Effect of Pesticide Residues on the Aromatic Composition of Red Wines

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The influence of pesticide residues on the aromatic composition (major and minor volatiles) of red wines made from *Vitis vinifera* (Monastrell var.) was studied by comparing the concentration of aromas in wines made from grapes subjected (or not) to phytosanitary treatment with chlorpyrifos, fenarimol, mancozeb, metalaxyl, penconazole, and vinclozolin, according to the agricultural practice of the area. The analytical determination of the major volatiles was made by gas chromatography using a flame ionization detector, while the minor volatiles were determined by adsorption−thermal desorption gas chromatography using a mass selective detector. There were significant differences between the ethyl acetate, methanol, isobutanol, and diethylacetal levels of the control wine and that containing chlorpyrifos residues, although only the ethyl acetate exceeded the olfactory threshold. With regard to the minor volatiles, significant differences were detected in the concentrations of some esters, aldehydes, and acids. However, only isoamyl acetate exceeded the olfactory threshold in wines containing residues of chlorpyrifos, fenarimol, and vinclozolin.

Keywords: *Aromatic compounds; pesticide residues; red wine*

INTRODUCTION

The smell or fragance of grape wine is one of the most important organoleptic characteristics and may be the reason for a wine being accepted or rejected. It therefore constitutes an interesting index for evaluating a wine's quality, which is based fundamentally on its aromatic fraction.

Wine aroma, which is made up of more than six hundred compounds of different chemical families (Etievant, 1991), can be classified into three groups (Cordonnier and Bayonove, 1981): primary aroma (varietal and prefermentative), which comes from the grape must; secondary or fermentative aroma, a group of volatile substances that appear during fermentation; and tertiary or postfermentative aroma, which develops during the aging and conservation of wines.

Any determination of the chemical nature of a varietal aroma is fraught with difficulties. The first step usually involves the isolation of grape and wine volatile components, while the second step involves analysis by means of gas chromatography−mass spectrometry (Sharpe and Chappell, 1991). Such a study is made easier if the crucial compound(s) occur(s) in volatile form in both grapes and wine. However, aroma compounds in wine may often exist in nonvolatile forms in the grapes. In addition, varietal aromas may originate from a particular combination of compounds, not a single unique compound. Extracting procedures can also influence the stability and isolation of potentially important compounds. When compounds of likely importance are isolated, they need to be identified and quantified. Only by comparing the concentration found in wine with its sensory threshold can the relative importance of a compound be assessed.

With regard to their comparative importance in aroma production, volatile ingredients have been classified as impactant (those that have a marked and distinctive effect on wine fragance), contributive (those that contribute to the overall complexity of the fragance of a wine), and/or insignificant (those without relevance on wine fragrance) (Jackson, 1994).

The evaluation of wine quality depends, to a great extent, on the consumer's experience and is often a very subjective process. However, quality does have components that can be to a greater or lesser extent quantified, although negative quality factors are generally easier to identify than positive quality factors, which tend to be more elusive. Some of the most obvious factors are the distinctive aromas derived from certain grape varieties, while recognizable modifications produced by viticultural practices, climate, wine making style, processing, and/or aging are also highly regarded. However, when too accentuated, these same features may be considered faults. The influence of these and other factors on wine quality has been studied by many researchers during recent years (Wilson et al., 1984; Bertrand and Torres-Alegre, 1984; Webster et al., 1993; Razungles et al., 1993; Salinas, 1996; Belancic, 1997; López-Tamames et al., 1997; Santos, 1997; Casp et al., 1998), although data in the scientific literature on the possible influence of pesticide residues on the aromatic composition of wines are scarce.

Partly for this reason, we have carried out a study of the influence that phytosanitary treatment may have on the wine aroma, not only as regards any interference

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Table 1. Mean Values ($n = 3$ Replicates) of General Parameters Found in Finished Wines (Given as $\bar{x} \pm$ SD)

parameter	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
density	994.3 ± 0.57	994.0 ± 0.00	995.0 ± 0.00	994.0 ± 0.00	995.3 ± 0.57	995.0 ± 0.00	994.6 ± 0.57	994.0 ± 0.00
pH	3.21 ± 0.06	3.22 ± 0.11	3.25 ± 0.13	3.22 ± 0.10	3.34 ± 0.17	3.16 ± 0.09	3.36 ± 0.16	3.35 ± 0.12
acidity[a]	7.59 ± 0.04	6.89 ± 0.49	8.19 ± 0.18	8.12 ± 0.16	8.12 ± 0.23	8.12 ± 0.16	8.17 ± 0.41	7.97 ± 0.64
volatile acidity	0.33 ± 0.06	0.12 ± 0.03	0.27 ± 0.05	0.17 ± 0.05	0.26 ± 0.08	0.25 ± 0.02	0.28 ± 0.05	0.26 ± 0.01
alcoholic grade	13.66 ± 0.55	12.90 ± 0.75	13.36 ± 0.73	13.43 ± 0.06	14.10 ± 0.20	13.23 ± 0.38	13.10 ± 0.61	14.13 ± 0.45
fermentation days	15.33 ± 1.52	11.00 ± 0.00	14.66 ± 2.88	12.00 ± 1.73	16.66 ± 4.04	14.00 ± 1.73	16.00 ± 2.64	15.66 ± 1.15

[a] As tartaric acid, g/L.

in the biological process of vine growth but also concerning the effect on typical biochemical processes during berry ripening. It is also necessary to keep in mind the influence that pesticide residues in harvested grapes might have on fermentation, because any interruption or delay in this process might alter the qualitative and quantitative distribution of the aromatic components of a wine. For this, we compared the aromatic composition (major and minor fractions) of red wines obtained from untreated grapes (blank), grapes treated during the vegetative period according to the coomon practice of the area (classic), and finally, grapes only treated the day before harvesting with an insecticide (chlorpyrifos) or five fungicides (fenarimol, mancozeb, metalaxyl, penconazole, and vinclozolin) commonly used by the vine growers of the Jumilla Appellation d'Origine in southeast Spain.

MATERIAL AND METHODS

Phytosanitary Treatments and Sampling. The experimental plots were situated in Jumilla, Murcia (southeast Spain) in a 15 year old plantation of *Vitis vinifera* (Monastrell variety) in full production. The vine-stocks had a plantation density of 2.5 × 2.5 m^2 and were in perfect nutritional and physiological condition. In September 1996, we carried out the phytosanitary treatments (three replications) with Topas, Rubigan, Ridomil Plus, Ronilan, Dursban, and Ventine as it is specified in a previous paper (Navarro et al., 1999). The samples were taken 24 h after the application.

On the other hand, during the 1996 vegetative period, the vine of the "classic" plot received three phytosanitary treatments. The active ingredients applied were fenarimol, trichlorfon, and metiram (June 2), fenarimol, trichlorfon, and captan (July 17), and captan (September 4).

Must and Wine Production. The grapes coming from each phytosanitary treatment (three replicates), once crushed, were introduced into independent vessels of fermentation. Wine-making was carried out according to the scheme shown in a previous paper (Navarro et al., 1999). In Table 1, the general parameters (density, pH, total and volatile acidity, alcoholic content, and fermantation days) of the finished wines are shown.

Analysis of Pesticide Residues. All the studied compounds were extracted from grapes, must, and wine by means of on-line microextraction, and their identification was confirmed by GC with EC and MS detectors according to the methodology described by Oliva et al. (1999) except for mancozeb, whose extraction and analysis were carried out following the method proposed by Keppel, 1971.

Analysis of Volatile Compounds. For sample conservation, 0.2 g of NaF (disinfectant) and 0.2 g of ascorbic acid (antioxidant) were added to 400 mL of decanted wine, which was then shaken until total dissolution of the added compounds. The samples were placed in a freezer (−30 °C) and kept at this temperature until used for analysis.

Aromatic analytical standards, at least 97% pure, were purchased from Sigma-Aldrich. Several dilutions were used to check the linearity of the response of the detector, in accordance with the methods used for determining major and minor volatiles. In all cases, the coefficients of lineal correlation were >0.9 and the coefficients of variability were <10%.

Highly Volatile Compounds (Major Volatiles). These compounds were determined through direct injection of the wine into a gas−liquid chromatograph with flame ionization detector (FID), following the method described by Huerta et al. (1995), in which, 1 mL of a 10% (v/v) solution of 3-pentanol (internal standard) in ethanol is diluted until 10 mL with the sample of wine. This sample is shaken until complete homogenization and then injected directly without previous distillation. The process was carried out in a cold chamber at 10 °C. A Perkin-Elmer OP-100 integrator was used in combination with the gas chromatograph (Perkin-Elmer 8310), which was provided with a PTV injector. A micropack column packed with 4% Carbowax 300 + bis-2-ethylhexylsebacate (92/8) on Volaspher A2 (100−120 μm) of 5 m length and 0.85 mm i.d. was used. The operating temperatures were as follows: initial PTV injector temperature, 100 °C; programming rate, 12 °C/min (100 to 40 °C); detector, 200 °C; column oven, initial, 33 °C, hold, 8.4 min, programming rate, 1.5 °C/min (33−70 °C), hold, 2 min, 4 °C/min (70−90 °C), and hold 10 min at 90 °C. The carrier gas was He 9.8 mL/min; detector gases, hydrogen 30 mL/min and air, 300 mL/min. Injection volume, 3 μL with split valve closed.

Low-Volatility Compounds (Minor Volatiles). A 1% (v/v) solution of methyl caprilate (internal standard) in ethanol in the amount of 3 μL was added to 50 mL of decanted wine. The minor compounds were then extracted and concentrated by adsorption−thermal desorption according to the method proposed by Salinas et al., 1994. The volatiles were isolated by purging with helium for 20 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 provided with a HP 5971 mass-selective detector (MSD). A 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, 8 min. The carrier gas was He at 22.6 psi. The sample was injected in 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), hold 2 min, to 200 °C and hold for 10 min.

Statistics. The descriptive statistics and nonparametric analysis of variance used to determine the relation between pesticide residues and the aroma concentration of the wines corresponded to SPSS, version 7.5 for Windows (Norusis, 1997).

RESULTS AND DISCUSSION

Only residues of captan (0.08 mg/kg) were detected in the crushed grapes from the classic plot. However, after pressing, no residues of this compound were detected. On the other hand, the evolution of residual levels during wine-making of the six compounds applied the day before havesting, have been published previously (Navarro et al., 1999).

Tables 2−6 show the mean values of the principal aromatic components (major and minor) found in the racked wines in the eight experiments carried out. Table 7 depicts the compounds showing significant differences

Table 2. Mean Values ($n = 3$ Replicates) of Major Volatiles (mg/L) Found in Finished Wine (Given as $\bar{x} \pm$ SD)

compounds	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
methyl acetate + ethyl formiate	23.01 ± 8.18	25.99 ± 5.07	16.13 ± 2.10	24.39 ± 8.63	23.56 ± 1.92	25.58 ± 8.32	24.56 ± 0.32	20.47 ± 4.22
ethyl acetate	82.97 ± 6.27	161.52 ± 33.89	159.20 ± 54.6	69.82 ± 12.70	66.18 ± 4.58	56.30 ± 12.53	73.38 ± 31.82	59.74 ± 6.81
diethylacetal	*a*	*a*	0.91 ± 0.15	1.31 ± 0.13	0.17 ± 0.30	0.22 ± 0.39	*a*	0.25 ± 0.43
methanol	54.52 ± 20.57	34.64 ± 7.41	78.93 ± 10.76	107.13 ± 34.91	61.50 ± 8.11	68.19 ± 6.55	57.49 ± 12.50	67.65 ± 3.09
1-propanol	35.36 ± 3.57	34.64 ± 4.85	34.89 ± 1.09	30.91 ± 4.76	40.64 ± 3.11	37.91 ± 2.48	35.67 ± 1.02	31.84 ± 1.47
isobutanol	66.87 ± 6.40	48.94 ± 1.84	63.61 ± 3.67	60.27 ± 10.84	69.25 ± 2.86	63.94 ± 3.86	70.75 ± 2.39	72.42 ± 3.41
1-butanol	0.75 ± 0.06	0.64 ± 0.07	0.56 ± 0.19	0.55 ± 0.12	0.73 ± 0.19	0.50 ± 0.17	0.74 ± 0.14	0.79 ± 0.10
2-methyl-1-butanol	18.68 ± 0.63	19.22 ± 0.97	16.92 ± 1.94	21.54 ± 3.02	21.67 ± 2.54	17.53 ± 1.84	15.63 ± 1.23	17.49 ± 1.90
3-methyl-1-butanol	84.05 ± 1.06	87.32 ± 4.30	76.16 ± 10.22	102.52 ± 18.75	89.24 ± 2.70	81.83 ± 9.68	73.26 ± 5.86	85.70 ± 7.86
∑ mg/L	367.21	412.91	447.31	408.44	372.94	352	351.48	356.35

[a] Not detected.

Table 3. Mean Values ($n = 3$ Replicates) of Minor Volatiles (Aldehydes, μg/L) Found in Finished Wine (Given as $\bar{x} \pm$ SD)

compounds	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
2-octanal	0.9 ± 0.2	0.23 ± 0.05	0.76 ± 0.05	0.83 ± 0.05	1.0 ± 0.20	0.86 ± 0.11	0.83 ± 0.15	1.0 ± 0.10
decanal	3.93 ± 0.90	2.50 ± 2.02	3.13 ± 0.21	2.96 ± 0.11	3.90 ± 1.49	2.83 ± 0.28	2.93 ± 0.05	3.26 ± 0.30
benzaldehyde	8.93 ± 3.26	4.2 ± 0.87	5.96 ± 2.03	6.23 ± 1.13	13.80 ± 3.29	7.80 ± 3.30	8.56 ± 4.39	12.36 ± 7.66
∑ μg/L	13.76	6.93	9.85	10.02	18.7	10.59	12.32	15.66

Table 4. Mean Values ($n = 3$ Replicates) of Minor Volatiles (Acids, μg/L) Found in Finished Wine (Given as $\bar{x} \pm$ SD)

compounds	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
isobutyric acid	1946.7 ± 756.5	1193.3 ± 257	2313.3 ± 496.6	1573.3 ± 274.7	2610 ± 1619.7	2630 ± 251.6	3176.7 ± 493.6	2430 ± 285.8
isovaleric acid	230 ± 65.6	276.6 ± 64.3	253.3 ± 11.5	323.3 ± 51.3	216.7 ± 23.1	350 ± 95.4	326.7 ± 51.3	260 ± 45.8
hexanoic acid	10 ± 1.28	163.3 ± 15.3	20 ± 10	26.7 ± 11.5	23.3 ± 15.3	33.3 ± 32.1	33.3 ± 5.8	20 ± 0.1
heptanoic acid	*a*	7.6 ± 5.8	*a*	*a*	*a*	*a*	*a*	*a*
octanoic acid	76.7 ± 11.5	220 ± 144.2	80 ± 26.5	73.3 ± 30.6	86.7 ± 20.8	66.7 ± 15.3	143.3 ± 25.2	66.7 ± 11.5
decanoic acid	140 ± 26.5	266.7 ± 66.6	146.7 ± 15.3	236.7 ± 115.9	216.7 ± 15.3	350 ± 260.6	193.3 ± 49.3	153.3 ± 32.1
∑ μg/L	2403.4	2127.6	2813.3	2233.3	3153.4	3430	3873.3	2930

[a] Not detected.

($P \leq 0.05$) in the seven wines with pesticide residues compared with the control wine.

Major Volatiles. Methanol, isobutanol, ethyl acetate, and diethylacetal levels present significant differences in some of the wines, while the other compounds show no significant differences at the probability level used.

The concentrations of methyl acetate and ethyl formiate lie within the range considered normal for red wines, although their values are among the highest found in the bibliography (González Raurich et al., 1985a; Pardo, 1995).

The concentration of ethyl acetate in our samples ranged from 59.74 to 83.97 mg/L in six of the eight wines. Concentrations above 150 mg/L are associated with wines of poor quality (Ribéreau-Gayon, 1978). The wines made with our "classic" grapes and those containing chlorpyrifos residues have higher concentrations of ethyl acetate than this threshold value. Such high values may stem from the accidental growth of oxidative yeasts which do not modify volatile acidity or from bacteria which oxidize the ethanol to acetic acid. Whatever the case, the wines have an off taste or taste of adhesive (Dubois, 1994a). The "classic" wines have a lower alcohol and volatile acidity levels than the other wines, suggesting that the ethanol may have been oxidized to acetid acid, which in turn has been esterified to give ethyl acetate. The high concentration of ethyl acetate in the wine containing chorpyrifos residues may be due to the increased quantity of nitrogen which this compound provides to feed the yeasts since it is derived from pyridine.

Only very low levels of diethylacetal were found, and in the control, "classic", and wines containing residues of penconazole it was not found at all. The highest concentration was found in the samples with the highest alcohol content.

Although methanol plays little role in the development of aroma, it has been widely studied for its alcoholic effect (Lee et al., 1975). Its concentration depends on the length of time the skins are left to macerate with the must and varies from 36 to 350 mg/L (Ribéreau-Gayon et al., 1980). The levels of methanol recorded in all the wines studied were within this normal range. Since the maceration time was the same in all cases, the higher concentrations found in the wine containing fenarimol residues may have been due to the greater activity of the enzyme pectinmethylesterase in their presence.

The major alcohols determined were: isoamylics (2-methyl-1-butanol, 3-methyl-1-butanol), 1-propanol, isobutanol (2-methyl-1-propanol), and 1-butanol. Some authors maintain that small quantities of major alcohols have a positive influence on wine quality, although excessively high levels may have the adverse effect (Bidan, 1975; Bertrand, 1981; Etievant, 1991). For example, Rapp and Mandery (1986) state that concentrations above 300 mg/L confer an unpleasant taste and Dubois, 1994a, suggests that a total concentration of 200

Table 5. Mean Values (n = 3 Replicates) of Minor Volatiles (Esters, μg/L) Found in Finished Wine (Given as $\bar{x} \pm$ SD)

compounds	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
isoamyl acetate	6.0 ± 10.3	*a*	266.3 ± 20.8	273.3 ± 101.2	45.1 ± 7.8	200 ± 40	40 ± 6.9	266.7 ± 30.6
ethyl hexanoate	106.8 ± 16.8	216.7 ± 58.6	97.4 ± 16.4	176.7 ± 61.1	84.5 ± 23.6	96.7 ± 15.3	93.3 ± 15.3	93.3 ± 15.3
hexyl acetate	3.40 ± 0.61	14.5 ± 6.1	7.6 ± 1.6	3.2 ± 0.15	3 ± 1.8	2.6 ± 0.32	2.4 ± 0.06	3.2 ± 0.42
ethyl heptanoate	3.70 ± 1.15	1.23 ± 0.40	3.66 ± 0.51	3.30 ± 0.20	3.30 ± 0.45	3.76 ± 0.80	3.93 ± 1.21	3.30 ± 0.70
ethyl lactate	933.3 ± 156.3	923.3 ± 115.9	893.3 ± 141.9	814.5 ± 60.6	1213.3 ± 146.4	966.7 ± 106.9	1006.7 ± 153.1	980.0 ± 202.2
ethyl octanoate	55.9 ± 7.7	226.7 ± 101.2	75.1 ± 19.7	201.5 ± 107.9	64.0 ± 31.6	68.8 ± 23.8	60.6 ± 15.0	67.1 ± 3.6
3-hydroxyethyl butanoate	150.3 ± 45.4	456.7 ± 130.5	193.2 ± 74.2	371.1 ± 120.2	185.5 ± 72.0	182.30 ± 72.7	157.3 ± 59.4	197.4 ± 49.0
ethyl nonanoate	1.30 ± 0.17	*a*	1.70 ± 0.79	1.53 ± 0.61	1.46 ± 0.90	1.46 ± 0.50	2.16 ± 0.90	1.23 ± 0.15
ethyl 2-furoate	2.53 ± 0.80	2.40 ± 0.75	2.96 ± 0.45	5.80 ± 4.17	2.43 ± 0.23	2.33 ± 0.72	4.23 ± 3.35	1.96 ± 0.15
ethyl decanoate	50.36 ± 26.67	9.46 ± 1.62	0.13 ± 0.05	59.53 ± 37.11	10.46 ± 3.06	12.00 ± 6.75	18.73 ± 4.48	12.90 ± 2.08
ethyl succinate	32.9 ± 5.79	29.63 ± 13.48	16.13 ± 4.39	36.33 ± 20.84	44.73 ± 10.19	29.33 ± 8.77	33.10 ± 6.94	33.90 ± 6.42
ethyl 9-decanoate	1046.7 ± 391.1	7810.0 ± 5843.3	1316.7 ± 533.5	5860.0 ± 3829.0	1790 ± 1712.6	1743.3 ± 976.2	1127.5 ± 271.9	1613.3 ± 265.0
2-phenylethyl acetate	23.90 ± 12.37	3.06 ± 1.65	11.10 ± 0.96	18.70 ± 1.05	23.13 ± 11.15	23.50 ± 1.76	28.73 ± 14.00	15.46 ± 5.79
methyl salicylate	1.56 ± 0.2	2.80 ± 0.43	1.56 ± 0.06	2.26 ± 0.25	1.50 ± 0.26	1.96 ± 0.4	1.90 ± 0.45	1.70 ± 0.36
ethyl dodecanoate	31.17 ± 20.9	96.17 ± 41.37	39.73 ± 16.07	119.77 ± 42.91	44 ± 3.82	27.73 ± 16.56	52.63 ± 10.30	37.63 ± 5.42
$\sum \mu$g/L	2503.81	9792.65	2926.97	7947.52	3516.41	3361.74	2632.81	3329.08

[a] Not detected.

Table 6. Mean Values (n = 3 Replicates) of Minor Volatiles (Alcohols, μg/L) Found in Finished Wine (Given as $\bar{x} \pm$ SD)

compounds	blank	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
1-pentanol	34.23 ± 15.86	37.30 ± 20.97	40.5 ± 6.64	32.4 ± 0.1	43.96 ± 1.89	38.46 ± 17.67	35.53 ± 4.89	29.63 ± 4.06
3-methyl-1-pentanol	12.5 ± 5.69	16.80 ± 6.06	8.46 ± 1.55	13.53 ± 4.84	10.43 ± 0.77	10.00 ± 1.75	12.53 ± 3.45	11.00 ± 2.32
1-hexanol	1370.63 ± 160.63	1585.36 ± 333.69	1106.6 ± 145.39	1189.13 ± 129.22	1226.96 ± 171.14	1265.20 ± 149.21	1338.06 ± 125.00	1021.8 ± 85.51
Z-3-hexen-1-ol	33.33 ± 4.20	47.13 ± 11.32	31.66 ± 7.91	32.30 ± 2.42	30.10 ± 5.19	34.83 ± 6.0	41.03 ± 12.53	37.23 ± 3.04
E-2-hexen-1-ol	11.56 ± 2.20	13.33 ± 1.55	9.73 ± 0.55	9.70 ± 1.51	8.40 ± 1.05	10.76 ± 2.50	11.63 ± 0.32	10.70 ± 0.85
1-octen-3-ol	11.53 ± 2.15	5.26 ± 1.23	10.96 ± 1.24	9.20 ± 2.20	14.06 ± 3.70	12.13 ± 1.25	13.00 ± 3.70	11.93 ± 2.55
1-heptanol	25.30 ± 2.97	63.00 ± 28.21	26.60 ± 2.56	54.20 ± 19.94	39.83 ± 5.60	33.76 ± 4.15	38.73 ± 6.61	28.70 ± 7.13
2-ethyl-1-hexanol	2.20 ± 0.36	1.93 ± 0.25	2.10 ± 0.17	2.00 ± 0.17	2.10 ± 0.30	2.26 ± 0.05	1.96 ± 0.15	2.23 ± 0.15
2-nonanol	0.40 ± 0.01	0.20 ± 0.01	0.36 ± 0.11	0.13 ± 0.05	0.46 ± 0.20	0.26 ± 0.05	0.26 ± 0.05	0.33 ± 0.05
linalool	18.50 ± 4.74	7.40 ± 2.02	15.96 ± 1.02	11.10 ± 4.59	15.56 ± 3.10	19.06 ± 3.50	18.46 ± 1.96	16.46 ± 0.90
1-octanol	9.83 ± 2.44	9.33 ± 2.29	10.06 ± 0.76	11.83 ± 0.65	17.40 ± 8.42	11.16 ± 2.54	13.33 ± 3.92	41.50 ± 4.99
1-decanol	1.76 ± 0.70	3.30 ± 2.16	1.86 ± 0.25	2.16 ± 0.46	2.56 ± 0.81	1.90 ± 0.60	2.33 ± 0.81	2.26 ± 0.83
geraniol	3.90 ± 1.21	2.06 ± 1.06	6.86 ± 0.68	4.23 ± 2.70	4.70 ± 2.39	7.60 ± 3.19	6.43 ± 2.05	5.90 ± 1.21
2-phenyl ethanol	18300 ± 2225.8	15973.3 ± 1302.7	17203.3 ± 678.8	16656.7 ± 638.8	19266.7 ± 1824.3	16430 ± 1441.2	15076.7 ± 2096.4	20133.3 ± 2242.9
nerolidol	1.50 ± 0.70	1.63 ± 0.15	1.16 ± 0.57	1.00 ± 0.1	0.73 ± 0.07	1.60 ± 0.88	1.00 ± 0.20	1.20 ± 0.52
$\sum \mu$g/L	19840	17769.3	18480.6	18031.8	20685.9	17881.3	16613.2	21357

Table 7. Volatile Compounds Showing Significant Differences ($P \leq 0.05$) When the Blank Wine Was Compared with Those Containing Pesticide Residues

	wine-making with pesticides						
aromatic compounds	classic	chlorpyrifos	fenarimol	mancozeb	metalaxyl	penconazole	vinclozolin
			Major Volatiles				
ethyl acetate	*	*					
diethylacetal		*	*	*	*		*
methanol			*				
isobutanol	*						
			Minor Volatiles				
hexanoic acid	*		*			*	*
heptanoic acid	*						
decanoic acid	*			*	*		
isoamyl acetate		*	*				*
hexyl acetate	*	*			*	*	
ethyl decanoate	*	*	*			*	
2-phenylethyl acetate	*						
ethyl dodecanoate			*				
1-octen-3-ol	*		*	*	*	*	
2-ethyl-1-hexanol	*		*				
1-octanol						*	

mg/L of superior alcohols is optimal for the aromatic quality of a wine. The concentration of higher alcohols in our wines was close to this value. Significant differences were observed between the isobutanol content of the "classic" wine (48.9 mg/L) and control (66.8 mg/L). A low concentration of isobutanol in a wine is due to a lower assimilation of the amino acid valine, a precursor of alcohol, by the yeast (MacDonall et al., 1984) or to alterations in the biosynthesis of this amino acid. The fact that the "classic" wine had undergone phytosanitary treatment during ripening of the grapes means that valine biosynthesis may indeed have been affected.

The maximum and minimum concentrations of the higher alcohols observed in our wines do not differ from the values observed by other authors. The ratio between the amylic alcohols (3-methyl-1-butanol/2-methyl-1-butanol) varies from 4.12 in the wine containing mancozeb residues and 4.89 in the wine with vinclozolin residues, values which can be considered normal and which indicate the absence of acetification (González Raurich et al., 1985a). The perception thershold (Salo, 1970) of the isoamylis alcohols (7 mg/L) is substantially exceeded in all the samples studied, while that of isobutanol (75 mg/L) is not reached by any.

Minor Volatiles. The minor volatiles heve been grouped into esters, aldehydes, acids, and alcohols.

Esters. Of the 15 compounds detected in our study, significant differences from the control values were noted in only five: isoamyl acetate, hexyl acetate, ethyl decanoate, phenylethyl acetate, and ethyl dodecanoate. Taken as a whole, the concentrations recorded in finished wines were within the range accepted as normal by other authors (Dubois, 1994b; Salinas et al., 1996; Santos, 1997).

However, significant differences from the control were observed in the wines showing residues of chlorpyrifos, fenarimol, and vinclozolin, all three exceeding the perception threshold of 200 mg/L (Peynaud, 1984). Although the presence of pesticide residues in the wines hindered sensorial analysis, there was a strong smell of banana in the wines treated with the above-mentioned active materials, pointing to a negative influence of the high ester concentration on the aromatic quality of the wines.

There were significant differences between the levels of hexyl acetate noted in the "classic" and chlorpyrifos containing wines and the levels in the control wine, although such differences were quantifiable from an analytical point of view rather than sensorial, since the levels encountered were well below the perception threshold of 2.4 mg/L (Etievant, 1991). Phenylethyl acetate levels were significantly higher in the "classic" wine than in the control wine, in which they were 8 times lower. The above three esters are mainly responsible for the fruity smell of wines (Tamborra, 1991).

Significant differences with the control were found in the ethyl decanoate levels of the "classic" wine and of those containing chlorpyrifos, fenarimol, and penconazole residues. According to Robicheaud and Noble (1990), this volatile and ethyl hexanoate have a strong influence on the aromatic profile of young wines.

Ethyl lactate, which is found in high concentrations in wines which have undergone malolactic fermentation, contributes to the loss of "freshness" in wine, although it cannot be considered as a negative organoleptic factor (Dubois, 1994a). The levels found in our wines indicate that the remains of the pesticides used do not influence malolactic fermentation since there were no differences between the wines as regards this substance.

Aldehydes. No statistical differences were observed in any of the three compounds found (2-octanal, decanal, and benzaldehyde). The concentrations fell within the range of those considered normal for quality wines.

Acids. Isobutyric and isovaleric acids are indices of bacterial activity and hence may be considered factors of poor quality (Bertrand, 1980). No significant differences were found in the wines studied, suggesting thet the insecticides used had no influence on the development of bacteria prejudicial to the organoleptic qualities of the wines elaborated in their presence. The values observed may be considered normal for red wines (Maarse and Visscher, 1989).

Hexanoic, octanoic, and decanoic acids act as quality-enhancing factors in wine-making. Shinohara (1985) studied their contribution to wine aroma and found that concentrations of 4−10 mg/L produced a rounded, smooth taste, while concentrations in excess of 20 mg/L led to an unpleasant taste. They are formed by the action of yeasts, in quantities which are proportional to the number of yeasts in the multiplication phase. In our case, the highest concentration of hexanoic acid was observed in the "classic" wine which had undergone a comparatively rapid fermentation due to the presence of a greater quantity of yeasts in the most intense

fermentation stage (Table 1). Significantly higher levels of this acid were observed in the "classic" wine and in those containing fenarimol, penconazole, and vinclozolin residues.

Significant differences were observed between the concentration of heptanoic acid in the "classic" wine and that in the control, while decanoic acid also showed differences between the control and the wines containing mancozeb and metalaxyl. No significant differences were found for octanoic acid. The levels of all these acids were below those reported by other authors in red wines (Dubois, 1994a; Salinas et al., 1996).

Alcohols. Of the 16 compounds detected, only 1-octanol and 1-octen-3-ol differed significantly in quantity, the former showing significant differences in the control and the wine containing penconazole residues, while the concentrations of the latter differed significantly from the control in the "classic" wine and those fermented in the presence of fenarimol, mancozeb, metalaxyl, and penconazole.

Both octanol and 1-octen-3-ol, are formed during ripening as a result of attack by gray mold and, if present in a high concentration, may be considered as a defect (Dubois, 1994b). However, all the concentrations recorded in our wines were below such a level.

The C_6 alcohols (1-hexanol, *E*-2-hexen-1-ol and *Z*-3-hexen-1-ol) bring herbaceous and astringent characteristics to a wine (Cordonnier and Bayonove, 1981). The low levels of residues in our wines showed no significat differences from the control.

The terpenic alcohols (linalool, genariol, and nerolidol) were also present in low concentrations, which were below the perception threshold in all cases, as was to be expected from wines made from Monastrell, a neutral variety (Salinas, 1998).

CONCLUSIONS

Ten major volatiles were determined, significant differences with respect to the control being observed in ethyl acetate, methanol, isobutanol, and diethylacetal. The "classic" wine and that containing chlorpyrifos had concentrations of ethyl acetate which were above the olfactory threshold. The wine containing fenarimol residues had the highest concentration of methanol, although still below the perception threshold. Fenarimol may have affected pectinesterase activity since all the wines were elaborated in the same way during maceration. The "classic" wine showed the lowest isobutanol concentration. The fact that the grapes used to make this wine were the only ones to receive phytosanitary treatment during ripening strongly suggests that such treatment affected valine synthesis or assimilation, this amino acid being a precursor of isobutanol.

A large group of minor volatiles were identified. These were grouped into 15 esters, 3 aldehydes, 6 acids, and 15 alcohols. The only esters to show significantly different concentrations between the wines were isoamyl, hexyl, and phenylethyl acetates and ethyl decanoate and dodecanoate. However, only isoamyl acetate exceeded the olfactory threshold in the wines containing residues of chlorpyrifos, fenarimol, and vinclozolin, in all of which a strong banana smell could be noted. The ethyl lactate concentrations recorded in the wines made from grapes treated with insecticide demonstrate that their active ingredients do not affect malolactic fermentation. Although some wines made from pesticide-treated grapes showed significantly higher concentrations of acids than the control wine, their concentration never exceeded the olfactory threshold level.

No significant differences existed between the concentrations of terpenoles and other alcohols.

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