

Assessment of the qualitative and quantitative characteristics of the grapes of grapevine cultivar Fokiano (*Vitis vinifera* L.) in Ikaria Island, under vineyard conditions

Katerina Biniari^{1,*}, Stavroula Nikolaou¹, Ioannis Daskalakis¹, Despoina Bouza¹, and Maritina Stavrakaki¹

¹Laboratory of Viticulture, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

Abstract. This study aimed at assessing the phenolic potential of indigenous Greek red grapevine cultivar Fokiano under different cultivation systems and altitudes, during the cultivation season 2019-2020, which could explore different approaches yielding better results in the same viticultural area, as is Ikaria Island. The samples of the present study were collected from productive (commercial) vineyards in the island of Ikaria, in the region of the North Aegean Sea. Samples of grapes were collected from 7 different vineyards at the northern part of Ikaria with different characteristics: (i) difference in the altitude of the vineyards and (ii) difference in the cultivation system (conventional or organic). The vineyards in question are located at an altitude of 200 m, 400 m, 600 m and 800 m. The samples were collected during the dates of harvest, which were determined according to the technological maturation of the grapes in combination with the biodynamic calendar. In all samples, the mechanical analyses of the grapes and berries took place and the characters of the must as well as the qualitative characters of the berries (must, skins, seeds) were studied. Total soluble solids of the must were calculated using a refractometer, the active acidity (pH) using a pH meter and the total acidity using a sodium hydroxide solution (NaOH). The mechanical analyses that were performed involved the weight of thirty (30) berries, the weight of the grape and the length and width of the berries and the grapes of each sample. The content of grape's skin in total anthocyanins, total phenolics, condensed tannins, total ortho-diphenols, total flavonoids, total flavanols, total flavonols and flavones and their antioxidant capacity with the use of FRAP and DPPH methods were determined using a spectrophotometer. The most important acids found in grapes were identified using High-Performance Liquid Chromatography (HPLC). The measurements in the grape seeds were made on the same compounds as the skins, except for total anthocyanins. The results of the present study showed that the altitude does not seem to have a significant effect on most of the qualitative and quantitative characters of the cultivar in Ikaria, however, in future studies, other factors that affect the qualitative characters of the grapes need to be taken into consideration and further evaluated. The microclimate of the highest altitude (800 m) had a positive effect regarding total phenolics, anthocyanins, tannins, flavonols, o-diphenol content and the antioxidant capacity of the skins according to FRAP method, but with opposite results in the case of the seeds. At the same time, it should be noted that no accurate conclusion can be drawn regarding the cultivation system (conventional or organic), since between the two pairs of conventional and organic vineyards (samples from conventional and organic vineyards at an altitude of 200 m - samples from conventional and organic vineyard at an altitude of 600 m), the measurements exhibit a variation in their results. The antioxidant capacity that was determined in the samples of Fokiano is also remarkable, when also compared with other indigenous red grapevine cultivars. In view of climate change, the exploitation of indigenous varieties under different soil and climatic conditions or even in the same viticultural region, like the one of the current experiment (different cultivation system, different altitude in the island of Ikaria) could unlock and highlight the full potential of such local varieties, depending on the final style of the wine produced.

*Corresponding author: kbiniari@aua.gr

1 Introduction

Throughout the Greek vineyards, there are more than 280 cultivated varieties for the production of wine grapes, table grapes, and raisin grapes. Many of these varieties still remain uncharted in terms of their phenolic potential, as is the case of grape cultivar Fokiano (*Vitis vinifera* L.). Another interesting aspect of Greek grapevine cultivars is their polyclonal nature [1], and as a result, a significant variability is observed within the same variety in terms of the ampelographic, genetic and phenolic characteristics [2,3] thus creating different biotypes of the same cultivar depending on the soil and climate conditions of where they are being cultivated.

The viticultural ‘terroir’ is a concept that refers to a specific sector where the knowledge of interactions between physical and biological environment as well as applied viticultural techniques, providing discrete characteristics to the products originating from a given area. Terroir includes a specific soil, topography, climate, and landmark characteristics, and all these factors interact one with the other [4].

The altitude of an area significantly affects the temperature variation as well as air circulation, particularly at slopes. The altitude has also an effect on the characters of the mesoclimate of an area, mainly in the distribution and frequency of extreme low temperatures and the thermal potential of the climate. It is reported that, depending on the latitude, an increase in altitude by 100 m brings about a decrease in temperature by 0.6-1 °C, which affects the heliothermic conditions in such a way as to cause a delay of 2-3 days in the ripening of the grapes [5].

Although most productive vineyards are located at an altitude between 350 and 650 m from sea level, there are many cases of grapevine cultivation at higher altitudes. In the soil and climate conditions of Greece, semi-mountainous (350-700 m) and mountainous (up to a certain extent) vineyards exhibit many advantages, especially for wine grape cultivars, and can be found up to altitudes of 1000 m [5].

Grape yield is associated with climate, therefore the changes to several climate factors, due to climate change, affects the composition of the grape as well as the organoleptic properties of the wine [6]. The light and temperature conditions that are formed around each vine depend on factors that influence canopy arrangement and management, such as the training system, the winter pruning for fruiting and green pruning, etc. [5].

The training system of the grapevines affects to a large extent the phenolic composition and content of the berries. The management of the annual vegetation, in combination with the different training systems, affects the microclimate of the grape growing zone, mainly because of the way the canopy is exposed to solar radiation [7].

At the same time, another important factor with a significant role in berry development and phenolic composition is temperature, which in combination with grapes exposure to solar radiation, enhances most phenolic compounds composition, including anthocyanins [8,9].

Also, it should be noted that the training system has an effect on the relationship between canopy leaf surface and the grapes, and consequently the distribution of solar radiation, and therefore, it can be concluded that the ripening process of the grapes and the concentration of phenolic compounds can differ between the training systems [10]. At the same time, the temperature of the shoots seems to affect the degree of synthesis of aromatic compounds [11].

Viticulture in Ikaria Island has a long tradition, dating back to the antiquity. Although the morphology of the island is quite difficult for cultivation (rocky landscape with high a large slope, the vines are located in runners in terracing slopes.

The aim of the present study was to investigate the phenolic variability of vines of grape cultivar Fokiano, originating from different vineyards in the island of Ikaria, and under different cultivation systems, training systems and altitudes, ultimately leading to its emergence and further exploitation.

2 Material and methods

2.1 Experimental design

The experiment took place in the cultivation season 2019-2020 on vines of grape cultivar Fokiano (*Vitis vinifera* L.), in commercial vineyards located in the northern part of Ikaria Island, in the eastern Aegean Sea region, in Greece (Fig. 1).



Figure 1. Location of vineyards, Ikaria Island.

The vineyards from the samples were collected as well as their characteristics are shown in Table 1, since the vineyards are characterized by differences in cultivation and training systems, as well as altitudes. In vineyards F1, F2, F4, F5 and F7, the vines are head-trained in the gobelet/bush vine form, with 1-2 node spurs per arm (3-5 arms). In vineyard F3, the vines are cordon-trained with 1-2 node spurs per arm, while in vineyard F6, the vines are own-rooted and trained in runners in terracing slopes, another characteristic of the island. It should be noted that the similar soil and climatic conditions prevail throughout the entire Ikaria Island and the various vineyards.

Table 1. Samples and vineyards' characteristics.

Sample	Vineyard characteristics
F1	Altitude 200 m, conventional vineyard, 20-year-old vines, grafted on R110 rootstock, head-trained
F2	Altitude 400 m, organic vineyard, 40-year-old vines, grafted on R110 rootstock, head-trained
F3	Altitude 600 m, organic vineyard, 10-year-old vines, grafted on R110 rootstock, cordon-trained
F4	Altitude 600 m, organic vineyard, 20-year-old vines, grafted on R110 rootstock, head-trained
F5	Altitude 600 m, conventional vineyard, 50-year-old vines, grafted on R110 rootstock, head-trained
F6	Altitude 800 m, organic vineyard, 100-year-old vines, own-rooted
F7	Altitude 200 m, organic vineyard, 20-year-old vines, grafted on R110 rootstock, head-trained

2.2 Grape sampling

At harvest, grapes were randomly selected from each vineyard. The sampling process, described in a previous study [2] involved the random selection of three (3) grapes from different vines of each vineyard and three (3) sampling processes, whereas each sampling was considered as one (1) replication.

2.3 Polyphenolic analysis

The phenolic potential as well as the antioxidant properties of the samples under study were determined, in terms of the qualitative and quantitative characters of grapes, berries and must, by carrying out the following: (i) mechanical analyses of grapes and berries, (ii) analyses on the must (pH, soluble solids content, total titratable acidity), (iii) determination of total phenols (in skins and seeds), (iv) determination of total anthocyanins (in skins), (v) determination of total flavonoid content (in skins and seeds), (vi) determination of total flavanols (in skins and seeds), (vii) determination of flavone and flavonol content (in skins and seeds), (viii) antioxidant activity with FRAP and DPPH methods (in skins and seeds), (ix) determination of individual organic acids and individual sugars (in must).

All reagents and chemicals as well as all procedures regarding the determination of mechanical analyses of grapes and berries, the analyses on the must, and the preparation of samples for spectrophotometric and HPLC analyses have been performed by following protocols described in a previous study [3].

2.4 Statistical analysis

All statistical analyses and correlations were obtained using the JMP v.10 statistical software (SAS Institute

Inc., Cary, NC, USA). The significance of the results was tested by Analysis of Variance (ANOVA) and the means of the values were compared by Tukey's range test at $P \leq 0.05$. Letters in columns denote statistically differences (Tukey-HSD, $P \leq 0.05$).

3 Results and Discussion

As mentioned above, during the technological maturity of the grapes, measurements related to the qualitative and quantitative characters of the grapes, berries and must were carried out in order to investigate possible differences between the different samples of grape cultivar Fokiano, taken from the selected vineyards of the current experiment.

The results (mean value and standard error) of each parameter measured and for each sample are shown in Tables 2-14. During the statistical analysis, the samples were separated in two categories. The statistically significant differences found between the samples, and for each parameter measured, have been highlighted and flagged with discrete letter. Moreover, a separate statistical analysis was performed between pairs of samples and the statistically significant differences found between these pairs have been highlighted and flagged with discrete capital letter. These pairs are: (i) F1 and F7 (samples from conventional and organic vineyard at an altitude of 200 m), (ii) F4 and F5 (samples from conventional and organic vineyards, head-trained vines, at an altitude of 600 m).

3.1 Mechanical properties of grapes and berries, and characters of the must

Regarding the grape length there was no statistically significant difference recorded between the samples under study (Table 2). Regarding the grape width and grape weight, F3 recorded the highest value with a statistically significant difference.

Table 2. Mechanical properties of the grapes.

Samples	Grape length (cm)	Grape width (cm)	Grape weight (g)
F1	16.03 ± 0.76 aA	8.14 ± 0.78 abA	149.43 ± 26.54 bA
F2	13.60 ± 0.92 a	8.00 ± 0.58 ab	210.90 ± 25.05 ab
F3	16.17 ± 0.59 a	9.92 ± 0.84 a	311.83 ± 47.92 a
F4	13.35 ± 1.02 aA	7.30 ± 0.53 abAB	157.70 ± 20.35 bB
F5	15.43 ± 0.73 aA	7.57 ± 0.57 abB	149.00 ± 16.32 bB
F6	14.17 ± 1.16 a	7.11 ± 0.63 ab	131.11 ± 20.57 b
F7	13.24 ± 0.54 aA	8.17 ± 0.42 abA	229.33 ± 20.05 abA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–b, A–B) are significantly different at significance level $p \leq 0.05$.

Regarding the mechanical properties of the berries, the samples did not exhibit statistically significant differences in terms of berry length and width (Table 3).

Regarding the weight of 30 berries, pairs F1-F7 and F4-F5 do not exhibit a statistically significant difference, although it seems that samples coming from organic vineyards record higher value. Sample F6 (own-rooted) at an altitude of 800 m recorded the lowest value with a statistically significant difference compared to all samples.

It should be noted that a tendency can be observed regarding the weight of berries from samples originating from different altitudes from the organic vineyards, according to which, as the altitude decreases, the weight of berries increases.

Table 3. Mechanical properties of the berries.

Samples	Berry length (cm)	Berry width (cm)	Weight of 30 berries (g)
F1	18.25 ± 1.37 aA	17.47 ± 1.12 aA	105.98 ± 3.32 aA
F2	17.57 ± 1.57 a	16.15 ± 1.34 a	93.08 ± 9.82 ab
F3	17.18 ± 2.09 aA	16.10 ± 1.76 aA	72.59 ± 3.09 bcB
F4	16.46 ± 1.57 aA	16.01 ± 1.32 aA	90.70 ± 4.58 abA
F5	16.56 ± 1.33 aA	16.47 ± 1.31 aA	88.64 ± 1.41 abA
F6	14.63 ± 2.77 a	14.77 ± 2.47 a	58.79 ± 5.65 c
F7	17.97 ± 1.72 aA	17.02 ± 1.48 aA	112.10 ± 1.41 aA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a-c, A-B) are significantly different at significance level $p \leq 0.05$.

There are statistically significant differences between the samples studied regarding both total soluble solids and total titratable acidity (Table 4). A more appropriate comparison should be made between samples coming from the same altitude. Sample F7 exhibited higher total soluble solids content, with a statistically significant difference compared to F1, while regarding the pH, F1 recorded the highest value, with a statistically significant difference compared to F7. No statistically significant differences were found in total titratable acidity.

Regarding samples F4 and F5, F4 exhibited lower total soluble solids, but higher pH and total titratable acidity compared to F5.

Table 4. Characters of the must.

Samples	TSS (Brix ^o)	Total titratable acidity (g L ⁻¹)	pH
F1	23.20 ± 0.00 b B	4.38 ± 0.33 eB	4.36 ± 0.00 aA
F2	22.40 ± 0.12 c	8.63 ± 0.00 b	3.81 ± 0.00 d
F3	20.00 ± 0.12 eC	11.38 ± 0.13 aA	3.70 ± 0.00 fB
F4	21.03 ± 0.12 dB	6.88 ± 0.25 cB	4.15 ± 0.00 bA
F5	23.13 ± 0.07 bA	5.75 ± 0.13 dC	4.01 ± 0.00 cB
F6	20.90 ± 0.06 d	7.63 ± 0.33 bc	3.77 ± 0.01 e
F7	24.67 ± 0.07 aA	3.75 ± 0.00 eB	4.01 ± 0.00 cB

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a-f, A-C) are significantly different at significance level $p \leq 0.05$.

3.2 Polyphenolic compounds

3.2.1 Total phenolics in skins and seeds

The results showed that the highest concentration of skin total phenolics was recorded in sample F6, with a statistically significant difference compared to the other samples (Table 5).

The highest concentration of seeds total phenolics was recorded in sample F1, with a statistically significant difference compared to F7 as well as compared to the other samples. In terms of altitude, no statistically significant difference is observed between samples coming from the organic vineyards F2 (400 m), F4 (600 m), F6 (800 m) and F7 (200 m).

Table 5. Total phenolics in skins and seeds.

Samples	Total phenolics skins (mg gallic acid / g FW)	Total phenolics seeds (mg gallic acid / g FW)
F1	2.63 ± 0.25 bA	72.147 ± 2.555 aA
F2	1.42 ± 0.01 d	48.907 ± 0.376 c
F3	1.63 ± 0.02 cdC	56.957 ± 3.831 bcA
F4	1.45 ± 0.01 dC	48.300 ± 1.692 cB
F5	1.91 ± 0.01 cdA	68.62 ± 2.05 abA
F6	4.48 ± 0.13 a	50.99 ± 0.38 c
F7	2.17 ± 0.13 bcA	54.43 ± 1.49 cB

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a-d, A-C) are significantly different at significance level $p \leq 0.05$.

3.2.2 Total flavanols in skins and seeds

The concentration in seeds total flavanols is generally higher than the one in skins (Table 6). The highest concentration in skin total flavanols was recorded in sample F7, with a statistically significant difference compared to the other samples.

The highest concentration in seed total flavanols was recorded in sample F5, which differed statistically significantly, compared to the other samples.

There is no direct correlation nor conclusion to be reached between the altitude and the total flavanols concentration.

Table 6. Total flavanols in skins and seeds.

Samples	Total flavanols skins (mg catechin / g FW)	Total flavanols seeds (mg catechin / g FW)
F1	4.14 ± 0.16 abcdA	29.56 ± 0.85 bA
F2	3.31 ± 0.07 d	21.19 ± 0.42 cd
F3	3.49 ± 0.12 cdB	29.51 ± 0.69 bA
F4	4.05 ± 0.21 bcdAB	21.74 ± 0.99 cdC
F5	4.26 ± 0.17 abcA	33.88 ± 0.68 aA
F6	4.52 ± 0.13 ab	20.14 ± 0.04 d
F7	4.95 ± 0.29 aAB	24.72 ± 0.36 cB

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–d, A–C) are significantly different at significance level $p \leq 0.05$.

3.2.3 Total flavonoids in skins and seeds

Regarding the skin total flavonoids (Table 7), sample F7 recorded the highest concentration, followed by sample F6, with no statistically significant difference observed. Grape cultivar Fokiano is characterized by significant higher concentration of skin total flavonoid when compared to grape cultivar Korinthiaki Staphis [2] and other red grape cultivars of the Greek vineyard [3,12].

Regarding the seeds total flavonoids, sample F5 scored the highest concentration, with a statistically significant difference compared to the other samples. As in the case of total flavonoids in skins, also in the case of total flavonoids in seeds, grape cultivar Fokiano is characterized by higher concentrations compared to other grape cultivars of the Greek vineyard [3, 12].

Table 7. Total flavonoids in skins and seeds.

Samples	Total flavonoids skins (mg catechin / g FW)	Total flavonoids seeds (mg catechin / g FW)
F1	22.56 ± 0.32 dB	186.48 ± 3.96 bcB

F2	18.96 ± 0.02 e	160.51 ± 0.67 d
F3	22.02 ± 0.61 dB	204.61 ± 1.65 bB
F4	21.91 ± 0.37 dA	164.64 ± 5.36 dC
F5	23.63 ± 0.12 cdA	248.78 ± 8.05 aA
F6	25.09 ± 0.16 bc	173.91 ± 1.95 cd
F7	26.76 ± 0.63 abA	202.84 ± 0.36 bA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–e, A–C) are significantly different at significance level $p \leq 0.05$.

3.2.4 Total flavone and flavonol content in skins and seeds

Regarding the skin total flavone and flavonol content, sample F6 recorded the highest concentration, with a statistically significant difference compared to the other samples (Table 8). The overall skin total flavone and flavonol content of the samples studied seems to be lower when compared to other varieties of the Greek vineyard [3].

On the contrary, the overall seed total flavone and flavonol of the samples studied seem to be higher compared to other varieties of the Greek vineyard [3]. More specifically and for the results of the present study, sample F7 scored the highest concentration, with a statistically significant difference compared to the other samples, followed by sample F5. The lower altitudes seem to favour the composition of total flavonols, since from the samples originating from organic vineyards F7, F2, F4 and F6 in altitudes 200 m, 400 m, 600 m and 800 m, it seems that the concentration of flavonols decreases as the altitude increases.

Table 8. Total flavones and flavonols in skins and seeds.

Samples	Total flavones and flavonols skins (mg rutin / g FW)	Total flavones and flavonols seeds (mg rutin / g FW)
F1	0.97 ± 0.04 deB	0.40 ± 0.03 cB
F2	0.91 ± 0.02 e	0.45 ± 0.02 bc
F3	1.19 ± 0.03 bcA	0.42 ± 0.03 bcB
F4	1.23 ± 0.04 bB	0.38 ± 0.02 cB
F5	1.41 ± 0.04 aA	0.52 ± 0.01 abA
F6	1.56 ± 0.04 a	0.39 ± 0.02 c
F7	1.19 ± 0.01 bcA	0.59 ± 0.01 aA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–e, A–B) are significantly different at significance level $p \leq 0.05$.

3.2.5 Condensed tannins in skins and seeds

Regarding the condensed tannins in skins, sample F6 displayed the highest concentration, with a statistically significant difference (Table 9).

When it comes to the condensed tannins in seeds, the concentrations overall are considerably higher than those in skins, for most of the samples studied. Sample F5 recorded the highest concentration, with a statistically significant difference, compared to the other samples.

Table 9. Condensed tannins in skins and seeds.

Samples	Tannins skins (mg catechin / g FW)	Tannins seeds (mg catechin / g FW)
F1	5.96 ± 0.02 cB	120.14 ± 12.76 bA
F2	4.03 ± 0.23 cd	84.83 ± 1.21 c
F3	2.97 ± 0.11 dC	134.06 ± 3.16 abA
F4	4.44 ± 0.17 cdA	116.49 ± 5.62 bB
F5	4.05 ± 0.24 cdA	146.57 ± 3.26 aA
F6	11.13 ± 0.03 a	15.78 ± 0.44 d
F7	9.82 ± 0.64 bA	37.11 ± 3.60 dC

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–d, A–C) are significantly different at significance level $p \leq 0.05$.

3.2.6 O-diphenol content in skins and seeds

Regarding the o-diphenol content in skins, sample F6 exhibited the highest concentration, with a statistically significant difference compared to the other samples (Table 10), while regarding the o-diphenol content in seeds, it was sample F7 that recorded the highest concentration, with a statistically significant difference compared to the other samples.

The highest concentration in skins o-diphenol content is observed where the highest concentrations of anthocyanins and tannins are observed.

Table 10. O-diphenol content in skins and seeds.

Samples	O-diphenols skins (mg catechin / g FW)	O-diphenols seeds (mg catechin / g FW)
F1	0.53 ± 0.01 abA	1.22 ± 0.03 dB
F2	0.48 ± 0.04 b	1.06 ± 0.01 e
F3	0.54 ± 0.02 abA	1.27 ± 0.04 dC
F4	0.51 ± 0.01 abA	1.28 ± 0.03 dA

F5	0.54 ± 0.03 abA	1.36 ± 0.07 cA
F6	0.61 ± 0.01 a	1.86 ± 0.02 b
F7	0.56 ± 0.02 abA	2.19 ± 0.07 aA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–e, A–C) are significantly different at significance level $p \leq 0.05$.

3.2.7 Antioxidant activity in skins and seeds

Regarding the antioxidant activity, it was determined with the FRAP and DPPH methods. Sample F6 exhibited the highest antioxidant capacity in skins, as measured with FRAP method, with a statistically significant difference compared to the other samples (Table 11). With the same method, the highest concentration of seed antioxidant capacity was recorded in sample F5, also with a statistically significant difference.

When the antioxidant capacity was determined with DPPH method, the highest concentrations in skins and seeds were recorded in samples F6 and F7, respectively, with statistically significant differences compared to the other samples.

The antioxidant capacity in skins using the FRAP method seems to be directly correlated with the concentration of flavonols and tannins, while the antioxidant capacity in seeds seems to be correlated with the one of flavanols.

Table 11. Antioxidant activity in skins and seeds.

Sam ples	FRAP (mg Trolox / g FW)		DPPH (mg Trolox / g FW)	
	Skins	Seeds	Skins	Seeds
F1	20.76 ± 0.85 cdAB	204.82 ± 8.31 bcA	18.16 ± 0.11 aA	37.58 ± 0.62 dB
F2	18.00 ± 0.32 d	170.78 ± 11.22 de	14.51 ± 0.23 d	31.11 ± 1.09 e
F3	24.16 ± 0.42 bA	193.98 ± 6.41 cdB	16.55 ± 0.17 abA	44.64 ± 2.05 cC
F4	21.64 ± 0.76 bcB	183.20 ± 4.19 cdB	16.15 ± 0.24 bcA	38.44 ± 0.17 dC
F5	22.82 ± 0.42 bcAB	238.49 ± 0.87 aA	14.07 ± 0.44 dB	70.81 ± 0.45 abA
F6	38.34 ± 0.55 a	148.84 ± 2.24 e	16.88 ± 0.18 a	67.58 ± 0.79 b
F7	22.59 ± 0.68 bcA	190.28 ± 0.33 cdA	14.91 ± 0.55 cdB	71.19 ± 0.57 aA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–e, A–C) are significantly different at significance level $p \leq 0.05$.

3.3 Total anthocyanins

Regarding total anthocyanin content, sample F6 recorded the highest concentrations in total anthocyanins with

statistically significant differences compared to the other samples (Table 12). It should be noted that this specific sample is own-rooted and comes from a vineyard almost 100 years old, and at an altitude of 800 m. As the altitude increases, so does the accumulation of anthocyanins, and these results are in agreement with previous studies [13,14], according to which the climate conditions that were observed in higher altitudes seem to favor the higher concentration of anthocyanins.

Among the other samples, no statistically significant differences were observed in total anthocyanins. It should be noted that after a preliminary analysis on individual anthocyanins, cyanidin seems to have the higher concentration, which contradicts the notion that the prevailing individual anthocyanin in *vinifera* varieties is malvidin [15-18], but more research is required (data not shown).

Table 12. Total anthocyanins.

Samples	Total anthocyanins (mg malvidin / g FW)
F1	2.104 ± 0.13 bcA
F2	1.69 ± 0.18 bc
F3	2.11 ± 0.07 bcA
F4	2.20 ± 0.09 bcA
F5	2.41 ± 0.17 bA
F6	5.63 ± 0.41 a
F7	2.28 ± 0.11 bcA

Values are the means of triplicates (± SE). Values on the same row carrying a different letter (a–c, A) are significantly different at significance level $p \leq 0.05$.

3.4 Individual sugars and organic acids in must

The results regarding the individual sugars showed that sample F7 and F5 recorded higher concentrations of fructose and glucose compared to F1 and F4, respectively, with a statistically significant difference (Table 13).

When comparing the relationship between individual sugars and altitude, it seems that we move higher in altitude, the concentration of sugars decreases. Sample F6 recorded the lowest value in fructose and glucose concentrations compared to all samples, with a statistically significant difference.

Table 13. Individual sugars in must.

Samples	Fructose (g/L must)	Glucose (g/L must)
F1	139.51 ± 3.70 cdB	154.84 ± 3.94 cdB

F2	149.07 ± 2.502 bc	161.60 ± 2.25 bc
F3	127.41 ± 2.44 dB	135.93 ± 2.58 eB
F4	128.10 ± 1.55 dB	140.51 ± 1.47 deB
F5	144.78 ± 2.46 cA	157.72 ± 2.54 cA
F6	91.77 ± 0.86 e	97.62 ± 0.93 f
F7	161.35 ± 2.63 abA	173.77 ± 3.09 abA

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–f, A–B) are significantly different at significance level $p \leq 0.05$.

The individual organic acid with the higher concentration was tartaric acid, followed by malic acid (Table 14). In the case of tartaric acid, the highest value was recorded in sample F6 (own-rooted, 800 m altitude), with a statistically significant difference, followed by sample F2 (400 m altitude). Samples F1-F7 and F4-F5 did not exhibit any statistically significant differences between them, respectively.

Regarding malic and ascorbic acids, sample F3 recorded the highest concentration, with a statistically significant difference when compared to the other samples.

Table 14. Individual organic acids in must.

Samples	Tartaric acid (µg/ mL must)	Malic acid (µg/ mL must)	Ascorbic acid (µg/ mL must)
F1	31570.41 ± 1072.55 bAB	4718.47 ± 258.29 eB	126.42 ± 8.63 dB
F2	35102.25 ± 1040.126 ab	9141.67 ± 563.52 bc	172.28 ± 7.24 d
F3	29751.99 ± 976.224 bA	12343.59 ± 573.29 aA	993.46 ± 40.01 aA
F4	29521.99 ± 520.97 bA	8948.43 ± 376.04 bcB	446.77 ± 26.53 cB
F5	29993.43 ± 907.25 bA	9644.31 ± 449.092 bB	217.50 ± 16.61 dC
F6	39379.78 ± 372.11 a	7476.546 ± 237.59 cd	164.31 ± 6.35 d
F7	30758.49 ± 291.30 bB	5804.77 ± 219.75 deB	150.61 ± 3.26dAB

Values are the means of triplicates (± SE). Values on the same column carrying a different letter (a–e, A–C) are significantly different at significance level $p \leq 0.05$.

4 Conclusions

Taking into consideration the results of the present study, Fokiano seems to be a promising indigenous grape cultivar with significant phenolic potential, depending on the cultivation technique which will be chosen in relation

to the altitude, such as training system. Evidently, more research is required in order to determine the ideal cultivation techniques, taking into consideration climate and soil conditions, that would result in the production of viticultural products of high quality.

In general, and given climate change, the use of native varieties and their biotypes in diverse cultivation systems will enable them to adapt to different altitudes and soil and climate conditions, and this could prove to be of great interest for vine growers and wine makers.

The authors would like to thank Afianes Wines for allowing the use of their vineyards for this research.

References

1. M. Stavrakaki, K. Biniari. *Sci. Hort.* **209**, 86-95 (2016)
2. M. Stavrakaki, K. Biniari, I. Daskalakis, D. Bouza, *Aust. J. Crop Sci.* **12**, 1927-1936 (2018)
3. K. Biniari, M. Xenaki, I. Daskalakis, D. Rusjan, D. Bouza, M. Stavrakaki. *Food Chem.* **307**, 125518 (2020)
4. C. van Leeuwen, J.P. Roby, L. de Resseguier. *OENO One* **52**(2), 173-178 (2018)
5. M.N. Stavrakakis, *Viticulture* (Embryo Publications, 2019)
6. G. Koufos, T. Mavromatis, S. Koundouras, G.V. Jones. *OENO One* **54**(4), 1201-1219 (2020)
7. R. Mota, D. Amorim, A., Favero, E., Purgatto, M., Regina. *Food Sci. Technol.* **31**, 967-972 (2011)
8. F. Mattivi, R., Guzzon, U., Vrhovsek, M., Stefanini, R., Velasco. *J. Agric. Food Chem.* **54**, 7692-7702 (2006)
9. E. Chorti, S., Guidoni, A., Ferrandino, L., Gangemi, V. Novello. *Quad. Sc. Spec. Vitic. Enol.* **29**, 155-167 (2007)
10. A.G. Reynolds, J., Vanden Heuvel. *Am. J. Enol. Vitic.* **60**(3), 251-268 (2009)
11. J. Bergqvist, N. Dokoozlian, N. Ebisuda. *Am. J. Enol. Vitic.* **52**, 1-7 (2001)
12. K. Biniari, O. Gerogiannis, I. Daskalakis, D. Bouza, D., M. Stavrakaki, *Not. Bot. Horti. Agrobo.* **46**(1) (2018)
13. N. Mateus, J.M. Machado, V.D. Freitas. *J. Agric. Food Chem.* **82**(14), 1689-1695 (2002)
14. T. Yue, M. Chi, C. Song, M. Liu, J. Meng, Z. Zhang, Z., M. Li. *Int. J. Food Prop.* **18**, 1584-1596 (2015)
15. E. Garcia-Beneytez, F.L. Cabello, E. Revilla. *J. Agric. Food Chem.* **51**(19), 5622-5629 (2003)
16. S. Kallithraka, A.A. Mohdaly, D.P. Makris, P. Kefalas. *J. Food Compost. Anal.* **18**(5), 375-386 (2005)
17. A. Teixeira, J. Eiras-Dias, S.D. Castellarin, H. Gerós. *Int. J. Mol. Sci.* **14**(9), 18711-18739 (2013)
18. G. González-Neves, G. Favre, D. Piccardo, G. Gil. *Int. J. Food Sci. Technol.* **51**, 260-267 (2015)