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Improvement of Cencibel Red Wines by Oxygen Addition after Malolactic Fermentation: Study on Color-Related Phenolics, Volatile Composition, and Sensory Characteristics

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Supporting Information

ABSTRACT: The objective of this paper was to check whether a micro-oxygenation technique applied after malolactic fermentation could improve the quality of Cencibel red wines. For that purpose, the color-related phenolics, volatile composition, and sensory characteristics during the micro-oxygenation treatment have been considered. The phenolic compounds more affected by the oxygen addition were hydroxycinnamic acids and their derivatives [(+)-catechin and (−)-epicatechin], flavonols (glycosilated forms), and anthocyanins-related pigments. The fact that the concentration of pyranoanthocyanins and hydroxyphenyl-pyranoanthocyanins was higher in treated red wines is closely related to their color stabilization. As a consequence, higher values of the yellow and red component of the color (b^* and a^*, respectively) were also observed in micro-oxygenated red wines. Red wine aroma quality was also improved in treated wines. A significant decrease in herbaceous notes, bitterness, acidity, and astringency was found, as well as higher scores of red fruits, plum, liquorice, and spicy attributes in oxygen-added red wines.

KEYWORDS: *color, micro-oxygenation, pigments, polyphenols, volatile compounds, sensorial analysis*

INTRODUCTION

The micro-oxygenation process was developed in 1991 in Madiran (France) with the aim of stabilizing the red wine color, improving the wine quality, and reproducing and/or accelerating the positive oxidative wine transformations that take place during oak barrel aging.[1] Small, continuous, and controlled quantities of oxygen are added to stainless steel tanks, by controlling the flow rate to avoid oxidation phenomena. Although the micro-oxygenation treatment is typically begun at the end of alcoholic fermentation and prior to malolactic fermentation,[2−4] this technique can be applied at any stage during the winemaking process (such as during alcoholic fermentation and after malolactic fermentation), as affirmed Parish et al.[5] However, scarce studies about the application of the micro-oxygenation treatment after malolactic fermentation have been developed.[6−9]

On the one hand, according to Cano-López,[8] the application of the micro-oxygenation technique after malolactic fermentation of red wines assures the occurrence of enough acetaldehyde that could promote the formation of anthocyanin-related pigments. In this way, Cano-López et al.[9] reported an increase in the concentration of A type vitisins and pinotin A in Monastrell and Cabernet Sauvignon micro-oxygenated wines once malolactic fermentation finished. However, a diminution of monomeric anthocyanins and ethyl-linked anthocyanin-tannin adducts was found, likely due to a conversion into more stable polymerized compounds.[6,9] It is highlighted that an excess of oxygen, when the micro-oxygenation technique is applied after malolactic fermentation, could provoke the formation of high molecular weight compounds, which, after precipitation, gave rise to a diminution of the color intensity of red wines.[8]

On the other hand, only few scientific studies about the effect of oxygen addition after malolactic fermentation on the sensorial profile of red wines have been developed. In this way, Cano-López et al.[8] affirmed that the application of the micro-oxygenation technique under these conditions made astringency and herbaceous notes decrease. Moreover, Pour-Nikfardjam et al.[10] reported that the development of fruity flavors was associated with the oxygen addition in Pinot Noir red wines. However, no scientific data about volatile composition have been previously reported in the aforementioned conditions.

Taking into account the scarce literature about the effects of micro-oxygenation technique applied after the malolactic fermentation of red wines, further research is necessary to elucidate the possible improvement of their quality. This study could be positive to the winemaking industry aiming at decreasing the unwanted astringency and bitterness of red wines after their malolactic fermentation.

Therefore, the objective of this research was to study in depth how the micro-oxygenation treatment applied after malolactic fermentation could improve the quality of a Cencibel red wine. Our interest was focused on several perspectives not previously considered in conjunction under these conditions: color, phenolic and volatile compositions, and sensory analysis. Therefore, a study of the evolution of the aforementioned parameters during the micro-oxygenation treatment has been

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carried out. In addition, authors would also elucidate the parameters that evidence a possible overoxygenation, to avoid it over the course of the micro-oxygenation treatment.

MATERIALS AND METHODS

Winemaking. Red wine made from *Vitis vinifera* grape cv. Cencibel, an indigenous variety of Castilla-La Mancha, was supplied by a local winery (Aquilice Cellar, Miguelturra, Castilla-La Mancha region, Spain) just at the end of malolactic fermentation. The wine was homogeneously distributed within four stainless steel tanks of 2000 L capacity, 2 m of height, and 115 cm of diameter, which guarantee the complete dissolution of oxygen in wine during the micro-oxygenation treatment. Two tanks were submitted to micro-oxygenation treatment, and the other two tanks contained untreated, control wine.

The micro-oxygenation treatment applied after the development of malolactic fermentation consisted of a total oxygen addition of 6 mL/L (20 °C) by means of a microdiffusion system (Laffort, Spain), distributed in three steps (Table 1). The dose supplied was appropriated according to the initial scores of astringency and bitterness and the manufacturer recommendations. First of all, an initial high oxygen dose was supplied during 7 days (3.5 mL/L) followed by a slight oxygen doses used for harmonizing and softening of red wines (0.5 mL/L). Subsequently, higher oxygen quantities were employed to provoke an overoxygenation (2 mL/L). Wines were analyzed during the oxygen addition until completion of the micro-oxygenation treatment, together with the control wine. All of the sample replicates were analyzed in duplicate. Conventional analyzes were performed by OIV official methods.[11]

Table 1. Scheme of Oxygen Doses Applied to Cencibel Red Wines

interval (days)	duration (days)	flow (mL/L/month)	oxygen dose (mL/L)
0−7	7	15	3.5
8−22	15	1	0.5
23−42	20	3	2
	42		6

Analysis of Wine Polyphenolic Compounds and Color Parameters. A Hewlett-Packard 8452A apparatus was used to the study of main phenolic families by spectrophotometry. Total polyphenolics, hydroxycinnamic acids, and their derivatives and flavonols[12] and flavan-3-ols[13] were measured. Also, the chromatic characteristics in the space CIELAB and color parameters were calculated.[14] The percentage contributions of copigmented and polymerized anthocyanins to the total wine color at pH 3.6 (degree of copigmentation and polymerization, respectively) were analyzed following the method described by Hermosín-Gutiérrez et al.[15]

An Agilent 1100 series system (Agilent, Waldbronn, Germany), equipped with a DAD photodiode detector (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI/MS^n) system, was used for the high-performance liquid chromatography (HPLC) separation, identification, and quantification of phenolic compounds, according to the method proposed by Castillo-Muñoz et al.[16] On the one hand, the anthocyanins, benzoic acid derivatives, and flavan-3-ols analysis was carried out by direct injection of wine samples, and quantification was made using the DAD chromatograms obtained at 520 (anthocyanins) and 280 nm (benzoic acid derivatives and flavan-3-ols). On the other hand, the isolation of wine flavonols and hydroxycinnamic acids and their derivatives was performed by the method proposed by Castillo-Muñoz et al.[16] The chromatographic method used was that proposed by Castillo-Muñoz et al.[17] and 360 (flavonols) and 320 nm (hydroxycinnamic acids and their derivatives) were used for quantification. For identification, the ESI-MS^n was used in positive mode for anthocyanins and flavan-3-ols, whereas

Table 2. Mean Values of Concentration (mg/L) and Standard Deviations (n = 2) of Hydroxycinnamic Acids and Their Derivatives (HCAD) Identified by HPLC-MS in Control (C) and Micro-oxygenated (M) Cencibel Red Wines during the Micro-oxygenation Treatment[a]

day		*t*-GRP	*t*-caftaric acid	*t*-coutaric acid	*c*-coutaric acid	*t*-fertaric acid	caffeic acid	*p*-coumaric acid	ferulic acid
0		25.8 ± 2.55	29.9 ± 0.84	17.8 ± 0.50	5.04 ± 0.07	12.5 ± 0.21	16.0 ± 0.41	7.01 ± 0.08	0.84 ± 0.08
3	C	20.8 ± 4.43	29.1 ± 2.28	17.1 ± 0.25	4.70 ± 0.03	11.1 ± 1.36	17.0 ± 0.33	7.27 ± 0.22	0.88 ± 0.04
	M	16.2 ± 4.35	27.9 ± 1.65	16.0 ± 0.21	4.52 ± 0.00	10.4 ± 1.07	15.5 ± 0.00	6.81 ± 0.19	0.70 ± 0.22
6	C	20.1 ± 1.53	24.1 ± 1.19	14.5 ± 0.72	3.96 ± 0.22	9.87 ± 0.18	17.6 ± 1.45	7.79 ± 0.60	0.94 ± 0.07
	M	16.2 ± 3.87	26.2 ± 1.80	14.9 ± 0.18	4.24 ± 0.18	10.6 ± 1.27	16.7 ± 0.07	7.45 ± 0.13	0.92 ± 0.04
7	C	16.4 ± 3.61	25.5 ± 1.98	14.1 a ± 0.10	4.18 ± 0.29	10.0 ± 0.93	17.8 b ± 0.36	7.61 b ± 0.08	0.98 ± 0.04
	M	16.3 ± 3.22	25.8 ± 1.13	14.9 b ± 0.06	4.41 ± 0.31	9.81 ± 0.52	16.4 a ± 0.33	7.08 a ± 0.04	0.88 ± 0.02
8	C	15.4 ± 2.54	23.2 ± 1.10	12.8 a ± 0.15	3.88 ± 0.33	8.96 ± 0.00	17.7 ± 0.56	7.58 ± 0.10	0.81 ± 0.17
	M	15.7 ± 2.19	26.1 ± 1.07	15.0 b ± 0.17	4.44 ± 0.32	9.49 ± 0.34	17.0 ± 0.48	7.41 ± 0.09	0.78 ± 0.18
18	C	17.3 ± 4.82	13.3 a ± 0.09	6.01 a ± 0.08	1.89 ± 0.43	7.85 ± 0.71	23.4 b ± 0.59	11.0 b ± 0.15	1.21 b ± 0.17
	M	16.8 ± 4.04	15.4 b ± 0.09	7.33 b ± 0.08	2.19 ± 0.34	6.29 ± 3.36	20.6 a ± 0.68	10.3 a ± 0.15	1.09 a ± 0.13
25	C	15.7 ± 3.09	6.95 a ± 0.34	2.49 a ± 0.24	0.92 ± 0.40	8.10 ± 0.49	26.2 b ± 0.10	13.1 b ± 0.03	1.73 ± 0.12
	M	15.4 ± 2.83	8.32 b ± 0.35	3.41 b ± 0.16	1.09 ± 0.29	7.08 ± 1.67	24.6 a ± 0.09	12.8 a ± 0.07	1.55 ± 0.10
28	C	22.0 ± 4.57	7.24 ± 0.09	1.42 a ± 0.05	0.77 ± 0.00	4.79 ± 0.18	26.9 b ± 0.05	13.6 ± 0.03	1.94 ± 0.04
	M	23.0 ± 4.64	7.58 ± 0.00	1.96 b ± 0.06	0.48 ± 0.13	5.02 ± 0.11	26.0 a ± 0.10	13.6 ± 0.02	1.89 ± 0.01
31	C	17.0 ± 1.20	4.88 a ± 0.04	0.93 ± 0.13	0.63 ± 0.03	4.34 ± 0.05	27.3 b ± 0.19	13.7 ± 0.06	2.09 ± 0.02
	M	20.1 ± 1.79	6.44 b ± 0.06	0.86 ± 0.04	0.57 ± 0.03	4.30 ± 0.09	26.4 a ± 0.22	14.0 ± 0.12	2.07 ± 0.03
36	C	18.3 ± 1.57	4.52 ± 0.16	0.36 ± 0.03	0.07 ± 0.01	3.87 ± 0.21	28.0 ± 0.32	14.0 ± 0.07	2.28 ± 0.07
	M	18.5 ± 2.12	4.92 ± 0.81	0.33 ± 0.02	0.07 ± 0.02	3.48 ± 0.66	27.6 ± 0.18	13.8 ± 0.02	2.07 ± 0.30
38	C	25.9 ± 2.65	4.91 ± 0.91	0.39 ± 0.05	0.10 ± 0.04	3.43 ± 0.63	28.3 b ± 0.14	14.3 ± 0.08	2.18 ± 0.31
	M	20.7 ± 0.59	5.27 ± 0.14	0.33 ± 0.03	0.06 ± 0.05	4.64 ± 1.43	26.5 a ± 0.03	14.1 ± 0.02	2.11 ± 0.29
42	C	17.0 ± 0.08	4.25 ± 0.12	0.40 ± 0.06	0.11 ± 0.06	4.49 ± 1.34	27.4 ± 0.15	13.7 a ± 0.01	2.11 ± 0.29
	M	19.1 ± 3.37	4.86 ± 0.60	0.37 ± 0.04	0.10 ± 0.05	4.13 ± 0.86	26.3 ± 0.33	14.0 b ± 0.03	2.13 ± 0.28

[a]For each compound and day, different letters indicate significant differences according to Student's test (p < 0.05) between control and micro-oxygenated red wines.

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Table 3. Mean Values of Concentration (mg/L) and Standard Deviations ($n = 2$) of Aglycone Flavonols Identified by HPLC-MS in Control (C) and Micro-oxygenated (M) Cencibel Red Wines during the Micro-oxygenation Treatment[a]

day		myricetin	quercetin	kaempferol	laricitrin	isorhamnetin	syringetin
0		2.75 ± 0.65	5.28 ± 1.31	0.59 ± 0.14	0.09 ± 0.04	0.16 ± 0.02	0.20 ± 0.01
3	C	2.45 ± 0.28	5.64 ± 1.49	0.61 ± 0.15	0.10 ± 0.05	0.15 ± 0.01	0.19 ± 0.03
	M	1.70 ± 0.66	3.56 ± 0.26	0.38 ± 0.04	0.08 ± 0.05	0.10 ± 0.02	0.12 ± 0.02
6	C	2.56 ± 0.46	4.27 ± 0.64	0.41 ± 0.08	0.10 ± 0.06	0.14 ± 0.03	0.17 ± 0.02
	M	2.15 ± 0.20	3.73 ± 0.18	0.39 ± 0.01	0.12 ± 0.00	0.13 ± 0.04	0.14 ± 0.01
7	C	2.07 ± 0.08	3.54 ± 0.19	0.40 ± 0.01	0.11 ± 0.03	0.10 ± 0.01	0.13 ± 0.02
	M	2.14 ± 0.07	3.63 ± 0.19	0.41 ± 0.00	0.09 ± 0.02	0.11 ± 0.00	0.12 ± 0.01
8	C	1.58 ± 0.14	2.76 ± 0.17	0.31 a ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
	M	1.79 ± 0.16	3.23 ± 0.17	0.38 b ± 0.01	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
18	C	1.67 ± 0.05	2.47 ± 0.17	0.23 b ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.08 ± 0.01
	M	1.53 ± 0.02	2.20 ± 0.13	0.20 a ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.09 ± 0.00
25	C	1.88 b ± 0.03	2.94 b ± 0.10	0.32 b ± 0.00	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
	M	1.56 a ± 0.01	2.41 a ± 0.10	0.25 a ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
28	C	1.87 ± 0.03	2.91 ± 0.16	0.32 ± 0.01	0.08 ± 0.04	0.10 ± 0.01	0.11 ± 0.01
	M	1.87 ± 0.04	2.95 ± 0.09	0.32 ± 0.01	0.07 ± 0.05	0.10 ± 0.00	0.11 ± 0.00
31	C	1.88 ± 0.05	3.08 ± 0.02	0.31 ± 0.01	0.09 a ± 0.00	0.10 ± 0.00	0.13 ± 0.02
	M	1.80 ± 0.11	2.94 ± 0.09	0.29 ± 0.01	0.11 b ± 0.00	0.10 ± 0.02	0.11 ± 0.01
36	C	2.11 ± 0.12	3.65 b ± 0.09	0.42 ± 0.03	0.08 ± 0.04	0.12 b ± 0.00	0.14 ± 0.02
	M	1.92 ± 0.14	3.30 a ± 0.07	0.37 ± 0.03	0.08 ± 0.05	0.10 a ± 0.00	0.11 ± 0.00
38	C	2.86 ± 0.02	4.84 ± 0.37	0.47 b ± 0.01	0.18 ± 0.01	0.16 b ± 0.02	0.18 ± 0.02
	M	1.75 ± 0.18	2.95 ± 0.09	0.29 a ± 0.02	0.09 ± 0.02	0.08 a ± 0.01	0.10 ± 0.01
42	C	1.85 ± 0.28	3.29 ± 0.12	0.35 ± 0.03	0.10 ± 0.04	0.09 ± 0.00	0.10 ± 0.03
	M	1.69 ± 0.39	3.06 ± 0.16	0.32 ± 0.03	0.09 ± 0.03	0.09 ± 0.00	0.08 ± 0.01

[a]For each compound and day, different letters indicate significant differences according to Student's test ($p < 0.05$) between control and micro-oxygenated red wines.

both positive and negative modes were used for flavonols and hydroxycinnamic acids and their derivatives.

Analysis of Wine Volatile Compounds. To quantify the major volatile compounds, the samples were centrifuged for 30 min at 12000 rpm and 4 °C and then passed through glass wool and spiked with 2-pentanol as internal standard (1 g/L) and directly injected (on split mode) in a Hewlett-Packard 5890 Series II Gas Chromatograph coupled to a flame ionization detector, according to the method proposed by Sánchez-Palomo et al.[18]

Minor volatile compounds of wines filtered through 0.45 μm Milli Pore filter were extracted in duplicate by solid-phase extraction (SPE) technique, according to the method proposed by the aforementioned authors.[18] The extracts were concentrated to 200 μL under a gentle stream of nitrogen and stored in a freezer (−20 °C) until chromatographic analysis in scan rate. A volume of 1 μL of extracts was injected in splitless mode into an Agilent Technology 6890N Network GC System equipped with an Agilent Technology 5973 inert Mass Selective Detector.

The identification was based on comparison of the mass spectra with those provided for authentic standards and by the NBS75K and Wiley A libraries. The response factor of each volatile compound was calculated by injection of commercial standard. For compounds in which commercial standards were not available, the response factors of compounds with similar chemical structures were used. All of the samples were injected in duplicate.

Descriptive Sensory Analysis. A panel of expert assessors (between 12 and 15) with experience in sensory analysis evaluated Cencibel red wines. Previously, by means of discriminative tests, assessors were training in descriptive sensory analysis during 16 sessions. Assessment took place in a standard sensory-analysis chamber,[19] equipped with separate booths and wine-testing glasses[20] covered with a watch glass to minimize the escape of volatile compounds. Wines were sniffed and tasted. Then, judges generated sensory terms individually. Finally, 11 attributes were selected by consensus: four olfactive (red fruits, plum/currant, liquorice, and spicy) and seven gustative and in-mouth feel attributes (red fruits, herbaceous, bitterness, body, acidity, astringency, and persistence intensity).

The panellists used a 10 cm unstructured scale to rate the intensity of each attribute. The left extreme of the scale indicated a null intensity of the descriptor, and the right extreme indicated the maximum value. Sensorial analysis was carried out every 7−10 days, coinciding with critical points of the micro-oxygenation treatment. All wine samples were evaluated in duplicate.

Statistical Analysis. Statistical analysis was carried out by using the SPSS version 15.0 for Windows statistical package. The Student's t test was applied to discriminate among the means of chemical and sensory data. Furthermore, a principal component analysis (PCA) was carried out with the aim of highlighting the main contributors to the variance among samples.

RESULTS AND DISCUSSION

General Parameters. The general composition of control (C) and micro-oxygenated (M) red wines was analyzed at the end of treatment. The results of analytical parameters of the Cencibel red wines revealed the correct development of the alcoholic fermentation [for both wines, alcohol strength (v/v) was 13.0, and the values for reducing sugars, fructose, and glucose were below 1.50, 1.20, and 0.20 g/L, respectively]. Therefore, these wines were considered as dry red wines. Also, the development of malolactic fermentation was optimal, due to the almost complete transformation of malic acid to lactic acid (below 0.10 and around 2 g/L, respectively). For both wines, the pH values were very similar (3.90 and 3.91, respectively), and volatile acidity values were below the limit established by CEE (1.2 g/L) (0.72 and 0.70 g/L, respectively). Correct values of free and total SO_2 were also obtained (around 20 and 64 mg/L for both wines, respectively).

Polyphenolic Compounds Identified in Cencibel Red Wines. A large extent of polyphenolic compounds have been identified, belonging to the different types of hydroxycinnamic acids and their derivatives, benzoic acids, flavan-3-ols, flavonols,

Table 4. Mean Values and Standard Deviations (n = 2) of Vitisin Type Pyranoanthocyanins and Hydroxyphenyl-pyranoanthocyanins Identified by HPLC-MS in Control (C) and Micro-oxygenated (M) Cencibel Red Wines during the Micro-oxygenation Treatment[a]

day		A type vitisin	Coum-A type vitisin	B type vitisin	Acet-B type vitisin	Coum-B type vitisin
0		0.52 ± 0.02	0.41 ± 0.13	3.21 ± 0.39	0.42 ± 0.06	1.33 ± 0.17
3	C	0.53 ± 0.14	0.39 ± 0.08	3.36 b ± 0.43	0.53 ± 0.07	1.30 ± 0.66
	M	0.35 ± 0.27	0.31 ± 0.18	2.24 a ± 0.00	0.27 ± 0.16	0.79 ± 0.05
6	C	0.76 ± 0.06	0.36 ± 0.06	3.53 ± 0.26	0.44 ± 0.21	1.19 ± 0.23
	M	0.68 ± 0.03	0.30 ± 0.13	3.11 ± 0.27	0.40 ± 0.15	1.07 ± 0.16
7	C	0.63 ± 0.08	0.29 ± 0.07	2.98 ± 0.37	0.38 ± 0.12	0.88 ± 0.03
	M	0.67 ± 0.12	0.40 ± 0.05	3.00 ± 0.42	0.42 ± 0.07	0.97 ± 0.08
8	C	0.49 ± 0.03	0.22 ± 0.10	2.40 ± 0.05	0.33 ± 0.08	0.61 ± 0.10
	M	0.57 ± 0.31	0.39 ± 0.15	2.73 ± 0.11	0.43 ± 0.06	0.89 ± 0.05
18	C	0.50 ± 0.02	0.21 ± 0.00	1.58 a ± 0.03	0.22 ± 0.05	0.43 ± 0.00
	M	0.59 ± 0.00	0.19 ± 0.02	2.13 b ± 0.03	0.34 ± 0.04	0.46 ± 0.09
25	C	0.51 ± 0.00	0.24 ± 0.11	1.47 a ± 0.01	0.21 ± 0.02	0.30 a ± 0.00
	M	0.59 ± 0.04	0.15 ± 0.01	1.95 b ± 0.00	0.30 ± 0.05	0.46 b ± 0.01
28	C	0.53 ± 0.02	0.14 a ± 0.02	1.39 a ± 0.03	0.21 a ± 0.02	0.35 ± 0.10
	M	0.62 ± 0.07	0.23 b ± 0.11	1.82 b ± 0.02	0.29 b ± 0.02	0.51 ± 0.05
31	C	0.55 ± 0.05	0.15 ± 0.03	1.31 a ± 0.01	0.20 ± 0.02	0.39 ± 0.02
	M	0.63 ± 0.07	0.17 ± 0.03	1.58 b ± 0.01	0.29 ± 0.05	0.44 ± 0.05
36	C	0.58 ± 0.07	0.25 ± 0.13	1.32 ± 0.00	0.30 ± 0.23	0.36 ± 0.06
	M	0.54 ± 0.05	0.23 ± 0.01	1.18 ± 0.02	0.22 ± 0.02	0.29 ± 0.03
38	C	0.63 ± 0.07	0.26 ± 0.07	1.52 ± 0.15	0.36 ± 0.09	0.56 b ± 0.07
	M	0.63 ± 0.10	0.25 ± 0.15	1.19 ± 0.06	0.11 ± 0.02	0.29 a ± 0.06
42	C	0.54 ± 0.06	0.24 ± 0.03	0.93 ± 0.09	0.18 ± 0.15	0.28 ± 0.01
	M	0.51 ± 0.07	0.32 ± 0.11	0.99 ± 0.30	0.11 ± 0.06	0.27 ± 0.03
		pinotin A	Mv-3-glc-4vp	Mv-3-acet-glc-4vp	Mv-3-coum-glc-4vp	Mv-3-glc-4vg
0		0.66 ± 0.13	0.81 ± 0.28	ND	ND	0.40 ± 0.13
3	C	0.65 ± 0.06	0.87 ± 0.35	0.12 ± 0.05	0.18 ± 0.09	0.42 ± 0.12
	M	0.41 ± 0.01	0.46 ± 0.02	0.09 ± 0.00	0.10 ± 0.01	0.22 ± 0.03
6	C	0.76 ± 0.09	0.66 ± 0.12	0.12 ± 0.02	0.16 ± 0.10	0.36 ± 0.14
	M	0.58 ± 0.01	0.56 ± 0.04	0.11 ± 0.02	0.12 ± 0.02	0.33 ± 0.01
7	C	0.51 ± 0.00	0.46 ± 0.03	0.07 ± 0.01	0.08 ± 0.01	0.27 ± 0.00
	M	0.49 ± 0.02	0.50 ± 0.02	0.07 ± 0.00	0.08 ± 0.01	0.28 ± 0.00
8	C	0.39 ± 0.00	0.33 ± 0.02	ND a	ND	0.15 ± 0.05
	M	0.47 ± 0.03	0.46 ± 0.00	0.08 b ± 0.01	0.08 ± 0.02	0.27 ± 0.01
18	C	0.43 b ± 0.01	0.31 ± 0.02	ND	ND	0.14 ± 0.00
	M	0.37 a ± 0.01	0.28 ± 0.02	ND	ND	0.12 ± 0.04
25	C	0.50 ± 0.03	0.34 b ± 0.00	0.07 b ± 0.01	0.05 ± 0.00	0.17 ± 0.01
	M	0.46 ± 0.03	0.31 a ± 0.00	ND a	ND	0.14 ± 0.03
28	C	0.57 a ± 0.01	0.38 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.20 ± 0.04
	M	0.64 b ± 0.02	0.42 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.21 ± 0.01
31	C	0.61 ± 0.01	0.39 ± 0.01	0.07 ± 0.01	0.04 ± 0.00	0.23 ± 0.01
	M	0.68 ± 0.04	0.41 ± 0.01	0.07 ± 0.00	ND	0.22 ± 0.02
36	C	0.77 b ± 0.00	0.49 b ± 0.01	0.09 ± 0.00	0.08 ± 0.00	0.27 ± 0.02
	M	0.67 a ± 0.01	0.41 a ± 0.00	0.07 ± 0.01	0.03 ± 0.00	0.24 ± 0.00
38	C	1.32 b ± 0.26	0.81 b ± 0.16	0.12 b ± 0.00	0.16 ± 0.05	0.56 b ± 0.12
	M	0.74 a ± 0.00	0.42 a ± 0.00	0.07 a ± 0.01	ND	0.27 a ± 0.05
42	C	0.70 a ± 0.02	0.42 a ± 0.00	ND	ND	0.31 b ± 0.07
	M	0.82 b ± 0.01	0.44 b ± 0.01	ND	ND	0.19 a ± 0.04

[a]For each compound and day, different letters indicate significant differences according to Student's test (p < 0.05) between control and micro-oxygenated red wines. coum, *p*-coumaroylated; acet, acetylated; Mv, malvidin; glc; glucoside; vp, vinyl-phenol; and vg, vinyl-guaiacol. ND, not detected.

and anthocyanins and anthocyanin-related red pigments (Tables 2−4 and Tables S1−S3 in the Supporting Information). The hydroxycinnamic acids and their derivatives, benzoic acids, and flavan-3-ols identified were the expected, well-known, compounds usually present in wine,[1,2] such as *t*-GRP, tartaric acid esters of *p*-coumaric acid, caffeic acid, and ferulic acid, and their respective acids, gallic acid, (+)-catechin, and (−)-epicatechin. Among the flavonols, both free (aglycone) and conjugated (3-glycosides) forms were found;[16,17] the complete series of the 3-glycosides (galactoside, glucuronide, and glucose) of both myricetin and kaempferol were detected, together with quercetin-3-glucuronide and quercetin-3-glucoside. Also, the 3-glucoside derivatives of laricitrin, syringetin, and isorhamnetin have been identified. All of the aforementioned flavonols have

Table 5. Mean Values and Standard Deviations (n=2) of Color Parameters by Spectrophotometic Measures in Control (C) and Micro-oxygenated (M) Cencibel Red Wines during the Micro-oxygenation Treatment[a]

day		color intensity	tonality	copigmentation (%)	polymerization (%)
0		31.5 ± 0.56	3.65 ± 0.03	20.2 ± 2.24	52.5 ± 2.25
3	C	28.7 ± 0.09	3.61 ± 0.01	17.1 ± 0.49	52.6 b ± 0.31
	M	30.3 ± 0.43	3.62 ± 0.04	14.2 ± 0.05	48.2 a ± 0.09
6	C	32.0 ± 0.08	3.54 ± 0.04	13.1 ± 0.88	56.4 ± 0.42
	M	35.4 ± 1.02	3.74 ± 0.19	12.7 ± 0.13	54.7 ± 0.19
7	C	29.4 a ± 0.07	3.63 a ± 0.01	14.7 ± 0.47	56.0 b ± 0.06
	M	30.2 b ± 0.00	3.72 b ± 0.00	14.2 ± 0.13	51.2 a ± 0.09
8	C	11.8 b ± 7.08	2.18 a ± 1.82	12.3 b ± 0.44	55.4 b ± 0.18
	M	6.82 a ± 0.01	3.52 b ± 0.00	9.47 a ± 0.56	51.4 a ± 0.35
18	C	8.78 b ± 0.02	3.46 a ± 0.00	8.77 b ± 0.56	58.5 b ± 0.22
	M	6.13 a ± 0.00	3.61 b ± 0.01	6.27 a ± 0.14	53.1 a ± 0.00
25	C	7.28 a ± 0.01	3.64 b ± 0.00	8.74 ± 0.36	56.8 a ± 0.02
	M	10.3 b ± 0.01	3.37 a ± 0.00	8.66 ± 0.05	61.8 b ± 0.03
28	C	6.46 a ± 0.00	3.58 b ± 0.00	11.1 b ± 0.07	58.4 a ± 0.12
	M	8.14 b ± 0.01	3.50 a ± 0.00	6.58 a ± 0.36	60.1 b ± 0.13
31	C	7.04 b ± 0.01	3.55 a ± 0.00	8.85 ± 0.43	58.2 ± 0.22
	M	6.29 a ± 0.00	3.69 b ± 0.01	7.28 ± 0.85	59.1 ± 0.09
36	C	7.57 b ± 0.01	3.52 ± 0.00	10.3 ± 0.50	60.0 ± 0.31
	M	6.23 a ± 0.02	3.53 ± 0.00	12.6 ± 0.98	59.1 ± 0.56
38	C	9.25 a ± 0.04	3.44 ± 0.00	9.70 b ± 0.09	62.4 ± 0.09
	M	9.63 b ± 0.05	3.49 ± 0.00	8.93 a ± 0.02	62.7 ± 0.15
42	C	7.56 a ± 0.04	3.49 ± 0.01	12.5 b ± 0.13	63.4 a ± 0.15
	M	8.61 b ± 0.03	3.49 ± 0.00	10.9 a ± 0.24	65.2 b ± 0.11

[a]For each compound and day, different letters indicate significant differences according to Student's test ($p < 0.05$) between control and micro-oxygenated red wines.

Table 6. Mean Values and Standard Deviations ($n = 2$) of CIELAB Chromatic Characteristics in Control (C) and Micro-oxygenated (M) Cencibel Red Wines during the Micro-oxygenation Treatment[a]

day		L^*	C^*	h^*	a^*	b^*
0		66.0 ± 0.53	31.7 ± 0.66	8.03 ± 0.54	31.3 ± 0.61	4.42 ± 0.39
3	C	68.6 ± 0.07	30.2 ± 0.55	8.07 ± 0.02	29.9 ± 0.54	4.23 ± 0.07
	M	67.4 ± 0.35	30.8 ± 0.47	7.70 ± 0.80	30.5 ± 0.53	4.12 ± 0.36
6	C	65.7 ± 0.00	32.0 ± 0.30	7.65 ± 0.02	31.7 ± 0.29	4.26 ± 0.02
	M	63.1 ± 0.49	32.8 ± 1.94	11.3 ± 1.45	32.1 ± 2.06	6.40 ± 0.43
7	C	67.9 ± 0.00	30.1 ± 0.15	8.02 a ± 0.28	29.8 ± 0.16	4.19 a ± 0.13
	M	67.5 ± 0.00	30.3 ± 0.04	9.80 b ± 0.16	29.8 ± 0.05	5.16 b ± 0.08
8	C	64.1 ± 0.07	34.1 ± 0.11	6.99 a ± 0.14	33.8 ± 0.13	4.15 ± 0.06
	M	64.3 ± 0.07	33.9 ± 0.08	7.87 b ± 0.21	33.6 ± 0.06	4.65 ± 0.13
18	C	56.6 a ± 0.07	38.8 b ± 0.01	8.25 a ± 0.08	38.4 b ± 0.02	5.57 b ± 0.05
	M	67.2 b ± 0.00	30.6 a ± 0.06	9.14 b ± 0.09	30.2 a ± 0.07	4.86 a ± 0.04
25	C	62.3 b ± 0.00	33.5 a ± 0.02	8.36 a ± 0.03	33.1 a ± 0.03	4.86 a ± 0.01
	M	51.6 a ± 0.07	42.6 b ± 0.03	9.93 b ± 0.06	42.0 b ± 0.04	7.35 b ± 0.04
28	C	65.6 b ± 0.00	31.8 a ± 0.00	7.69 a ± 0.06	31.5 a ± 0.01	4.26 a ± 0.04
	M	59.2 a ± 0.07	37.1 b ± 0.01	9.32 b ± 0.06	36.6 b ± 0.01	6.01 b ± 0.04
31	C	63.3 a ± 0.07	34.0 b ± 0.04	8.03 a ± 0.01	33.6 b ± 0.04	4.74 a ± 0.01
	M	66.7 b ± 0.07	30.3 a ± 0.01	9.76 b ± 0.03	29.9 a ± 0.00	5.14 b ± 0.01
36	C	61.2 a ± 0.07	35.1 b ± 0.11	7.79 b ± 0.14	34.8 b ± 0.08	4.76 b ± 0.10
	M	66.6 b ± 0.07	31.3 a ± 0.03	7.00 a ± 0.13	31.0 a ± 0.02	3.81 a ± 0.07
38	C	55.1 b ± 0.21	39.4 ± 0.13	8.30 a ± 0.11	39.0 ± 0.13	5.69 a ± 0.09
	M	54.0 a ± 0.14	39.5 ± 0.08	9.89 b ± 0.09	38.9 ± 0.07	6.78 b ± 0.08
42	C	61.3 b ± 0.21	35.6 a ± 0.21	7.97 a ± 0.16	35.2 a ± 0.20	4.93 a ± 0.13
	M	57.7 a ± 0.07	38.6 b ± 0.04	10.0 b ± 0.08	38.0 b ± 0.03	6.72 b ± 0.06

[a]For each compound and day, different letters indicate significant differences according to Student's test ($p < 0.05$) between control and micro-oxygenated red wines. L^*, lightness; C^*, chroma; h^*, hue; a^*, red contributor to the wine color (negative values indicate green contribution, while positive values indicate red contribution); and b^*, yellow contributor to the wine color (negative values indicate blue contribution, while positive values indicate yellow contribution).

been also identified as free aglycones. In addition, native grape anthocyanins were detected in Cencibel red wines,[2,3,21] including nonacylated, acetylated, and *p*-coumaroylated anthocyanins (anthocyanidin 3-glucosides) of the five expected wine

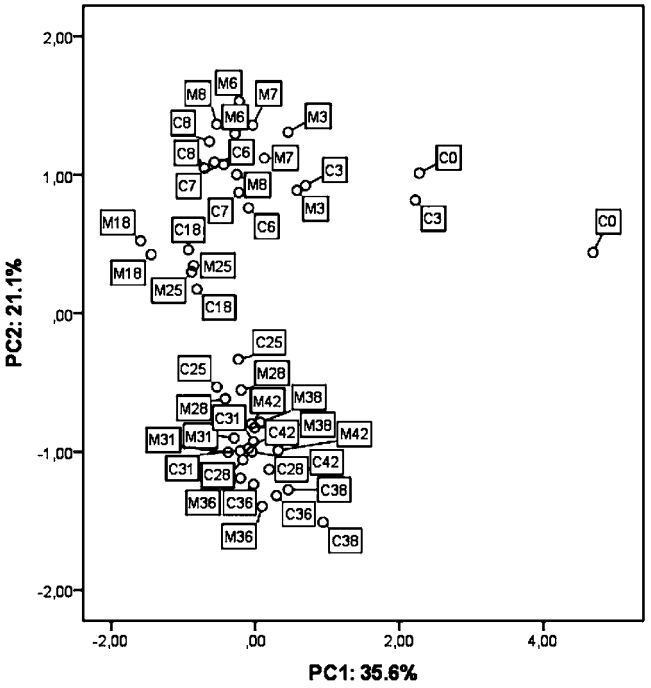

Figure 1. Plot of Cencibel red wine samples in the space defined by PCs PC1 vs PC2, with regard to polyphenolic compounds and color parameters: control (C) and micro-oxygenated (M) red wines in each studied point of micro-oxygenation treatment.

anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, and malvidin), together with caffeoylated malvidin 3-glucoside. It is remarkable that a high number of anthocyanin-related red pigments were detected in the studied wines:[2,3,22] pyranoanthocyanins derived from yeast metabolites (vitisins A and B) and hydroxyphenyl-pyranoanthocyanins derived from hydroxycinnamic acids (e.g., pinotin A). The complete series of vitisin B (the nonacylated, acetylated, and *p*-coumaroylated derivatives) have been identified in Cencibel red wines, as well as nonacylated and *p*-coumaroylated derivatives of vitisin A[23] (Table 4). Among hydroxyphenyl-pyranoanthocyanins, the nonacylated, acetylated, and *p*-coumaroylated derivatives of malvidin-3-glucoside-4-vinyl-phenol have been quantified, together with pinotin A (malvidin-3-glucoside-4-vinyl-catechol) and malvidin-3-glucoside-4-vinyl-guaiacol.[24]

Micro-oxygenation Effect on Phenolic Composition and Color Parameters. *Hydroxycinnamic Acids and Their Derivatives.* The main hydroxycinnamic acid derivative in Cencibel red wines was *trans*-caftaric acid (Table 2), followed by *trans*-coutaric and *trans*-fertaric acids. The content of *cis*-coutaric acid was lower than the respective isomer, according to the results obtained by Hermosín-Gutiérrez et al.[25] in Cabernet Sauvignon, Cencibel, and Syrah red wines.

During the time, a hydrolysis of tartaric esters of hydroxycinnamic acids (*trans*-caftaric, *trans* and *cis*-coutaric, and *trans*-fertaric acids) was observed, increasing in parallel the concentration of their respective released hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids, respectively). A high level of hydrolysis of tartaric esters of *p*-coumaric acid was observed (diminution nearly 97%), followed by *trans*-caftaric acid (85%) and *trans*-fertaric acid (62%). However, this diminution was not correlated with the increase of the respective acids. This fact could be due to hydroxycinnamic acids that participate in several chemical reactions, such as their reaction with monomeric anthocyanins, giving rise to hydroxyphenyl-pyranoanthocyanins, thus decreasing their concentration.

trans-Caftaric acid contributes to the formation of 2-*S*-glutathionylcaftaric acid [also called grape reaction product (GRP)], resulting in the nucleophilic addition of glutathione to the quinone of *trans*-caftaric acid. The variability of the content of GRP during the time could be due to the glutathione initially available. *trans*-GRP is already formed during crushing, in which a high amount of oxygen is present. Therefore, the little doses of oxygen applied during the micro-oxygenation treatment did not significantly affect to *trans*-GRP (Table 2).

Contrarily to its respective acid, the content of *trans*-caftaric acid was significantly higher in micro-oxygenated red wines than in untreated ones, above all from the second part of the micro-oxygenation treatment (Table 2). A similar evolution of *trans*- and *cis*-coutaric acids was observed until the second stage of micro-oxygenation treatment. In the third part of the treatment, the increase in the oxygen amount made the content of those phenolic compounds decrease more in micro-oxygenated red wines than that observed in control wines. As a consequence, no significant differences were observed between both treated and untreated red wines.

On the basis of the results obtained, the extent of the hydrolysis of tartaric esters of hydroxycinnamic acids in micro-oxygenated red wines after malolactic fermentation seemed to be not as high as control wines, as suggested the significantly higher concentration of *trans*-caftaric and *trans*-coutaric acids and lower of their respective acids (Table 2). As far as we know, no previous study about the influence of micro-oxygenation treatment after malolactic fermentation on hydroxycinnamic acids and their derivatives has been reported.

Flavan-3-ols and Benzoic Acids. The main flavan-3-ol present in Cencibel red wines was (+)-catechin (Table S1 in the Supporting Information), whose concentration increased during the time.[25] The differences in flavan-3-ols content could be attributable to oxidation reactions, and tannin−tannin and anthocyanin−tannin polymerizations (or tannin−anthocyanin). The lack of variations of (−)-epicatechin and gallic acid concentration suggested that hydrolysis reactions of (−)-epicatechin gallate were practically inexistent.

Highlighted are the scarce significant differences on flavan-3-ol compounds in relation to the micro-oxygenation effects, according to the Student's *t* test ($p < 0.05$) (Table S1 in the Supporting Information). However, although without significant differences, the content of (+)-catechin was slightly higher in treated red wines, as well as the amount of (−)-epicatechin in the majority of the studied points, until the second part of the micro-oxygenation treatment. From that moment, an increase in the amount of oxygen provoked the contrary effect, above all in the case of (−)-epicatechin.[6] Therefore, micro-oxygenated red wines had a significantly lower percentage of polymerization until the second part of the treatment (Table 5), which could positively influence the diminution of astringency according to Pour-Nikfardjam et al.[7]

Flavonols. Myricetin-3-glucoside, followed by the 3-glucuronide and 3-glucoside of quercetin, were the most abundant flavonols present in Cencibel red wines after malolactic fermentation (Table 3 and Table S2 in the Supporting Information), according to Cheynier et al.[26] A diminution in flavonol content was observed during the time in both control and micro-oxygenated red wines, in agreement with Hermosín-Gutiérrez et al.[25] (among 20−30% in glycosilated flavonols and 30−50% in their aglycones) (Table 3 and Table S2 in the

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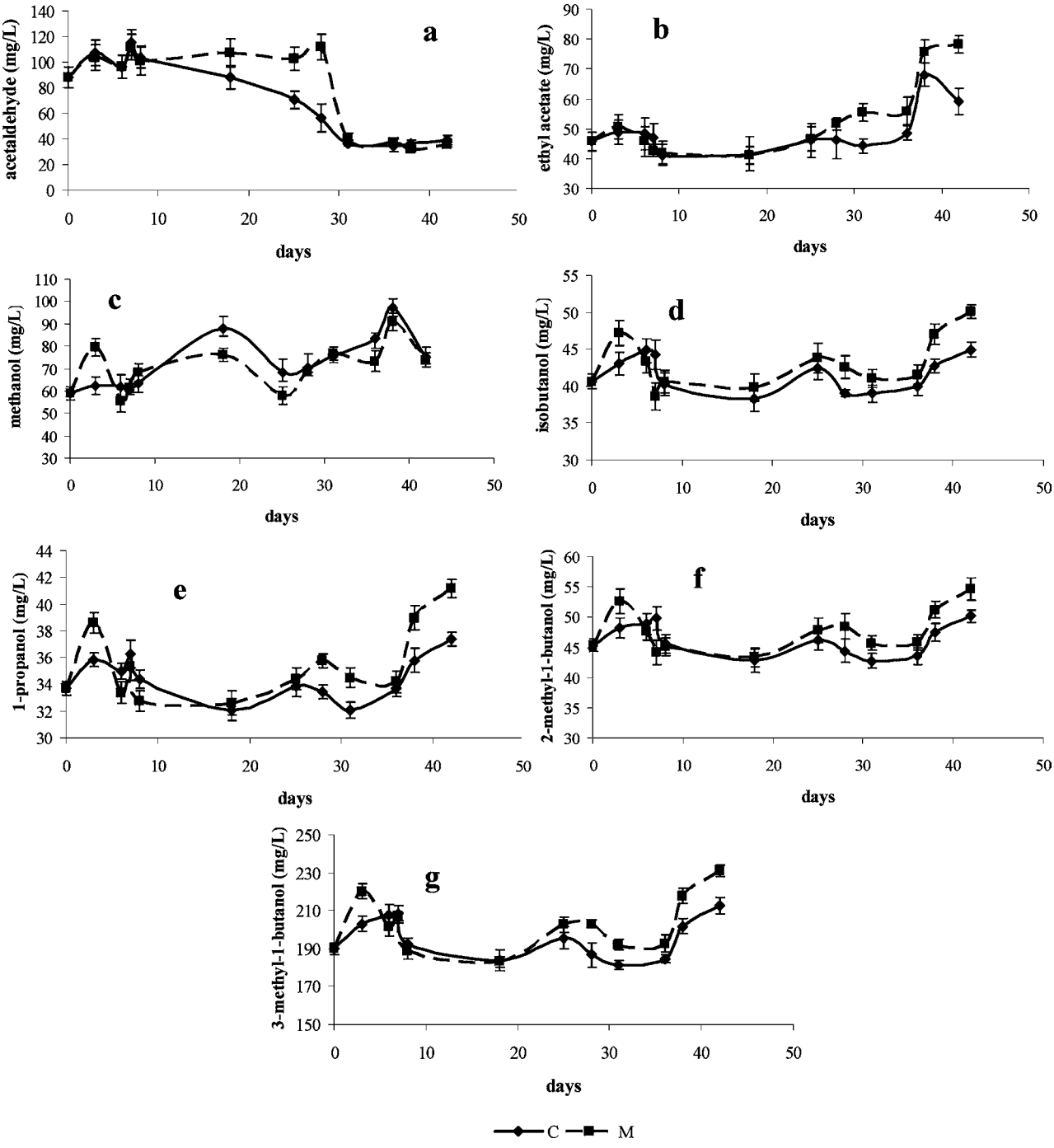

Figure 2. Mean values of concentration (mg/L) and standard deviations of major volatile compounds identified by GC-MS in control (C) and micro-oxygenated (M) Cencibel red wines during the micro-oxygenation treatment.

Supporting Information). This fact could be due to different reasons: hydrolysis reactions of glycosylated flavonols, oxidation reactions, and/or precipitation of aglycones due to their insolubility in the hydro-alcoholic medium.

A scarce effect of micro-oxygenation treatment on flavonol compounds, according to Student's *t* test, was observed. The micro-oxygenation treatment only made flavonol content decrease minimally during the first and second part of the treatment, above all glycosilated forms (Table S2 in the Supporting Information). Moreover, punctual significant differences in aglycones (mainly kaempferol, quercetin, and isorhamnetin) were observed between control and treated red wines during all of the micro-oxygenation treatment (Table 3), in agreement with results found by Castellari et al.[27] in aged Sangiovese wines.

On the basis of the results, although the scarce significant differences, micro-oxygenated red wines suffered a slightly faster and higher hydrolysis of the glycosidic flavonols than untreated wines, with slower and more lasting hydrolysis. Similar results were obtained in Cabernet Sauvignon red wines by McCord.[6]

Anthocyanic Compounds. A diminution in the content of monomeric anthocyanins during the time was observed, according to several authors[28,29] (Table S3 in the Supporting Information). Among these, the nonacylated 3-glucosides of

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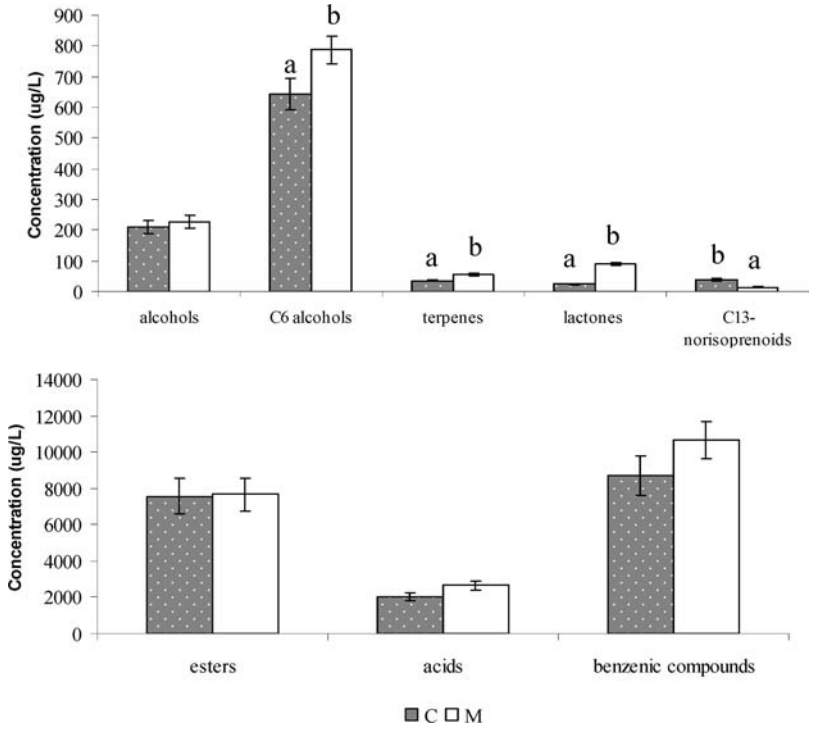

Figure 3. Volatile compound concentrations (μg/L) and standard deviations belonged to different chemical families of control (C) and micro-oxygenated (M) Cencibel red wines at the end of the micro-oxygenated treatment (day 42). Different letters indicate significance of $p < 0.05$ according to Student's t test.

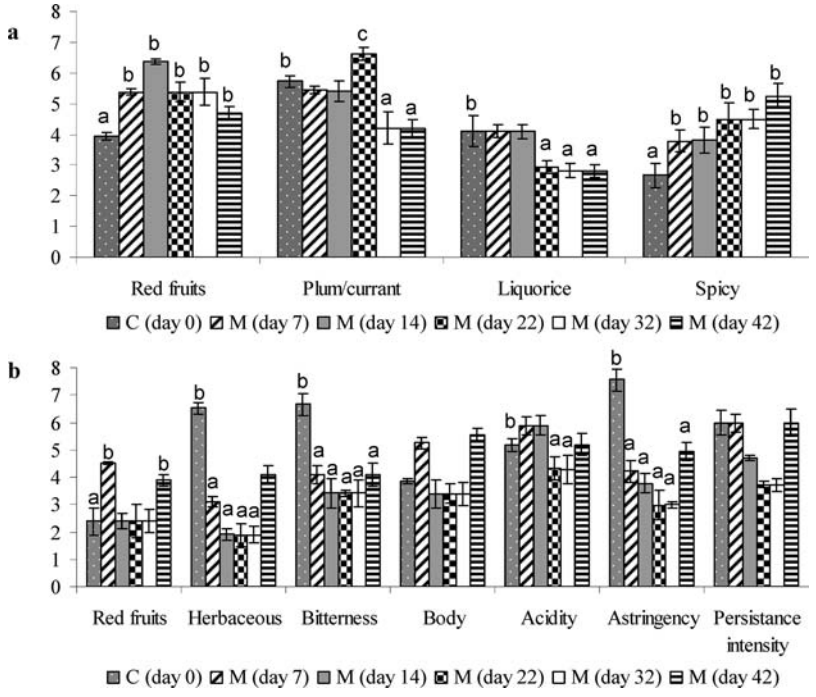

Figure 4. Olfactive (a) and gustative and in-mouth feel (b) attributes mean scores and standard deviations of control (C) and micro-oxygenated (M) Cencibel red wines in different points of the micro-oxygenation treatment. Different letters indicate significance of $p < 0.05$ according to Student's t test between control (C, day 0) and micro-oxygenated (M) wines in each studied points.

petunidin and malvidin (30 and 45%, respectively) and the nonacylated and *p*-coumaroylated 3-glucosides of delphinidin (23 and 10%, respectively) and peonidin (10%) were the anthocyanins with higher percentage of reduction. As a consequence, a decrease in % copigmentation and color intensity occurred in both wines during the time, contrary to the increase in their % polymerization[28] (Table 5). The latter fact could be caused by the formation of oligomeric and polymeric pigments of high molecular weight by reaction between monomeric anthocyanin and flavan-3-ols.[30,31]

According to the Student's t test applied to the set of data, scarce significant differences in the anthocyanin fraction between

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control and micro-oxygenated red wines were found (Table S3 in the Supporting Information). The content of *p*-coumaroylated derivatives of the 3-glucosides of delphinidin, petunidin, peonidin, and malvidin was significantly lower in treated red wines in the second and the third part of micro-oxygenation treatment (with the exception of the *trans* isomer of the last one). Therefore, the % polymerization increased in oxygen-added red wines, above all at the end of the micro-oxygenation treatment. According to Pour-Nikfardjam et al.,[7] higher values of the % polymerization indicate a possible overoxygenation of red wines (Table 5). Moreover, it is well-known that copigmentation provokes a bathochromic shift from reddish to bluish hues.[32] Therefore, a possible consequence of the diminution of % copigmentation in micro-oxygenated red wines could be the increase in the yellow component of the color (b^*)[8,29] (Table 6).

Pyranoanthocyanins and Hydroxyphenyl-pyranoanthocyanins. B type vitisins are formed by means of the reaction between acetaldehyde and anthocyanins, being that derived from malvidin-3-glucoside (the so-called vitisin B) the major pyranoanthocyanin present in Cencibel red wines (Table 4). The evolution of the content of nonacylated, acetylated, and *p*-coumaroylated derivatives of vitisin B during the time for both wines (control and micro-oxygenated) is shown in Table 4.

At the beginning of the micro-oxygenation treatment, a significant decrease in the concentration of vitisin B derivatives was observed (Table 4). In the period of 18−31 days, the decrease in B type vitisins was lower in the micro-oxygenated red wines than in control wines. As a consequence, a significantly higher content of vitisin B derivatives was observed in micro-oxygenated red wines in that period, in comparison with the values obtained in control wines, increasing as well the values of the red color contribution (a^*). After that, in the third part of the micro-oxygenation treatment, the content of B type vitisins of treated red wines fell to a greater extent than that observed in control wines, and as a consequence, no significant differences were observed between both wines.

The formation of A type vitisins takes place by reaction between pyruvic acid and anthocyanins. Although less pronounced, vitisin A (reaction product between pyruvic acid and malvidin-3-glucoside) suffers a similar evolution than vitisin B derivatives (Table 4). As a consequence, the orangey tonalities of micro-oxygenated red wines were higher, above all at the end of the second part of micro-oxygenation treatment (Table 6). This fact is in agreement with several authors,[31] who affirmed that the increase in the content of vitisin A indirectly increase the values of the yellow component of the color (b^*), due to this compound have an absorbance maximum at 510 nm.[33]

The reaction between anthocyanins and hydroxycinnamic acids (or their respective 4-vinyl-phenols) produces the formation of hydroxyphenyl-pyranoanthocyanins, which have been previously described by several authors.[22,34] Pinotin A (malvidin-3-glucoside-4-vinyl-catechol, from the reaction with caffeic acid) and malvidin-3-glucoside-4-vinyl-phenol (from the reaction with *p*-coumaric acid) were the most abundant hydroxyphenyl-pyranoanthocyanins identified in Cencibel red wines (Table 4). As shown in Table 4, the evolution of hydroxyphenyl-pyranoanthocyanins during the time and micro-oxygenation treatment was consistent with that of hydroxycinnamic acids, due to the latter ones participate in their formation.[22] This fact suggests the already formation of hydroxyphenyl-pyranoanthocyanins during alcoholic fermentation, because of the enzymatic hydrolysis of tartaric esters of hydroxycinnamic acids and subsequently enzymatic decarboxylation of the released acids, or even by the direct reaction between free hydroxycinnamic acids and anthocyanins. Following the significant increase in the content of hydroxycinnamic acids in control wines at the end of micro-oxygenation treatment, the formation of the respective hydroxyphenyl-pyranoanthocyanins was reactivated (Table 4). Therefore, this fact reinforces the suggested pathway involving the direct reaction between hydroxycinnamic acids and malvidin-3-glucoside, according to several authors.[22,35]

The content of hydroxyphenyl-pyranoanthocyanins decreased in oxygen-added wines during the first part of micro-oxygenation treatment. This tendency was also observed during the second part of the treatment, with a higher diminution in control wines (Table 4). Later, their content in untreated wines increased until being similar to the initial values, with the exception of malvidin-3-glucoside-4-vinyl-phenol. In the case of micro-oxygenated wines, the amount of hydroxyphenyl-pyranoanthocyanins increased and was similar to control wines in the first part of the treatment and continued increasing when higher amounts of oxygen were added (third part of the treatment). The concentration of the main hydroxyphenyl-pyranoanthocyanins in the final micro-oxygenated wines was significantly higher than in control wines (Table 4); however, some of them disappeared.

PCA. PCA was applied to extract useful information from the complex matrix of phenolic compounds data and color parameters corresponding to control and micro-oxygenated red wines in each studied point of micro-oxygenation treatment. A total of nine significant principal components (PCs) arose according to Kraiser's criterion (eigenvalues >1). With these factors, 89.76% of the total variance is explained. The first three PCs explained nearly 70% of the total accumulated variance. The first PC (PC1), which explains 35.6% of the total variance, mainly contains monomeric anthocyanins (nonacylated, acetylated, *p*-coumaroylated, and caffeoylated 3-glucosides) and some aglycone flavonols (kaempferol and quercetin), with a positive sign. In the case of PC2, which explains 21.1% of the total variance, hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids) with a negative sign, and tartaric esters of hydroxycinnamic acids and B type vitisin with a positive sign are the main contributors. Figure 1 shows the samples to the plane defined by these two PCs. As can be seen, PC1 mainly separated the initial control samples (day 0), whereas PC2 organized them according to the time sequence. No significant differences were observed between control and micro-oxygenated in each studied point of the micro-oxygenation treatment by means of PCA, being that the previously applied Student's *t* test more indicated for that purpose.

Micro-oxygenation Effect on Volatile Compounds. A large extent of volatile compounds has been identified in Cencibel red wines, which belonged to different chemical families (esters, acids, alcohols, terpenes, C_{13}-norisoprenoids, lactones, and benzenic compounds). On the one hand, Figure 2 shows the evolution of the concentration of major volatile compounds during the micro-oxygenation treatment, for both control and treated red wines. The amount of acetaldehyde showed an important decrease in control wines (Figure 2a). However, its content in treated red wines increased until almost the end of the micro-oxygenation (day 30) and in the final phase of the treatment was maintained at the same value than the control wine. According to several authors,[36,37] this fact could be caused by the acetaldehyde formation from the oxidation of ethanol due to the oxygen addition.

The content of ethyl acetate in control and micro-oxygenated red wines suffered a little increase during the third part of the treatment (days 23−42) and even more in micro-oxygenated red wines, probably because of the formation of acetic acid in the final of malolactic fermentation. According to Dupuy et al.,[38] the esterification reaction occurs when small quantities of oxygen are presented. The evolution in the content of the major alcohols formed during the fermentation showed an increase of the levels respect to the initial value. This fact could be the consequence of some residual activity of fermentation yeast, especially in micro-oxygenated wines (Figure 2c−g). Although these values were below the sensorial threshold that produces aroma defects,[39] the increase in their content at the end of the micro-oxygenation treatment could suggest an overoxygenation.

With regard to the minor volatile compounds, the addition of oxygen by means of micro-oxygenation treatment produced a slightly increase in the concentration of some chemical families at the end of micro-oxygenation treatment, being significant in the case of C_6 alcohols, terpenes, and lactones (Figure 3). The content of C_6 alcohols such as 1-hexanol and (*E*)-2-hexen-1-ol was significantly higher in micro-oxygenated red wines [1-hexanol, 528 μg/L in control wines (C)/660 μg/L in micro-oxygenated wines (M); (*E*)-2-hexen-1-ol, 3.68 μg/L C/6.53 μg/L M], as well as the C_6 acids (hexanoic acid and 2-hexenoic acid) and long-chain acids (2-hexenoic acid, 7.19 μg/L C/18.89 μg/L M). In addition, the concentration of *trans*-geraniol and its respective acid significantly increased (5.68 μg/L C/10.83 μg/L M), as well as lactones by effect of the oxygen addition. However, highlighted is the disappearance of β-damascenone and α-ionol in the treated red wines. According to Silva-Ferreira et al.[40] and du Toit et al.,[41] this fact could be due to an overoxygenation of micro-oxygenated red wines. With regard to esters, there were not remarkable differences in the short- and medium-chain ethyl fatty acid esters between both red wines, but the content of long-chain ones was significantly higher in micro-oxygenated red wines [diethyl malate (26.72 μg/L C/36.99 μg/L M), diethyl monosuccinate (146.40 μg/L C/232.79 μg/L M), and ethyl glutarate (9.39 μg/L C/20.47 μg/L M)]. Similar results were found by Brock et al.[42] and Silva-Ferreira et al.[43] in Sherry and Oporto wines, respectively. Finally, among benzenic compounds, those whose concentration was significantly higher in micro-oxygenated red wines were guaiacol (18.21 μg/L C/34.54 μg/L M), 4-vinyl-guaiacol (414.34 μg/L C/1012.19 μg/L M), eugenol (4.49 μg/L C/5.40 μg/L M), and acetovanillone (64.76 μg/L C/156.62 μg/L M).

Descriptive Sensorial Analysis. The attributes selected in the descriptive sensorial analysis of the samples, together with the mean scores for each one, are shown in Figure 4. Sensorial analysis was carried out every 7−10 days, coinciding with critical points of the micro-oxygenation treatment.

With regard to the olfactory analysis, red fruits and plum/currant were the most remarkable attributes present in Cencibel red wines. The red fruits notes significantly increased during the first part of the micro-oxygenation treatment and until day 14, while the plum/currant attribute suffered a significant increase at the end of the second part of the micro-oxygenation treatment, in comparison with the values of control wines (Figure 4a). This fact is in agreement with that of Hernández-Orte et al.[4] in Cabernet Sauvignon red wines. During the third part of the treatment, the scores of these attributes showed an important diminution, but they were significantly above of the values obtained in control wines in the case of red fruits. The liquorice attribute presented in micro-oxygenated red wines had significantly lower scores than control wines at the end of the treatment. Contrarily, an important increase in the values of the spicy attribute was observed in micro-oxygenated red wines during the whole treatment, being significantly higher than the scores obtained in control wines (Figure 4a). This fact could be closely related to the high content of eugenol and 4-vinyl-guaiacol in these wines.

With regard to the gustatory and in-mouth feel attributes, herbaceous, bitterness, acidity, and astringency notes significantly decreased as a consequence of the oxygen addition, in agreement with Ortega-Heras et al.[44] and Parpinello et al.[45] (Figure 4b) in Mencía, Tinta del Toro, and Cabernet Sauvignon red wines. The diminution of the astringency could be attributable to the aforementioned decrease in the content of (+)-catechin and (−)-epicatechin, as well as the formation of anthocyanin-flavan-3-ol pigments.[30] During the last part of the oxygenation treatment (days 32−42), as a consequence of the oxygen addition, herbaceous, bitterness, and astringency notes slightly increased, in parallel to the high content of C_6 alcohols and flavan-3-ols but maintaining significantly lower than in control wines. Taking these facts into account and also the increase in the percentage of polymerization previously described, an overoxygenation could probably take place in Cencibel red wines during the third part of the micro-oxygenation treatment.[7,46] Red fruits and body attributes were related to the supplied oxygen dose. In conclusion, the best valued red wines were those obtained after the second part of the micro-oxygenation treatment, negatively affecting the third part of the treatment due to the increase of astringency and herbaceous notes.

In summary, the application of micro-oxygenation treatment to red wines after their malolactic fermentation was advantageous, taking into account the color stabilization, the increase in the concentration of numerous volatile compounds, and an improvement of their sensorial quality. In this way, the application of micro-oxygenation treatment reached the pursued intention, which was to reduce the excess of astringency, herbaceous notes, and bitterness of red wines after their malolactic fermentation. Moreover, the numerous studied parameters permitted us to detect the probable overoxygenation that could happen if oxygen is added after malolactic fermentation. As a result, this study could be very useful for oenological industry to obtain good quality red wines and resolve specific winemaking problems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of mean values of concentration and standard deviations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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