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Effect of Fungicide Residues on the Aromatic Composition of White Wine Inoculated with Three *Saccharomyces cerevisiae* Strains

MARÍA ANGELES GARCÍA,[†] JOSÉ OLIVA,*[,†] ALBERO BARBA,[†]
MIGUEL ÁNGEL CÁMARA,[†] FRANCISCO PARDO,[§] AND EVA MARÍA DÍAZ-PLAZA[#]

Departamento de Química Agrícola, Geología y Edafología, Facultad de Química,
Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain; Bodega San Isidro,
Ctra. de Murcia s/n, 30520 Jumilla (Murcia), Spain; and Departamento de Ciencia y Tecnología
Agroforestal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha,
Avenida de España s/n, 02071 Albacete, Spain

The effects of three fungicide residues (cyprodinil, fludioxonil, and pyrimethanil) on the aromatic composition (acids, alcohols, and esters) of *Vitis vinifera* white wines (var. Airén) inoculated with three *Saccharomyces cerevisiae* strains (syn. *bayanus*, *cerevisiae*, and syn. *uvarum*) are studied. The aromatic exponents were extracted and concentrated by adsorption−thermal desorption and were determined by gas chromatography using a mass selective detector. The addition of the three fungicides at different doses (1 and 5 mg/L) produces significant differences in the acidic fraction of the aroma, especially in the assays inoculated with *S. cerevisiae,* although the final contents do not exceed the perception thresholds. The lower quality wines, according to isomeric alcohol content [(*Z*)-3-hexen-1-ol and 3-(methylthio)propan-1-ol] are those obtained by inoculation with *S. cerevisiae* (syn. *bayanus*) and addition of cyprodinil. The addition of fungicides in the assays inoculated with *S. cerevisiae* (syn. *bayanus*) produces an increase in the ethyl acetate and isoamyl acetate contents, which causes a decrease in the sensorial quality of the wine obtained.

KEYWORDS: **Fermentative aroma; fungicide residues; white wine; *Saccharomyces cerevisiae***

INTRODUCTION

The aroma of a wine represents one of the most important sensorial qualities, as on many occasions it will be instrumental in the wine's being accepted or rejected by the consumer. It therefore constitutes an index of interest when the quality of a wine is evaluated.

The aroma of a wine is highly complex; it is made up of a mixture of >500 volatile parts belonging to a large number of chemical families, including aldehydes, acetones, alcohols, acids, esters, terpenes, phenols, etc. (*1*, *2*).

The aromatic composition of a wine depends on different types of factors: varietal (*3*−*8*), environmental (*9*), agronomic (*10*−*12*), and technological (*13*−*19*). Although there is a wealth of literature on the factors that influence the aroma of wines, there are few studies on the effects of pesticide residues in this fraction (*20*−*23*).

One of the most influential factors in wine aroma is the alcoholic fermentation because this is responsible for the sensations of wine (major volatiles) that make up the aromatic base common to all wines. The remaining aromas are to be found in the minority volatiles, including varietal, prefermentative, and fermentative, as well as those generated during preservation and aging processes (*24*, *25*).

In recent years the trend has been to use selected yeasts for the alcoholic fermentation. These yeasts have specific fermentative characteristics that guarantee the smooth development of the process (*26*, *27*) but are also capable of generating positive aromas for the organoleptic properties of the wine.

It is for all of the above reasons that we study herein the effects of three commonly used fungicides in vine growing (cyprodinil, fludioxonil, and pyrimethanil) on the aromatic composition of white wines obtained by inoculation of sterile must with three *Saccharomyces cerevisiae* strains.

MATERIALS AND METHODS

Chemicals and Reagents. Cyprodinil [*N*-(4-cyclopropyl-6-methylpyrimidim-2-yl)aniline], fludioxonil [4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile], and pyrimethanil [*N*-(4,6-dimethylpyrimidin-2-yl)aniline] analytical standards used were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and were certified to be at least >99% pure. Standard stock solutions (∼100 μg/mL) were prepared in water/ethanol (9:1 v/v). Working standard solutions (1 and 5 μg/mL) were obtained by dilution in the same solvent. Ethanol was a pure reagent (Panreac,

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* Corresponding author (e-mail josoliva@um.es; fax +34968364148).
[†] Universidad de Murcia.
[§] Bodega San Isidro.
[#] Universidad de Castilla-La Mancha.

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Table 1. Perception Threshold Values of Several Aromatic Compounds in Wines

compound	perception threshold (mg/L)	compound	perception threshold (mg/L)	compound	perception threshold (mg/L)
2-methylpropanoic	8.1[a]	(*Z*)-3-hexen-1-ol	0.07[b]	ethyl hexanoate	0.08[a]
3-methylbutanoic	0.7[a]	3-(methylthio)propan-1-ol	4[a]	hexyl acetate	0.58[a]
octanoic acid	13[b]	2-phenylethanol	7.5[c]	ethyl octanoate	0.51[a]
decanoic acid	10[b]	ethyl acetate	17[a]	ethyl decanoate	2.4[a]
isoamylic alcohol	7[a]	ethyl butyrate	4[b]	2-phenylethyl acetate	0.65[a]
1-hexanol	6.2[a]	isoamyl acetate	0.2[a]		

[a] Reference *1.* [b] Reference *34.* [c] Reference *44.*

Barcelona, Spain). Water used was purified with a Millipore Milli-Q RG (Molsheim, France).

Plant Materials. White grapes, *Vitis vinifera* var. Airen, were harvested in September 2001 in an experimental plot in Jumilla, Murcia (southeastern Spain). The nutritional state and physiological conditions of the grape were perfect. The grapes were not treated with any pesticides during the growing season.

Obtaining the Sterile Must. The grape was crushed in the winery and was pressed 6 h later to obtain the free-run must. The processes below were applied to obtain the sterile must: clarification of the free-run must using cellulose plaque filters (3 μm lx) on a laboratory scale and amicrobic filtration in a vacuum of the clarified must using 0.45 μm Millipore filters.

Inoculation and Fermentation. *Yeasts.* The yeasts used belong to the *S. cerevisiae* strain, and all present affinity toward white grape varieties. The yeast strains were *S. cerevisiae* (syn. *bayanus*), *S. cerevisiae*, and *S. cerevisiae* (syn. *uvarum*). All yeast strains were obtained from commercially prepared active dry yeasts supplied by Lallemand-Agrovin (Ciudad Real, Spain).

Fermentation. One and a half liters of sterile must without addition of sulfur dioxide was placed in 2 L glass vessels, in which fermentations were carried out. The inoculation of the sterile must was performed at 30 g/hL (10^6 cell number/mL) of active dry yeast. The preculture of these yeasts was carried out in a glucose solution (5%), which was constantly stirred on a rotating stirrer (model Unimixer Lab-Line Biomedical) set at 200 rpm and for 24 h. The fungicides were added separately in two doses (1 and 5 mg/L) and were dissolved in a water/ethanol (9:1 v/v) solution in the sterile must, to which, moments before, one of the selected yeasts had been inoculated. All assays were performed three times. Nutrients (ammonium phosphate and thiamin) were also added to each fermentation flask at a dosage of 30 g/hL. Fermentation was performed at a controlled temperature of 24 °C during 7−9 days.

Volatile Compounds Analyzed. The main aromatic compounds produced during fermentation have been analyzed. Acids included 2-methylpropanoic, 3-methylbutanoic, octanoic, and decanoic. Alcohols included isoamylic, 1-hexanol, (*Z*)-3-hexen-1-ol, 3-(methylthio)propan-1-ol, and 2-phenylethanol. Esters included ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, and 2-phenylethyl acetate.

Analysis of Volatile Compounds. *Preservation of Samples.* NaF (0.2 g) (a disinfectant) and 0.2 g of ascorbic acid (an antioxidant) were added to 400 mL of decanted wine. The sample was then stirred until the added preservatives were completely dissolved. The samples were then placed in a freezer at −30 °C, and the temperature was kept steady until analysis.

Linearity. Aromatic analytical standards, at least 97% pure, were purchased from Sigma-Aldrich (Dorset, U.K.). Several dilutions were used to check the linearity of the response of the detector, in accordance with the methods used for determining the aromatic compounds. In all cases, the coefficients of lineal correlation were >0.98 and the coefficients of variability <10%.

Sample Preparation, Apparatus, and Chromatography. Three microliters of a 1% (v/v) solution of methyl caprylate (internal standard) in ethanol was added to 50 mL of wine. The aromatic compounds were then extracted and concentrated by adsorption−thermal desorption following the method proposed by Salinas et al. (*28*) and Salinas and Alonso (*29*). The volatiles were isolated by purging with helium for 20 min at ambient temperature and 40 mL/min and retained in a tube with Tenax TA (60−80 mesh). The packed tube was introduced into a Spantech TD-4 thermal desorber (Perkin-Elmer) coupled to a Hewlett-Packard 6890 gas chromatograph equipped with an HP 5973 mass-selective detector (MSD). An SGE 50 m × 0.22 mm i.d. fused silica capillary column coated with a 0.25 μm layer of cross-linked BP-21 was used. The injector and interface were operated at 200 and 280 °C, respectively. The operating conditions were as follows: acquisition mode, scan (35−500); voltage, 1016 mV; ionization foil temperature, 230 °C; quadrupole temperature, 150 °C; solvent delay, 3 min. The carrier gas was He at 1.50 mL/min. The sample was injected in EPC split mode (50 mL/min), and the oven temperature was programmed as follows: 50 °C for 0 min, raised to 180 °C (2.5 °C/min), held for 2 min, raised to 200 °C, and held for 10 min.

Statistics. The descriptive statistics and nonparametric analysis of variance used to determine the relationship between pesticide residues and the aroma concentration for each yeast corresponded to SPSS version 11.0 for Windows.

RESULTS AND DISCUSSION

Table 1 shows the perception threshold values of the aromatic compounds studied. **Tables 2−4** show the average values of the aromatic compounds detected in the different assays and the significant differences between the wines with pesticides and the control wines.

Acids. Acids with five or fewer carbon atoms (2-methylpropanoic and 3-methylbutanoic acids) present in the wine are indices of low quality and may indicate alterations due to bacteria action. On the other hand, those acids containing more than five carbon atoms (octanoic and decanoic acids) contribute positively to the aroma of the wine when their concentrations are between 4 and 10 mg/L. At concentrations of >20 mg/l, however, the smell is unpleasant (*15*, *30*−*32*).

In our experiments, the highest 2-methylpropanoic acid contents were produced in the wines inoculated by *S. cerevisiae* (**Table 3**). Significant differences exist for this yeast for the three pesticides studied (in both doses) with respect to the blank, where this acid was not detected. Nevertheless, in the case of the other two yeasts, we also found significant differences between the blank and the experiments with fungicide addition, although there were lesser variations between the two. For pyrimethanil in winemaking with inoculation with *S. cerevisiae* (syn. *bayanus*) (**Table 2**) and for cyprodinil and fludioxonil in those inoculated with *S. cerevisiae* (syn. *uvarum*) (**Table 4**) lower quantities are observed compared with those obtained for the blank. In any case, this increase is not sufficient as to have an effect on the sensorial quality of the wine, because the concentrations are below the perception threshold (8.1 mg/L) (*1*).

In the case of 3-methylbutanoic acid, the characteristic aroma of which is fairly unpleasant (sweaty and rotten), the values found in the three assays were very low, despite the significant

Table 2. Aromatic Compounds (Milligrams per Liter) in Finished Wines by a Selected Strain of *S. cerevisiae* (Syn. *bayanus*) to Fungicide Supplementation in Filtered Must (Mean Values and SD, $n = 3$ Replicates)

	winemaking							
compound	blank	cyprodinil 1 (a)	cyprodinil 5 (b)	fludioxonil 1 (c)	fludioxonil 5 (d)	pyrimethanil 1 (e)	pyrimethanil 5 (f)	SD,[a] $p \leq 0.05$
acids								
2-methylpropanoic	2.69 ± 0.04	3.35 ± 0.09	2.78 ± 0,18	2.21 ± 0.20	2.68 ± 0.11	2.48 ± 0.46	1.94 ± 0.10	a, c, f
3-methylbutanoic	0.12 ± 0.02	nd[b]	nd	nd	nd	nd	nd	a–f
octanoic	3.20 ± 0.12	2.95 ± 0.51	4.23 ± 0.91	3.66 ± 0.97	3.54 ± 0.21	3.13 ± 0.60	3.47 ± 0.37	ns[c]
decanoic	0.67 ± 0.08	0.91 ± 0.21	1.77 ± 0.67	1.17 ± 0.60	2.15 ± 0.18	1.14 ± 0.11	1.07 ± 0.10	b, d
alcohols								
isoamylic	47.7 ± 3.05	72.59 ± 4.7	68.88 ± 2.5	74.39 ± 5.2	44.99 ± 0.4	67.5 ± 0.85	69.9 ± 16.4	a–c, e, f
1-hexanol	0.71 ± 0.05	0.63 ± 0.07	0.33 ± 0.32	0.76 ± 0.04	0.76 ± 0.01	0.66 ± 0.01	0.76 ± 0.05	b, d, e
(*Z*)-3-hexen-1-ol	0.01 ± 0.00	0.10 ± 0.01	0.73 ± 0.58	nd	0.01 ± 0.01	0.06 ± 0.02	nd	a–c, e, f
3-(methylthio)propan-1-ol	2.11 ± 0.21	3.12 ± 0.40	4.39 ± 1.77	2.94 ± 1.44	2.23 ± 0.40	2.30 ± 0.70	3.21 ± 1.07	b
2-phenylethanol	18.6 ± 2.47	29.69 ± 1.3	22.64 ± 2.4	20.52 ± 0.6	17.18 ± 2.9	24.27 ± 3.8	18.85 ± 8.9	a
esters								
ethyl acetate	61.2 ± 0.48	113.3 ± 12.5	104.7 ± 5.7	92.6 ± 4.06	92.2 ± 3.96	91.9 ± 2.55	110.6 ± 8.6	a–f
ethyl butyrate	0.16 ± 0.02	0.11 ± 0.02	0.24 ± 0.03	0.16 ± 0.03	0.29 ± 0.02	0.17 ± 0.04	0.27 ± 0.02	b, d, f
isoamyl acetate	1.52 ± 0.09	1.82 ± 0.40	2.22 ± 0.42	1.15 ± 0.10	1.75 ± 0.16	1.64 ± 0.61	2.01 ± 0.29	b
ethyl hexanoate	0.55 ± 0.02	0.31 ± 0.03	0.36 ± 0.08	0.20 ± 0.03	0.16 ± 0.05	0.34 ± 0.08	0.21 ± 0.07	a–f
hexyl acetate	0.18 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.19 ± 0.02	0.17 ± 0.03	0.21 ± 0.02	0.21 ± 0.02	ns
ethyl octanoate	0.38 ± 0.05	0.37 ± 0.01	0.34 ± 0.04	0.34 ± 0.08	0.29 ± 0.04	0.32 ± 0.04	0.34 ± 0.12	ns
ethyl decanoate	0.36 ± 0.09	0.15 ± 0.02	0.23 ± 0.01	0.21 ± 0.04	0.29 ± 0.02	0.30 ± 0.02	0.12 ± 0.05	a, f
2-phenylethyl acetate	0.38 ± 0.03	0.09 ± 0.01	0.18 ± 0.02	0.21 ± 0.05	0.11 ± 0.01	0.23 ± 0.01	024 ± 0.11	a–e

[a] SD = significant differences. [b] nd= not detected. [c] ns = not significant.

Table 3. Aromatic Compounds (Milligrams per Liter) in Finished Wines by a Selected Strain of *S. cerevisiae* to Fungicide Supplementation in Filtered Must (Mean Values and SD, $n = 3$ Replicates)

	winemaking							
compound	blank	cyprodinil 1 (a)	cyprodinil 5 (b)	fludioxonil 1 (c)	fludioxonil 5 (d)	pyrimethanil 1 (e)	pyrimethanil 5 (f)	SD,[a] $p \leq 0.05$
acids								
2-methylpropanoic	nd	7.74 ± 1.92	5.58 ± 1.60	5.14 ± 1.49	2.38 ± 0.15	4.16 ± 0.57	7.78 ± 1.23	a–f
3-methylbutanoic	0.10 ± 0.01	nd[b]	0.76 ± 0.20	0.95 ± 0.31	nd	nd	nd	a–f
octanoic	1.69 ± 0.01	5.37 ± 1.32	3.56 ± 1.52	6.15 ± 0.97	1.75 ± 0.19	1.44 ± 0.07	5.51 ± 0.45	a–c, f
decanoic	0.60 ± 0.01	1.74 ± 0.58	0.77 ± 0.41	2.74 ± 0.29	0.89 ± 0.23	0.69 ± 0.26	1.25 ± 0.49	a, c
alcohols								
isoamylic	50.13 ± 0.5	60.8 ± 9.17	56.91 ± 8.2	44.1 ± 11.5	42.8 ± 0.76	45.05 ± 3.9	59.52 ± 6.8	ns[c]
1-hexanol	0.19 ± 0.01	0.38 ± 0.12	0.26 ± 0.09	0.44 ± 0.11	0.28 ± 0.01	0.21 ± 0.01	0.25 ± 0.02	a, c–f
(*Z*)-3-hexen-1-ol	0.06 ± 0.01	0.19 ± 0.04	0.10 ± 0.04	0.01 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	nd	a–c, f
3-(methylthio)propan-1-ol	0.31 ± 0.02	1.34 ± 0.57	1.10 ± 0.36	0.96 ± 0.35	0.40 ± 0.06	0.97 ± 0.31	0.32 ± 0.02	a–c, e
2-phenylethanol	9.74 ± 0.91	26.60 ± 7.4	22.64 ± 0.3	24.66 ± 6.5	11.38 ± 1.9	19.21 ± 1.2	24.7 ± 1.24	a–c, e, f
esters								
ethyl acetate	40.9 ± 0.57	39.0 ± 7.83	38.2 ± 0.98	53.1 ± 10.6	47.7 ± 0.67	29.36 ± 4.5	40.3 ± 21.6	ns
ethyl butyrate	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	b, d
isoamyl acetate	0.06 ± 0.01	0.11 ± 0.01	0.19 ± 0.05	0.11 ± 0.01	0.28 ± 0.02	0.07 ± 0.02	0.07 ± 0.04	a–d
ethyl hexanoate	0.72 ± 0.04	0.13 ± 0.01	0.21 ± 0.06	0.16 ± 0.03	0.28 ± 0,02	0.12 ± 0.07	0.16 ± 0.05	a–f
hexyl acetate	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0,01	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	ns
ethyl octanoate	0.90 ± 0.01	0.66 ± 0.01	0.60 ± 0.04	0.57 ± 0.09	0.75 ± 0.03	0.63 ± 0.02	0.67 ± 0.12	a–f
ethyl decanoate	0.34 ± 0.02	0.15 ± 0.02	0.18 ± 0.04	0.27 ± 0.04	0.28 ± 0.03	0.15 ± 0.01	0.25 ± 0.07	a, b, e
2-phenylethyl acetate	0.32 ± 0.01	0.34 ± 0.07	0.38 ± 0.03	0.28 ± 0.07	0.24 ± 0.02	0.29 ± 0.01	0.31 ± 0.06	ns

[a] SD = significant differences. [b] nd = not detected. [c] ns = not significant.

differences found, and in the majority of the cases it was not possible to detect its presence.

The results obtained for octanoic acid (with a smell of rancid butter) show that the wines fermented in the presence of *S. cerevisiae* and with the addition of fungicides are those which present the greatest differences with respect to the blank (**Table 3**). There were no significant differences in the assays inoculated with *S. cerevisiae* (syn. *bayanus*) (**Table 2**), whereas with *S. cerevisiae* (syn. *uvarum*) differences appear with only the fludioxonil and pyrimethanil fungicides and at the higher dose (**Table 4**).

All of the fungicides studied produce a decrease in the decanoic acid concentrations with respect to the blank in the assays inoculated with *S. cerevisiae* (syn. *uvarum*) (**Table 4**). In contrast, in the assays with *S. cerevisiae* (syn. *bayanus*) and *S. cerevisiae* the cyprodinil and fludioxonil fungicides produce

Table 4. Aromatic Compounds (Milligrams per Liter) in Finished Wines by a Selected Strain of *S. cerevisiae* (Syn. *uvarum*) to Fungicide Supplementation in Filtered Must (Mean Values and SD, $n = 3$ Replicates)

	winemaking							
compound	blank	cyprodinil 1 (a)	cyprodinil 5 (b)	fludioxonil 1 (c)	fludioxonil 5 (d)	pyrimethanil 1 (e)	pyrimethanil 5 (f)	SD,^{a} $p \leq 0.05$
acids								
2-methylpropanoic	1.10 ± 0.07	nd^{b}	nd	nd	1.67 ± 0.02	2.38 ± 0.16	1.79 ± 0.58	a–e
3-methylbutanoic	nd	nd	nd	nd	0.44 ± 0.21	nd	nd	d
octanoic	1.92 ± 0.03	2.32 ± 0.87	1.54 ± 0.96	1.26 ± 0.03	0.62 ± 0.57	1.07 ± 0.19	0.70 ± 0.08	d, f
decanoic	1.62 ± 0.03	0.88 ± 0.34	1.07 ± 0.78	0.60 ± 0.15	0.28 ± 0.10	0.60 ± 0.13	0.28 ± 0.12	a, c–f
alcohols								
isoamylic	3.72 ± 0.02	41.1 ± 2.5	28.78 ± 9.1	37.8 ± 4.37	47.03 ± 0.5	29.7 ± 2.95	33.1 ± 13.3	a–f
1-hexanol	0.44 ± 0.03	0.36 ± 0.12	0.30 ± 0.13	0.68 ± 0.05	0.65 ± 0.04	0.56 ± 0.04	0.49 ± 0.16	b–e
(*Z*)-3-hexen-1-ol	nd	0.13 ± 0.04	0.10 ± 0.08	0.06 ± 0.04	0.06 ± 0.03	0.01 ± 0.00	0.06 ± 0.04	a, b
3-(methylthio)propan-1-ol	nd	nd	nd	nd	0.62 ± 0.21	nd	nd	d
2-phenylethanol	8.40 ± 0.31	14.32 ± 2.8	10.74 ± 3.8	9.95 ± 0.56	8.69 ± 0.90	12.3 ± 1.58	9.78 ± 2.35	a, e
esters								
ethyl acetate	53.7 ± 0.61	64.8 ± 3.4	62.0 ± 6.7	57.2 ± 15.0	50.8 ± 11.5	65.85 ± 1.8	62.1 ± 10.2	ns^{c}
ethyl butyrate	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	0.07 ± 0.04	0.05 ± 0.02	0.03 ± 0.01	ns
isoamyl acetate	0.07 ± 0.01	0.12 ± 0.03	0.11 ± 0.05	0.13 ± 0.05	0.18 ± 0.09	013 ± 0.04	0.12 ± 0.02	d
ethyl hexanoate	0.24 ± 0.06	0.13 ± 0.03	0.16 ± 0.05	0.07 ± 0.02	0.11 ± 0.01	0.08 ± 0.01	0.07 ± 0.02	a–f
hexyl acetate	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	ns
ethyl octanoate	0.15 ± 0.02	0.18 ± 0.03	0.15 ± 0.05	0.14 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.18 ± 0.03	ns
ethyl decanoate	0.05 ± 0.01	0.07 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.02	ns
2-phenylethyl acetate	0.02 ± 0.00	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	ns

[a] SD = significant differences. [b] nd = not detected. [c] ns = not significant.

higher levels than in the blank and, hence, just as occurs with the values found for octanoic acid, the indication is that the presence of these products is not going to affect the sensorial quality of the wine in an unfavorable way. The decrease in the levels of both acids in the assays with *S. cerevisiae* (syn. *uvarum*), as compared to the control, may be due to the fact that this physiological race is most affected by the presence of the fungicides. Thus, the initial biocide effect produced by these products leads to a lower number of viable yeasts being obtained in the exponential phase and, hence, to a lower production of these aromatic compounds. (*33*).

The volatile acid levels are in all cases below the threshold perception level (13 and 10 mg/L for octanoic and decanoic acids, respectively) (*34*). The concentrations of these acids found in our wines are similar to those reported by other researchers in white wines (*34*).

Other works on the effects on the acidic fraction of the aroma of pesticide residues, such as the antibotritic fungicides carbendazim, dichlofuanid, iprodione, procymidone, and vinclozolin (*20*) and fenarimol, penconazole, metalaxyl, mancozeb, vinclozolin, and chlorpyrifos (*23*), conclude that no significant differences exist between the wines with the addition of antibotritic fungicides and the blank. Although in the experiment performed by Oliva et al. (*23*) analytical differences exist in the assays with mancozeb and metalaxyl residues, these do not negatively affect the sensorial quality of the wines.

Alcohols. The capacity to produce volatile alcohols is a common characteristic of all yeasts, but the quantity varies according to the genus, species, and strain. It is, moreover, a hereditary trait and can be used in genetic improvements (*35*). These compounds are formed in the yeast cells and from their precursor amino acids and are then transferred to the wine. Thus, the content of these compounds is closely related to those factors that affect fermentation, such as the variety of grape, yeasts, sugars, fermentation temperature, and presence of pesticides (*21*−*23*, *26*, *34*, *36*−*40*).

In small quantities alcohols have a positive effect on the quality of the wine, but in large quantities they are considered to be unpleasant compounds and may drastically decrease the aromatic quality of the wine (*30*, *41*). Of the five alcohols identified, only 2-phenylethanol bestows positive qualities on the wines. Isoamylic alcohols (2-methyl-1-butanol and 3 methyl-1-butanol) contribute to a greater extent to the intensity of the smell rather than to the quality of the aroma (*1*, *42*), whereas the C$_6$ [1-hexanol and (*Z*)-3-hexen-1-ol] alcohols give the wine a herbaceous and astringent character (*1*, *43*). 3-(Methylthio)-propan-1-ol produces a negative aroma with a smell similar to that of boiled cauliflower, which arises following the addition of sulfur to the must (*44*).

The data obtained show that in the assays with *S. cerevisiae* (syn. *bayanus* and syn. *uvarum*) the pesticides studied produce, with both doses, an increase in the concentrations of isoamylic alcohols with respect to the blank. All of the values are above the perception threshold (7 mg/L) (*1*), a fact which indicates that the presence of the fungicides during fermentation causes a decrease in the quality of the wine. The behavior observed suggests that for the yeast *S. cerevisiae* (**Table 3**) no significant differences exist. This is due to the fact that the fungicides studied do not affect the fermentative kinetics. The values obtained in our winemakings range from 14.73 to 74.39 mg/L, below the range in the literature of 20−400 mg/L (*24*, *34*). These low values are due to the fact that the Airen variety produces wines of low aromatic content (*45*).

The final 1-hexanol levels show significant differences with respect to the control for all of the products and yeasts used. However, they are of an analytical type and could never be detected by a panel of wine-tasters because the perception threshold stands at 6.2 mg/L for 1-hexanol and the maximum values reached for this alcohol are 0.76 mg/L. Nevertheless, the presence of cyprodinil in both doses and for the three yeasts increases the (*Z*)-3-hexen-1-ol level to above the perception threshold (0.07 mg/L) (*34*). Hence, the presence of this fungicide during fermentation produces lower quality wines because it is this alcohol that is chiefly responsible for the herbaceous smell in wines.

From the results for 3-(methylthio)propan-1-ol we can observe that of all the wines obtained, the only one to be affected from a sensorial viewpoint would be that obtained from the addition of *S. cerevisiae* (syn. *bayanus*) and cyprodinil (5 ppm) (**Table 2**), because this value is above the olfactory perception threshold (4 mg/L). In the other assays in which significant differences are observed, values never exceed the threshold cited.

With all of the fungicides an increase in 3-(methylthio)propan-1-ol is produced with respect to the blank for the assays inoculated with *S. cerevisiae* (syn. *bayanus*) and *S. cerevisiae*. This increase is independent of the dose of the fungicide added. The highest level of this alcohol is produced in assays inoculated with *S. cerevisiae* (syn. *bayanus*), which indicates that this yeast produces inferior quality wines.

Because sulfite has not been added to the must, the appearance of this alcohol may be due to the presence of sulfur in the harvested grape. As the grape used is ecological, no organic synthesis chemical can be used, and only treatments with inorganic products such as sulfur and copper may be used to prevent diseases.

2-Phenylethanol bestows a very agreeable and heavy aroma of flowers (old roses). This is the aroma perceived in many wines once the glass is empty. Its perception threshold stands at 7.5 mg/L according to Salo (*46*). In our study the values obtained in all of the assays exceed the perception threshold and the levels of the blank and, hence, there is no negative effect on the quality of the aroma from these fungicides.

The final values of this alcohol found in our study are lower than the averages reported by other researchers in white wines (35 mg/L) (*34*).

In contrast, studies by Aubert et al. (*21*) on the effects of fluxilazole on the aromatic fraction of the wine highlight its effect on the levels of C_6 compounds and isoamylic alcohols. The same study reports a decrease in the levels of 1-hexanol, (*Z*)-3-hexen-1-ol, 2-phenylethanol and isoamylic alcohols, which increases as the treatment dose is increased, with the suggestion that this may be due to the effect of the fluxilazole on the metabolism of the yeasts. This fungicide, which belongs to the family of triazoles, is known to be an inhibitor of sterols biosynthesis (*47*−*51*).

Another study by Oliva et al. (*23*) finds no significant differences for these compounds between the blank and those to which the pesticides fenarimol, penconazole, metalaxyl, mancozeb, vinclozolin, and chlorpyrifos were added.

Esters. These constitute the major group of volatile compounds that contribute to the aroma of the wine. Most of them are already present in the grape and are partially extracted in the must (*4*, *52*). The acids and alcohols in free state react together to form esters, but the reaction is both slow and reversible in an aqueous medium. Esters are also formed during alcoholic fermentation and in the aging of wines (*1*, *2*, *53*, *54*). The principal esters are formed by yeasts through enzymatic formation of between free alcohols and the acil-S-CoA (*55*).

Some of the main factors that influence the concentration of esters in wines are the variety of grape (*4*, *56*), fermentation conditions (*16*, *57*, *58*), aging (*19*, *59*), and phytosanitary treatments of the grape (*20*−*23*).

Of the eight compounds identified in our study (ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, and 2-phenylethyl acetate), ethyl acetate is the most important. Furthermore, it plays an important role in the quality of the aroma because at concentrations >160 mg/L it is unpleasant and is responsible (rather than acetic acid) for the characteristic smell of pricked wines (*60*−*62*). It is not distinguishable at lower concentrations, but it contributes to the rough character of red wines, whereas in whites it bestows a rough, astringent element. In lower measures, <80 mg/L, it contributes to the pleasant smell of the wine (*63*).

From the results obtained for this ester it is observed that only in those assays inoculated with *S. cerevisiae* (syn. *bayanus*) do levels exceed 80 mg/L, the maximum acceptable value for optimum quality wines. These high levels may be due to the accidental development of the oxidative yeasts, which do not modify the volatile acidity, or that of bacteria which oxidize the ethanol to acetic acid. In both cases, unpleasant sensations of glue or pricked wine are produced (*34*).

For some researchers the biosynthesis of ethyl acetate is inversely related to the biosynthesis of lipids in *S. cerevisiae*, because if the latter stops, an increase of the ester is produced (*64*). Hence, as *S. cerevisiae* is, in our study, the least affected by the presence of fungicides, the final values are the lowest for the three yeasts.

In the case of ethyl butyrate, all levels are below the perception threshold (0.4 mg/L) (*34*).

Isoamyl acetate, with a banana aroma, gives values below the perception threshold (0.2 mg/L) in all of the winemakings inoculated with *S. cerevisiae* (syn. *bayanus*) and, moreover, for all of the fungicides studied. The presence of pesticide residues leads to an increase in isoamyl acetate and affects the aromatic quality negatively because it is too fruity an aroma and bestows an unpleasant hint on the wine (*65*). The levels of the other two yeasts are below the perception threshold.

Ethyl hexanoate (with a smell of apples, violets, and green fruit), ethyl octanoate (pineapple and pear), and ethyl decanoate (pineapple) strongly affect the aromatic character of the young wines (*66*).

Significant differences exist in the case of ethyl hexanoate for the three yeasts used in all of the assays with the addition of the fungicides studied. A decrease occurs in the final level of this compound with respect to the blank. Differences also exist for ethyl octanoate in the assays inoculated with *S. cerevisiae* and for ethyl decanoate in those inoculated with *S. cerevisiae* (syn. *bayanus*) and *S. cerevisiae*.

In general, the total levels of the three esters decrease with respect to the control when the fungicides are added, and this leads to less fruity wines.

The levels found for the three esters are situated within the range (0−3.4 mg/L) reported by other researchers into white wines (*31*, *34*).

Finally, in the case of hexyl acetate (cherries and pears) no significant differences exist between the different assays. With 2-phenylethyl acetate (roses and violets) differences occur only for assays inoculated with *S. cerevisiae* (syn. *bayanus*) with addition of the three fungicides, when the level of the compound is reduced to half. However, these analytical differences do not affect the sensorial quality as the perception threshold stands at 0.65 mg/L.

Other studies on the effects of other pesticides on the esters in the aromatic fraction indicate that fungicides of the family of triazoles affect the levels of these compounds in the wines (*21*, *22*).

Insecticides such as chlorpyrifos also lead to higher ethyl acetate contents, which may be due to the type of nitrogen composition that it may confer on the must. When used by the yeasts, it has a negative effect on the final sensorial quality of the wine. Furthermore, the isoamyl acetate levels are higher in winemakings with traces of chlorpyrifos residues, and to a lesser extent in the cases of fenarimol and vinclozolin, and hence they confer an excessively fruity hint to the wines (*23*).

Wines obtained in the presence of dichlofuanid produce an increase in the ethyl acetate and a decrease in overall ester levels (*20*).

In conclusion, the results obtained indicate that although significant differences are produced between the winemakings with fungicides and the blank, these do not affect the sensorial quality of the wine in the majority of the aromatic compounds studied, because the final levels do not exceed the perception threshold.

The lowest quality wine is that obtained by inoculation with *S. cerevisiae* (syn. *bayanus*) and addition of cyprodinil in the larger dose.

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