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Effect of Some Viticultural Parameters on the Grape Carotenoid Profile

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The effect of some viticultural parameters on the grape carotenoid profile was investigated. Grape cultivar, ripeness stage, sunlight and shade exposure, altitude, and vegetative height were studied. Differences between cultivars were observed in eight different black grape varieties: Touriga Brasileira (TBR), Tinta Barroca (TB), Tinta Amarela (TA), Souzão (S), Touriga Franca (TF), Touriga Nacional (TN), Tinta Roriz (TR), and Tinto Cão (TC), from the Douro region. TA and TBR clearly produced higher concentrations of carotenoids. Results showed that carotenoid content decreased during ripening. Decreases of lutein were observed until 66%, whereas β-carotene slowly decreased, having a constant level until the harvest date. Carotenoid contents were consistently higher in grapes exposed to shade than in those exposed to direct sunlight in both studied white grape varieties, Maria Gomes (MG) and Loureiro (L). In the Douro Valley, high-elevation terraces, which presented a lower temperature and higher humidity during the maturation period, appeared to produce grapes with higher carotenoid values. Grapes grown with higher vegetative height seem to have higher carotenoid levels; furthermore, grapes grown with lower vegetative height had higher weight and sugar concentrations.

KEYWORDS: ***Vitis vinifera*; carotenoids; cultivars; sunlight and shade; altitude; vegetative height; berry growth**

INTRODUCTION

In the Douro region of Portugal grape quality is determined by taking into account several viticultural parameters. The existence of several types of quality wine in the Demarcated Region of Douro determined the need for a criteria that are applied to selecting and sharing the musts that are produced in the region. Thus, of the entire amount of land under viticulture, only 26000 ha is authorized for Port Wine. The vines that are considered to be appropriate for this wine are selected according to criteria of quality based on a scoring method and classified according to a scale of quality that ranges from A to F. This method considers soil, climatic, and agricultural parameters that are important in determining the quality potential of each vineyard. Furthermore, the "benefício" coefficient, which is attributed to a vineyard, is calculated on the basis of the registered characteristics of each vineyard. Before a vineyard can be planted on the very steep slopes, the land has to be shaped to form terraces. The manner by which the vines are trained is the one that best overcomes the restraints imposed upon them by the climate, the soil, the needs of the plants, and the production goals (*1*).

It is well-known that carotenoid contents in plants are related to the metabolic processes of plant cells, which are dependent on climatic factors, agricultural practices, and plant variety (*2*). Carotenoids are mostly synthesized from the first stage of fruit formation until veraison and then degrade between veraison and maturity to produce C13-norisoprenoid compounds (*2*), which have been reported as odor-active substances responsible for the typical aromas of some grape varieties (*2*−*5*). Effects on carotenoid concentrations in grapes due to climatic conditions and sunlight exposure have already been studied (*3*, *6*−*8*). In general, the highest carotenoid levels occurred in grapes produced in hot regions. Nevertheless, grapes exposed to sunlight seem to have lower carotenoid concentrations than shaded grapes, at maturity.

In previous work has been shown a relationship between carotenoid contents in grapevine berries and plant water status (*9*). It was observed that irrigated treatment seems to contribute to lower carotenoid levels in grapes, when vines are planted in a lower water retention capacity soil. However, in a higher water

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retention capacity soil, irrigated treatment seems to have no effect on carotenoid contents when compared with nonirrigated treatment.

An altitude effect on proanthocyanidins and anthocyanins has been reported in grape skins (*10*−*12*). Anthocyanin concentrations were greater in skins of grapes grown at higher altitude, and proanthocyanidins, in skins and seeds, were higher at lower altitudes. It was reported that aroma potential, given by monoterpenes and norisoprenoid concentrations, showed higher values from grapes grown at lower altitude (*13*). Conversely, wines from grapes grown at lower altitude suggested higher wine quality (*11*, *13*), as determined by scoring methods (*1*).

As grape quality is determined by viticultural parameters (*1*), the aim of this study was to examine the impact of some viticultural parameters such as altitude and vegetative height on the grape carotenoid profile to be able to correlate carotenoid content with grape quality. Stage of ripening, climatic conditions, sunlight exposure, and cultivars were studied, as well, to provide some new insights into the understanding of the changes in carotenoid compounds, knowing that carotenoids are precursors of several aroma compounds.

MATERIALS AND METHODS

Plant Materials and Treatments. The effect of *grape cultivar* on carotenoid contents was studied in varieties of *Vitis vinifera* from one grape-growing subregion from Douro, Cima Corgo (CC), in eight different grape varieties: Touriga Brasileira (TBR), Tinta Barroca (TB), Tinta Amarela (TA), Souzão (S), Touriga Franca (TF), Touriga Nacional (TN), Tinta Roriz (TR), and Tinto Cão (TC). The study was conducted in two consecutive years, 2001 and 2002. Samples were analyzed during the last month of maturation. Vines were spaced 1.1 m in rows 2.2 m apart with a north-northwest orientation, trained to a bilateral cordon.

The effect of *sunlight and shade exposure* was investigated with two white grape varieties: Maria Gomes (MG) from the Bairrada region and Loureiro (L) from the Vinho Verde region. In MG grapes, vine spacing was 1.2 m in east-west-oriented rows, with 2.5 m between rows and vines trained to a bilateral cordon. Samples analyzed were taken September 3, 10, and 18. In L grapes, vine spacing was 1.5 m in northwest-southeast-oriented rows, with 3.0 m between rows and vines trained to a single cordon. Samples analyzed were taken September 13 and 20 and October 1. Grape berries were exposed to direct sunlight and kept in the shade by vegetation protection.

The effect of *altitude* was investigated with two grape varieties of *V. vinifera* from the CC subregion of Douro: Touriga Franca and Touriga Nacional. The vineyard system consisted of 4 m wide horizontal terraces containing two rows of vines each. The vines were spaced 1.1 m in rows 2.0 m apart, trained to a double-guyot system and pruned to 8−10 nodes per vine for TF and 12−15 nodes per vine for TN. Two terraces were situated at low altitudes, between 85 and 90 m (above sea level), two terraces were situated at medium altitudes, between 145 and 155 m, and two additional terraces were situated at high altitudes, between 180 and 210 m, for TF and TN, respectively.

The effect of *vegetative height*, the height of the plants, was investigated with one grape variety of *V. vinifera*, TN, from the CC subregion of Douro. The vines were spaced 1.0 m in rows 2.0 m apart, trained to a single-guyot system. One terrace had 60 cm of vegetative height and another had 100 cm of vegetative height.

°*Brix* was measured using a refractometer, LEICA model 7530. *Berry weight* was estimated by an average of 50 g of fresh berries.

Extraction and Determination of Carotenoids. *Grape Material.* Approximately 50 g of fresh berries, without seeds, was homogenized using a Turrax homogenizer at 9500 rpm for 15 min. This procedure provided 40 g of sample that was spiked with 200 μL of internal standard, 170 mg/L of β-apo-8′-carotenal (Fluka) (10810), and diluted with 40 mL of water (18.3 MΩ/cm). Extraction was carried out with 40 mL of ether/hexane (1:1, v/v) of HPLC grade (Merck), agitated for 30 min. The extraction was repeated two more times with 20 mL of ether/hexane (30 min each). The final combined extract was concentrated to dryness (rotavapor) and resuspended in 1 mL of acetone/hexane (1:1, v/v) for HPLC determination. Light exposure was minimized during sample preparations to avoid photoisomerization.

Table 1. Carotenoid Concentrations in Eight Different Cultivars from CC Douro Subregion, at Harvest Date, in 2001 and 2002[a]

grape	neoxanthin	violaxanthin	luteoxanthin	lutein	chlorophyll *a*	β-carotene
			2001			
TBR	21	31	47	702	166	769
TB	7	51	35	993	157	795
TA	8	98	4	654	172	910
S	10	74	18	458	117	456
TF	10	71	5	511	120	606
TN	10	44	9	383	91	463
TR	8	19	8	386	105	750
TC	13	65	5	423	116	549
			2002			
TBR	47	9	2	386	51	537
TB	31	5	4	279	43	495
TA	44	14	6	410	65	621
S	20	3	2	231	65	495
TF	35	4	2	297	54	530
TN	51	10	5	365	57	402
TR	40	7	4	259	49	567
TC	19	3	3	218	67	617

[a] Carotenoid concentration is expressed in μg/kg of berry. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein.

HPLC. A Beckman model 126 quaternary solvent system, equipped with System 32 Karat software and a 168 rapid-scanning, UV−visible photodiode array detector, was used. The absorption spectra were recorded between 270 and 550 nm.

(a) Stationary phase HPLC was performed on a Nova-Pack C18, 60 Å, 4 μm particles (3.9 × 300 mm), Waters.

(b) Mobile Phase HPLC was performed with solvent A, ethyl acetate (Merck pure grade), and solvent B, acetonitrile/water (9:1 v/v) (Merck pure grade and pure water), flow rate = 1 mL/min. The following gradient was employed: 0−31 min (0−60% A); 31−46 min (60% A); 46−51 min (60−100% A); 51−55 min (100% A); 55−60 min (100−0% A); 60−65 min (0% A). t_r values were as follows: neoxanthin, 5.5 min; violaxanthin, 6.0 min; luteoxanthin, 6.2 min; lutein, 13.6 min; chlorophyll, 30.2 min; and β-carotene, (32.4 min *(14)*.

Identification. Carotenoids were identified by comparison with commercially available standards: β-carotene (Sigma 95%, synthetic) (C-9750), lutein (Sigma 70%, from alfalfa) (X-6250), neoxanthin (0234.1) and violaxanthin (0259) from (CaroteNature GmbH), and chlorophyll *a* (Aldrich, from spinach) (25,825-3). Luteoxanthin was identified by comparison of retention time and UV−visible photodiode array spectra (*15*).

Statistical Analysis. Principal component analysis (PCA) was carried out using an XLSTAT-Pro version 6.1.9. The PCA method shows similarities between samples projected on a plane and makes it possible to determine which variables determine these similarities and in what way. An analysis of variance (ANOVA) using Excel software from Windows 98 v 7.0 was applied to the experimental data; the results were considered to be significant if the associated p value was <0.05.

RESULTS AND DISCUSSION

Effect of Grape Ripening and Cultivar on Carotenoid Contents. Table 1 shows the concentration of carotenoids in different cultivars from the CC Douro subregion, at harvest date. The study was conducted in two consecutive years, 2001 and 2002, for eight different grape varieties: Touriga Brasileira, Tinta Barroca, Tinta Amarela, Souzão, Touriga Franca, Touriga Nacional, Tinta Roriz, and Tinto Cão. Differences between cultivars can be observed **(Table 1)**. For the first year of the study (2001), the ANOVA treatments of the data showed differences between cultivars and between the different com-

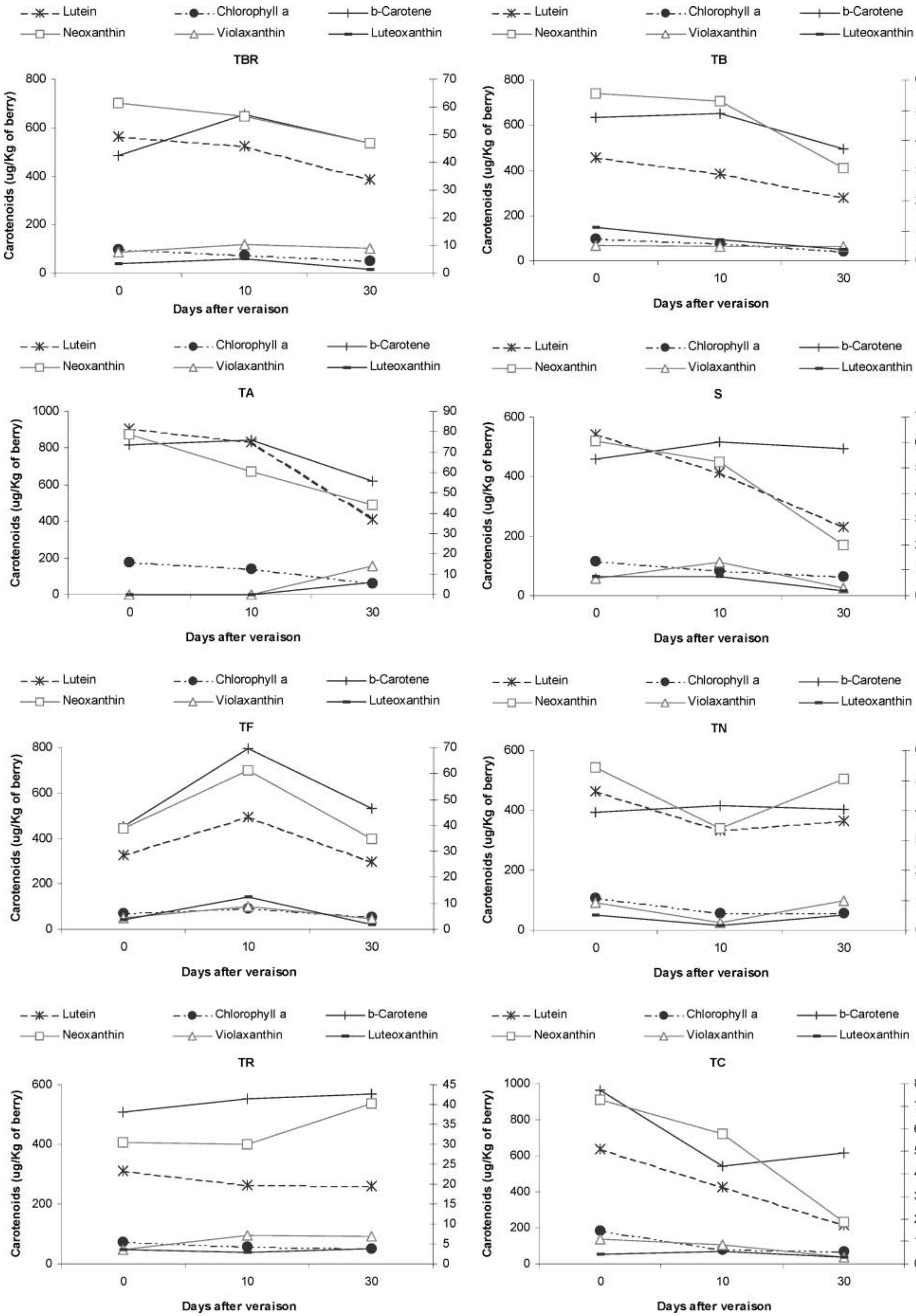

Figure 1. Evolution of the levels of carotenoids during the last month of maturation for the eight cultivars, in the last year of study (2002): lutein, chlorophyll *a*, and β-carotene in the first *yy*-axis; neoxanthin, violaxanthin, and luteoxanthin in the second *yy*-axis.

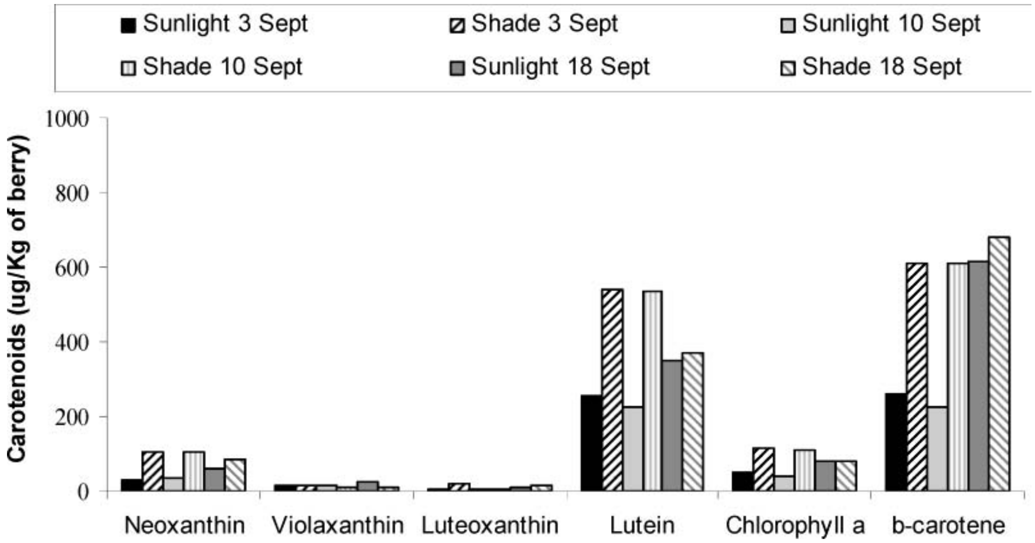

Figure 2. Effect of degree of ripeness, sunlight, and shade on carotenoid concentration in Maria Gomes grapes. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein.

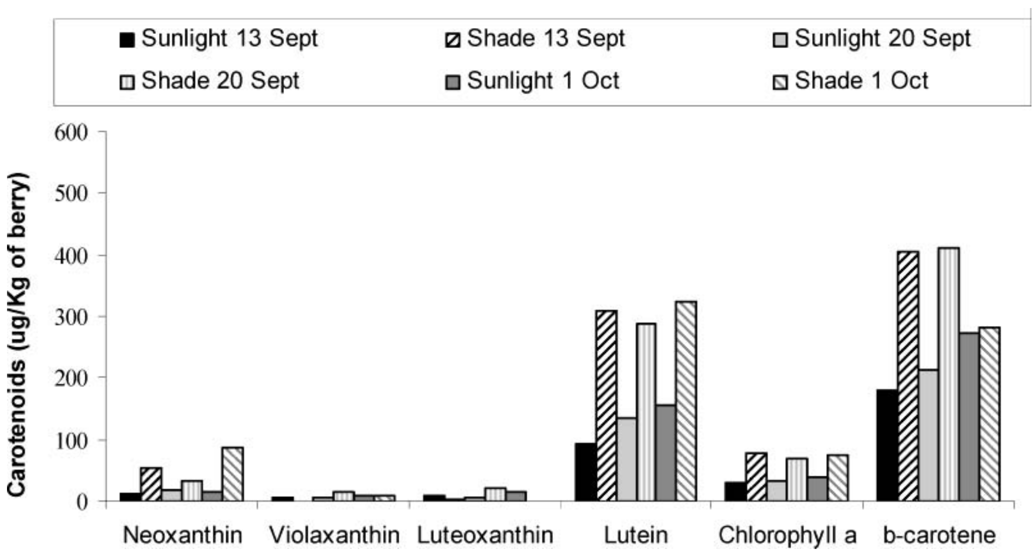

Figure 3. Effect of degree of ripeness, sunlight, and shade on carotenoid concentration in Loureiro grapes. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein.

pounds ($p = 3.99E-02$) and ($p = 4.04E-17$), respectively, at the 95% level, although no significant differences were observed for the last year of the study (2002). TA and TBR clearly produced higher concentrations of carotenoids in both years. Along with these grape varieties TB had, as well, higher carotenoid levels in 2001, although this observation was not evident for 2002.

The levels of carotenoids were followed during the last month of maturation for the eight cultivars, in the last year of study (2002) (**Figure 1**). In general, results showed that, from all plots and experimentation, carotenoid content decreased during ripening, which was previously noted by other authors (*16*−*18*). An increase was observed between veraison and the 10 days after veraison in TF. This behavior was not expected because carotenoids are mostly synthesized from the first stage of grape formation until veraison and then degrade between veraison and maturity. The largest percentage decreases were observed for lutein and chlorophyll *a*, to 66 and 64%, respectively. β-Carotene slowly decreased and, in some cases, reached a constant level until the harvest day. Neoxanthin, violaxanthin, and luteoxanthin seem to have slightly decreased during this period. These results are in agreement with previous work in which the presence of carotenoids in grape berries demonstrated that β-carotene and several xanthophylls are abundant before veraison, with decreasing levels during ripening. These decreases were less prominent during maturation (*9*, *16*−*18*).

Effect of Sunlight and Shade on Carotenoid Concentrations. The effect of sunlight and shade on carotenoid levels in Maria Gomes and Loureiro grapes is illustrated in **Figures 2** and **3**, respectively. Samples were analyzed from September 3 to September 18, harvest date, for MG grapes and from September 13 to October 1, harvest date, for L grapes. Carotenoid levels were consistently higher in grapes protected from direct sunlight exposure than in those exposed to direct sunlight, in both grape varieties. Nevertheless, this difference was less evident for the last stage of ripeness. The proposed carotenoid compounds occurred in higher concentrations during the early stages of ripening. Decreases in the proposed carotenoid compounds over the sampling period were to 39% for MG **(Figure 2)** and to 31% for L **(Figure 3)**.

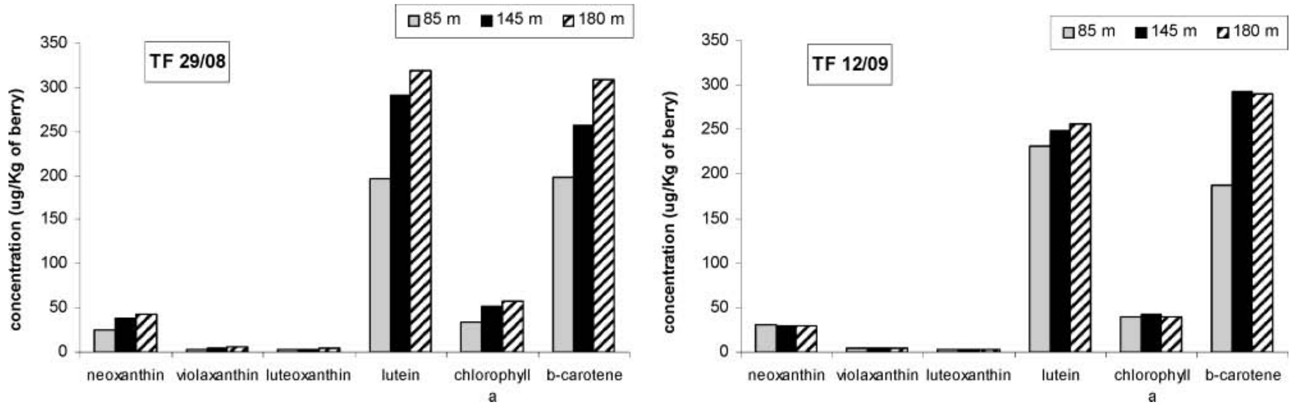

Figure 4. Effect of altitude on carotenoid levels in Touriga Francesa grapes. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein.

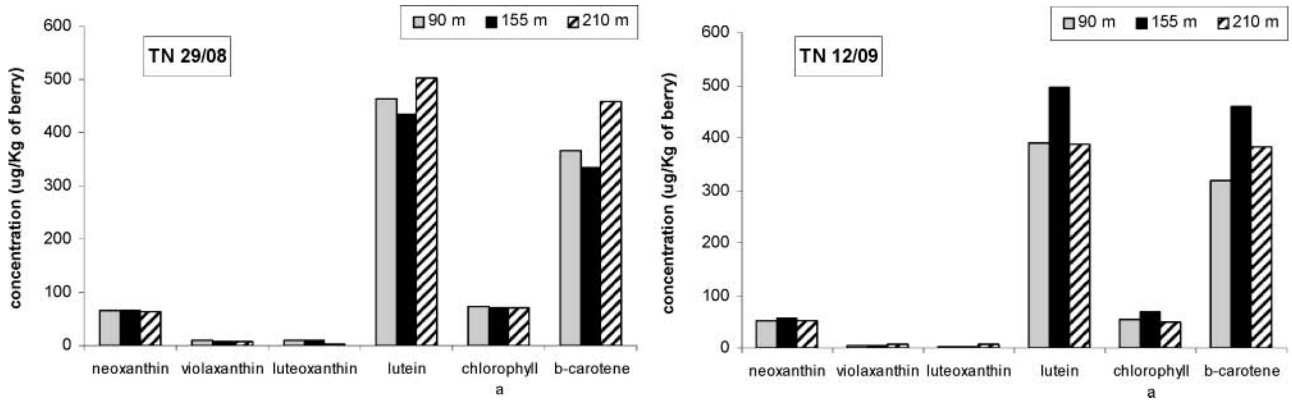

Figure 5. Effect of altitude on carotenoid levels in Touriga Nacional grapes. Neoxanthin, violaxanthin, and luteoxanthin are expressed in equivalents of lutein.

ANOVA treatments of the data showed differences between cultivars and between the different compounds ($p = 2.07E-02$ and $p = 1.31E-10$, respectively, at the 95% level for MG and $p = 1.30E-02$ and $p = 8.90E-11$), respectively, at the 95% level for L). These results were consistent with the previous observation that carotenoid concentration decreases with an increase of ripeness and grapes exposed to sunlight have lower carotenoid levels than shaded grapes (*3*, *6*−*8*).

Effect of Altitude on Carotenoid Concentration. The effect of altitude on changes in carotenoid contents in Touriga Franca and Touriga Nacional grapes, from the CC Douro subregion, is shown in **Figures 4** and **5**, respectively. Grapes were sampled from three different terraces of Douro vineyards with different altitudes on two sample dates (29/08 and 12/09, harvest date). Altitudes ranged from 85 to 145 to 180 m and from 90 to 155 to 210 m for TF and TN, respectively. In this study all terraces were chosen from the same vineyard and had similar cultivation conditions.

Altitude can strongly affect climatic conditions due to it direct impact on temperature, humidity, and other environment factors that affect grape maturity. Indeed, in the Douro Valley the temperature is lower and the humidity higher on hillsides in comparison with low-elevation terrace sites situated nearer the Douro River (*12*). **Table 2** shows berry growth by measurement of the berry weight and °Brix, whereas **Figure 6** gives the factor scores (factor score plot 1−2 accounts for 85% of total variance) from the principal components study carried out with data from **Figures 4** and **5** and **Table 2**. From a study of **Figure 6**, it can be concluded that two different groups can be seen, a first group with the TF grape variety and a second group with the TN grape variety. TF grapes have higher berry weight and lower carotenoid concentrations. On the contrary, TN grapes have lower berry weight and higher carotenoid concentrations.

Table 2. Berry Growth in TF and TN Grapes

sample[a]	altitude (m)	wt/berry (g)	°Brix
TF 29/08	85	2.2	18
	145	2.2	18
	180	1.7	18
TF 12/09	85	2.2	18
	145	2.3	20
	180	1.9	19
TN 29/08	90	1.3	20
	155	1.1	20
	210	1.0	19
TN 12/09	90	1.3	20
	155	1.2	20
	210	1.1	21

[a] TF, Touriga Franca; TN, Touriga Nacional.

For TF, grape growth at lower altitude (TF I_85 m and TF II_85 m) is well grouped, having the lowest carotenoid concentrations. Conversely, for the same variety, grape growth at higher altitude (TF I_145 m, TF II_145 m, TF I_180 m, and TF II_180 m) is, as well, grouped and seems to have higher carotenoid levels. High altitude, which presented a lower temperature and higher humidity during the maturation period, appeared to reflect higher carotenoid values in the TF grape variety (**Figure 6**). This observation was not so evident in TN grapes. The highest carotenoid values are grouped in TN I_210 m and TN II_155 m (**Figure 6**), whereas the other plots have different behaviors.

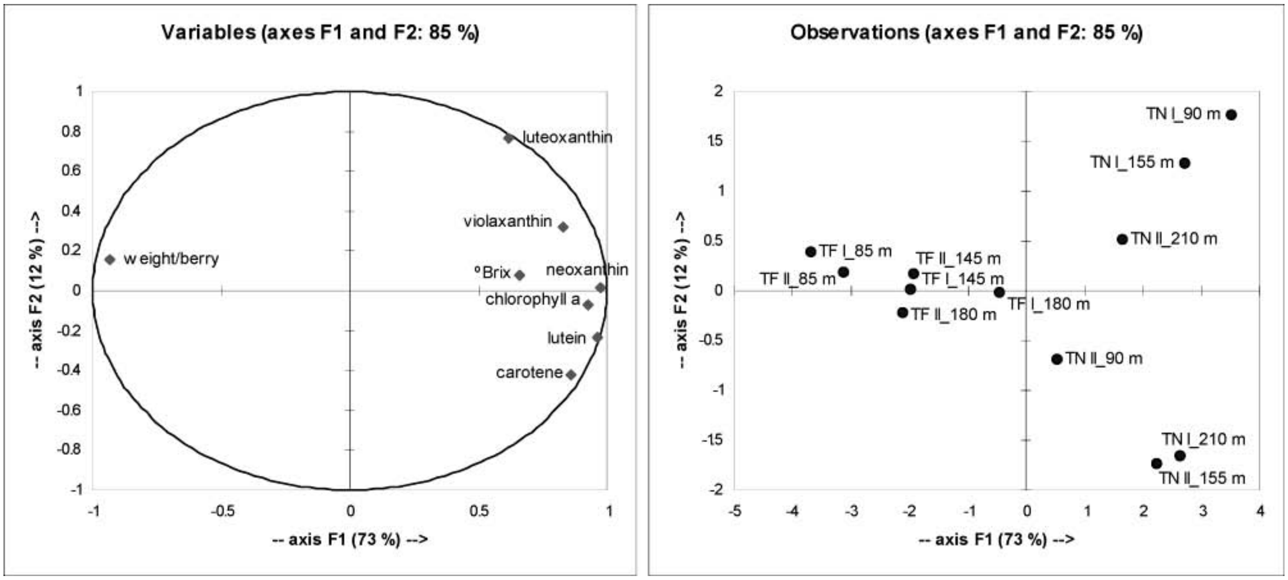

Figure 6. Principal components diagram of the carotenoid contents with different altitudes in the two analyzed cultivars (TF and TN): factor score plot 1–2. Components 1 and 2 account for 85% of the total variance. TF I, TF 29/08; TF II, TF 12/09; TN I, TN 29/08; TN II, TN 12/09.

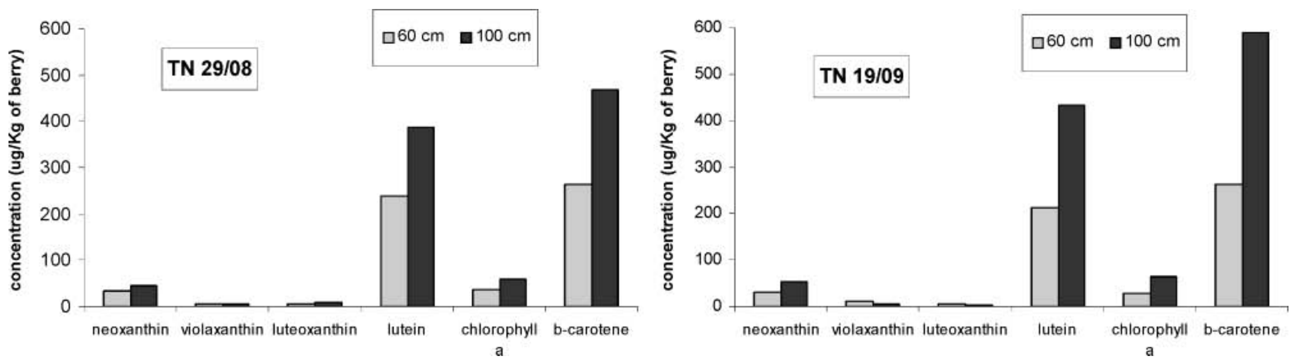

Figure 7. Effect of vegetative height on carotenoid levels in TN grapes.

Table 3. Berry Growth in TN Grapes

sample[a]	vegetative height (cm)	wt/berry (g)	°Brix
TN 29/08	60	1.2	21
	100	1.0	18
TN 19/09	60	1.1	24
	100	0.8	19

[a] TN, Touriga Nacional.

Altitude contributes to a decrease of berry size, but no significant differences were observed in °Brix in cultivars TF and TN (**Table 2**). The lower temperature associated with a lower berry growth may decrease carotenoid degradation during the maturation period and could explain the higher carotenoid values in the high-elevation terrace sites. On the nother hand, carotenoids are proportionally more concentrated in small-berry samples than in big-berry samples as they are present in higher amounts in skin than in pulp (*14*).

Effect of Vegetative Height on Carotenoid Concentration. The effect of vegetative height on carotenoid contents was investigated in Touriga Nacional grapes from the CC Douro subregion for two sample dates, 29/08 and 19/09, harvest date, as shown in **Figure 7**. **Table 3** shows the berry growth in TN grapes by measurement of the berry weight and °Brix. Results showed that grapes grown to higher vegetative height seem to have higher carotenoid levels **(Figure 7)**. Furthermore, grapes grown to lower vegetative height had higher weights and sugar concentrations **(Table 3)**. This could be explained by an effect of canopy density and a consequent bunch exposure to sunlight, for example, grapes grown to lower vegetative height are less protected from sunlight exposure than grapes grown to higher vegetative height and consequently have lower carotenoid concentrations. On the nother hand, the lower berry growth in grapes with higher vegetative height may decrease the carotenoid degradation during the maturation period and could explain the higher carotenoid values in the higher vegetative height.

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