JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of Elevated CO_2 on Grapevine (*Vitis vinifera* L.): Volatile Composition, Phenolic Content, and in Vitro Antioxidant Activity of Red Wine

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The impact of elevated carbon dioxide concentration ($[CO_2]$) on the quality of berries, must, and red wine (with special reference to volatile composition, phenolic content, and antioxidant activity) made from Touriga Franca, a native grape variety of *Vitis vinifera* L. for Port and Douro wine manufacturing grown in the Demarcated Region of Douro, was investigated during 2005 and 2006. Grapevines were grown either in open-top chambers (OTC) with ambient (365 ± 10 ppm) or elevated (500 ± 16 ppm) $[CO_2]$ or in an outside plot. In general, the increase of $[CO_2]$ did not affect berry characteristics, especially the total anthocyan and tannin concentrations. However, the total anthocyan and polyphenol concentrations of the red wine were inhibited under elevated $[CO_2]$. The antioxidant capacity of the wines was determined by DPPH, ABTS, and TBARS assays and, despite the low concentrations of phenolics, the elevated $[CO_2]$ did not significantly change the total antioxidant capacity of the red wines. Thirty-five volatile compounds belonging to seven chemical groups were identified: C_6 alcohols, higher alcohols, esters, terpenols, carbonyl compounds, acids, volatile phenols, and C_{13} norisoprenoids. Generally, the same volatile compounds were present in all of the wines, but the relative levels varied among the treatments. The effect of elevated $[CO_2]$ was significant because it was detected as an increase in ethyl 2-methylbutyrate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, butyric acid, and isovaleric acid concentrations and a decrease in ethyl acetate concentration when compared to wines produced in ambient $[CO_2]$ in 2005. In elevated $[CO_2]$, wines from 2006 had lower methionol, 1-octanol, and 4-ethylguaiacol and higher ethyl lactate and linalool concentrations. The increase in $[CO_2]$ did not significantly affect C_6 alcohols, citronellol, carbonyl compounds, and β-damascenone concentrations. This study showed that the predicted rise in $[CO_2]$ did not produce negative effects on the quality of grapes and red wine. Although some of the compounds were slightly affected, the red wine quality remained almost unaffected.

KEYWORDS: Grapes;*Vitis vinifera*; red wine; antioxidant activity; polyphenols; anthocyans; volatile compounds; elevated $[CO_2]$; climate change

INTRODUCTION

Carbon dioxide (CO_2) is the most important greenhouse gas, and its concentration has been increasing since the beginning of the industrial revolution, mainly as a result of the burning of fossil fuels. Compared with preindustrial levels, the CO_2 concentration ($[CO_2]$) has increased by 34%, with an accelerated rise since 1950. If no climate-driven policy measures are implemented, it is expected that CO_2 will exceed 550 ppm by the middle of the 21st century (*1*). The continued increases in CO_2 and other greenhouse gases in the atmosphere are expected to induce an additional 1−4.0 °C increase in average global surface temperatures by the year 2100 (*1*, *2*), leading to the scenario of future higher evaporative demand and increase in drought frequency and intensity (*1*). The predicted changes in $[CO_2]$ are expected to increase C_3 plant carbon assimilation (*3*) and, therefore, increase growth rate and yield of plants. According to Hoch and collaborators (*4*), elevated $[CO_2]$ leads to an increased concentration of nonstructural carbon compounds (mainly free carbohydrates and starch), but the extent of the change varies, depending on the species and growth conditions. Bindi and collaborators (*5*) studied the response of grapevine to CO_2 enrichment and found that wine quality and specifically

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10.1021/jf8020199 CCC: $40.75
Published on Web 12/10/2008

the concentrations of acids and sugars varied during ripening, but by harvest those differences had vanished.

Aroma, polyphenol content, and antioxidant activity are some of the most important aspects determining wine character and quality, which are dependent on plant genetic information and environmental factors. Wine aroma depends on numerous compounds, mainly higher alcohols (isobutyl and isoamyl alcohol), organic acids, esters (ethyl acetate, isoamyl acetate, ethyl hexanoate, and octanoate) and, to a lesser extent, aldehydes, which constitute the *fermentation bouquet* of wine (*6*−*10*).

Grapes and red wine are considered to be good sources of phenolic compounds, which are responsible for the astringency, color, taste (*11*, *12*), and, presumably, also the antioxidant properties of wines. Phenolic grape and wine compounds can be divided into two groups: nonflavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols, and flavonols) (*13*). The concentration of phenolic compounds in grapes depends on the grape cultivar, maturity stage, yield, production area, and environmental conditions (*14*−*17*).

During the past two decades, epidemiological studies have shown that coronary heart diseases are less prevalent in countries where a regular and moderate consumption of wine is widespread (*18*). Particularly, red wine is an important source of polyphenols, which are capable of inhibiting the processes behind coronary artery disease (*19*). In addition, red wine effects include inhibition of chronic inflammation and thrombotic tendencies (*20*). The inhibition of human low-density lipoproteins (LDL) in vitro was demonstrated by the addition of the mixture of polyphenols from wine (*21*). It has recently been revealed that the consumption of wine by humans leads to an increase in the antioxidant capacity of plasma (*22*−*26*).

Red wine produced in the Demarcated Douro Region is one of the most important products in the Portuguese economy. To our knowledge, information about the changes in volatile composition, phenolic content, and antioxidant activity of red wines produced at elevated [CO_2] is limited. However, this information is relevant to predict the quality of red wine in a future scenario of climate change. The aims of this study were to (1) describe the results of a two-year open-top chamber (OTC) experimental campaign carried out to collect information on the impact of elevated [CO_2] (500 ppm) on grapes, must, and red wine quality, with special reference to volatile composition, phenolic content, and antioxidant activity; and (2) assess the variations caused by natural harvest fluctuations in different vintages.

MATERIALS AND METHODS

Plant Material and Experimental Design. Sound red grapes of the Portuguese *Vitis vinifera* L. cv. Touriga Franca (one of the finest grapes for Port and Douro wines), grafted on 1103P, were obtained from a vineyard located in Vila Real (campus of the University of Trás-os-Montes e Alto Douro, 41° 17′ 10″ N, 7° 44′ 8″ W, 470 m above mean sea level, *Baixo Corgo* subregion of the Demarcated Douro Region, northern Portugal) during 2005 and 2006. The vines, planted in a typical schistous soil during 1997, were spur pruned on a bilateral cordon system (10−12 buds per vine). Plants were managed without irrigation and grown according to a commercial protocol as applied by commercial farmers.

Grapevines were grown in ambient (365 ± 10 ppm) or elevated CO_2 (500 ± 16 ppm) under two rectangular open-top chambers (OTC ambient and OTC CO_2, respectively) separated from one another by about 6 m. Each OTC (12.0 × 2.5 × 2.5 m in dimension), enclosing 10 plants growing in similar soil, air temperature, and photosynthetic photon flux density (PPFD) conditions, was constructed of 1 mm polyethylene film with a 75% light transmittance. CO_2 was fumigated from sunrise to sunset between budbreak and harvest. Other grapevines were grown in an outside OTC (exterior, CO_2 = 365 ± 10 ppm) to separate the CO_2 effect from any temperature increase or other changes related to OTC effect. Several sensors connected to a logger from deltaT Devices were installed to monitor the climate variables inside and outside the OTCs and to control the carbon dioxide level inside OTC CO_2. The CO_2 injector operated in on−off mode control with a time sampling of 30 s. The CO_2 injection was performed acting over an electronic valve linked to a pure industrial CO_2 reservoir (maintained by Air Liquid Portugal). The distribution tube (polyvinyl chloride), with several emission holes separated by 20 cm, was located along the vineyard row at the base of the grapevine canopies. CO_2 concentration was monitored with an infrared gas analyzer (GMP111, Vaisala, Finland). The climate variables were acquired and/or controlled with a sampling interval of 30 s, the storage time being 5 min. Briefly, during summer daylight period, the OTC mean air temperatures were similar in ambient and elevated CO_2, whereas within the OTCs it was 3.30 (±0.09 SE) °C higher than outside. On the other hand, within the OTCs the average relative humidity was higher (+3.1%) than outside during the night and lower (−4.2%) at the midday period.

Berry Analysis. The maturity was assessed on 30 berries by the following indices: fruit, pulp, and skin weights and skin color. Color was measured with a tristimulus colorimeter (Minolta CR-200B Chroma Meter) having an 8 mm diameter viewing area. Chromatic analyses were carried out following the CIE (Commission International de l'Eclairage) system of 1976, as described in Gonçalves and co-workers (*27*). Values of L^*, a^*, and b^* were measured to describe a three-dimensional color space. The vertical axis L^* is a measure of lightness, where values range from completely opaque (0) to completely transparent (100), a^* is a measure of redness (or $-a^*$ of greenness), and b^* is a measure of yellowness (or $-b^*$ of blueness) on the hue circle (*28*). From the a^* and b^* values, the hue angle (H^*) or tonality, which expresses the color nuance, can be calculated from $H^* = \text{arctg}(b^*/a^*)$ (*28*,*29*). The chroma, a measure of chromaticity (C^*), indicating the purity or saturation of the color can be obtained as $C^* = (a^{*2} + b^{*2})^{1/2}$ (*28*). The data of each measurement are the average of triplicate measures on equidistant points of each fruit.

Samples of 100 grape berries were collected from each of the two OTC treatments and the outside plot at different stages of fruit maturity during the growing season. Samples were placed in polyethylene bags and frozen for later analyses. Grape analyses were conducted by thawing frozen samples in a refrigerator at 4 °C overnight. Berries were manually separated from the stems, and the sample's juice was separated from skins and seeds in a juice extractor. Grape quality was assessed as probable alcohol, °Brix, pH, titratable acidity, total anthocyans, tannins, malic acid, tartaric acid, glucose, and fructose according to OIV methods (*30*).

Wine and Must Analysis. At the end of the maturation period, grapes were harvested and made into wine. Wine was made using a standardized microvinification procedure. For each single microvinification, approximately 40 kg of grapes was used. The destemmed and crushed grapes were pumped into stainless steel fermentation vessels, 50 mg L^{-1} sulfur dioxide was added at the crusher, and a few hours later a pure yeast starter was added. The grape juice fermented with the cap of grape skins, being plunged twice daily to completion of fermentation. At completion of fermentation the wines were pressed, and 50 mg L^{-1} SO_2 was added. Wines were stored in glass carboys for 2 months for cold stabilization, being racked once before bottling. At bottling, 50 mg L^{-1} SO_2 was added. Samples were collected during the fermentative process (fermentation days: 0 and 8), ethanol was added, at the final concentration of 20% (v/v), to stop the fermentation, and the samples were placed at −20 °C.

Total monomeric anthocyanins were determined by the pH-differential method, and the total polyphenols were determined by measuring the absorbance at 280 nm (*31*). Total acidity, volatile acidity, fixed acidity, pH, density, °Brix, and alcoholic strength by volume were determined according to OIV methods (*30*). All of the analyses were performed in triplicate.

Thiobarbituric Acid Reactive Substances (TBARS) Assay. The deoxyribose method was performed for determining the rate of reaction of the hydroxyl radical with antioxidant (*32*). Reaction mixtures in a

final volume of 1.0 mL contained deoxyribose (60 mM), KH_2PO_4−KOH buffer (pH 7.4, 20 mM), $FeCl_3$ (100 μM), EDTA (100 μM), H_2O_2 (1 mM), and ascorbic acid (100 μM). Solutions of $FeCl_3$ and ascorbic acid were made up immediately before use. After incubation at 30 °C for 1 h, the color was developed by adding 1 mL of 1% thiobarbituric acid (TBA) (w/v) and 1 mL of 25% (v/v) HCl, which was then heated in a boiling water bath for 15 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. All of the assays were conducted in triplicate.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Cation Decolorization Assay. The DPPH• assay was used to measure the free radical scavenging capacity of the must (day 0) and wine (day 8) (*33*, *34*). Used as reagent, DPPH• offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. Two hundred and eighty microliters of 0.004% DPPH• methanolic solution was pipetted into each well of a 96-well plate followed by 100 μL of sample, Trolox, or solvent for the blank. The mixture was incubated at 30 °C for 1 h, and the absorbance at 515 nm was measured with a microplate reader. All of the assays were conducted in triplicate.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Cation Decolorization Assay. $ABTS^{\bullet+}$ was generated through a chemical oxidation reaction with potassium persulfate (*35*). The concentration of ABTS radical was adjusted with methanol to an initial absorbance of 0.700 ± 0.020 at 734 nm. To 280 μL of this solution of $ABTS^{\bullet+}$ was added 20 μL of sample or Trolox or solvent upon a 96-well plate. The mixture was incubated for 5 min at 30 °C, and the absorbance at 734 nm was measured with a microplate reader. The radical stock solution was freshly prepared, and all of the analyses were performed in triplicate.

Volatile Compounds Analysis. Solid-Phase Microextraction Procedure (SPME). Volatile compounds were sampled from wines and standard solutions using SPME with a DVB/CAR/PDMS fiber (50/30 μm) from Supelco (Bellefonte, PA). To ensure that any contaminants that might be present were removed prior to use, the SPME fiber was conditioned by exposing it to the hot GC injection port (270 °C) for at least 1 h until no peaks were detected in blank analyses. Ten milliliters of sample and 10 mL of internal standard solution (2-octanol, 100 μg L^{-1}) plus 4 g of NaCl were transferred to a 40 mL vial and capped with PTFE-faced silicone seals. The sample solution in the vial was allowed to equilibrate for 10 min while magnetically stirring at 1300 rpm. The SPME fiber was introduced into the vial headspace and held for 60 min under constant temperature (20 ± 1 °C) and stirring. All of the analyses were executed in triplicate.

GC-MS Analysis. Volatile compounds were separated using an Agilent 6890 N gas chromatograph equipped with a 5973N mass selective detector and an Innowax capillary column (30 m × 0.25 mm × 0.5 μm; Agilent, Santa Clara, CA). A 0.75 mm liner was used, and the analysis was performed in the splitless mode. The injector temperature was 270 °C, and the desorption time was 10 min. The column was maintained at 40 °C for 5 min after injection, ramped at 4 °C/min to 200 °C, and then ramped at 10 °C/min to 240 °C, at which it was held for 15 min. Helium was used as the carrier gas at 34 cm/s average linear velocity. All mass spectra were acquired in electron impact (EI) mode at 70 eV, using full scan with a scan range of 26−250 amu, at a rate of 6.12 scans/s. Sample compound spectral identification was aided by the use of the Wiley database (*36*). When possible, identification was confirmed by comparing mass spectra and retention indices with those of authentic standards.

Preparation of Standards. Single standard stock solutions (1% v/v) of the volatile compounds were prepared by spiking each compound in pure ethanol (Merck LiChrosolv). Working solutions, prepared just before use, were made from the stock solutions by spiking and mixing them with a hydroalcoholic solution (11.5% vol), to which had been added tartaric acid (3 g L^{-1}) and potassium hydrogentartrate (3 g L^{-1}), and adjusting the pH to 3.2 with 6 M NaOH.

Statistical Analysis. The data are presented as means ± standard deviations. Data were tested using a one-way ANOVA to determine the main effects of CO_2 treatment (comparing OTC CO_2 and OTC ambient values) and the main effects of OTC (comparing OTC ambient and exterior values). A probability value of <0.05 was considered to be significant.

Table 1. Berry Weights, at the Optimal Ripeness Stage, of Cv. Touriga Franca Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

treatment	berry wt (g)	pulp wt (g)	skin wt (g)	skin/pulp wt ratio
		Year 2005		
OTC CO_2	2.37 ± 0.43	2.00 ± 0.38	0.37 ± 0.10	0.19 ± 0.05
OTC ambient	2.40 ± 0.56	2.05 ± 0.51	0.35 ± 0.07	0.17 ± 0.03
exterior	2.47 ± 0.36	2.17 ± 0.33	0.30 ± 0.05	0.14 ± 0.02
P value (CO_2 effect)	*0.835*	*0.639*	*0.210*	*0.104*
P value (OTC effect)	*0.558*	*0.297*	*0.003*	*<0.001*
		Year 2006		
OTC CO_2	2.99 ± 0.91	2.44 ± 0.85	0.54 ± 0.12	0.24 ± 0.07
OTC ambient	2.85 ± 0.58	2.28 ± 0.52	0.57 ± 0.11	0.25 ± 0.06
exterior	2.71 ± 0.52	2.19 ± 0.46	0.52 ± 0.12	0.24 ± 0.06
P value (CO_2 effect)	*0.503*	*0.376*	*0.306*	*0.214*
P value (OTC effect)	*0.306*	*0.452*	*0.098*	*0.374*

[a] Mean values ± SD (n = 30) for the years 2005 and 2006.

RESULTS AND DISCUSSION

Elevated CO_2 Effects. The ranges of quality indices of berries for each treatment are shown in **Tables 1−3**. The analyses of variance revealed no significant differences in berry, pulp, and skin weights and skin/pulp weight ratios of berries produced by both OTC treatments (**Table 1**). Between years, the weights and ratios were always greater in 2006 than in 2005 ($P < 0.01$). Generally, the increase of [CO_2] did not affect berry color, except the increase of the color parameters b^* ($P < 0.001$) and C^* ($P < 0.01$) for the 2006 harvest (**Table 2**). The parameter C^* is used as color criterion, where the higher the value of C^*, the better the quality of grapes and wine (*37*). On average, higher values of L^* and b^* and lower a^* ($P < 0.01$) were determined in the 2006 wines compared to 2005 wines.

The control of maturation was conducted between August 23 and September 27 in 2005, and the means of the different parameters are shown in **Table 3**. In elevated [CO_2], berries had higher fructose concentration and higher pH at the beginning of grape maturation control, compared to berries produced in ambient [CO_2]. Probable alcohol, °Brix, total acidity, total anthocyans, tannins, malic acid, tartaric acid, and glucose concentrations of the berries were not significantly affected by an increase in [CO_2].

The quality indices, °Brix, total acidity, and pH of the different musts in the two years of study are presented in **Table 4**. The effect of [CO_2] was detected ($P < 0.05$) only in total acidity in 2006 musts, for which values were considerably higher (12%) than in OTC ambient musts. Higher values of °Brix and pH ($P < 0.05$) were always determined in the 2006 harvest than in 2005 wines.

Wine classic analyses were conducted to ensure that the vinification was carried out properly, with results shown in **Table 5**. The analyses of variance revealed no significant differences in total, volatile, and fixed acidity of wines from both OTC treatments. Wines produced in elevated [CO_2] in 2005 had considerably ($P < 0.01$) higher alcohol and lower pH than wines in ambient [CO_2]. The density of wine in 2006 decreased significantly ($P < 0.001$) by the rise of [CO_2]. Wines produced in ambient [CO_2] in 2005 were richest (371%) in anthocyans. The same tendency was observed for wines produced in 2006, although the differences were not as great. The major anthocyanin present in red wine was malvidin 3-*O*-glucoside (data not shown). Similar results were obtained by Gómez-Alonso and collaborators (*13*) for Cencibel red wine. In addition, the concentrations of total polyphenols were also lower in wines produced in elevated [CO_2] than in wines produced in ambient

Table 2. Chromatic Parameters of Cv. Touriga Franca Berries, at the Optimal Ripeness Stage, Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

treatment	luminosity (L^*)	a^*	b^*	hue angle (H^*)	chroma (C^*)
		Year 2005			
OTC CO_2	25.1 ± 0.7	0.41 ± 0.35	0.04 ± 0.59	73.6 ± 49.1	0.68 ± 0.42
OTC ambient	25.1 ± 0.7	0.42 ± 0.28	0.00 ± 0.61	80.7 ± 49.2	0.70 ± 0.36
exterior	25.6 ± 0.6	0.49 ± 0.26	−0.26 ± 2.31	84.6 ± 58.5	0.86 ± 2.23
P value (CO_2 effect)	*0.799*	*0.719*	*0.617*	*0.333*	*0.759*
P value (OTC effect)	*<0.001*	*0.105*	*0.308*	*0.633*	*0.492*
		Year 2006			
OTC CO_2	25.6 ± 0.9	0.40 ± 0.50	0.49 ± 0.25	67.2 ± 39.5	0.77 ± 0.34
OTC ambient	25.5 ± 1.0	0.40 ± 0.44	0.26 ± 0.30	68.9 ± 54.1	0.62 ± 0.35
exterior	26.1 ± 1.0	0.25 ± 0.48	0.12 ± 0.46	79.9 ± 54.5	0.60 ± 0.36
P value (CO_2 effect)	*0.593*	*0.946*	*<0.001*	*0.814*	*0.006*
P value (OTC effect)	*<0.001*	*0.023*	*0.016*	*0.176*	*0.643*

[a] Mean values ± SD ($n = 90$) for the year 2005 and 2006.

Table 3. Ripening Stages of Cv. Touriga Franca Berries at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

	date	OTC CO_2	OTC ambient	exterior	*P* value (CO_2 effect)	*P* value (OTC effect)
probable alcohol (v/v)	Aug 23	7.86 ± 0.21	7.96 ± 0.03	6.85 ± 0.26	*0.241*	*0.002*
	Sept 27	9.56 ± 0.49	10.0 ± 0.4	8.48 ± 0.66	*0.457*	*0.022*
°Brix	Aug 23	14.6 ± 0.4	14.8 ± 0.1	12.9 ± 0.4	*0.246*	*0.002*
	Sept 27	17.3 ± 0.8	18.0 ± 0.6	15.6 ± 1.0	*0.457*	*0.020*
pH	Aug 23	3.22 ± 0.06	3.20 ± 0.08	3.12 ± 0.01	*0.010*	*0.126*
	Sept 27	3.48 ± 0.07	3.66 ± 0.01	3.54 ± 0.03	*0.783*	*0.005*
total acidity (g L^{-1})	Aug 23	7.13 ± 0.53	6.57 ± 0.21	7.36 ± 0.33	*0.167*	*0.024*
	Sept 27	4.46 ± 0.23	4.11 ± 0.28	4.00 ± 0.25	*0.167*	*0.632*
total anthocyans (g L^{-1})	Aug 23	2.31 ± 0.24	2.12 ± 0.01	1.83 ± 0.42	*0.195*	*0.298*
	Sept 27	1.81 ± 0.30	1.52 ± 0.15	1.82 ± 0.58	*0.242*	*0.425*
tannins (mg L^{-1})	Aug 23	132 ± 17	99.7 ± 19.8	123 ± 32	*0.320*	*0.337*
	Sept 27	99.0 ± 10.0	81.8 ± 24.4	91.8 ± 24.9	*0.096*	*0.645*
malic acid (g L^{-1})	Aug 23	4.07 ± 0.24	3.79 ± 0.57	3.77 ± 0.13	*0.506*	*0.964*
	Sept 27	2.16 ± 0.30	2.30 ± 0.15	2.49 ± 0.75	*0.475*	*0.690*
tartaric acid (g L^{-1})	Aug 23	2.96 ± 0.08	2.65 ± 0.28	2.62 ± 0.30	*0.331*	*0.919*
	Sept 27	2.71 ± 0.14	2.88 ± 0.23	2.41 ± 0.21	*0.132*	*0.059*
glucose (g L^{-1})	Aug 23	67.6 ± 7.3	71.2 ± 12.5	53.7 ± 6.8	*0.741*	*0.100*
	Sept 27	83.0 ± 13.1	78.8 ± 15.9	80.5 ± 3.7	*0.690*	*0.861*
fructose (g L^{-1})	Aug 23	74.2 ± 9.0	73.3 ± 13.6	56.7 ± 8.4	*0.007*	*0.145*
	Sept 27	104 ± 4	89.9 ± 3.0	92.1 ± 2.0	*0.927*	*0.343*

[a] Mean values ± SD ($n = 3$) for the year 2005.

Table 4. Quality Indices of Cv. Touriga Franca Musts from Grapes Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

treatment	°Brix	total acidity (g L^{-1})	pH
	Year 2005		
OTC CO_2	18.9 ± 0.7	4.40 ± 0.71	3.54 ± 0.27
OTC ambient	17.8 ± 0.9	4.60 ± 0.28	3.33 ± 0.08
exterior	18.0 ± 0.5	4.38 ± 0.50	3.40 ± 0.11
P value (CO_2 effect)	*0.294*	*0.746*	*0.391*
P value (OTC effect)	*0.671*	*0.565*	*0.447*
	Year 2006		
OTC CO_2	20.8 ± 0.4	4.80 ± 0.14	3.67 ± 0.09
OTC ambient	21.0 ± 0.4	4.30 ± 0.00	3.71 ± 0.08
exterior	18.5 ± 0.6	5.17 ± 0.06	3.40 ± 0.09
P value (CO_2 effect)	*0.629*	*0.038*	*0.684*
P value (OTC effect)	*0.016*	*<0.001*	*0.034*

[a] Mean values ± SD ($n = 3$) for the years 2005 and 2006.

[CO_2]. In contrast, high [CO_2] growing conditions significantly enhanced the strawberry fruit content of anthocyanin and phenolic content in accordance with Wang and co-workers (*38*).

When the two years of study were compared, wines from 2006 had, on average, higher density, pH, volatile acidity, total anthocyans, and total polyphenols and lower alcohol, total acidity, and fixed acidity than wines from 2005 ($P < 0.05$).

What could have caused the absence of effects of CO_2 on anthocyans in the fruit but still result in differences in the wine? As is well-known, anthocyans are located in the skins of red cultivars within vacuoles. Extraction requires that the thick cell walls be degraded and the thin vacuole membranes broken for the contents to diffuse into the wine. This wall/membrane degradation is maximized when the pH is very low (pH 1.0; i.e., the pH of the anthocyanin extraction solution), but the wall maceration during winemaking conditions tends to take place at pH values closer to 3.6 (*39*) and in our study between 3.4 and 3.9.

In summary, the chemical analysis of the wine showed, with a few exceptions, no significant differences among the wine compounds obtained from control and enriched areas in both seasons. These exceptions regarded total anthocyan concentrations that in both years were statistically inhibited under elevated

Table 5. General Composition of Cv. Touriga Franca Wines Obtained from Berries Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

treatment	density	alcohol (vol %)	pH	total acidity[b] (g L^{-1})	volatile acidity[c] (g L^{-1})	fixed acidity (g L^{-1})	total anthocyans (mg L^{-1})	total phenolics (index at 280 nm)
				Year 2005				
OTC CO_2	0.992 ± 0.00	12.1 ± 0.0	3.40 ± 0.01	6.34 ± 0.26	0.330 ± 0.04	5.93 ± 0.21	23.9 ± 4.0	33.4 ± 3.0
OTC ambient	0.993 ± 0.00	10.9 ± 0.1	3.53 ± 0.01	6.87 ± 0.11	0.495 ± 0.06	6.25 ± 0.18	112 ± 9	41.8 ± 0.3
exterior	0.992 ± 0.00	10.5 ± 0.2	3.62 ± 0.07	5.11 ± 0.30	0.545 ± 0.04	4.43 ± 0.28	61.2 ± 37.2	31.1 ± 3.9
P value (CO_2 effect)	*1.000*	*0.002*	*0.007*	*0.117*	*0.923*	*0.237*	*0.006*	*0.060*
P value (OTC effect)	*0.383*	*0.081*	*0.091*	*<0.001*	*0.180*	*<0.001*	*0.101*	*0.006*
				Year 2006				
OTC CO_2	0.996 ± 0.00	11.0 ± 0.2	3.84 ± 0.04	4.39 ± 0.21	0.570 ± 0.04	3.68 ± 0.16	240 ± 2	43.8 ± 0.6
OTC ambient	0.995 ± 0.00	11.6 ± 0.2	3.91 ± 0.01	4.06 ± 0.06	0.545 ± 0.01	3.38 ± 0.05	282 ± 13	51.4 ± 0.1
exterior	0.996 ± 0.00	9.3 ± 0.2	3.65 ± 0.08	4.82 ± 0.21	0.497 ± 0.12	4.20 ± 0.09	187 ± 9	41.7 ± 1.4
P value (CO_2 effect)	*<0.001*	*0.106*	*0.108*	*0.166*	*0.498*	*0.118*	*0.047*	*0.003*
P value (OTC effect)	*0.272*	*0.001*	*0.020*	*0.163*	*0.615*	*0.001*	*0.002*	*0.003*

[a] Mean values ± SD ($n = 3$) for the years 2005 and 2006. [b] Expressed as tartaric acid. [c] Expressed as acetic acid.

Table 6. Total Antioxidant Activity of Cv. Touriga Franca Wines from Berries Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

	TBARS (nmol mg^{-1} of protein)		DPPH (μg mg^{-1} of protein)		ABTS (μg mg^{-1} of protein)	
treatment	day 0	day 8	day 0	day 8	day 0	day 8
OTC CO_2	6.73 ± 0.24	5.81 ± 0.45	0.40 ± 0.02	0.53 ± 0.02	84.5 ± 6.3	85.4 ± 5.4
OTC ambient	8.39 ± 0.33	6.71 ± 0.80	0.36 ± 0.01	0.52 ± 0.03	75.8 ± 3.4	84.0 ± 6.0
exterior	6.31 ± 0.28	5.60 ± 0.39	0.41 ± 0.01	0.54 ± 0.04	83.6 ± 7.6	85.3 ± 6.4
P value (CO_2 effect)	*0.002*	*0.163*	*0.045*	*0.480*	*0.104*	*0.784*
P value (OTC effect)	*0.001*	*0.098*	*0.008*	*0.537*	*0.179*	*0.819*

[a] Mean values ± SD ($n = 3$) for the year 2005.

[CO_2]. In contrast, Bindi and co-workers (*5*) described a tendency for higher concentration of these pigments in the enriched [CO_2] wine.

In our study, the DPPH radical scavenging assay and ABTS and TBARS assays were performed to compare the antioxidant capacity of various wine samples, with values presented in **Table 6**. Data from the three assays indicated that the elevated [CO_2] did not significantly change the total antioxidant capacity of the red wine. Notwithstanding the fact that no statistical differences were observed in the antioxidant capacity of wine in the different environmental conditions (OTC CO_2, OTC ambient, and exterior), we observed that variations in the wine antioxidant capacity found by the methods of DPPH and ABTS were quite similar. Furthermore, the conditions which show better antioxidant capacities are the same that show a higher protective effect against deoxyribose, showing therefore lower TBARS values.

However, some data showed a slight decrease in the total antioxidant capacity in the very first stage of fermentation, pointing to the conclusion that probably elevated CO_2 decreased the total antioxidant capacity of grapes but the final product was not affected, despite the lower concentration of total polyphenols and total anthocyans (**Table 5**). Our results showed that grapes and red wine contain large concentrations of phenolic compounds and, according to Macheix and co-workers (*40*), are mostly flavonoids, which may contribute to the inhibition of human low-density lipoprotein (LDL) oxidation and, therefore, higher antioxidant activity (*41*).

According to several authors (*23, 42*), the antioxidant potency of wines and grapes on human LDL in test tube assays has furthermore been shown to correlate to the presence of distinct types of phenols and, in turn, to their relative abundance in the particular sample being tested. Several papers have documented that the antioxidant activity of phenolic mixtures may exceed the expected activity as calculated from the sum of the antioxidant activities of the individual phenols, which indicates that synergistic effects may occur among phenolics in mixtures (*43*). On the other hand, antagonistic interactions cannot be ruled out either (*44*).

The ethanol extracts of wines were analyzed by HS-SPME-GC-MS, and example chromatograms are shown in **Figure 1**. The volatile compounds present in all wines were generally the same, but the relative levels varied among the three treatments. Seven groups of volatile compounds were identified: C_6 alcohols, higher alcohols, esters, terpenols, carbonyl compounds, acids, volatile phenols, and C_{13} norisoprenoids. **Table 7** shows the volatile composition of Touriga Franca wines, with most of the compounds analyzed from fermentation. A total of 35 free aroma compounds were quantified in the 2005 and 2006 wines (**Table 7**). The main C_6 alcohol was identified as 1-hexanol, followed by *cis*-3-hexenol, and the third compound was *trans*-3-hexenol. The increase in [CO_2] did not significant affect C_6 alcohol concentrations. However, the concentrations of these three compounds varied with the year ($P < 0.01$), with higher values in 2006 wines. These compounds are the most important substances responsible for grassy or herbaceous flavor in wines (*45, 46*).

The chromatograms revealed that Touriga Franca wines contained at least five higher alcohols: isoamyl alcohol, 1-octanol, benzyl alcohol, 2-phenylethanol, and methionol (**Figure 1**). In elevated [CO_2], wines had lower ($P < 0.05$) methionol and 1-octanol concentrations than wines produced in ambient CO_2 in 2006 (**Table 7**). The concentrations of the other higher alcohols did not vary between treatments, but with years. In fact, in the 2006 wines, the concentrations of isoamyl alcohol, 1-octanol, benzyl alcohol, 2-phenylethanol, and methionol were significantly higher ($P < 0.001$). Alcohols were the most abundant compounds, but the concentrations were much lower than 400 mg L^{-1}, thus contributing in a positive way to wine aroma (*9, 47*). Among the aliphatic alcohols, isoamyl alcohol

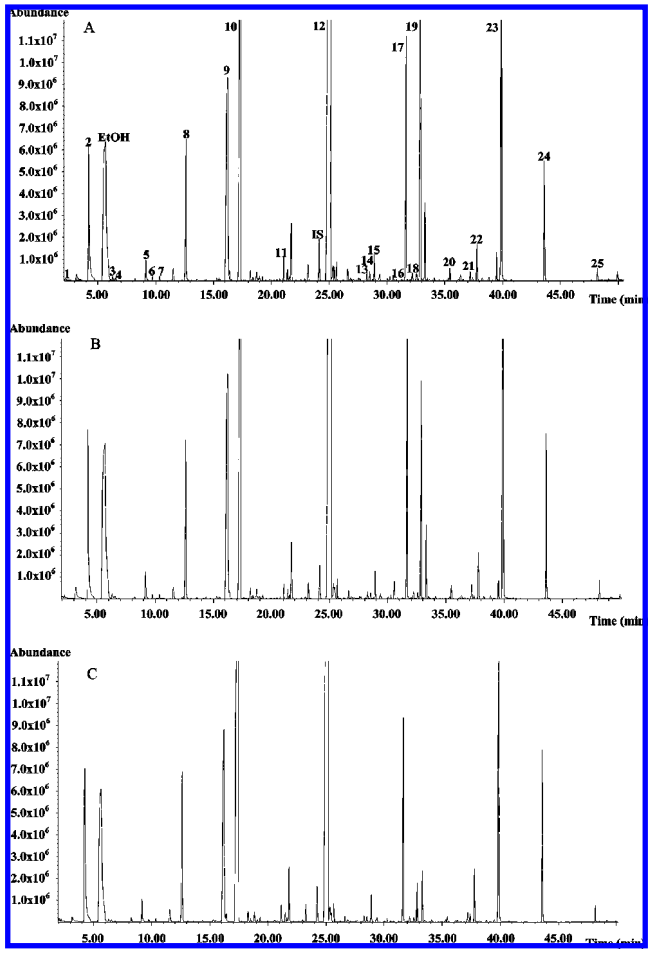

Figure 1. Chromatograms of HS-SPME-GC-MS analysis of wine samples from 2006: (**A**) OTC CO_2; (**B**) OTC ambient; (**C**) exterior. Peaks: (1) acetaldehyde; (2) ethyl acetate; (3) ethyl propionate; (4) ethyl isobutyrate; (5) ethyl butyrate; (6) ethyl 2-methylbutyrate; (7) ethyl isovalerate; (8) isoamyl acetate; (9) isoamyl alcohol; (10) ethyl hexanoate; (11) ethyl lactate; (12) ethyl octanoate; (13) benzaldehyde; (14) linalool; (15) 1-octanol; (16) butyric acid; (17) ethyl decanoate; (18) isovaleric acid; (19) diethyl succinate; (20) citronellol; (21) 2-phenylethyl acetate; (22) hexanoic acid; (23) 2-phenylethanol; (24) octanoic acid; (25) decanoic acid; IS (internal standard).

showed the highest concentration in both years ranging from 137 to 170 mg L^{-1} and from 226 to 248 mg L^{-1}, in 2005 and 2006 wines, respectively. Another alcohol, present at very high concentration (>12.7 mg L^{-1}), was 2-phenylethanol. These two alcohols are characterized by malty and floral (rose, lavender) attributes, respectively (*48*). The 2-phenylethanol content of all wines was higher than its odor threshold (7.5 mg L^{-1}) (*49*) and, therefore, it contributed pleasantly to wine aroma (*50*). In the 2006 wines, the levels of this alcohol were higher (>27.7 mg L^{-1}) than in the 2005 wines (<16.0 mg L^{-1}).

With regard to ester compounds, high levels were observed for ethyl acetate (>24.9 mg L^{-1}) and ethyl lactate (>1.8 mg L^{-1}) (**Table 7**). In fact, all of the wines showed high values of ethyl acetate (classified as acetate or ethyl ester); nevertheless, its content was below the level considered to be negative (100 mg L^{-1}) (*51*). Among the rest of the acetic esters, isoamyl acetate was the most abundant (>0.5 mg L^{-1}), with values higher than its odor threshold (0.03 mg L^{-1}) (*52*). Several authors consider that these are among the key compounds in the fruity flavors of wines (e.g., refs *9*, *10*, and *53*), and they are synthesized during must fermentation (*48*). The effect of elevated [CO_2] was significant ($P < 0.05$) because increases in ethyl 2-methylbutyrate, isoamyl acetate, ethyl hexanoate, and ethyl octanoate and a decrease in ethyl acetate concentration were detected when compared to wines produced in ambient [CO_2] in 2005. In 2006, elevated [CO_2] led to higher ethyl lactate concentration ($P < 0.05$) compared to wines produced in ambient [CO_2]. In general, 2006 wines had higher ($P < 0.01$) concentrations of ethyl acetate, ethyl isobutyrate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl isovalerate, isoamyl acetate, ethyl heaxanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, and 2-phenylethyl acetate and a lower concentration of ethyl propionate than the 2005 wines.

The syntheses of alcohols (through β-oxidation of fatty acids) and esters (via esterification of alcohols and carboxylic acids) are oxygen-dependent processes (*54*−*56*). Consequently, the production of some alcohols and esters by grape is reduced at elevated [CO_2] (*57*, *58*).

On the other hand, the increase in soluble sugars in the fruit arising from elevated [CO_2] treatment may therefore result in an increase in the availability of precursors able to produce aromatic compounds. High [CO_2] growing conditions significantly enhanced the strawberry fruit content of methyl butanoate, ethyl butanoate, methyl hexanoate, ethyl hexanoate, hexyl hexanoate, hexyl acetate, Furaneol, linalool, and methyl octanoate (*59*).

Among monoterpene compounds, citronellol and linalool were detected in all wines (**Table 7**), which is consistent with the results obtained by Selli and collaborators (*48*). The citronellol concentration was not affected ($P > 0.05$) by elevated [CO_2], in either year. In contrast, the 2006 wines from OTC CO_2 had significantly more ($P < 0.05$) linalool, but the level of this compound was lower than its perception limit. The terpene alcohols such as linalool and citronellol are positioned in the group with flowery notes together with other monoterpenes, benzyl alcohol and 2-phenyletanol, in accordance with literature odor descriptions (e.g., refs *60* and *61*).

With regard to the fatty acid composition, hexanoic, octanoic, and decanoic acids occurred abundantly (**Table 7**). Similar results were obtained by González-Marco and co-workers (*8*), especially in Chardonnay wine fermented in oak barrels. The contents of 6-, 8-, and 10-carbon fatty acids were in agreement with those found by Selli and collaborators (*48*). These three acids are not associated with wine quality but play an important role in the complexity of the aroma (*62*). Isovaleric and butyric acids were also identified in low amounts. In elevated [CO_2], wines from 2005 had higher butyric and isovaleric acid concentrations than wines produced in ambient [CO_2]. Wines from 2006 had significantly ($P < 0.001$) higher concentrations of all five fatty acids than wines produced in 2005.

The carbonyl compound benzaldehyde was identified in low concentrations (**Table 7**) in all of the wines, and it can contribute to aroma with a bitter almond or cherry flavor (*37*, *63*). In addition, acetaldehyde and diacetyl were also detected. The diketone, diacetyl, is a major flavor metabolite produced by lactic acid bacteria and imparts a buttery aroma and flavor to the wines (*64*). The CO_2 did not affect the concentration of these compounds in the differently treated wines. With regard to the effect of the year, acetaldehyde concentration was higher ($P < 0.05$) in 2006 wines, but benzaldehyde and diacetyl concentrations were not affected by year.

The volatile phenols of this group found at significant levels were guaiacol, 4-ethylguaiacol, and 4-ethylphenol. In elevated [CO_2], wine from 2006 had lower 4-ethylguiacol concentration than wine produced in ambient [CO_2]. The effect of year was not significant in 4-ethylguaiacol and 4-ethylphenol concentra-

Table 7. Volatile Compounds of Cv. Touriga Franca Wines from Berries Grown at Elevated (OTC CO_2) and Ambient (OTC Ambient) CO_2 and Exterior[a]

compound (μg L^{-1})	OTC CO_2	OTC ambient	exterior	*P* value (CO_2 effect)	*P* value (OTC effect)	OTC CO_2	OTC ambient	exterior	*P* value (CO_2 effect)	*P* value (OTC effect)
			Year 2005					Year 2006		
C_6 alcohols										
1-hexanol	1963 ± 14	1850 ± 162	1634 ± 93	*0.429*	*0.030*	2000 ± 16	1886 ± 117	2292 ± 144	*0.306*	*0.047*
trans-3-hexenol	nd	14.9 ± 2.1	5.40 ± 1.01	*0.423*	*0.347*	36.5 ± 0.5	33.8 ± 1.5	33.7 ± 2.3	*0.142*	*0.953*
cis-3-hexenol	127 ± 7	146 ± 9	136 ± 12	*0.138*	*0.311*	137 ± 4	123 ± 9	251 ± 35	*0.173*	*0.017*
higher alcohols										
isoamyl alcohol	170390 ± 1	149640 ± 9	137270 ± 7	*0.082*	*0.053*	226740 ± 16	226290 ± 9	247790 ± 48	*0.976*	*0.595*
1-octanol	8.78 ± 0.38	7.60 ± 1.10	8.09 ± 1.04	*0.289*	*0.574*	33.5 ± 2.0	41.4 ± 1.8	41.5 ± 5.3	*0.045*	*0.977*
benzyl alcohol	77.5 ± 7.3	85.3 ± 13.1	61.2 ± 6.4	*0.534*	*0.004*	404 ± 31	331 ± 31	373 ± 98	*0.144*	*0.616*
2-phenylethanol	13165 ± 1	16070 ± 3	12763 ± 2	*0.276*	*0.072*	33735 ± 6	33830 ± 4	27723 ± 7	*0.988*	*0.343*
methionol	2550 ± 0	1855 ± 0	1650 ± 0	*0.143*	*0.215*	2870 ± 0	3250 ± 0	3477 ± 1	*0.014*	*0.633*
esters										
ethyl acetate	26415 ± 1	31640 ± 0	24944 ± 7	*0.013*	*0.253*	40675 ± 3	65405 ± 29	61050 ± 11	*0.353*	*0.816*
ethyl propionate	144 ± 5	173 ± 19	167 ± 18	*0.179*	*0.674*	133 ± 5	129 ± 8	125 ± 21	*0.591*	*0.824*
ethyl isobutyrate	28.1 ± 1.0	28.3 ± 0.6	23.0 ± 3.4	*0.830*	*0.071*	42.0 ± 5.6	33.7 ± 1.3	49.3 ± 6.3	*0.177*	*0.046*
ethyl butyrate	76.8 ± 1.0	60.2 ± 6.7	55.7 ± 3.6	*0.075*	*0.210*	180 ± 2	214 ± 19	249 ± 31	*0.134*	*0.247*
ethyl 2- methylbutyrate	4.97 ± 0.21	4.09 ± 0.13	2.88 ± 0.24	*0.037*	*<0.001*	16.4 ± 1.3	14.0 ± 1.4	12.1 ± 1.1	*0.218*	*0.186*
ethyl isovalerate	4.84 ± 0.47	4.05 ± 0.07	3.08 ± 0.26	*0.146*	*0.001*	10.7 ± 1.3	8.80 ± 0.42	9.42 ± 0.91	*0.185*	*0.451*
isoamyl acetate	639 ± 20	502 ± 9	479 ± 38	*0.013*	*0.443*	537 ± 57	674 ± 49	610 ± 21	*0.124*	*0.121*
ethyl hexanoate	131 ± 2	109 ± 3	102 ± 5	*0.012*	*0.113*	290 ± 11	322 ± 23	396 ± 65	*0.209*	*0.235*
ethyl lactate	1825 ± 0	1830 ± 1	2019 ± 2	*0.994*	*0.886*	47500 ± 2	36640 ± 1	42580 ± 2	*0.022*	*0.043*
ethyl octanoate	65.1 ± 1.1	56.0 ± 0.6	52.2 ± 3.6	*0.010*	*0.186*	160 ± 27	182 ± 30	215 ± 50	*0.513*	*0.482*
ethyl decanoate	71.4 ± 7.7	60.9 ± 11.2	52.6 ± 6.4	*0.390*	*0.181*	61.7 ± 13.7	193 ± 57	156 ± 90	*0.087*	*0.651*
diethyl succinate	20.0 ± 0.0	20.0 ± 0.0	26.0 ± 0	*1.000*	*0.811*	7110 ± 2	2960 ± 1	847 ± 0	*0.142*	*0.004*
2-phenylethyl acetate	12.0 ± 0.5	11.6 ± 3.0	10.2 ± 2	*0.880*	*0.395*	14.3 ± 1.6	20.3 ± 5.8	13.7 ± 2.9	*0.289*	*0.174*
terpenols										
linalool	1.11 ± 0.03	1.22 ± 0.10	1.12 ± 0.07	*0.270*	*0.121*	5.15 ± 0.26	3.99 ± 0.00	5.03 ± 0.34	*0.025*	*0.027*
citronellol	7.58 ± 0.14	7.65 ± 1.28	6.40 ± 0.69	*0.950*	*0.079*	13.7 ± 1.3	20.2 ± 1.9	9.57 ± 2.58	*0.055*	*0.016*
carbonyl compounds										
benzaldehyde	4.55 ± 2.86	1.02 ± 0.45	0.65 ± 0.65	*0.227*	*0.479*	6.25 ± 0.75	0.79 ± 0.06	1.14 ± 0.57	*0.412*	*0.469*
acetaldehyde	1645 ± 0	1360 ± 0	2894 ± 1	*0.408*	*0.116*	3350 ± 3	2215 ± 1	21317 ± 13	*0.696*	*0.139*
diacetyl	1055 ± 0	465 ± 0	428 ± 0	*0.063*	*0.705*	600 ± 1	505 ± 0	853 ± 0	*0.861*	*0.301*
acids										
butyric acid	135 ± 0	110 ± 0	100 ± 0	*0.038*	*0.111*	250 ± 0	250 ± 0	327 ± 0	*1.000*	*0.134*
isovaleric acid	410 ± 0	305 ± 0	253 ± 0	*0.011*	*0.016*	530 ± 0	440 ± 0	400 ± 0	*0.086*	*0.068*
hexanoic acid	510 ± 0	450 ± 0	389 ± 0	*0.051*	*0.124*	1545 ± 0	1735 ± 0	2608 ± 0	*0.112*	*0.014*
octanoic acid	325 ± 0	305 ± 0	268 ± 0	*0.228*	*0.256*	1335 ± 0	1795 ± 0	2537 ± 0	*0.063*	*0.167*
decanoic acid	200 ± 0	200 ± 0	174 ± 0	*1.000*	*0.593*	310 ± 0	760 ± 0	870 ± 0	*0.076*	*0.771*
volatile phenols										
guaiacol	0.23 ± 0.03	0.64 ± 0.02	0.56 ± 0.08	*0.211*	*0.261*	1.17 ± 0.36	1.35 ± 0.16	1.15 ± 0.42	*0.574*	*0.581*
4-ethylguaiacol	nd	7.87 ± 0.88	0.14 ± 0.09	*0.335*	*0.014*	0.55 ± 0.12	1.81 ± 0.23	0.68 ± 0.21	*0.020*	*0.011*
4-ethylphenol	0.06 ± 0.08	31.3 ± 3.8	0.30 ± 0.19	*0.367*	*0.020*	0.68 ± 0.19	1.37 ± 0.12	1.12 ± 0.39	*0.050*	*0.475*
C_{13} norisoprenoids										
β-damascenone	0.31 ± 0.06	0.71 ± 0.13	0.90 ± 0.11	*0.063*	*0.055*	1.02 ± 0.01	0.95 ± 0.03	3.06 ± 0.78	*0.089*	*0.036*

[a] Mean values ± SD ($n = 3$) for the years 2005 and 2006. nd, not detected.

tions, but guaiacol concentration was higher ($P < 0.001$) in 2006 wines than in 2005 wines.

The C_{13} norisoprenoid β-damascenone was quantified in all of the wines (**Table 7**). β-Damascenone is a potent wine flavorant derived from grape carotenoids (*65*, *66*), and it has fruity odor (*67*). This compound did not vary among the treatments, but with the year; the wines from 2006 had more ($P < 0.001$) β-damascenone than wines from 2005.

Open-Top Chamber Effects. Briefly, we present the impact of chambers on the quality of grapes, musts, and wines, because several researchers have recognized that chambers interfere with natural micrometeorological conditions of wind flow and radiation exchange (*68*). Also, our results confirmed that the environment inside OTCs, independent of CO_2 effect, was slightly modified, mainly lower PPFD and higher temperature and vapor pressure deficit, with relevant effects on vine physiology, productivity (data not shown), grapes, and red wine quality.

Open-top chambers induced a significant increase in skin weight ($P < 0.01$) and skin/pulp weight ratio ($P < 0.001$) of berries in 2005, compared to berries produced exterior (**Table 1**). Berries grown in OTC ambient had slightly lower L^* ($P < 0.001$) in both years and higher a^* and b^* ($P < 0.05$) in 2005 (**Table 2**). Therefore, wines from exterior vineyards had a brighter color (highest value of L^*). During the maturation control, increases were noted in alcohol and °Brix ($P < 0.005$) of the berries produced in OTC than in the exterior (**Table 3**). In addition, higher pH ($P < 0.01$) on September 27 and lower total acidity ($P < 0.05$) on August 23 were also observed. In 2006, musts obtained from grapes grown in OTC ambient had higher °Brix and pH ($P < 0.05$), but lower total acidity ($P < 0.001$), than the musts made with grapes grown in the exterior (**Table 4**). A chamber effect was observed in the increase of total acidity and fixed acidity ($P < 0.001$) and decreased total polyphenols ($P < 0.01$) of the 2005 wines (**Table 5**). Moreover, higher alcohol ($P < 0.001$), pH ($P < 0.05$), and total anthocyan and polyphenol ($P < 0.01$) concentrations and lower fixed acidity ($P < 0.001$) were determined in OTC ambient wines produced in 2006. In general, there were no significant differences in total antioxidant activity between wines produced in the exterior or in OTC ambient (**Table 6**).

With regard to the volatile composition, wines from 2005 produced in OTC ambient had higher concentrations of 1-hexanol ($P < 0.05$), benzyl alcohol ($P < 0.01$), ethyl 2-methylbutyrate ($P < 0.001$), ethyl isovalerate ($P < 0.01$), isovaleric acid ($P < 0.05$), 4-ethylguaiacol ($P < 0.05$), and 4-ethylphenol ($P < 0.05$) than wines from grapes produced in the exterior (**Table 7**). Furthermore, wines from 2006 produced in OTC ambient

had higher concentrations ($P < 0.01$) of diethyl succinate, citronellol, and 4-ethylguaiacol and lower concentrations of *cis*-3-hexenol, linalool, ethyl lactate, and hexanoic acid.

In summary, this study showed that the predicted rise in [CO_2] might strongly stimulate grapevine photosynthesis and yield (data not shown) without causing negative impacts on the quality of grapes and red wine. In fact, our data based on the analyses of wine following fermentation and the informal sensorial analysis carried out by the researchers showed that, although some of the compounds were slightly affected by elevated [CO_2], wine quality remained almost unaffected.

The Mediterranean basin is the main cultivation area of vine in Europe, which is characterized by summer droughts that deeply influence vineyard growth and productivity. As with the future scenario of global climate change, the precipitation and soil moisture in the Mediterranean regions would be reduced in a climate of elevated [CO_2], the positive CO_2 effects on grapevine yield may be completely canceled. Our data may be used in scaling up models that can predict the extent of vine responses to climate change in the Mediterranean region. However, in the near future it is important to study the interactive effects of elevated [CO_2], water availability, and high temperature before more conclusions can be drawn.

ACKNOWLEDGMENT

We thank Air Liquide Portugal for the technical [CO_2] enrichment. Helena Ferreira, Natália Teixeira, Rui Martins, and José Baltazar Carvalho are also acknowledged for their field and laboratorial assistance.

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Received for review July 2, 2008. Revised manuscript received October 28, 2008. Accepted November 9, 2008. Financial support from Fundação para a Ciência e Tecnologia (Lisboa, Portugal), project no. POCTI/AGG/47938/2002, is gratefully acknowledged.

JF8020199