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## Phenolic Composition in Grape (*Vitis vinifera* L. cv. Malbec) Ripened with Different Solar UV-B Radiation Levels by Capillary Zone Electrophoresis

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The responses of *Vitis vinifera* L. cv. Malbec to different solar ultraviolet-B radiation (UV-B) levels were assessed in two contrasting situations, under sunlight with full UV-B (+UV-B) and filtered UV-B (-UV-B), in three different locations at 500, 1000, and 1500 m above sea level (asl). To evaluate the effects of radiation, a simple, accurate, and rapid method for the separation and simultaneous determination of representative phenolic compounds in grape berry skins by capillary zone electrophoresis was developed. Separation was carried out in less than 20 min with 20 mM sodium tetraborate buffer containing 30% methanol, pH 9.00. The procedure is fast and reliable, and extracted grape berry skins can be directly analyzed without prior sample cleanup procedure. Berry skins from the +UV-B treatment at 1500 m asl showed the highest levels of total polyphenols anthocyanins, and resveratrol, compared with the -UV-B treatment at this altitude.

KEYWORDS: Phenolic composition; UV-B radiation; grapevines; *Vitis vinifera* L. cv. Malbec; capillary zone electrophoresis

#### INTRODUCTION

Solar ultraviolet-B radiation (UV-B; wavelength range = 280-315 nm) is mostly absorbed by stratospheric ozone and atmospheric gases; however, a small amount reaches the earth's surface. Such UV-B is biologically important and potentially harmful to plants, depending on the fluence rate, daily dosage, species, cultivar, and relative quantity of photosynthetically active radiation (PAR) (*1–3*). It induces diverse morphological, physiological, and biochemical responses. High fluences of UV-B photons can cause direct cellular damage by generating photoproducts of DNA, proteins, and lipids or by overproduction of reactive oxygen species (ROS). Nevertheless, moderate levels of UV-B stimulate transcription of genes involved in protective

responses (4). In fact, some genes of the phenylpropanoid and flavonoid biosynthetic pathways are up-regulated by this radiation (5–8), promoting the accumulation of UV-B-absorbing compounds.

Phenolic compounds are components of wine with a great impact on the sensorial characteristics of red wine, especially color and flavor. They have also shown beneficial effects for human health, particularly as antioxidants (9). As these molecular species are localized in the cell vacuoles of the solid parts of the berries (including skin, seeds, and brush) and are extracted during winemaking (10), the phenolic composition of wines is correspondingly dependent on the composition of grapes (11). Phenolics are classified as non-flavonoids and flavonoids. The former include the benzoic and cinnamic acids, which are colorless, with no particular flavor or odor, and stilbenes such as resveratrol that plays a role in defensive mechanisms against the attack of pathogens, diverse injuries, and UV-B (12, 13). Resveratrol is the most promoted, and apparently protects grape and wine consumers from cardiovascular diseases (14). Flavonoids include anthocyanins (malvidin, peonidin, etc.), which are the red pigments in grapes and wines. Also, flavan-3-ols (monomeric catechins and oligopolymeric proanthocyanins),

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which are mainly responsible for the astringency, bitterness, and structure of wines, and flavonols (quercetin, myricetin, kaempferol, and their glycosides), yellow pigments that seem to contribute to bitterness and stabilize and increase wine color through copigmentation with anthocyanins (15). Flavonoids are important factors for winemaking as well as for UV protection and as insect-feeding deterrents (16, 17). The grape berries accumulate them during the ripening stage, and the anthocyanin accumulation in the skin begins after the phenological stage known as *veráison*, the onset of ripening characterized by the appearance of color in varieties for red wine. Their biosynthesis is primarily dependent upon varietal factors, and it is influenced by a combination of environmental and viticulture factors (18). Their concentration in wine is also affected by berry size, because it depends on the relationship between the surface of the skin and the volume of the berry (15).

The analysis of phenolic compounds is routinely performed by high-performance liquid chromatography (HPLC) and other chromatographic techniques (19–21). Over the past two decades, capillary electrophoresis (CE) and related techniques have rapidly developed into powerful analytical techniques for the separation of a wide range of analytes ranging from large protein molecules to small inorganic ions (22, 23). Moreover, CE techniques have seen a significant increase in applications in food analysis in recent years (24-27). The use of capillary zone electrophoresis (CZE) for the analysis of the phenolic profile in samples of viticultural interest can have benefits in terms of robustness and ruggedness, versatility, cost, and time (28). On the other hand, gas chromatography (GC), HPLC, or chromatographic CE modes such as micellar electrokinetic chromatography (MECK) or capillary electrochromatography (CEC) are time-consuming.

The cultivar used in this study, Malbec, is representative of Argentinean wines, and in Mendoza province its cultivation extends from areas at 500 m above sea level (asl) up to very high altitudes, near 1500 m asl. The environmental differences according to height (UV-B, PAR, temperature regime, and type of soil) make the grapes from high areas the latest to ripen, possibly due to the greater thermal amplitude. The methodology employed in this study consisted of the exclusion of solar UV-B using filters, without modifying other variables. The purpose of the present paper is to evaluate the effect of different solar UV-B levels during ripening stage on the phenolic accumulation and composition of *Vitis vinifera* L. cv. Malbec grape berry skins and wines cultivated at different altitudes by UV–vis spectrophotometry and CZE.

#### MATERIALS AND METHODS

**Cultivation.** *Plant Material.* The experiment was carried out during 2006, in three commercial vineyards of *V. vinifera* L. cv. Malbec of selected clones planted without roostock, at different altitudes in the province of Mendoza, Argentina. They are located approximately at 500, 1000, and 1500 m asl in La Libertad (68° 28' W and 33° 12' S), Ugarteche (68° 54' W and 33° 13' S), and Gualtallary (69° 77' W and 33° 22' S).



**Figure 2.** Transmittance spectra: polyester filter, -UV-B treatment; polyethylene film, +UV-B treatment.

The grapevines were trained on a vertical trellis and pruned as Guyot, arranged in north–south oriented rows spaced 2 m apart, with a distance of 1.20 m between two consecutive plants of each row. The three vineyards differed in the origin and the age of plants, type of soil, and irrigation system (500 m asl, 50-year-old plants, sandy soils, and unpressurized irrigation; 1000 m asl, 14-year-old plants, silty soils, and drip irrigation; 1500 m asl, 8-year-old plants, sandy soils, and drip irrigation). Also, due to their location in different altitudes, they have different regimens of temperature and solar radiation.

*Experimental Field Procedures. UV Treatment.* Two radiation regimens were set by covering grapevines from approximately 15 days before berry véraison stage (when berry color began to change) until harvest, a period of approximately 90–105 days depending on the location, with specific plastic sheeting.

Solar UV-B radiation was removed to produce the minus UV-B treatment (-UV-B) using clear polyester filters (100  $\mu$ m, Oeste Aislante, Buenos Aires, Argentina). This polyester filter absorbed 95% of UV-B without significantly affecting UV-A or PAR. To manage differences caused by wind, temperature, or humidity under plastic sheeting, low-density polyethylene was used (40  $\mu$ m). This polyethylene transmitted most radiation from sunlight and was designated the full UV-B treatment (+UV-B). **Figure 2** shows the transmittance spectra for both filters.

The plastic sheeting covered the east and west facing sides of the canopy at an angle of 45° with respect to the soil, and it was placed 30 cm above the grapevines. The treatments were protected by antihail nets (black polyethylene) that produce a 17% shade (measured by means of an LI-250 light meter with an LI-190SA quantum sensor; LI-COR Inc., Lincoln, NE).

A randomized complete block (RCB) design was used, with two treatments and five replications for each vineyard. The experimental unit consisted of five plants; two were used for grape berry sampling, and the berries of the five plants were used for microvinification.

**Sampling.** Fifty grape berries per experimental unit were collected in nylon bags (10 berries from 5 clusters), at the moment of harvest, when sugar concentration reached 24.50 °Brix. In the field, samples were kept in ice to prevent dehydration. At the laboratory, they were frozen and conserved at -20 °C until analysis.

Grape Analysis. Apparatus. A Varian Cary UV-vis spectrophotometer was used to perform the absorptiometric measurements, with

 Table 1. Linear Regression Data for the Analysis of Resveratrol (R),

 (+)-Catechin (C), and Quercetin (Q)

| analyte | concn range (mg/L) | $r^2$ ( $n = 6$ ) | LOQ (mg/L) | LOD (mg/L) |
|---------|--------------------|-------------------|------------|------------|
| R       | 0.06-100           | 0.998             | 0.18       | 0.07       |
| С       | 0.20-100           | 0.996             | 0.32       | 0.12       |
| Q       | 0.20-100           | 0.997             | 0.28       | 0.08       |
|         |                    |                   |            |            |

1 and 10 mm optical path cells for wine analysis and grape skin, respectively. The pH values were measured with an Orion 940 pH-meter, equipped with a glass-combined electrode.

A Beckman P/ACE MDQ instrument (Beckman Instruments, Inc., Fullerton, CA) equipped with a diode array detector and a data handling system comprising an IBM personal computer and P/ACE System MDQ software was used for CE analysis. Detection was performed at 280 and 520 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corp. and had the following dimensions: 67 cm total length, 50 cm effective length, 75  $\mu$ m i.d., 375  $\mu$ m o.d. The temperature of the capillary and the samples was maintained at 15 °C. Samples were pressure-injected at the anodic side at 0.50 psi for 5 s.

*CZE Analysis.* (a) Regeneration of Capillary and Its Maintenance. Capillary preparation was carried out by rinsing with 0.10 mol/L NaOH for 5 min and then with water for 5 min, and it was finally conditioned with running electrolyte for 10 min before sample injection. To achieve high migration time reproducibility and to avoid solute adsorption, the capillary was washed between analyses with NaOH for 2 min, followed by water for 2 min, and then equilibrated with the running buffer for 4 min.

(b) Reagents and Solutions. For CZE, the background electrolyte (BGE) solutions were composed of 20 mM sodium tetraborate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>•10H<sub>2</sub>O) buffer (pH 9.00) containing 30% (v/v) methanol. Electroosmotic flow (EOF) determination was performed by using acetone as an EOF marker. The EOF marker was prepared by diluting 1 mL of acetone with the BGE up to 50 mL and ultrasonication for 5 min prior injection.

The structures of the compounds studied are shown in **Figure 1**. Resveratrol was purchased from Sigma Chemical Co. (St. Louis, MO); (+)-catechin and quercetin were purchased from Extrasynthese (Genary, France). Stock standard solutions for the construction of calibration curves were prepared in ethanolic solution and then suitably diluted (final ethanol concentration = 15% v/v) to obtain standard solutions within the concentration range of 0.06–100 mg/L.

Ultrapure water (resistivity = 18.3 M $\Omega$ ·cm) was obtained from Barnstead EASY pure RF water system (Dubuque, IA). All other reagents and solvents were of analytical grade quality. All solutions were degassed by ultrasonication (Testlab, Argentina). Running electrolytes and samples were filtered through a 0.45  $\mu$ m Titan syringe filter (Sri Inc., Eaton Town, NJ).

*CZE Procedure.* The electrolyte solution was prepared daily. All solutions were filtered through a 0.45  $\mu$ m membrane prior to injection. At the beginning of the day, the capillary was conditioned with 0.10

mol/L NaOH for 5 min, followed by water for 5 min, and then with running electrolyte for 10 min before sample injection. To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with sodium hydroxide for 2 min, followed by water for 2 min, and then equilibrated with the running buffer for 4 min. Samples were pressure-injected at the anodic side at 0.50 psi for 3–7 s. A constant voltage was used for all experiments.

Total Anthocyanin and Polyphenol Contents. UV–vis determinations were performed according to the method of Riou and Asselin (28), a modification of the Puissant and Leon method (29). Samples (50 grape berries) were defrosted at room temperature, and skins were separated from pulp and seeds by hand; 50 mL of extracting synthetic solution (ethanol, 12%; tartaric acid, 6 mg/mL; SO<sub>2</sub>, 100  $\mu$ g/mL; pH 3.2) was added. The system was kept in the dark for 3 h at 70 °C. The liquid fraction was centrifuged for 5 min at 600 rpm (181.81g), and the supernatant was collected.

For anthocyanin content, the supernatant was diluted 1:50 (v/v) with acidified distilled water (1% v/v HCl), and the absorbance was measured at 520 nm against a blank of reagents.

For polyphenol content, the supernatant was diluted 1:100 (v/v) with distilled water, and the absorbance was measured at 280 nm against a blank of reagents.

Wine Analysis. *Microfermentations*. To obtain three replicates for each treatment, winemaking was carried out according to the following procedure. The grapes were separated manually from the clusters and placed in 25 L plastic tanks with the addition of 30 mg/L potassium metabisulfite. The maceration was carried out at 5 °C for 3 days. Then the grapes were crushed and inoculated with 800 mg/L of selected commercial *Saccharomyces cervisiae bayanus* yeast. During fermentation the temperature was kept at 25 °C and density was measured daily. When the alcoholic fermentation was completed, the solid parts were separated and sulfur dioxide (60 mg/L) was added as potassium metabisulfite. Finally, the temperature was maintained at 5 °C during 7 days, and the upper fraction was bottled.

UV-Vis Analysis. Total phenol index (TPI) and color intensity (CI) were spectrophotometrically analyzed according to the method of Ribéreau-Gayon et al. (30). For all cases, samples under study were 1-month-old wines. For TPI determinations wines were diluted 1:10 (v/v) with double-distilled water and measured at 280 nm against a blank of reagents. The CI values were calculated for undiluted wines and absorbance measurements at 420, 520, and 620 nm.

**Statistical Analysis.** One-way analyses of variance (ANOVA) and Fisher's multiple comparison of means to discriminate between the averages by the minimum difference, with a significance level of  $P \le 0.05$ , were applied. Analysis was performed by means of Statgraphics Centurion XV version 15.0.10.

#### **RESULTS AND DISCUSSION**



areas, research on the impact on grape of UV-B, or even the whole UV that reaches the earth's surface (including UV-A and

Despite potential benefits for both scientific and commercial

**Figure 3.** Total polyphenolic and anthocyanin contents in grape skin by UV–vis spectrophotomety. Conditions were as described under Materials and Methods. Values are means  $\pm$  SEM; n = 5. Different letters indicate significant statistical differences between the treatments using Fisher's multiple test with a significance level of  $P \leq 0.05$ . Factors tested: total polyphenolics, absorbance<sub>280 nm</sub>/50 berries; total anthocyanins, absorbance<sub>520 nm</sub>/50 berries.

Phenolics in Grapes with Different Solar UV-B Levels



**Figure 4.** Resveratrol, catechin, and quercetin concentrations in grape skin by CZE. Conditions: 20 mM sodium tetraborate buffer, pH 9.00 containing 30% (v/v) methanol; capillary, 67 cm full length, 50 cm effective length, 75  $\mu$ m i.d., 375  $\mu$ m o.d.; hydrodynamic injection at 0.50 psi, 5 s; 30 kV constant voltage, capillary temperature, 15 °C; detection by UV absorbance at 280 nm. Values are means  $\pm$  SEM; n = 5. Different letters indicate significant statistical differences between the treatments using Fisher's multiple test with a significance level of  $P \le 0.05$ . Factor tested: analyte concentration, mg/L.

| Table | 2. | Recovery | Test |
|-------|----|----------|------|
|-------|----|----------|------|

|             | base value<br>(mg/L) | quantity<br>added (mg/L) | quantity<br>found <sup>a</sup> (mg/L) | recovery <sup>b</sup><br>(%) |
|-------------|----------------------|--------------------------|---------------------------------------|------------------------------|
| aliquot I   |                      |                          |                                       |                              |
| Ŕ           |                      | 0.00                     | 2.42                                  |                              |
| С           |                      | 0.00                     | 6.80                                  |                              |
| Q           |                      | 0.00                     | 3.20                                  |                              |
| aliquot II  |                      |                          |                                       |                              |
| Ŕ           | 2.42                 | 5.00                     | 7.63                                  | 104.20                       |
| С           | 6.80                 | 0.00                     |                                       |                              |
| Q           | 3.20                 | 0.00                     |                                       |                              |
| aliquot III |                      |                          |                                       |                              |
| Ŕ           | 2.42                 | 0.00                     |                                       |                              |
| С           | 6.80                 | 5.00                     | 11.69                                 | 97.80                        |
| Q           | 3.20                 | 0.00                     |                                       |                              |
| aliquot IV  |                      |                          |                                       |                              |
| Ŕ           | 2.42                 | 0.00                     |                                       |                              |
| С           | 6.80                 | 0.00                     |                                       |                              |
| Q           | 3.20                 | 5.00                     | 8.08                                  | 97.60                        |
|             |                      |                          |                                       |                              |

<sup>a</sup> Mean value (n = 6). <sup>b</sup> 100[(found - base)/added].

UV-B), has not received much attention. Most results have been obtained under unrealistic conditions with different experimental designs and/or UV treatments (31-34). There are two basic experimental approaches to assess the effects of UV-B on plants: enhancing UV-B using lamps and reducing solar UV-B using filters. The experimental design employed for the development of the present paper is considered to be the most realistic approach (35). Reduction studies are most appropriate to evaluate the effects of present-day UV-B radiation because they do not alter the biologically effective radiation as much as in lamp supplementation (36). Nevertheless, very few studies of that kind are available (34, 37-39). It has to be pointed out

that, to our awareness, field studies on the effects of UV-B in berries in Malbec or other cultivars used for red wine production have not been carried out before.

**Method Development.** Rapid and trustworthy analysis is needed when physiological responses are being evaluated for a vast number of biological samples. CZE can meet many of the requirements concerning the achievement of these goals due to the high efficiency, low cost, reproducibility, rapidity, and selectivity related to the technique.

To propose a specific and accurate way of analyzing phenolic compounds extracted from grape berry skins by using CZE, it is essential to find the best experimental conditions in which the analytes can be separated from each other. The optimization was performed using a synthetic ethanolic 12% (v/v) solution mixture containing resveratrol, (+)-catechin, and quercetin. The following parameters were consecutively optimized: sample conditioning, pH, BGE composition and concentration, sample and capillary temperatures, and other electrophoretic parameters such as separation voltage, injection mode, and length.

*Effect of pH.* The buffer pH plays an important role in improving selectivity in CZE, especially for closely related compounds, because it affects both the overall charges of the solute and the EOF. The effect of the buffer pH was investigated within the range of 300-1000. Satisfactory resolution was achieved at low pH (pH range = 3.00-4.20) and at high pH (pH range = 8.20-10.00). Nevertheless, it was found that when the pH was increased, resolution and reproducibility also increased, whereas time analysis decreased. A pH value of 9.00 was chosen as optimal.

*Effect of Buffer Composition and Concentration.* Buffer concentration has also a significant effect on the separation performance through its influence on the EOF and the current

Table 3. Total Phenol Index (TPI) and Color Intensity (CI) in Wines by UV-Vis Spectrophotometry<sup>a</sup>

|           | 1500   | 1500 m asl   |                                      | 1000 m asl                           |  | 500 m asl  |  |
|-----------|--|--|--------------------------------------|--------------------------------------|--|--|--|
|           | +UV-B  | -UV-B  | +UV-B                                | -UV-B                                | +UV-B  | -UV-B  |  |
| TPI<br>CI | $\begin{array}{c} \text{42.90} \pm \text{1.26 a} \\ \text{1.81} \pm \text{0.07 a} \end{array}$ | $\begin{array}{c} \text{41.22} \pm \text{1.34 a} \\ \text{1.63} \pm \text{0.08 a} \end{array}$ | $34.24 \pm 1.26$ b $1.72 \pm 0.07$ a | $36.62 \pm 1.26$ b $1.65 \pm 0.07$ a | $\begin{array}{c} \text{24.65} \pm \text{1.26 c} \\ \text{0.51} \pm \text{0.07 b} \end{array}$ | $\begin{array}{c} \text{25.83} \pm \text{1.26 c} \\ \text{0.50} \pm \text{0.07 b} \end{array}$ |  |

<sup>a</sup> Conditions as described under MaterialsI and Methods. Values are means  $\pm$  SEM; n = 3. Different letters indicate significant statistical differences between the treatments using the Fisher's multiple test with a significance level of  $P \le 0.05$ .

produced in the capillary. Different BGEs have been tested such as boric acid, sodium tetraborate, phosphate, and Tris, but the one producing the best results considering selectivity, reproducibility, baseline, and current performance was sodium tetraborate, pH 9.00.

While the other parameters were kept constant (pH 9.50, 30 kV, 15 °C) the buffer concentration was varied from 5 to 75 mM. Increases in migration times as well current were observed when the concentration of buffer increased. Resolution also improved for higher buffer concentrations, but no appreciable improvements were observed for buffer concentrations above 20 mM. Methanol was used as an organic modifier to enhance the resolution. Various concentrations of methanol (5, 10, 15, 20, 25, and 30% v/v) were added into the 20 mM sodium tetraborate buffer, pH 9.00. The compounds were baseline separated when 30% (v/v) of methanol was added. Therefore, a 20 mM sodium tetraborate buffer containing 30% methanol, pH 9.00, was chosen as the BGE as it gave a full separation of the analytes of interest in <20 min.

*Injection Parameters.* The injection mode giving the best response concerning reproducibility and linear range was hydrodynamic mode. Reproducibility and linear range were not satisfactory for electrokinetic injection mode. Injection parameters were optimized by varying the lengths of sample (3–7 s) and pressure injection until optimum conditions were reached. The best results were obtained for the following experimental parameters: hydrodynamic injection mode, 0.50 psi, 5 s.

Analytical Performance. The calibration plots were measured under the optimal experimental conditions over the concentration range of 0.20-100 mg/L. The migration times for resveratrol, (+)-catechin, and quercetin were 8.04, 10.50, and 18.80 min, respectively. They were obtained representing the ratio of the corrected areas versus concentration. Six points of the calibration curve were determined, and three replicate injections of standards at each concentration level were performed. The calibration equations were calculated by the least-squares linear regression method, and unknown concentrations were calculated by interpolation. The detection and quantitation limits were calculated as the analyte concentrations that gave rise to peak heights with signal-to-noise ratios of 3 and 10, respectively. The limits of detection (LOD) and quantification (LOQ) were determined by injecting standard combined solutions at three different concentrations for each analyte (0.08, 4.00, and 20 mg/L). Table 1 shows the concentration ranges for calibration curves of each analyte and limits of detection and quantitation.

To determine the repeatability (within-day precision) of the method, replicate injections (n = 6) of 20 mg/L combined solution containing resveratrol, (+)-catechin, and quercetin were carried out. In all cases, the precision was better than 0.82% for the migration time and 2.96% for the peak area. Good peak area precision was achieved without adding any internal standard.

Intermediate precision (between-day precision) was also evaluated over 3 days by performing six injections each day. Intermediate precision (relative standard deviation, RSD) on the basis of migration time and peak area was better than 0.62 and 2.44%, respectively.

Repeatability of the method was performed by two analysts (n = 6) using the proposed method and the same instrumentation. The results showed no significant differences: 0.50% (RSD).

Analysis of Grape Skin. Figure 3 shows total polyphenolic and anthocyanin contents obtained by UV–vis spectrophotometry. Once the conditions for separation and quantification were established, the CZE method was applied to the determination of resveratrol, (+)-catechin, and quercetin in real grape skin samples. The results are shown in Figure 4. CZE has proven to be a very effective and robust technique for the individual determination of phenols in grape for a large number of samples.

Considering that the three vineyards differed in the origin and age of plants, type of soil, and irrigation system and different regimens of temperature and solar radiation were present, conclusions on phenolic accumulation could only be ascribed for +UV-B and -UV-B treatments within each altitude.

The results suggested that UV-B stimulated the transcription of some genes involved in protective responses at high altitudes. Berry skins from 1500 m asl showed the highest levels of total polyphenols, anthocyanins, resveratrol, and (+)-catechin. Indeed, at this altitude, differences between the +UV-B and -UV-B treatments were statistically significant for total polyphenols, anthocyanins, and resveratrol. Bearing in mind that these compounds play a major role in enological quality, potential commercial improvements may be possible for vineyards located at higher altitudes, where different factors, including enhanced levels of solar UV-B radiation, are found. Interestingly, a differential regulation for resveratrol as compared to (+)catechin and quercetin was observed under the same conditions, suggesting that its biosynthetic pathway is UV-B dependent. The biosynthesis of (+)-catechin and quercetin in berry skins could be regulated by different factors apart from UV-B, whereas their contents were enhanced at higher altitudes even for the -UV-B treatment.

Method Validation. To determine the accuracy of this method, 5 mL of the sample solution (Ugarteche vineyard 1000 m asl, +UV-B treatment) was collected and divided into 10 portions of 0.50 mL each. The proposed method was applied to six portions, and the average concentrations determined for each compound [resveratrol, (+)-catechin, and quercetin] were taken as a base value. Then, known quantities of the analytes were added to the other aliquots, and the phenolic compounds were determined following the recommended procedure (Table 2).

Analysis of Wines. Table 3 shows the results for TPI and CI obtained by UV-vis spectrophotometry. The highest TPI levels were obtained at 1500 m asl, whereas the lowest CI levels was found for 500 m asl. Undoubtedly, several other factors apart from UV-B affect those indices due to the fact that their levels were never diminished for the exclusion treatment (-UV-B). It has to be considered that many other enological variables play important roles in wine quality.

#### **ABBREVIATIONS USED**

BGE, background electrolyte; C, (+)-catechin; CE, capillary electrophoresis; CEC, capillary electrochromatography; CI, color intensity; CZE, capillary zone electrophoresis; EOF, electroosmotic flow; GC, gas chromatography; HPLC, high-performance liquid chromatography; i.d., inner diameter; LOD, limit of detection; LOQ, limit of quantification; MECK, micellar electrokinetic chromatography; o.d., outer diameter; PAR, photosynthetically active radiation; Q, quercetin; R, resveratrol; RCB, randomized complete block; ROS, reactive oxygen species; RSD, relative standard deviation; TPI, total phenol index; UV-A, ultraviolet A radiation (wavelength range = 315–400 nm); UV-B, ultraviolet B radiation (wavelength range = 280–315 nm).

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