ± 0.1gh	4.2 ± 0.2h	3.0 ± 0.7f	3.9 ± 0.1h	3.8 ± 0.2gh
3	2,6-dimethyl-1,7-octadiene-3,6-diol <sup>a</sup>	2.2 ± 0.2g	2.9 ± 0.5h	1.2 ± 0.4f	1.2 ± 0.1f	1.5 ± 0.1f	1.4 ± 0.2f	1.5 ± 0.1f	1.6 ± 0.1f
4	α-terpineol	1.2 ± 0.1f	1.5 ± 0.2fg	1.4 ± 0.1fg	1.7 ± 0.1g	2.1 ± 0.3h	2.6 ± 0.1i	3.4 ± 0.2j	3.5 ± 0.1j
5	β-citronellol	5.6 ± 0.8g	8.3 ± 0.2h	4.7 ± 1.8g	4.8 ± 0.1g	5.2 ± 1.1g	2.5 ± 0.3f	2.4 ± 0.2f	2.6 ± 0.8f
6	nerol	3.4 ± 0.1j	4.0 ± 0.6j	1.4 ± 0.2gh	1.8 ± 0.3hi	2.2 ± 0.2i	0.19 ± 0.07f	nd f	1.2 ± 0.2g
7	neric acid <sup>a</sup>	1.6 ± 0.4f	4.1 ± 0.2g	3.4 ± 1.0g	8.3 ± 0.8i	9.0 ± 0.4i	3.7 ± 0.1g	7.8 ± 0.6hi	7.0 ± 0.6h
Norisoprenoids									
8	vitispirane A <sup>a</sup>	1.1 ± 0.1f	0.95 ± 0.07f	1.9 ± 0.4gh	1.5 ± 0.1fg	1.9 ± 0.1gh	2.3 ± 0.3h	3.5 ± 0.6i	2.5 ± 0.1h
9	vitispirane B <sup>a</sup>	0.93 ± 0.03fg	0.80 ± 0.07f	1.1 ± 0.4fg	1.2 ± 0.1fg	1.5 ± 0.1gh	2.0 ± 0.4h	2.9 ± 0.4i	2.0 ± 0.2h
10	Riesling acetal <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	8.4 ± 0.3g	10 ± 1h	10 ± 0.2h
11	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) <sup>a</sup>	0.68 ± 0.06f	0.65 ± 0.03f	0.86 ± 0.17f	0.82 ± 0.10f	0.78 ± 0.17f	1.6 ± 0.1g	1.9 ± 0.3g	1.6 ± 0.1g
12	tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) <sup>a</sup>	nd f	nd f	nd f	nd f	nd f	0.13 ± 0.03g	0.26 ± 0.04h	0.24 ± 0.04h
13	β-damascenone	4.8 ± 0.2i	6.2 ± 0.2j	3.5 ± 0.4h	4.9 ± 0.2i	4.0 ± 0.2h	2.0 ± 0.3f	3.0 ± 0.1fg	2.2 ± 0.1f
14	3-oxo-β-ionone <sup>a</sup>	0.50 ± 0.11f	1.5 ± 0.2h	0.93 ± 0.19g	1.9 ± 0.2i	3.0 ± 0.2j	2.8 ± 0.1j	7.0 ± 0.2k	7.8 ± 0.2l
15	β-ionone	0.46 ± 0.16gh	0.51 ± 0.06h	0.26 ± 0.06f	0.34 ± 0.05fg	0.24 ± 0.04f	0.38 ± 0.15fh	0.30 ± 0.04fg	0.35 ± 0.05fh
16	actinidols <sup>a</sup>	0.86 ± 0.08f	2.8 ± 0.3gh	1.6 ± 0.4fg	3.4 ± 0.3hi	5.2 ± 0.7j	4.6 ± 0.5ij	13 ± 1k	15 ± 2l
Volatile Phenols									
17	guaiacol	1.9 ± 0.1h	2.0 ± 0.1h	1.2 ± 0.2fg	1.1 ± 0.1f	1.4 ± 0.1g	1.3 ± 0.1fg	1.3 ± 0.1g	1.4 ± 0.2g
18	4-ethylguaiacol	0.29 ± 0.01g	0.26 ± 0.08g	0.16 ± 0.04f	0.14 ± 0.03f	0.20 ± 0.05fg	0.54 ± 0.04i	0.44 ± 0.04h	0.50 ± 0.03hi
19	eugenol	1.6 ± 0.1fg	2.0 ± 0.1h	1.5 ± 0.2fg	1.3 ± 0.3f	1.3 ± 0.1f	1.5 ± 0.1fg	1.8 ± 0.2gh	1.8 ± 0.1gh
20	4-ethylphenol	0.56 ± 0.17h	0.23 ± 0.02g	0.54 ± 0.14h	0.06 ± 0.01f	0.07 ± 0.01fg	0.43 ± 0.05h	0.17 ± 0.03fg	0.17 ± 0.09fg
21	4-vinylguaiacol	728 ± 174i	307 ± 43gh	326 ± 166h	58 ± 2f	86 ± 9f	147 ± 38fg	98 ± 13f	110 ± 18f
22	4-vinylphenol	3620 ± 867i	1180 ± 171gh	1594 ± 755h	224 ± 11f	259 ± 74f	553 ± 158fg	353 ± 113f	223 ± 36f
23	4-allyl-2,6-dimethoxyphenol	3.6 ± 0.6fg	3.2 ± 0.3f	4.0 ± 0.3fh	3.5 ± 0.2fg	4.2 ± 0.2gh	4.7 ± 0.3hi	5.1 ± 0.3i	5.5 ± 0.8i
Vanillin Derivatives									
24	vanillin	11 ± 0.5gh	9.9 ± 0.2fg	8.4 ± 0.8f	11 ± 1g	9.1 ± 0.1fg	9.9 ± 3.0fg	13 ± 1h	11 ± 0.4fgh
25	methyl vanillate	3.9 ± 0.3f	26 ± 1k	3.3 ± 0.1f	8.4 ± 0.4g	19 ± 1i	3.4 ± 0.1f	11 ± 0.2h	21 ± 1j
26	ethyl vanillate	12 ± 1f	50 ± 4h	18 ± 1f	46 ± 1h	65 ± 3i	37 ± 8g	91 ± 2j	130 ± 3k
27	acetovanillone	78 ± 4i	123 ± 1k	50 ± 2f	53 ± 2fg	77 ± 2i	57 ± 5g	65 ± 3h	91 ± 4j
28	zingerone	43 ± 2gh	64 ± 2i	31 ± 2f	32 ± 1f	45 ± 2h	32 ± 4f	36 ± 4fg	48 ± 7h
29	homovanillyl alcohol	5.6 ± 0.7f	9.4 ± 2.0ij	5.3 ± 0.1f	7.6 ± 0.3gi	5.6 ± 0.4fg	6.4 ± 0.3fgh	7.9 ± 0.6hi	10 ± 2j
30	syringaldehyde	9.9 ± 1.6fg	13 ± 1hi	8.0 ± 0.1f	15 ± 2ij	11 ± 0.1gh	9.6 ± 0.3fg	21 ± 1k	16 ± 1i
31	homovanillic acid <sup>a</sup>	87 ± 1g	117 ± 22h	83 ± 10g	120 ± 9h	119 ± 20h	55 ± 11f	75 ± 6fg	79 ± 3fg
32	acetosyringone	16 ± 1hi	39 ± 2k	11 ± 0.2f	14 ± 1gh	27 ± 2j	12 ± 2fg	18 ± 2i	29 ± 1j
Benzenes									
33	benzaldehyde	7.3 ± 0.7g	7.4 ± 0.1g	5.8 ± 1.8fg	5.6 ± 0.5fg	4.5 ± 0.1f	5.7 ± 1.5fg	5.2 ± 1.0f	4.4 ± 0.2f
34	phenylacetaldehyde	4.9 ± 2.6g	4.4 ± 0.4g	2.4 ± 0.2f	2.2 ± 0.2f	2.1 ± 0.1f	3.4 ± 0.9fg	3.1 ± 0.3fg	3.4 ± 0.1fg
35	ethyl dihydrocinnamate	0.28 ± 0.06h	0.25 ± 0.07gh	0.18 ± 0.01fg	0.19 ± 0.03fg	0.24 ± 0.01gh	0.13 ± 0.02f	0.15 ± 0.01f	0.19 ± 0.05fg
36	ethyl cinnamate	0.59 ± 0.01f	1.1 ± 0.1g	0.65 ± 0.09f	1.2 ± 0.2gh	1.3 ± 0.1gh	0.73 ± 0.21f	1.6 ± 0.1i	1.4 ± 0.2hi
37	2-phenoxyethanol	4.0 ± 0.2hi	4.7 ± 1.2i	3.1 ± 0.4gh	3.1 ± 0.2g	2.5 ± 0.3fg	2.2 ± 0.2fg	2.1 ± 0.2f	2.3 ± 0.2fg
38	1,2-dimethoxy-4-propylbenzene <sup>a</sup>	16 ± 1gh	32 ± 2j	12 ± 1f	15 ± 1g	25 ± 2i	13 ± 1fg	18 ± 1h	30 ± 1j
Lactones									
39	δ-octalactone	3.5 ± 0.4i	2.3 ± 0.3h	1.9 ± 0.4gh	1.7 ± 0.1gh	1.5 ± 0.3fg	1.6 ± 0.5fh	1.2 ± 0.2f	1.1 ± 0.1f
40	γ-nonolactone	2.8 ± 0.2g	3.2 ± 0.2h	1.7 ± 0.1f	1.9 ± 0.2f	2.5 ± 0.1g	1.8 ± 0.2f	1.9 ± 0.1f	2.5 ± 0.1g
41	δ-nonolactone	nd f	nd f	nd f	nd f	nd f	0.31 ± 0.01g	0.34 ± 0.06g	0.31 ± 0.01g
42	γ-decalactone	1.3 ± 0.4ij	1.7 ± 0.3j	0.88 ± 0.24gh	0.96 ± 0.10h	0.94 ± 0.01ghi	0.37 ± 0.01f	0.60 ± 0.13fg	0.63 ± 0.05fh
43	δ-decalactone	68 ± 6g	61 ± 9g	44 ± 6f	40 ± 2f	37 ± 4f	40 ± 5f	38 ± 3f	36 ± 5f
Miscellaneous									
44	(Z)-3-hexen-1-ol	156 ± 18h	141 ± 13h	68 ± 9fg	70 ± 4fg	73 ± 8g	59 ± 8fg	59 ± 5fg	53 ± 1f
45	(E)-2-hexen-1-ol	11 ± 2g	11 ± 2g	nd f	nd f	nd f	0.42 ± 0.13f	0.79 ± 0.16f	0.74 ± 0.15f
46	3-methylbutyric acid	41 ± 3g	37 ± 8g	16 ± 3f	21 ± 1f	21 ± 1f	18 ± 6f	16 ± 2f	14 ± 1f
47	2-methylbutyric acid	28 ± 6g	25 ± 9g	10 ± 1f	16 ± 2f	14 ± 1f	12 ± 2f	12 ± 2f	8.9 ± 1.7f

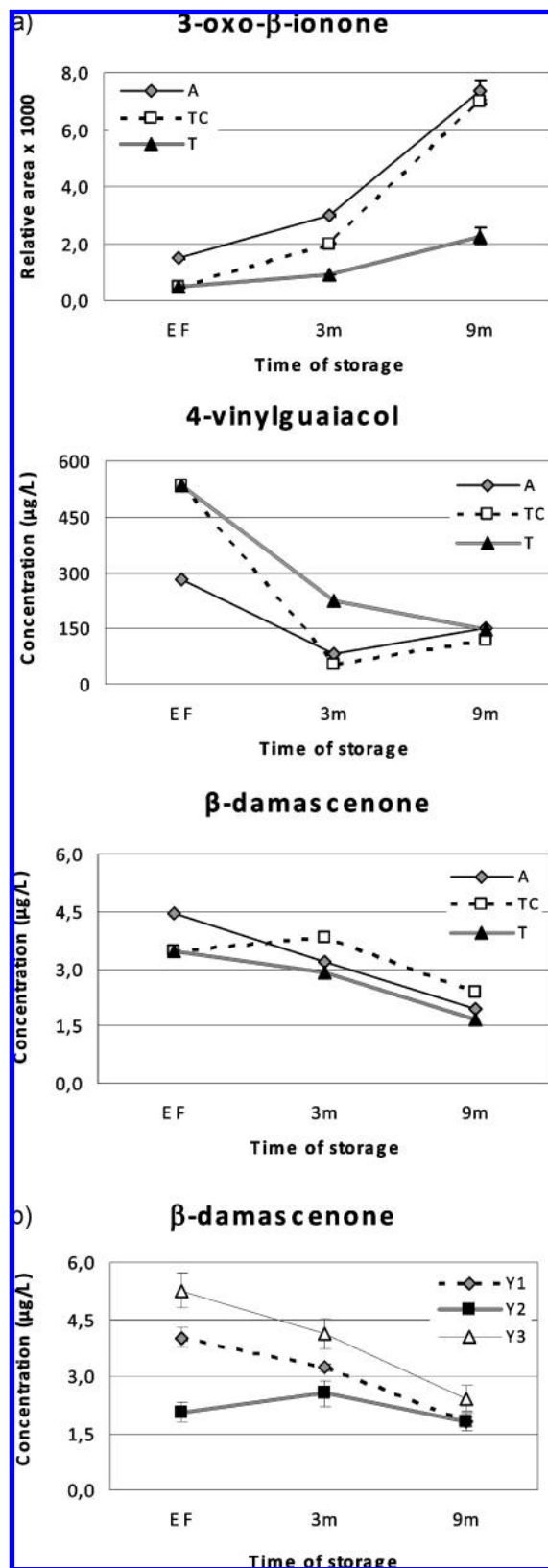
<sup>a</sup>Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). Different letters indicate significant differences (significant level 95%). <sup>b</sup>T, wines obtained with unsupplemented must; TC, wines supplemented after fermentation; A, wines obtained with supplemented must. <sup>c</sup>EF, end of fermentation; 3m, 3 months of storage on lees; 9m, 9 months of storage on lees. <sup>d</sup>nd, not detected. <sup>e</sup><DL, under the detection limit.



**Figure 1.** Principal component plot showing the scores for the 25 samples (average of two replicates) considered in the present study. Y1, Y2, and Y3: yeast strains 1, 2, and 3, respectively. T, wine obtained from the unsupplemented must; TC, wine supplemented with grape flavor precursors after alcoholic fermentation; A, wine obtained from supplemented grape juice; EF, end of fermentation; 3m, 9m, after 3 and 9 months of storage, respectively.

these weights can be seen in the principal component plot shown in **Figure 1**. The plot reveals that even if the three factors have an influence, the highest weight is due to the time of storage, because samples are ranked along the first component according to time. The second factor in importance is the addition of precursors, as all the samples produced from grape musts supplemented with precursors have comparatively highest scores on the second component, whereas unsupplemented wines have the lowest scores on this component. Wines supplemented with precursors after fermentation lay halfway between these two kinds of samples and tend to converge with the samples that were supplemented with precursors before fermentation. It should be noted that the plot indicates that differences due to the presence of precursors tend to increase with the time of storage. Finally, the factor with the smallest weight is the strain of yeast used in the fermentation. The influence of this factor is less evident in the plot, although it can be observed that samples produced with yeast 2 have slightly smaller scores of component 1 than the other equivalent samples and higher scores of component 2 (except at time 0). Differences introduced by yeast also tend to become higher after the aging process, particularly for samples supplemented with precursors.

**Evolution of the Concentration of the Volatile Compounds during Storage Time.** The trends followed by the different compounds are, generally speaking, relatively independent of the addition of precursors and of the strain of yeast. Three of the terpenes (linalool,  $\alpha$ -terpineol, and neric acid), all of the norisoprenoids (except  $\beta$ -damascenone and  $\beta$ -ionone), 4-allyl-2,6-dimethoxyphenol, ethyl vanillate, syringaldehyde, and ethyl cinnamate increase during storage,  $\beta$ -ionone and vanillin remain constant, whereas the rest of compounds tend to decrease during the process. Some authors also reported an increase of the concentration of some of these compounds. For example, Riu-Aumatell et al. (24) found an increase of the levels of TDN and vitispiranes during cava aging on yeast lees, whereas Bueno et al. (22) reported an increase of linalool and  $\alpha$ -terpineol in Airen wines, but a decrease in Macabeo wines. All of these changes make samples after storage on lees quite different from the original samples, as indicated in **Figure 1**. It is worth mentioning that these results are worse than expected, because one of our hypotheses was that aging on lees would promote mainly increasing changes in



**Figure 2.** Evolution of the average concentrations of some components during storage, showing differences related to the level of supplementation with precursors (a) or to the strain of yeast (b). TC, wine supplemented with grape flavor precursors after alcoholic fermentation; A, wine obtained from supplemented grape juice; EF, end of fermentation; 3m, 9m, after 3 and 9 months of storage, respectively.

nearly all compounds from precursors, at least in those samples that had been supplemented.

**Table 6.** Rate of Production of Volatile Compounds Derived from a Fixed Amount of Precursors in Wine in Contact with Lees or in Synthetic Media (Control)

		yeast strain 1 <sup>b</sup>		yeast strain 2 <sup>b</sup>		yeast strain 3 <sup>b</sup>		control <sup>c</sup>	
		3m	9m	3m	9m	3m	9m	3m	9m
Compounds with Increasing Trends									
2	linalool	1.0	0.06	0.95	0.88	0.61	0.98	0.64	1.6
4	$\alpha$ -terpineol	0.44	0.93	0.48	1.1	0.32	0.84	0.12	1.0
7	neric acid <sup>a</sup>	2.6	3.2	5.6	4.4	4.9	4.1	2.4	3.3
8	vitispirane A <sup>a</sup>	-0.12	1.3	1.1	1.1	-0.33	1.2	0	0
9	vitispirane B <sup>a</sup>	0.24	0.74	1.0	1.0	0.06	0.85	0	0
10	Riesling acetal <sup>a</sup>	0	3.6	0	3.1	0	1.9	0	0.90
11	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) <sup>a</sup>	0.001	0.67	0.10	0.36	-0.04	0.28	0	0
12	<i>tert</i> -1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) <sup>a</sup>	0	0.22	0	0.23	0	0.13	0	0.07
14	3-oxo- $\beta$ -ionone <sup>a</sup>	1.1	5.1	1.2	4.8	1.0	4.2	0.33	2.3
16	actinidols <sup>a</sup>	1.9	8.2	2.0	8.4	1.8	8.6	0.79	4.3
23	4-allyl-2,6-dimethoxyphenol	-1.3	1.1	-0.19	1.1	-0.59	0.40	0	0
26	ethyl vanillate	28	64	32	86	28	55	4.8	6.1
30	syringaldehyde	9.5	3.4	4.2	5.6	6.7	11	27	43
36	ethyl cinnamate	0.62	0.33	0.89	1.1	0.60	0.87	0	0
Compounds with Decreasing Trends									
5	$\beta$ -citronellol	-2.6	-1.6	0.02	-0.11	0.10	-0.19	3.7	0
6	nerol	-3.6	-0.44	0.79	0.71	0.46	-0.19	0	0
1	nerol oxide <sup>a</sup>	-0.30	0.06	-0.11	0.04	-0.30	-0.07	0.22	0
3	2,6-dimethyl-1,7-octadiene-3,6-diol	-0.24	0.02	0.47	0.56	0.03	0.12	1	0.77
13	$\beta$ -damascenone	0.25	0.34	1.1	0.79	1.4	1.1	0.61	1.3
15	$\beta$ -ionone	0.25	-0.13	0.09	0.05	0.07	-0.09	0	0.27
17	guaiacol	-0.23	-0.14	-0.04	0.03	-0.08	0.03	0.35	0.42
18	4-ethylguaiacol	-0.10	-0.07	0.07	-0.07	-0.02	-0.11	0	0
20	4-ethylphenol	-0.25	-0.05	0.16	-0.03	-0.48	-0.26	0	0
21	4-vinylguaiacol	-230	-19	-12	-17	-268	-48	4.0	3.8
22	4-vinylphenol	-990	-146	15	76	-1370	-201	8.0	5.7
24	vanillin	-2.6	-2.9	2.3	1.9	2.6	3.3	15	18
25	methyl vanillate	4.8	7.8	7.3	8.9	5.2	7.3	4.1	4.0
27	acetovanillone	-2.3	3.2	14	24	3.3	7.6	4.2	4.0
28	zingerone	-8.5	-1.5	5.2	11	1.3	3.7	1.6	1.0
29	homovanillyl alcohol	1.7	3.5	0.68	1.5	2.3	1.6	0	0
31	homovanillic acid <sup>a</sup>	4.8	13	39	20	36	20	25	14
32	acetosyringone	2.7	5.6	7.9	8.1	3.2	5.9	2.7	2.7
33	benzaldehyde	-6.4	-11	0.75	1.1	-0.20	-0.57	2.8	2.5
34	phenylacetaldehyde	-2.7	-0.73	1.6	0.76	-0.18	-0.26	8.4	9.1
35	ethyl dihydrocinnamate	-0.01	-0.02	0.08	-0.01	0.01	0.02	0	0
37	2-phenoxyethanol	0.40	-0.42	0.24	0.17	-0.08	-0.10	4.1	1.9
38	1,2-dimethoxy-4-propylbenzene <sup>a</sup>	4.9	7.4	7.5	8.1	3.4	5.2	0.59	0.45
39	$\delta$ -octalactone	0.30	0.02	-0.13	-0.25	-0.14	-0.47	0	0
40	$\gamma$ -nonalactone	-2.9	-2.5	0.49	0.57	0.27	0.10	0	0.46
41	$\delta$ -nonalactone	-0.16	-0.24	0	0.38	0	0.03	0	0
42	$\gamma$ -decalactone	-4.2	-3.5	0.30	0.18	0.08	0.24	1.9	0.25
43	$\delta$ -decalactone	4.1	5.4	0.16	-2.9	-4.0	-2.5	0	0
44	( <i>Z</i> )-3-hexen-1-ol	0.09	2.3	-23	-3.8	1.4	-0.33	0	0.04
45	( <i>E</i> )-2-hexen-1-ol	0	0.57	0	0.06	0	0.37	0	0
46	3-methylbutyric acid	-7.7	-5.9	-6.4	-2.9	4.6	-1.9	0	0.86
47	2-methylbutyric acid	-5.9	-4.3	-6.1	-2.1	5.4	0.07	0	0.43

<sup>a</sup> Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone  $\times$  1000). <sup>b</sup> Data are the subtraction between the concentrations found in wines supplemented after alcoholic fermentation and those found in the unsupplemented wines. <sup>c</sup> Concentration of volatiles released in synthetic media supplemented with grape flavor precursors.

In the cases of increasing tendencies, the highest increase is most often observed from 3 to 9 months, especially for Riesling acetal and *tert*-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), which were found only after 9 months of storage. As the rate of increase is in most cases higher in supplemented samples, differences in the levels of these compounds related to the supplementation tend to be higher at the end of the storage, as can be seen in the case of 3-oxo- $\beta$ -ionone shown in **Figure 2a**.

To determine whether the increases should be attributed to hydrolytic procedures specifically induced by the yeast lees or can

be explained by normal acid hydrolysis, a comparison between the rates observed in two media (wine with lees or synthetic wine without lees) was carried out. Results can be seen in **Table 6**. As shown in the table, the amount of volatile compound formed by simple acid hydrolysis is in most cases much smaller (or even null) than that formed in the presence of lees. This clearly indicates that lees are taking an active part in the aroma formation from precursors. However, in the cases of linalool and  $\alpha$ -terpineol, the increments observed in the absence of lees are comparable or even higher. This result is in agreement with that reported by Bautista et al. (23), who also found an increase of the



concentration of both compounds after 7 months of wine storage in samples stored either with or without lees. In the case of syringaldehyde, the increment in the samples stored without lees is much higher than that observed in the presence of lees. Even considering the adsorptive power of lees, this result suggests that lees are promoting the transformation of the precursor to some other molecule.

In the cases of decreasing tendencies, the decrease was generally higher in the first 3 months of storage. In most cases, the decreases observed throughout the whole process are not higher than 40%, but there are five remarkable exceptions. As can be seen in **Tables 3–5**, the levels of nerol, 4-vinylguaiaicol, 4-vinylphenol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol decrease in percentages between 60 and 80, reaching even 100% for (*E*)-2-hexen-1-ol.

It is important to evaluate whether all of those decrements are specifically caused by the lees or whether they are due to just any other cause of natural degradation not related to the presence of lees, such as oxidation, reaction, or chemical rearrangement. This can be assessed in part from the results presented in **Table 7**. The table gives the evolution of some compounds in a synthetic wine containing or not yeast lees. Leaving aside some compounds such as neric acid, 4-allyl-2-methoxyphenol, 1,2-dimethoxy-4-propylbenzene, and vanillin derivatives, which increase surely because the natural aroma solution contained precursor molecules not completely hydrolyzed during its preparation (see methods), the table shows that many volatile compounds are fairly unstable in a synthetic wine. Half-lives were estimated from the corresponding plots (graphical interpolation of data in **Table 7**) as stability criteria. It is noteworthy that in the absence of yeast lees only  $\alpha$ -terpineol,  $\beta$ -ionone, 3-oxo- $\beta$ -ionone, actinidols, guaiacol, phenylacetaldehyde, ethyl cinnamate,  $\gamma$ -nonalactone, and methylbutyric acids can be considered to be relatively stable in the synthetic wine. Although the instability of some compounds is well documented in the scientific literature (37–40), those data should not be directly extrapolated to real wines in which stability will be surely higher, and we should focus on the effect of lees. It can be observed that half-lives in samples incubated with lees are, in general, smaller than those observed in the samples aged without lees, particularly in the cases of linalool,  $\beta$ -citronellol,  $\beta$ -damascenone,  $\beta$ -ionone, guaiacol, vanillin, benzaldehyde, ethyl cinnamate, 2-phenoxyethanol, and methylbutyric acids. In all of these cases, the volatile compound remaining in the samples with yeast lees is less than half of that remaining in samples without yeast lees and in some cases can be less than 10%. A smaller level in the samples stored on lees is also observed in the cases of  $\alpha$ -terpineol, syringaldehyde, homovanillic acid, phenylacetaldehyde, and  $\gamma$ -nonalactone. This implies that the decreasing trends observed in the general experiment for some of these compounds are to a large extent due to the presence of lees, whereas in the rest of the cases, particularly 2,6-dimethyl-1,7-octadiene-3,6-diol and vinylphenols, natural instability seems to be the major cause. Finally, it is worth mentioning that the existence of a decreasing tendency causes in a few cases yeast-related differences observed in recently fermented samples to become nearly canceled. The case of  $\beta$ -damascenone is shown in **Figure 2b** as an example.

#### Effect of the Addition of the Grape Flavor Precursor Extract.

The addition of precursors has as a consequence a significant increase in the levels of four terpenes (linalool,  $\alpha$ -terpineol, 2,6-dimethyl-1,7-octadiene-3,6-diol, and neric acid), of all the norisoprenoids and vanillin derivatives (vanillin and  $\beta$ -ionone showing particular behaviors), and of ethyl cinnamate and 1,2-dimethoxy-4-propylbenzene. On the contrary, the addition of precursors has a surprising significant negative effect on the

levels of ethylphenol and vinylphenols, benzaldehyde, lactones, (*Z*)-3-hexen-1-ol, and 2- and 3-methylbutyric acids (see **Tables 3–5**). In all of these cases there are well-known nonglycosidic precursors such hydroxycinnamic acids, fatty acids, or amino acids, and the results suggest that something present in the precursor fraction added to the must inhibits the synthesis of these compounds by yeast or catalyzes their degradation. The case of vinyl and ethyl phenols is even more striking because the addition of precursors after fermentation also brings about a significant reduction on the levels of these compounds (see **Figure 2a**).

As for the moment at which the supplementation takes place, it can be said that in general it does not have much importance and that the levels of samples supplemented after fermentation are smaller than, but converge with, those of samples supplemented before fermentation. There are, however, some remarkable exceptions. The most relevant is  $\beta$ -damascenone, for which supplementation after fermentation seems to be more effective at any time (see **Figure 2a**). Something similar happens to vanillin and syringaldehyde. These observations could be explained because, on average, the natural acid hydrolysis seems to be more effective than the enzyme-driven hydrolysis for the formation of these compounds.

**Role of Yeast.** As was aforementioned, the role of yeast in this experiment is quantitatively less important. However, a closer look at the data reveals that the strain of yeast not only affects the formation of some volatile compounds from precursors during fermentation, in accordance with previous results (36, 41) but in some cases the lees from different strains also may show a differential ability to form compounds from precursors. This can be best answered with the help of data in **Table 6**. As can be seen, lees from yeast strain 2, which was not particularly active forming aroma compounds from precursors during fermentation, formed slightly higher levels of some compounds such as linalool,  $\alpha$ -terpineol, vitispirane, 4-vinylphenol, most vanillin derivatives, ethyl cinnamate, 1,2-dimethoxy-4-propylbenzene, and  $\gamma$ -nonalactone than the lees from the other strains, and all of these small changes together explain the observation made in the PCA plot of **Figure 1** about the progressive differentiation of the wines made and aged with this yeast strain. The experimental setup does not allow extracting clear conclusions about whether this was caused by specific enzymatic activities of the lees of this strain or whether it is a question of the amount of volatile absorbed by the biomass formed, although the fact that different compounds were affected at different levels suggests that the behavior of this strain was really different.

In conclusion, it can be said that the aging of wine on yeast lees causes important changes in the concentration levels of the volatile compounds derived from precursors and that, in fact, is after an aging time when differences linked to the level of precursors in the must become more evident. Three terpenes, all norisoprenoids except  $\beta$ -damascenone and  $\beta$ -ionone, 4-allyl-2,6-dimethoxyphenol, ethyl vanillate, syringaldehyde, and ethyl cinnamate increase during the process,  $\beta$ -ionone and vanillin remain constant, and the rest of the compounds decrease. Lees take an active part not only in the formation of some aroma molecules from precursors but also in the sorption or degradation of aroma molecules. As the presence of lees accelerates both the formation and degradation rates at different levels for each compound, aging on lees must be considered as an accelerated and differential aging process from the aroma formation point of view. It has also been found that the addition of the precursor fraction makes the levels of vinylphenols decrease. Finally, it has also been shown that lees from different yeast strains may have slightly different abilities to release volatile compounds derived from precursors.

**Table 7.** Evolution of the Concentration (in Micrograms per Liter, Except Where Indicated<sup>a</sup>) of Volatile Compounds during the Aging of Model Wines with and without Yeast Lees (Data Are the Average of the Three Replicate Samples)

		WA				WLA-WL <sup>c</sup>			half-lives <sup>d</sup>	
		0	2w	6w	17w	2w	6w	17w	without lees	with lees
Terpenes										
1	nerol oxide <sup>a</sup>	0.19 ± 0.02	0.18 ± 0.03	0.11 ± 0.01	0.58 ± 0.09	0.13 ± 0.02	0.11 ± 0.01	0.57 ± 0.05		
2	linalool	9.5 ± 0.4	3.8 ± 0.1	4.0 ± 0.2	6.4 ± 0.3	2.6 ± 0.1	2.4 ± 0.2	2.6 ± 0.7	1.7	1.0
3	2,6-dimethyl-1,7-octadiene-3,6-diol <sup>a</sup>	0.52 ± 0.01	0.74 ± 0.04	0.54 ± 0.03	0.28 ± 0.02	0.75 ± 0.05	0.53 ± 0.02	0.26 ± 0.01	19	19
4	α-terpineol	6.5 ± 0.2	6.6 ± 0.1	5.8 ± 0.1	5.1 ± 0.2	4.8 ± 0.1	4.3 ± 0.1	3.5 ± 0.1	>50	18
5	β-citronellol	1.7 ± 0.04	0.98 ± 0.04	0.90 ± 0.13	1.1 ± 0.1	0.42 ± 0.09	0.16 ± 0.06	nd	4.0	1.0
6	nerol	0.58 ± 0.07	0.48 ± 0.06	0.50 ± 0.06	nd	0.53 ± 0.05	0.44 ± 0.03	nd	11	9.0
7	neric acid <sup>a</sup>	2.6 ± 0.1	3.3 ± 0.2	3.9 ± 0.2	4.3 ± 0.6	4.7 ± 0.4	5.5 ± 0.5	5.4 ± 0.3		
Norisoprenoids										
8	vitispirane A <sup>a</sup>	6.1 ± 0.1	2.3 ± 0.1	0.46 ± 0.04	0.20 ± 0.02	1.1 ± 0.01	0.72 ± 0.04	0.42 ± 0.03	1.5	1.0
9	vitispirane B <sup>a</sup>	5.1 ± 0.04	1.8 ± 0.03	0.39 ± 0.04	0.22 ± 0.05	0.80 ± 0.03	0.56 ± 0.04	0.28 ± 0.01	1.0	1.0
10	Riesling acetal <sup>a</sup>	3.9 ± 0.1	4.0 ± 0.2	3.6 ± 0.1	3.5 ± 0.1	3.4 ± 0.03	3.1 ± 0.01	3.0 ± 0.2	50	40
11	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) <sup>a</sup>	1.2 ± 0.04	0.29 ± 0.02	0.29 ± 0.01	0.22 ± 0.01	0.06 ± 0.01	0.15 ± 0.03	0.13 ± 0.02	1.0	0.80
12	tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd		
13	β-damascenone	12 ± 0.2	10 ± 0.1	7.5 ± 0.2	1.7 ± 0.1	3.3 ± 0.04	2.0 ± 0.1	0.44 ± 0.03	8.0	1.0
14	3-oxo-β-ionone <sup>a</sup>	12 ± 0.1	13 ± 0.3	13 ± 0.2	12 ± 1	14 ± 0.4	15 ± 0.2	11 ± 1	>50	>50
15	β-ionone	1.9 ± 0.02	1.6 ± 0.01	1.7 ± 0.01	1.9 ± 0.1	0.07 ± 0.04	nd	nd	>100	0.90
16	actinidols <sup>a</sup>	14 ± 0.2	14 ± 0.3	15 ± 0.3	13 ± 1	17 ± 1	17 ± 0.2	14 ± 1	>51	>51
Volatile Phenols										
17	guaiacol	1.2 ± 0.2	0.83 ± 0.01	0.71 ± 0.01	0.74 ± 0.03	0.34 ± 0.03	0.16 ± 0.02	0.12 ± 0.01	30	1.0
18	4-ethylguaiacol	0.22 ± 0.01	0.23 ± 0.02	0.24 ± 0.04	nd	0.22 ± 0.01	0.22 ± 0.01	nd	12	12
19	eugenol	nd	nd	nd	nd	nd	nd	nd		
20	4-ethylphenol	0.57 ± 0.01	0.58 ± 0.05	0.61 ± 0.11	0.23 ± 0.04	0.49 ± 0.01	0.49 ± 0.02	0.17 ± 0.01	15	14
21	4-vinylguaiacol	14 ± 1	8.4 ± 0.4	1.9 ± 0.2	1.8 ± 0.3	9.3 ± 0.3	2.0 ± 0.2	1.1 ± 0.1	2.5	2.8
22	4-vinylphenol	47 ± 5	22 ± 2	11 ± 1	13 ± 2	22 ± 0.4	13 ± 1	8.6 ± 0.8	1.7	2.0
23	4-allyl-2,6-dimethoxyphenol	0.37 ± 0.04	0.47 ± 0.04	0.54 ± 0.04	0.82 ± 0.09	0.53 ± 0.05	0.56 ± 0.01	0.74 ± 0.02		
Vanillin Derivatives										
24	vanillin	0.72 ± 0.03	0.83 ± 0.02	1.3 ± 0.04	1.2 ± 0.1	0.61 ± 0.10	0.84 ± 0.06	0.54 ± 0.06		
25	methyl vanillate	0.47 ± 0.02	0.65 ± 0.06	0.55 ± 0.04	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	2.4 ± 0.1		
26	ethyl vanillate	0.85 ± 0.04	1.2 ± 0.1	1.1 ± 0.04	1.7 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	1.8 ± 0.1		
27	acetovanillone	<DL	<DL	<DL	0.15 ± 0.05	1.3 ± 0.2	2.0 ± 0.2	6.1 ± 0.3		
28	zingerone	0.97 ± 0.05	1.2 ± 0.03	1.3 ± 0.05	1.4 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.8 ± 0.1		
29	homovanillyl alcohol	nd	nd	nd	nd	nd	nd	nd		
30	syringaldehyde	2.6 ± 0.1	4.2 ± 0.2	3.7 ± 0.04	4.5 ± 0.3	3.0 ± 0.2	2.4 ± 0.2	3.0 ± 0.1		
31	homovanillic acid <sup>a</sup>	7.1 ± 0.4	8.8 ± 0.4	28 ± 3	26 ± 4	8.7 ± 0.3	12 ± 2	19 ± 2		
32	acetosyringone	2.4 ± 0.1	3.2 ± 0.2	2.9 ± 0.04	3.3 ± 0.2	3.2 ± 0.2	3.5 ± 0.2	4.8 ± 0.3		
Benzenes										
33	benzaldehyde	7.5 ± 0.3	5.8 ± 0.2	6.6 ± 0.4	7.8 ± 0.5	0.35 ± 0.15	nd	0.18 ± 0.49		0.90
34	phenylacetaldehyde	1.6 ± 0.3	1.7 ± 0.2	1.3 ± 0.3	1.4 ± 0.04	1.7 ± 0.6	0.66 ± 0.01	0.77 ± 0.20	>50	17
35	ethyl dihydrocinnamate	0.44 ± 0.01	0.45 ± 0.04	0.46 ± 0.08	nd	0.39 ± 0.01	0.39 ± 0.01	nd	13	12
36	ethyl cinnamate	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.90 ± 0.01	0.11 ± 0.01	0.19 ± 0.02	0.21 ± 0.02	>50	0.90
37	2-phenoxyethanol	8.2 ± 0.4	4.2 ± 0.2	3.5 ± 0.3	6.3 ± 0.5	0.84 ± 0.69	nd	nd		0.90
38	1,2-dimethoxy-4-propylbenzene <sup>a</sup>	0.21 ± 0.01	0.19 ± 0.03	0.27 ± 0.06	0.22 ± 0.04	2.6 ± 0.3	3.5 ± 0.2	4.7 ± 0.3	>50	
Lactones										
39	δ-octalactone	nd	nd	nd	nd	nd	nd	nd		
40	γ-nonolactone	1.9 ± 0.03	2.0 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.3 ± 0.04	1.4 ± 0.1	0.95 ± 0.1	>50	17
41	δ-nonolactone	nd	nd	nd	nd	nd	nd	nd		
42	γ-decalactone	nd	nd	nd	nd	nd	nd	nd		
43	δ-decalactone	nd	nd	nd	nd	nd	nd	nd		
Miscellaneous										
44	(Z)-3-hexen-1-ol	nd	nd	nd	nd	nd	nd	nd		
45	(E)-2-hexen-1-ol	nd	nd	nd	nd	6.4 ± 0.2	4.3 ± 0.2	4.1 ± 0.2		
46	3-methylbutyric acid	1.0 ± 0.03	0.70 ± 0.02	0.78 ± 0.03	0.86 ± 0.03	0.18 ± 0.07	nd	0.09 ± 0.08	>50	0.90
47	2-methylbutyric acid	0.40 ± 0.04	0.20 ± 0.03	0.23 ± 0.04	0.32 ± 0.05	nd	nd	nd	>50	1.0

<sup>a</sup>Chemical standard not available. Tentatively identified. Data are the relative areas (to 4-hydroxy-4-methyl-2-pentanone × 1000). <sup>b</sup>WA, model wine supplemented with aroma compounds without yeast lees; WLA, model wine supplemented with aroma compounds and yeast lees; WL, model wine supplemented with yeast lees without aromas. <sup>c</sup>Subtraction of the concentration of the volatile compounds released from yeast lees. <sup>d</sup>Half-lives were calculated by graphical interpolation

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