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Effect of Oak Extract Application to Verdejo Grapevines on Grape and Wine Aroma

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ABSTRACT: Volatile compounds from a commercial aqueous oak extract application to white Verdejo grapevines at veraison have been studied. Treated grapes under two types of formulation (25% and 100%) have been analyzed at the optimum maturation time, and winemaking was then subsequently carried out. The volatile compounds were analyzed by stir bar sorptive extraction−gas chromatograpy−mass spectrometry. The results suggest that after the grapevine treatments, grapes store the volatiles in the form of nonvolatile precursors, and some of the volatiles are released during the winemaking process, especially six months after the alcoholic fermentation. The sensory analysis shows that wines maintain the typical aroma properties of Verdejo wines at the end of fermentation; but after six months, the wine color is greener and more astringent, and, in terms of aroma, it has wooden notes as if the wine has been aged in oak barrels.

KEYWORDS: volatiles, oak extracts, grapevines, grapes, wines, Verdejo

INTRODUCTION

Varietal aroma of wines is constituted by a complex group of substances that can occur in both forms: as volatile molecules (so-called odor-active compounds), or as odorless precursors. The latter are related to wine aroma potential, as during the winemaking process and aging, they can be transformed into odor active-compounds.[1,2]

The concentration of varietal volatile compounds in wines varies depending on the grape variety and the "terroir effect", which is related to the soil, climate, viticulture, and environment in which the plant grows. The results of such effects produce wines with a characteristic and identifiable origin.[3] With regard to the impact of external factors on the aroma, although still a matter of controversy, the scent of eucalyptus in wines from vineyards near eucalyptus forests may be due to the absorbtion of the aroma by the grape plants. However eucalyptol, a compound that possesses the characteristic odor of eucalyptus, is a terpene that may originate from chemical transformations of other terpenes in the grape[4] as well as from the combination of certain wine components.[5] On the other hand, there is evidence that certain fungicide treatments applied to the vineyard can influence the aroma of wines, especially the varietal component.[6]

In recent years, some research groups have studied how grapes from grapevines exposed to smoke from forest fires produced wines with smoke sensorial notes.[7−10] Smoke applications on grapevines showed that volatile compounds from smoke such as guaiacol, 4-methylguaiacol, 4-ethylphenol, furfural, and eugenol were absorbed by the plant and then transmitted to the must and wine during the winemaking process,[7] especially when smoke application was made seven days after veraison.[9] Further, the sensory characteristics of wines from grapevine exposure to smoke for 1 h was of the same order as that resulting from wines in contact with oak, material that contains some compounds that are also in the smoke.[10]

Indeed, oak barrels have been used for a long time to age wines, especially red wines, as it improves wine characteristics, especially the aroma. In this sense, the wines in contact with oak wood extract volatile compounds, which proportionate aromatic notes of "wood", "coconut", "spices", "toasted", and "smoke" associated with compounds such as oak lactones, eugenol, vanillin, guaiacol, etc.[11,12] Some of these compounds are already present in green wood, and others are formed during the toasting process of cooperage.[13]

The aforementioned observations suggest that grapevines may absorb the aroma compounds present within oak extracts. Among the constituents of oak are some of the compounds also in smoke, such as eugenol and guaiacol, which are absorbed by grape plants and transmitted to their wines. In addition, the aroma compounds of oak extracts have characteristics of aged wines and therefore add value to the wines.

Vitis vinifera cv. Verdejo is an important Spanish white cultivar, the base of Rueda Denomination of Origin wines, which produces young white wines with fruity attributes (citrus and tropical characteristics) with hints of green fruit.[14] The tropical fruit character of Verdejo is related to the presence of 3-mercaptohexyl acetate.[15] Is it possible to transfer the aromas of oak extracts to this young wine grape variety? Without a doubt, this would provide an innovative viticultural strategy in order to obtain a different type of wine. As a consequence, the aim of this work was to study the impact of different formulations of a commercial aqueous oak extract applied to Verdejo grapevines in relation to the oak volatile composition of grapes and their respective wines.

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MATERIALS AND METHODS

Oak Extract. The aqueous oak extract chosen for this study was provided by Protea France S.A. (Gensac la Pallue, France). This extract is a food additive utilized in spirits and fruit juices, which guarantees that no toxicity risk exists. It was produced by macerating French toasted oak chips (*Quercus sessilis*) from natural seasoning for at least 18 months in demineralized water at 100 °C for 32 h. Before treatment of the grapevines, the absence of phytotoxicity was confirmed in *Solanum lycopersicum* var. Micro Tom tomato (INRA, Montpellier, France), a cultivar used as a quickly grown plant.

Grapevine Treatments. White grapes from *Vitis vinifera* variety Verdejo grown in the La Mancha region (Albacete province, southeast Spain) during the year 2009 were used. The annual average temperature was 13 °C, with a minimum of −15 °C (January) and a maximum of 40 °C (August). Grapevines were grown on a trellis system with drip irrigation system to ensure the plants' water needs, as this region has 300−400 mm of rainfall per year.

Oak extract treatments were applied to the grapevines during veraison, when the green color of the grape was more transparent and the flexibility of the skins was high, presenting this aspect in at least half of the clusters. At veraison, the metabolic activity of the plant is very important and it has been shown that the volatile composition can be affected by external phenomena such as smoke.[9] Formulations of each of the treatments were prepared with 0.5 mL of the adjuvant Fluvius (BASF, Germany) per liter; this is a wetting agent typically used for foliar herbicide treatment. The extract without dilution (100% treatment) and diluted with water at 25% (25% treatment) were applied only once on the seventh day after veraison. In addition, on the same day, a third treatment consisted of an aqueous solution of eugenol plus guaiacol (E+G treatment) standard compounds (Sigma-Aldrich, Gillingham, England) (6 g/L of each compound) was applied. For each of the treatments, a row of 188 plants was used. A total of 752 plants was necessary, with two untreated rows between different applications to avoid contamination. Also, a row of 188 plants was not treated (control). Around 230 mL of each formulation was applied evenly per plant by spraying over leaves. The treatments were carried out when the ambient temperature was below 20 °C, at approximately 7 a.m. Several hours before harvest, grape sampling was carried out, starting with the first grapevine of each row, by taking a cluster from every fifth grapevine, for the entire row (188 plants). Clusters with northern and southern distribution were alternatively picked, making a total of 11 kg of grapes for each treatment as well as for the control. Grapes from all clusters were destemmed and mixed. From this mixture, 300 grapes were randomly taken to obtain a weight of 100 berries (triplicate analysis). The remianing grapes were frozen at −20 °C for later volatile composition analysis.

Winemaking. White Verdejo grapes were harvested on August 27 at the technological ripening moment when the degrees Baumé/titratable acidity ratios were between 2.5 and 3. These grapes were picked at night with a harvesting machine, the temperature of the grapes being 19 ± 2 °C. Ten grams of potassium metabisulfite per 100 kg of grapes was added. For each treatment, all 188 plants were harvested. Grapes were first destemmed, followed by a pressing process with 55% yield. The must from each treatment, without skin contact, was put in a 200 L stainless steel tank. One liter of must was removed for oenological parameters analysis. *Saccharomyces cerevisiae* strain QA23 was inoculated at a dose of 20 g/hL according to the recommendation of Lallemand (Spain). The alcoholic fermentation temperature was maintained around 13 °C, and the density was measured daily with a densimeter. The alcoholic fermentation was completed when the reducing sugars were below 2.5 g/L. At the end of the alcoholic fermentation, the free SO_2 concentration was corrected to 25−35 mg/L. The wines were stored for six months in the tanks at 17 °C protected from oxygen. For each tank, three different wine samples were taken at the end of the alcoholic fermentation and also six months later and then frozen at −20 °C until analysis.

Oenological Parameters Analysis. Degrees Baumé, reducing sugars, titratable acidity, volatile acidity, pH, alcohol degree, and yeast assimilable nitrogen (YAN) from the different samples were measured following the methods established by ECC.[16] Grape yield for the plant was calculated by dividing the total mass production (kg) by the number of plants (188).

Extraction of Volatile Compounds from Grapes and Oak Extract by HS-SBSE. We used as a reference the methods proposed by Weldegergis and Crough[17] and Callejón et al.,[18] which described the analysis of wines and wine vinegars, respectively, and adapted them to our samples. Grapes randomly picked from the three different treatments and the control were defrosted, crushed, and macerated for 2 h and then strained with a colander (must). One aliquot of strained grapes was centrifuged at 176*g* for 30 min (centrifuged must). These two sample types (must and centrifuged must) were used in order to choose the best extraction conditions for the volatile compounds studied: *cis*-oak lactone, *trans*-oak lactone, furfural, 5-methylfurfural, eugenol, guaiacol, vanillin, acetovanillone, 6-methoxyeugenol, methyl vanillate, 4-vinylguaiacol, 4-ethylguaiacol, and 4-ethylphenol (Aldrich, Gillingham, England), characteristic compounds of oak wood, by headspace-stir bar sorptive extraction (HS-SBSE). The variables studied were vial volume, 50 and 20 mL, and retention temperature, 40 °C and 60 °C. In the 50 mL vial, 22 mL of sample was added, so the headspace was 28 mL, and in the 20 mL vial, 9 mL of sample was added, so the headspace was 11 mL. In all cases, 0.1 g of NaCl was added per milliliter of sample. Also, 10 μL of internal standard γ-hexalactone (Sigma-Aldrich) solution at 1 μL/mL in absolute ethanol (Merck, Damstard, Germany) was added per milliliter of sample.[19] A polydimethylsiloxane-coated stir bar (twister, 0.5 mm film thickness, 10 mm length, Gerstel, Mülheim, and der Ruhr, Germany) was inserted into the twister-headspace vial and hermetically closed. The vial was introduced into a heater (Selecta, Barcelona) at the appropriate temperature and was stirred with a common magnetic stirrer during 1 h at 500 rpm. Next, the twister was removed, rinsed with distilled water, dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC−MS analysis. To check the method, samples were analyzed in triplicate. Once volatile extraction was optimized, the method proposed was applied to analyze the oak extract and the different grape samples. Grapes were separated into three lots, and each of them was crushed and macerated and the volatile compounds were extracted (n = 3).

The precision of the method was calculated with the coefficient of variation, where six extractions were performed on a sample of grapes (control). Recovery was studied by spiking two concentrations of the target compounds to the grape matrix. Compounds were then extracted and quantified according to the extraction method, and their recovery was calculated. The limit of quantification (LOQ) and limit of detection (LOD) were estimated as the concentration of the analyte of a standard that produced a signal-to-noise ratio of 10 and 3 times, respectively.

Extraction of Volatile Compounds from Wines by Immersion SBSE. Volatile wine compounds were extracted according to Marín et al.[19] The twister was introduced into 10 mL of sample to which 100 μL of the same internal standard γ-hexalactone was added. Samples were stirred at 500 rpm at room temperature for 1 h. The twister was then removed from the sample, rinsed with distilled water, dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC−MS analysis. For each wine, three samples were analyzed (n = 3).

Analysis of Volatile Compounds by GC-MS. In the thermal desorption tube, the volatile compounds were desorbed from the twister under the following conditions: oven temperature, 330 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow, 45 mL/min. The compounds were transferred into a Hewlett-Packard LC 3D

Table 1. Oenological Parameters in Grapes after the Different Grapevine Treatments ($n = 3$)[a]

treatments	yield (kg/plant)	weight of 100 berries	degrees Baumé	YAN (mg N/L)	titratable acidity (g/L)	degrees Baumé/TA	pH
control	3.93	100.7 ± 0.2	12.4 ± 0.1	196 ± 3	4.5 ± 0.1	2.8 ± 0.1	3.6 ± 0.1
25%	3.26	112.7 ± 0.8	12.2 ± 0.2	168 ± 4	4.2 ± 0.2	2.9 ± 0.1	3.6 ± 0.0
100%	3.90	119.3 ± 0.6	12.4 ± 0.0	210 ± 7	4.4 ± 0.2	2.8 ± 0.2	3.6 ± 0.0
E+G	3.72	121.4 ± 0.3	12.4 ± 0.1	182 ± 5	5.0 ± 0.3	2.5 ± 0.1	3.6 ± 0.2

[a] Control: untreated grapes; 25%: grapevines treated with oak extract diluted to quarter strength; 100%: grapevines treated with oak extract without dilution; E+G: grapevines treated with eugenol plus guaiacol solution. TA: titratable acidity. YAN: yeast assimilable nitrogen.

Table 2. Relative Area × 10^{-3} of the Grape Volatile Compounds under the Different Conditions Studied[a,b]

	must				centrifuged must			
	vial volume 50 mL		vial volume 20 mL		vial volume 50 mL		vial volume 20 mL	
	temp 60 °C	temp 40 °C	temp 60 °C	temp 40 °C	temp 60 °C	temp 40 °C	temp 60 °C	temp 40 °C
furfural	10 ± 1b	10 ± 1b	5.3 ± 0.5a	5.2 ± 0.5a	21 ± 2d	12 ± 1b	18 ± 2c	20 ± 2 cd
5-methylfurfural	5.9 ± 0.8b	6.7 ± 0.7bc	1.6 ± 0.2a	2.1 ± 0.3a	26 ± 2e	8 ± 1 cd	9 ± 1d	9.1 ± 0.9d
eugenol	5.6 ± 0.8bc	6.0 ± 0.8c	2.8 ± 0.4a	1.8 ± 0.2a	10 ± 1e	4.6 ± 0.6b	8 ± 1d	6.3 ± 0.9c
guaiacol	4.4 ± 0.4b	6.6 ± 0.7e	1.8 ± 0.2a	2.6 ± 0.4a	6.6 ± 0.9e	6.2 ± 0.6de	5.5 ± 0.5 cd	4.7 ± 0.6bc
vanillin	3.4 ± 0.4c	3.2 ± 0.3bc	2.8 ± 0.3bc	1.6 ± 0.2a	4.6 ± 0.6d	2.7 ± 0.3b	2.8 ± 0.3bc	2.6 ± 0.3b
acetovanillone	9.3 ± 0.9e	4.1 ± 0.4bc	2.5 ± 0.2a	3.6 ± 0.4ab	11 ± 1f	7.1 ± 0.8d	7.9 ± 0.9d	5.2 ± 0.6c
methyl vanillate	2.5 ± 0.3e	0.89 ± 0.09ab	1.4 ± 0.1c	0.61 ± 0.08a	1.9 ± 0.3d	1.2 ± 0.1bc	0.9 ± 0.2ab	0.75 ± 0.08a
4-vinylguaiacol	2.1 ± 0.2de	2.0 ± 0.2 cd	1.3 ± 0.1b	0.85 ± 0.09a	3.2 ± 0.3 g	2.5 ± 0.3f	2.4 ± 0.2ef	1.7 ± 0.2c
4-ethylguaiacol	2.1 ± 0.3c	4.6 ± 0.5d	0.52 ± 0.05a	0.76 ± 0.08ab	2.1 ± 0.2c	1.1 ± 0.2b	1.0 ± 0.1b	0.82 ± 0.08ab
4-ethylphenol	1.7 ± 0.2c	2.9 ± 0.3d	1.7 ± 0.2c	0.85 ± 0.08b	1.0 ± 0.1b	1.0 ± 0.1b	1.0 ± 0.1b	0.54 ± 0.05a
total	47 ± 2b	47 ± 2b	21.7 ± 0.8a	19.9 ± 0.9a	87 ± 3e	47 ± 2b	57 ± 3d	52 ± 3c

[a] All parameters are given with their standard deviation ($n = 3$). Different letters indicate significant differences (level of significance: $p < 0.05$) between columns. [b] Grapes randomly picked from the four different clusters were used.

mass detector (Palo Alto, CA) with a fused silica capillary column (BP21 stationary phase 30 m length, 0.25 mm i.d., and 0.25 μm film thickness; SGE, Ringwood, Australia). The chromatographic program was set as follows: 40 °C (held for 5 min), raised to 150 °C by 5 °C/min, and then raised to 230 °C by 10 °C/min (held for 5 min). The total analysis time was 40 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used and the detection and quantification were carried out in the selected ion monitoring (SIM) mode. The m/z of ions monitored in the SIM runs were (italic ions are those used for quantification) as follows: *cis*-oak lactone *99*, 101, 132, 156; *trans*-oak lactone *99*, 101, 132, 156; furfural 39, 67, 95, *96*; 5-methylfurfural 53, 81, 109, *110*; eugenol 121, 131, 149, *164*; guaiacol 53, 81, *109*, *124*; vanillin *151*, 152, 155, 156; acetovanillone 108, 123, *151*, *166*; 6-methoxyeugenol 81, 119, 131, *194*; methyl vanillate 123, *151*, 167, *182*; 4-vinylguaiacol 77, 107, *135*, *150*; 4-ethylguaiacol 91, 122, *137*, *152*; 4-ethylphenol 77, 91, *107*, *122*. The detector temperature was 150 °C. Identification was carried out by comparison with the mass spectrum and retention index of chromatographic standards and data found in the literature. Two calibrations were performed, one for the headspace extraction, using the optimum conditions of the method, and one for extraction by immersion. For all of these compounds, the concentrations of the standards (Sigma-Aldrich) were between 0.05 and 3500 μg/L in a 12% ethanol (v/v) solution at pH 3.6, and the quantification was based on five-point calibration curves ($R^2 > 0.9$ for both extraction methods). Grape results are given in μg/kg, taking into account the 85% must yield.

Sensory Analysis. A panel of eight expert judges (three females and five males, with ages between 25 and 50 years old) participated in the study. At the end of the alcoholic fermentation and six months later, judges evaluated each wine in triplicate, which were randomly presented. The sensory analysis was performed by modifying the classic questionnaire of Verdejo wines from Rueda Spanish Origin Apellation, but adding the wood and spicy attributes. Thus, the analysis was composed of 17 attributes or descriptors grouped by visual phase (color intensity, yellow and green tones), olfactory phase (odor intensity, fermentatives, varietals, fruity, florals, herbaceous, wood, and spicy), and gustatory phase (mouthfeel, acidity, bitterness, astringency, persistence, and balance). Panelists rated each attribute on a scale from 1 (absence) to 7 (maximum presence). The sensory analysis of wines from E+G grapevine treatment was not carried out because, as they are not natural products, there could be a health risk to the judges.

Statistical Analysis. The statistical elaboration of the data was performed using SPSS Version 17.0 statistical package for Windows (SPSS, Chicago, IL). Volatile compound data were processed using variance analysis (ANOVA). Differences between means were compared using the least significant differences (LSD) test at 0.05 probability level. Two variance analyses were carried out, one of them related to the different oak extract treatments, and the other to the E+G solution treatment. Discriminant analyses of the volatiles composition in the control wine and the wines obtained from grapes treatment with oak extract were performed, as well as analyses of their sensory attributes, at the end of alcoholic fermentation and after six months.

RESULTS AND DISCUSSION

The effect of two oak extract applications to Verdejo cultivar grapevines has been studied. In addition, another grapevine treatment with a standard solution of eugenol and guaiacol was followed, as recently studied in the literature.[20] These two

Table 3. Coefficient of Variation (CV (%)), Recovery Indices (R1, R2), Limit of Detection (LOD), and Limit of Quantification (LOQ) for Each Compound Analyzed[a]

	CV (%)	C1 (μg/kg)	R1 (%)	C2 (μg/kg)	R2 (%)	LOD (μg/kg)	LOQ (μg/kg)
cis-oak lactone	7.1[b]	4.0	72	8.0	89	0.45	1.52
trans-oak lactone	8.0[b]	7.0	78	14.0	80	0.85	2.83
furfural	15.8	45.0	73	90.0	79	7.21	24.03
5-methylfurfural	5.9	0.6	72	1.2	81	0.16	0.53
eugenol	1.4	0.8	84	1.6	104	0.20	0.66
6-methoxyeugenol	4.1[b]	0.2	82	0.4	95	0.03	0.09
guaiacol	16.2	0.4	72	0.8	78	0.13	0.41
4-vinylguaiacol	14.3	0.4	77	0.8	92	0.11	0.35
4-ethylguaiacol	0.8	1.2	82	6.0	94	0.35	1.18
4-ethylphenol	16.4	0.8	75	4.0	89	0.23	0.78
vanillin	16.5	0.04	75	0.24	79	0.01	0.03
acetovanillone	15.5	0.05	73	0.30	73	0.01	0.04
methyl vanillate	15.9	0.05	76	0.30	81	0.01	0.04

[a] C1, C2: two different concentrations of each compound added to the grapes. [b] Such compounds are not found in grapes, so their CV was calculated from the samples that were spiked with them.

compounds are present in the oak extract studied, in oak-aged wines, and in grapes and wines from smoke applications to grapevines, which opens a new research field on plant responses to exogenous agents.

Grape Oenological Parameters. The oenological parameters of grapes from the different treatments (control, 25%, 100%, and E+G) are shown in Table 1. The control and 100% treatment produced the highest fruit yields. Also, 100% treatment grapes showed a high grape weight, but without important differences with the grapes from the E+G treatment, which showed the highest values for this parameter. These small differences observed could be due to the plants' natural variation. There were no differences between the degrees Baumé, titratable acidity, and pH, and therefore neither in the degrees Baumé/titratable acidity ratio. Thus, we conclude that none of the treatments affected oenological parameters. In all the cases, YAN was higher than 140 mg N/L, which is the concentration needed to complete alcoholic fermentation.[21]

Selection of HS-SBSE Extraction Conditions. Table 2 shows the grape volatile composition under the different extraction conditions. The extraction was higher for centrifuged must, with the exception of methyl vanillate, 4-ethylphenol, and 4-ethylguaiacol, regardless of temperature and vial volume. The centrifuged must was more limpid than the original must, so the interchange of the different volatile compounds between the liquid and the gas phase could be facilitated, improving the extraction process. Regarding the vial volume, for a sample type (must and centrifuged must) and temperature given, the extraction of the volatile compounds was higher when a 50 mL vial volume was used compared to a 20 mL volume, in most of the cases. Also, Delgado et al.[22] found that when vial volume was increased, extraction was improved. Significant differences have been found for a 50 mL volume; when the temperature was increased, the volatiles absorption increased considerably. Theoretically, high temperatures will increase the partial vapor pressure of analytes in the headspace[23] but only up to a limit, as high temperatures (above 75 °C) will decrease the absorption of the volatile compounds onto the twister.[18] Therefore, the optimum extraction conditions of the volatile compounds studied from grapes are as follows: crushed, strained, and centrifuged, vial volume of 50 mL, with 22 mL of sample, and 60 °C retention temperature. Table 3 shows the coefficient of variation, recovery index, and limits of detection (LOD) and quantification (LOQ) for each compound. The precision of the method was calculated with the coefficient of variation, and the results fluctuated between 1% and 16% for the different quantified compounds. The recovery index was used in order to find out the accuracy of the method. This index fluctuated between 72% and 104% depending on the different compounds analyzed. Because of the lack of grape analysis references to these compounds by HS-SBSE, no comparison has been carried out. However, LOD and LOQ values seem to be adequate for grape analysis.

Table 4. Volatile Composition of the Aqueous Oak Extract[a]

	concentration (μg/L)
cis-oak lactone	5.6 ± 0.4
trans-oak lactone	11.3 ± 0.9
furfural	2819 ± 200
5-methylfurfural	15.1 ± 0.2
eugenol	9.8 ± 0.6
6-methoxyeugenol	0.97 ± 0.04
guaiacol	15.2 ± 0.5
4-vinylguaiacol	1.4 ± 0.4
4-ethylguaiacol	27 ± 3
4-ethylphenol	4 ± 1
vanillin	2.3 ± 0.2
acetovanillone	0.9 ± 0.1
methyl vanillate	1.5 ± 0.5

[a] All parameters are given with their standard deviation (n = 3).

Volatile Compounds in Oak Extract. The optimized HS-SBSE method was applied to the extraction of volatile compounds from aqueous oak extract; the results are shown in Table 4. Note that the extract used in this study comes from toasted wood chips macerated with water by heating at 100 °C for 32 h (according to the Protea SA procedure). However, up to now, the literature on wood volatile composition refers to their extraction by different hydroalcoholic solutions. Lactones are characteristic compounds of oak wood, which are also generated

Table 5. Concentration of Volatile Compounds (μg/kg) in Grapes from the Different Grapevine Treatments[a]

	control	25% extract	100% extract	E+G
cis-oak lactone	nd	nd	nd	nd
trans-oak lactone	nd	nd	nd	nd
furfural	65.1 ± 11.0a	73.3 ± 25.3a	76.1 ± 15.0a	70.3 ± 0.2a
5-methylfurfural	0.84 ± 0.05ab	1.01 ± 0.32b	0.64 ± 0.07a	0.61 ± 0.11a
eugenol	1.48 ± 0.02b	1.12 ± 0.03ab	0.82 ± 0.22a	1.50 ± 0.21b
6-methoxyeugenol	nd	nd	nd	nd
guaiacol	0.50 ± 0.10ab	0.43 ± 0.06a	0.42 ± 0.09a	0.61 ± 0.02b
4-vinylguaiacol	0.70 ± 0.11b	0.69 ± 0.07b	0.64 ± 0.05b	0.37 ± 0.01a
4-ethylguaiacol	4.78 ± 0.04c	1.20 ± 0.20a	2.21 ± 0.60b	1.45 ± 0.03a
4-ethylphenol	2.6 ± 0.5c	2.5 ± 0.3c	1.4 ± 0.1b	0.8 ± 0.1a
vanillin	0.170 ± 0.051b	0.049 ± 0.001a	0.053 ± 0.002a	0.043 ± 0.008a
acetovanillone	0.23 ± 0.07b	0.13 ± 0.02a	0.12 ± 0.01a	0.10 ± 0.01a
methyl vanillate	0.300 ± 0.051b	0.087 ± 0.008a	0.094 ± 0.001a	0.071 ± 0.010a

[a] All parameters are given with their standard deviation ($n = 3$). nd: not detected. Different letters indicate significant differences (level of significance: $p < 0.05$) between columns. Control: untreated grapevines; 25%: grapevines treated with oak extract diluted to quarter strength; 100%: grapevines treated with oak extract without dilution; E+G: grapes treated with eugenol plus guaiacol solution.

during the toasting process.[13] These compounds correspond to the "toasted", "wood", or "coconut" aroma characters of the commercial oak extracts. *cis*-Oak lactone concentration is higher than the *trans* isomer concentration in American oak, but the concentrations of both lactones are closer to one another in French oak.[24−26] The concentration of *trans*-oak lactone in the extract was twice the concentration of *cis*-oak lactone. This fact may be attributed to the water maceration process at high temperature for the toasted wood, which would imply a partial loss of the *cis*-oak lactone because it is the most volatile isomer.[26] Among the compounds found in the wood that is subjected to high temperatures, the furanic compounds are the most abundant, emphasizing a larger furfural content,[25] which is generated as a result of the pentose Maillard reaction.[26] It is also the most abundant compound in the extract used, and its content is higher than that reported in the literature on analysis of the wood used in wine aging. 5-Methylfurfural comes from the hexose Maillard reaction, and the concentrations of the extract are within the range reported in the literature for ethanolic extracts of French oak.[25] The volatile phenols such as eugenol, 6-methoxyeugenol, guaiacol, vanillin, acetovanillone, and methyl vanillate are formed by lignin degradation during the toasting process, although eugenol and vanillin are also present in green wood.[27] 4-Vinylguaiacol is in the range indicated by the literature mentioned above, while 4-ethylguaiacol, guaiacol, and eugenol are present in higher concentrations and 6-methoxyeugenol in lower concentrations. Note that guaiacol content is greater than eugenol content, a result which coincides with the findings of Guillén and Manzanos[28] in aqueous oak smoke preparations. The concentration of 4-ethylguaiacol in the extract is almost seven times higher than that of 4-ethylphenol. Vanillin and derivatives such as acetovanillone and methyl vanillate were found in very low concentrations compared to the other compounds.

Volatile Compounds in Grapes. In the literature, we did not find reports on the volatile composition of Verdejo grapes. This is a nonaromatic grape variety used for the production of young wines, which are characterized by a typical floral and fruity aroma.[14] This study focuses on the compounds present in the oak extract that may have been transmitted to the grapes and their respective wines by the treatments. Neither oak lactones nor 6-methoxyeugenol were detected in any of the grape samples (Table 5). However, other compounds such as eugenol, guaiacol, 4-ethylguaiacol, 4-vinylguaiacol, 4-ethylphenol, vanillin, methyl vanillate, and acetovanillone were found, which have also been reported in Verdejo wines,[29] suggesting that they may come from the grapes. Compounds such as furfural and 5-methylfurfural are generally not the focus of studies in aromas of young wines; however, the presence of furfural has been reported in Macabeo young white wines and could therefore come from the grapes.[30] The concentrations of furfural, guaiacol, and 4-vinylguaiacol are similar in the control grapes and in those treated with the extract (Table 5), but in the latter they contain significantly lower concentrations of 4-ethylguaicaol and the three vanillin derivatives analyzed compared to the control. The presence of 4-ethylphenol in grapes has not been studied; however, its existence in grapes as precursors has been suggested.[31] When the grapes from the two oak extract treatments are compared, similar concentrations are observed for all compounds, except for 5-methylfurfural and 4-ethylphenol, which are in higher concentrations when the treatment applied was 25%, and for 4-ethylguaiacol, which was higher in the 100% treatment. Eugenol is a volatile compound found in Chardonnay grape skins[32] and in young white wines, therefore contributing to the varietal aroma with hints of aromatic clove spices.[30]

Grapes treated with the solution of eugenol and guaiacol (E+G treatment) do not exhibit an increase in the concentrations of these compounds, contrary to what one might expect, because no significant differences were found compared to the control (Table 5). Among the other compounds, furfural and 5-methylfurfural are found in concentrations similar to that in the control but the other compounds tested are in lower concentrations. Flavor compounds in grapes can be present as their free, odor-active form, or as nonvolatile precursors, mainly glycoconjugates, releasing the aglycone during the winemaking process.[1,2,33] Glycosides of guaiacol were found in both grapes and wine,[10,20,34] and glycosides of eugenol were reported in grapes.[35] Therefore, eugenol and guaiacol, added to the grapevines through the E+G treatment, may have been stored by the berries as glycosylated precursors, as no increment was observed because this study focuses only on the volatile compounds or

Table 6. Oenological Parameters in Wines at the End of the Alcoholic Fermentation and after Six Months[a]

grapevine treatment	pH	titratable acidity (g/L)	volatile acidity (g/L)	alcohol degree (v/v %)
		End of Alcoholic Fermentation		
control	3.5 ± 0.0	4.3 ± 0.2	0.3 ± 0.0	13.1 ± 0.2
25%	3.5 ± 0.1	4.2 ± 0.1	0.2 ± 0.0	13.3 ± 0.2
100%	3.5 ± 0.1	4.2 ± 0.2	0.2 ± 0.0	13.5 ± 0.1
E+G	3.5 ± 0.0	4.2 ± 0.0	0.2 ± 0.1	13.2 ± 0.3
		Six Months after Alcoholic Fermentation		
control	3.6 ± 0.1	4.0 ± 0.1	0.3 ± 0.1	13.8 ± 0.2
25%	3.6 ± 0.0	4.0 ± 0.2	0.3 ± 0.0	13.6 ± 0.3
100%	3.6 ± 0.0	4.0 ± 0.1	0.3 ± 0.0	14.0 ± 0.1
E+G	3.6 ± 0.1	3.9 ± 0.1	0.3 ± 0.1	13.6 ± 0.0

[a] All parameters are given with their standard deviation (n = 3). Control: untreated grapevines; 25%: grapevines treated with oak extract diluted to quarter strength; 100%: grapevines treated with oak extract without dilution; E+G: grapes treated with eugenol plus guaiacol solution.

Table 7. Concentration of Volatile Compounds (μg/L) in Wines from the Different Grapevine Treatments[a]

	control wine		wine from 25% treatment		wine from 100% treatment	
	end of alcoholic fermentation	six months after alcoholic fermentation	end of alcoholic fermentation	six months after alcoholic fermentation	end of alcoholic fermentation	six months after alcoholic fermentation
cis-oak lactone	nd	nd	1.8 ± 0.1a	10.3 ± 0.5c	2.8 ± 0.6a	9 ± 2b
trans-oak lactone	nd	nd	4.8 ± 0.3a	22 ± 3d	8 ± 1b	19 ± 2c
furfural	29.1 ± 4.31a	30.0 ± 1.21a	30.1 ± 2.11a	29.1 ± 3.03a	30.1 ± 2.01a	29.5 ± 0.01a
5-methylfurfural	3.40 ± 0.31a	6.6 1 ± 0.32b	9.22 ± 0.91c	7.91 ± 0.41c	8.80 ± 0.50bc	8.40 ± 1.03c
eugenol	4.0 ± 0.3a	11.3 ± 0.5b	14.0 ± 1.1b	31.2 ± 3.4c	14.5 ± 0.9b	28.1 ± 4.1c
6-methoxyeugenol	2.01 ± 0.20a	0.90 ± 0.04a	1.31 ± 0.11a	17.20 ± 2.01c	1.30 ± 0.13a	8.21 ± 0.41b
guaiacol	2.9 ± 0.2a	3.0 ± 0.1a	8.0 ± 0.6c	4.1 ± 0.2b	8.0 ± 0.6c	3.8 ± 0.1b
4-vinylguaiacol	566 ± 43b	426 ± 17a	533 ± 37b	391 ± 35a	528 ± 21b	446 ± 16a
4-ethylguaiacol	15.2 ± 1.0a	14.8 ± 0.6a	20.3 ± 1.1bc	22.4 ± 2.1 cd	23.0 ± 1.2d	18.1 ± 0.8b
4-ethylphenol	3.0 ± 0.2ab	2.0 ± 0.1a	5.0 ± 0.8bc	4.9 ± 0.7b	3.0 ± 0.2ab	6.1 ± 1.0c
vanillin	0.50 ± 0.04b	0.44 ± 0.02ab	1.02 ± 0.11c	0.70 ± 0.06b	1.13 ± 0.12d	0.40 ± 0.02a
acetovanillone	2.0 ± 0.2a	1.7 ± 0.1a	2.1 ± 0.2a	7.6 ± 0.4c	2.4 ± 0.1a	6.1 ± 0.3b
methyl vanillate	0.26 ± 0.02a	0.36 ± 0.01c	0.26 ± 0.01a	1.10 ± 0.01e	0.29 ± 0.02ab	0.67 ± 0.01d

[a] All parameters are given with their standard deviation (n = 3). nd: not detected. Different letters indicate significant differences (level of significance: $p < 0.05$) between columns. Control: untreated grapevines; 25%: grapevines treated with oak extract diluted to quarter strength; 100%: grapevines treated with oak extract without dilution.

aroma-free forms. If such glycosylation has taken place, the biosynthesis of other volatile compounds from these grapes could have been affected and therefore presents significant differences from that of the control grapes.

Wines Oenological Parameters. Table 6 shows the oenological parameters of wines at the end of the alcoholic fermentation and after six months. All values were normal for wines from healthy grapes.[36] The treatments did not affect the parameters analyzed because the values were similar to those of the control wine. Only the alcohol degree slightly increased at the six month sampling in comparison with the end of alcoholic fermentation.

Wine Volatile Composition. Neither of the two oak lactones was found in the control wine, so their origin in other wines can be attributed to the oak extract treatments (Table 7). At the end of fermentation, the concentrations of *cis*-oak lactone were similar in the wines from the two extract treatments and less than that of the *trans*-oak lactone, and the highest concentration was found in the 25% treatment wine. In these wines, the *cis*/*trans* lactone ratio was between 0.37 (25% treatment) and 0.34 (100% treatment), slightly lower than the ratio of the extract, however maintaining the pattern of the extract because *trans*-oak lactone predominates over the *cis*-oak lactone.

After six months, both lactone isomer concentrations increased significantly in both types of wines but more importantly in the grapes from 25% treatment, but without exceeding the respective perception thresholds (20−23 μg/L, *cis* isomer; 140 μg/L, *trans* isomer).[37,38] The *cis*/*trans* ratio is still more favorable to the *trans*-oak lactone in the 25% treatment than in 100% treatment, as it remains between 0.46 (25% treatment) and 0.44 (100% treatment). The reason for increment oak lactones should be investigated in future studies; however, these results suggest that the plant accumulates part of these lactones in the berries as

Table 8. Concentration of Volatile Compounds (μg/L) in Wines from E+G Grapevine Treatment[a]

	control wine		wine from E+G treatment	
	end of alcoholic fermentation	six months after alcoholic fermentation	end of alcoholic fermentation	six months after alcoholic fermentation
cis-oak lactone	nd	nd	3.3 ± 0.2a	17 ± 2b
trans-oak lactone	nd	nd	8 ± 1a	15 ± 3b
furfural	29.1 ± 4.31a	30.0 ± 1.21a	29.0 ± 1.1a	29.4 ± 0.1a
5-methylfurfural	3.40 ± 0.31a	6.6 1 ± 0.32b	9.21 ± 0.01c	8.92 ± 0.22c
eugenol	4.0 ± 0.3a	11.3 ± 0.5b	42.1 ± 2.0c	158.0 ± 22.3d
6-methoxyeugenol	2.01 ± 0.20a	0.90 ± 0.04a	0.90 ± 0.05a	23.21 ± 1.10b
guaiacol	2.9 ± 0.2a	3.0 ± 0.1a	7.9 ± 0.4b	8.0 ± 0.6b
4-vinylguaiacol	566 ± 43b	426 ± 17a	474 ± 24b	362 ± 1a
4-ethylguaiacol	15.2 ± 1.0a	14.8 ± 0.6a	20.1 ± 2.0b	103.2 ± 24.3b
4-ethylphenol	3.0 ± 0.2ab	2.0 ± 0.1a	5.0 ± 0.3b	11.1 ± 2.0c
vanillin	0.50 ± 0.04b	0.44 ± 0.02ab	0.60 ± 0.03a	0.70 ± 0.02b
acetovanillone	2.0 ± 0.2a	1.7 ± 0.1a	2.3 ± 0.1a	17.3 ± 3.1b
methyl vanillate	0.26 ± 0.02a	0.36 ± 0.01c	0.31 ± 0.02b	1.31 ± 0.30d

[a] All parameters are given with their standard deviation ($n = 3$). nd: not detected. Different letters indicate significant differences (level of significance: $p < 0.05$) between columns. Control: untreated grapevines; E+G: grapes treated with eugenol plus guaiacol solution.

nonvolatile precursor forms and later, as a result of the wine-making process and the chemical hydrolysis at the low pH of wines, are released at the end of the alcoholic fermentation and mainly after six months. There is no possibility for the formation of glycoconjugates of the oak lactone ring molecules; although oak lactone precursors have been described as ring-opened *cis*- and *trans*-oak lactone glucosides and gallates that can undergo the lactonization process at wine pH.[39]

The concentration of furfural and 5-methylfurfural in all samples (Table 7) were below the values found by other authors in wine aged in contact with oak[24,40] and lower than their olfactory threshold (88 mg/L and 20 mg/L, respectively).[41,42] The furanic compounds give the wine a bitter almond aroma and are considered to enhance the aroma of the lactones.[43] Extract treatments did not affect the furfural content but increased 5-methylfurfural content, although there were no differences between the two oak extract formulations used. After six months, there was a significant increase in 5-methylfurfural in the control wine while it remained constant in the other wines.

In the control wine a significant increase of eugenol is seen after six months (Table 7), which shows that it is released from the soluble precursor forms that must be in the wine. The wines from the grapevines treated with extracts contain significantly higher amounts of eugenol than the control wine. After six months, its concentration increased substantially, exceeding its olfactory threshold (15 μg/L according to Cutzach et al.[44]). These facts suggest the presence in wines of soluble eugenol precursors from which eugenol is released with age, and that grapes from oak extract treatments accumulate eugenol as nonvolatile precursors. In aged wines kept for one year in bottles, a decrease in eugenol has been described.[45] These results suggest an important difference among wines from grapes treated with oak extracts and aged wines, given that eugenol from the latter decreases over time, as its presence in the wine is due to its extraction from the wood of the barrel in the form of the free compound, while in the wines from the grapes treated with oak extract, eugenol is probably mainly in a nonvolatile precursor form biosynthesized in grapes, which could be released with age.

6-Methoxyeugenol has a spicy aroma and increased significantly after six months due to the effect of the two oak treatments (Table 7); a greater proportion comes from 25%, exceeding its olfactory threshold (12 μg/L[46]).

Lower guaiacol content was found in the control wine than in the treated grapes, not exceeding its olfactory threshold (9.5 μg/L, smoke aroma descriptor, according to Ferreira et al.[42]); in any of the cases six months after the end of alcoholic fermentation, the concentrations of guaiacol significantly decreased in the wines from treated grapes, so its behavior is different from that observed for eugenol. The major compound in all wines is 4-vinylguaiacol, having concentrations within the range described for white wines[30] and exceeding its olfactory threshold (10 μg/L according to Guth[47]). The concentration of 4-vinylguaiacol is higher than the ethylphenols concentration in white wines, contrary to what happens in red wines,[48] and decreases significantly after six months, independently of the grapevine treatment (Table 5). Ethylphenols content remains constant in the control wine over time, and in wines made with treated grapes, their concentrations are slightly higher. The ethylphenols, 4-ethylphenol and 4-ethylguaiacol, may come from the grapes (Table 5), but they can also be formed by vinylphenol enzymatic reduction.[49] In wines from grapevine oak extract treatments, the concentrations are higher than in the control wine (Table 7) which may be due to the contribution of the extracts, suggesting that there are no soluble precursors, as there is no release after six months, which is the case especially predominant with eugenol.

After the alcoholic fermentation, the concentrations of vanillin, acetovanillone, and methyl vanillate, that contributed the spicy aromas and vanilla, were very low and below those found by other authors in wines in contact with oak.[40,45] After six months, a significant increase in acetovanillone and methyl vanillate was observed, which was higher in wines from the 25% treatment, although none of them exceeded the olfactory threshold (60 μg/L for vanillin, 1000 μg/L for acetovanillone, and 3000 μg/L for methyl vanillate).[50] Therefore, these two compounds could probably be incorporated into the plant by the oak extract treatments and stored in the form of nonvolatile precursors, only to be released in the wines with time.

Eugenol and guaiacol are common compounds in aged wines, which are extracted from the oak wood into the wine.[40,50] These

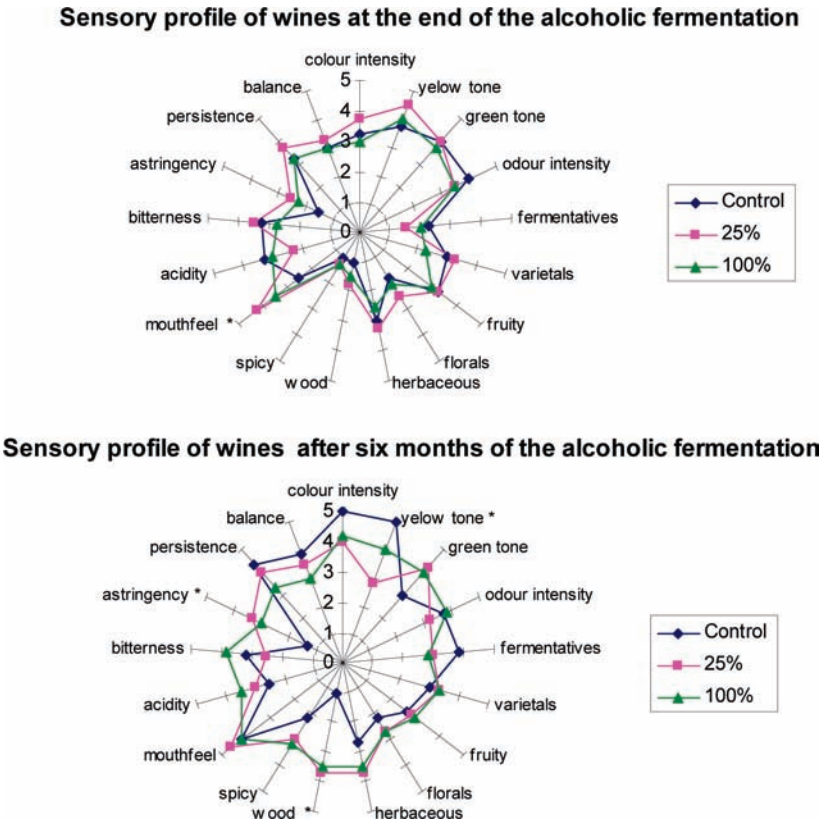

Figure 1. Sensory profiles of wines at the end of the alcoholic fermentation and six months after the alcoholic fermentation. *Level of significance: $p < 0.05$ with control wine.

compounds have also been subject to studies related to the smoke taint of wines from grapevines exposed to smoke.[7,8] This effect is due to the volatile phenols released from smoked grapes throughout the winemaking process, making use of a solution of eugenol and guaiacol as a benchmark to check the evolution of these compounds in grapes and wines (Table 8). Both eugenol and guaiacol significantly increase their concentration compared to its control once the fermentation has finished, with higher eugenol content than guaiacol. After six months, the content of eugenol increased to concentrations four times higher, while guaiacol remained constant. In view of these results and those observed within the grapes (Table 5), we believe that eugenol and guaiacol are probably assimilated by grapes mainly in the form of nonvolatile compounds and that during the winemaking process both compounds are probably released from their precursors, eugenol being released in greater proportion than guaiacol (Table 7). Furthermore, in wines at the end of the fermentation, a large part of the soluble nonvolatile precursors of eugenol is maintained and released with time. These results confirm earlier comments regarding the wines from the grapevines treated with oak extracts. Also, Hayasaka et al.[34] showed that berries and leaves from grapevines exposed to smoke stored guaiacol from smoke, like β-D-glucopyranoside, and transfer it to the must where the aglycone is released by enzymatic and chemical hydrolysis. The application of the E+G solution to the grapevine has shown increases of other compounds in relation to the control wine at the end of the alcoholic fermentation, especially the two lactones, 4-ethylguaiacol and 4-ethylphenol. It is possible that the application of the E+G solution modifies the biosynthesis of 4-ethylguaiacol and 4-ethylphenol, as they are shikimic derivatives.[31] In addition, 4-ethylguaiacol could also come from the enzymatic reduction of 4-vinylguaiacol,[49] since its content significantly decreased. However, we cannot provide any explanation for the increase in *trans*- and *cis*-oak lactones (*cis/trans* ratio of 0.41) so it will be the subject of future research.

Six months after the end of fermentation, there was a significant increase in 6-methoxyeugenol, suggesting an increase in the formation of its putative nonvolatile precursor from eugenol, as it was not detected in grapes (Table 5). Moreover, increases in the concentrations of the lactones were also observed, but the *cis/trans* ratio is higher than 1, which favored the formation of the *cis* isomer. The concentrations of ethylphenols showed a significant increase that could be ascribed to the significant decrease of vinylphenols. The vanillin derivatives also increased significantly, especially acetovanillone, indicating that there were also adjustments in the formation of soluble precursors of these compounds in the berries due to the E+G treatment. In summary, the results obtained as a consequence of the E+G treatment for the grapevines are new and should be confirmed in future studies, given their relevance.

Sensory Analysis. Figure 1 shows the sensory analysis of the control wines and those made from grapevines treated with oak extracts. At the end of alcoholic fermentation, the wines showed all the characteristics of young Verdejo wines, finding significant differences due to the oak extracts only in the "mouthfeel" attribute that was greater when the wines came from grapevines treated with 25% oak extract. This attribute is one of six that have been evaluated in the gustatory phase and has the highest average value followed by the "persistent" and "bitterness" attributes,

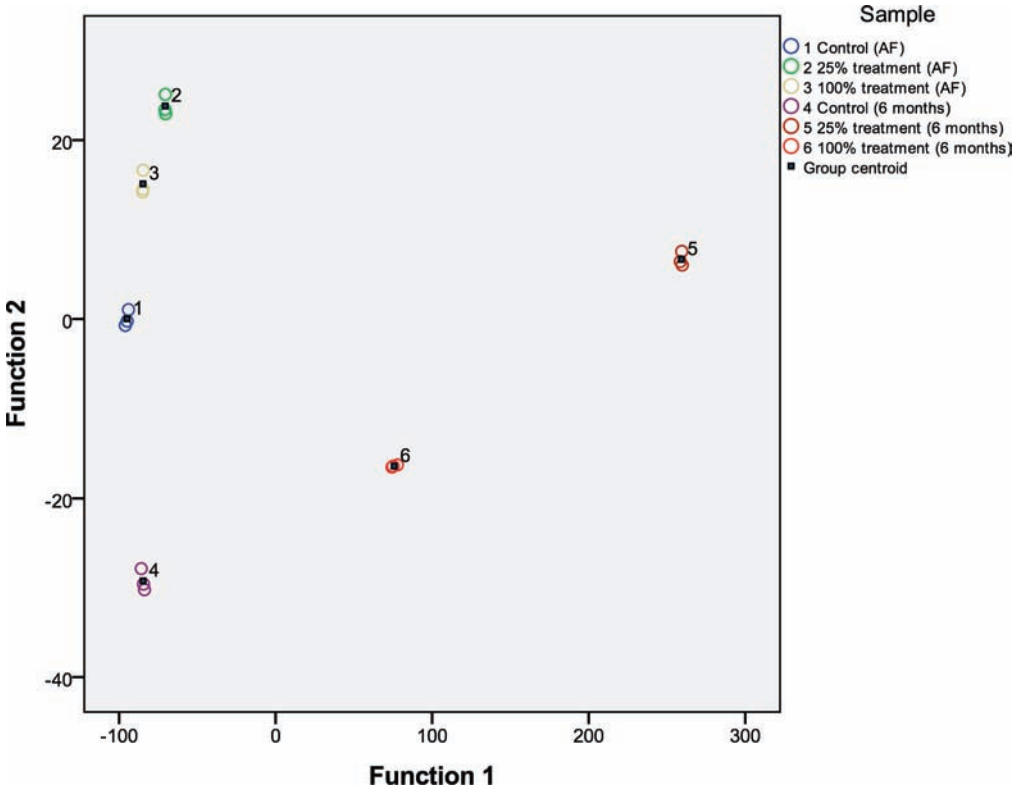

Figure 2. Canonical discriminant analysis of volatile compound concentrations and sensory attributes in wines at the end of the alcoholic fermentation (AF) and after six months.

both showing no significant differences among the three wines. The predominant attribute of the visual phase was "yellow tone", and the lowest attribute was the "colour intensity". Among the attributes of the olfactory phase, the highest average scores were for "fruity," "varietals", and "herbaceous", typical for wines from this grape variety.[14] After six months, wines from the grapevines treated with oak extracts suffer a major sensory change that is highlighted in the three sensory phases. Thus, the "yellow tone" is significantly higher in the control wine, which in turn has the lowest values for the "green tone" attribute. These results show a significant improvement in color quality of the wines from treated grapevines, because in young Verdejo wines the color green is associated with higher quality. In the olfactory phase, there is a significant increase in the "wood" attribute, especially when the 25% formulation was used. These results are consistent with those obtained in the study of the volatiles composition of these wines (Table 7), because their concentrations of *cis*- and *trans*-oak lactones responsible for the "wood" aromatic note were high, although their olfactory threshold was not exceeded. However, synergic effects between these compounds and others such as furfural and 5-methylfurfural have been described, increasing the "lactone" aromatic perception.[43] In the gustatory phase, two attributes, "mouthfeel" and "astringency", increased significantly compared to the control wine, which are slightly higher in wines from the treatment of the grapevines with the 25% extract formulation. Also, grapes treated with this 25% formulation had the highest average values for "persistence" and "balance" attributes. Consequently, treatments with oak extracts on grapevines produce sensory attributes in the wines that are revealed six months after the alcoholic fermentation, being characteristic of wines that have been aged in oak barrels.[14]

The discriminant analysis applied to wines (control, and 25% and 100% oak extract treatment) in the two sampling times (at the end of the alcoholic fermentation and after six months of this) (Figure 2) was carried out by taking into account the volatile compounds concentration and their sensory attributes. Sample differentiation was achieved by two canonical functions; the first explained the 97.1% of the total variance and the second explained the 1.9%. The most important discriminating variables were 4-ethylguaiacol, 6-methoxyeugenol, guaiacol, methyl vanillate, and 4-vinylguaiacol, followed by the attributes of yellow tone, wood, and astringency. After the alcoholic fermentation, the wines are quite similar, while the wines after six months are clearly separated in the graph, thus showing the full extent of the effect of oak extract treatment. This statistical analysis corroborates that the wines are differentiated in relation to their aroma composition only after time and not at the end of alcoholic fermentation.

In conclusion, the application of aqueous oak extracts to grapevines of the white Verdejo cultivar affects the aroma composition of grapes and wines. The results suggest that berries store volatiles, which come from the oak extract formulations, as nonvolatile precursors, some of which are released during the winemaking process. This is especially evident after six months, when the highest release of these volatiles occurs, significantly impacting the aroma. Sensory analysis shows that wines from grapevines treated with oak extracts maintain the typical aroma character of Verdejo wines at the end of fermentation, but after six months, the color is greener, they are more astringent, and the aroma of oak wood is highlighted like that of wines aged in barrels.

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