“Developing Identity ON Yield, SOil and Site”
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3.5.2 (a) Grapes Analysis Results

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Introduction

The aim of the study is to evaluate the potential of some indigenous/rare grape varieties of Greece to produce high quality wines. The grapevine varieties (Batiki white, Ligarida, Voulgaroudi, Papadies, Bougialmas, Bogiajides, Karnachalias) were sourced from the region of east Macedonia/ Thrace and more specifically from Soufli region. Grapes and juices were analyzed and then using the grapes of these selected varieties vinifications were carried out under controlled situations (using the same vinifications protocols) and the produced wines were bottled in order to with the analyzes.

Each sample was analyzed twice and the results of the analysis are presented after statistical treatment

Study Area: The Terrain of the East Macedonia/ Thrace region is mostly mountainous, with mountain ranges extended into the sea. The territory is characterized by elongated mountainous ranges, plains to the coasts and large rivers flowing through the area. The climate is different from the rest of Greece, because of the north and northwest winds during winter. Moreover, the temperature goes down low in the mountain regions, the rain level is higher than the rest of the Greek mainland, while the snow is much more and does not melt before mid-spring. In coastal areas, the climate is clearly milder, typically temperate with Mediterranean features, with warm and dry summer and during winter there are several rains.

Plant material: A total of 7 varieties was planted at an equal number of plants from Bellas vineyard (Alania area, Soufli), and in the varietal collection of "Stafilos" at the wider area of Soufli, Greece, reflecting the varietal diversity. It is important to mention that the samples were collected on the basis of the empirical identification. Two bins of grapes were collected and transported to Oenolysis experimental winery, Pallini Athens, in order to make wines separately from each variety (double vinification).

Analytical Methods

Classical analyses (pH, Total Acidity, Brix) were carried according the OIV guidelines methods. At the time of harvest, a sub-sample of 300 berries was randomly selected and the essential analytical berry parameters were determined. Initially for each vineyard sample, berry weight and berry volume of one hundred 100 berries was measured. For berry volume a 1000 ml measuring cylinder was filled with exactly 500 ml of distilled water and one hundred (100) berries. Reading was recorded and the volume of water was subtracted. Berry weight of 100 berries was measured on an electronic scale. Total soluble solids were measured by hydrometry in Baume scale; pH was measured using a HANNA portable pH meter (HI 991003) and titratable acidity (TA) was measured by neutralization with sodium hydroxide, all methods according to OIV guidelines.

Total anthocyanins and phenolics in red grape berries

This method was applied in order to estimate total anthocyanins in red grape berries based on the methods described by Illand et al. (2000) and involves extraction with ethanol of these compounds from a known weight homogenized grape sample. A portion of the ethanol extract is then acidified at low pH and quantification based on the absorbance in visible region of the light spectrum. Malvidine is the major anthocyanin in *Vitis vinifera* grapes but is not the only and the results are expressed in malvidin-equivalents for comparative purposes only. In addition, measurement of the absorbance at 280 nm provides an estimate of the concentration of total phenolics in the solution.

The procedure requires homogenizing of fifty (50) randomly selected berries in a blender (24.000 rpm for 30 s). In 10 ml centrifuge tubes are brought 1 ml of grape extract and 10 ml of 50% Ethanol/H_2O solution. Tubes were covered with aluminum foil and stirred at 240rpm for 1 hour at 20_oC. Samples were then centrifuged (10 min at 4.000 rpm) and 0.5 ml of the extract was brought in glass tube containing 10 ml HCL 1N. Tubes were kept in dark for 3 hours and absorbance at 520 nm (1 mm glass cuvette, halogen lamp) and 280 nm (10mm quartz cuvette, UV light lamp) was recorded.

Anthocyanins mg/berry =

$$\frac{A_{520} \text{ x dilution factor x final extract volume (ml) x berry weight (g) x } 1000}{500 \text{ x } 100 \text{ x homogenate weight (g)}}$$

Anthocyanins mg/ gr of berry = Anthocyanins mg/berry / (weight of 50 berries/ 50)

Total phenolic (au): A_{280} x 100

Amino acid analyses of grape must and wine

The concentration of individual AA was determined by high performance liquid chromatography (HPLC) after derivatization with O-phthalaldialdehyde (OPA) according to the method described by Bena-Tzourou (1999). Initially the frozen grapes (-20^{o}C) were crushed and grape juice was extracted and filtered through No. 4 Whatman paper and then diluted (1:10 v/v) with internal standard solution (Norvaline) at 62.5 mmole/l in 0.1 M HCl. This solution was again filtered through a disposable 0.45-mm filter before it was injected into the chromatographic column. Wine samples were prepared in the same way, but excluding the initial step of filtration. The autosampler was programmed to add 5-mL sodium borate buffer (0.4N, pH 10.4) to 1-ml OPA reagent and 1-ml sample. The reaction mixture was then agitated during 6 cycles (10 sec/cycle) before adding 1-ml FMOC-Cl reagent. The mixture was agitated during 3 cycles and finally injected (injection volume was 8 mL). The chromatographic system was Hewlett Packard 1090 Series II/M AminoQuant liquid chromatograph including: column oven, binary eluent system DR5, autosampler; a programmable Hewlett Packard 1046A fluorescence detector, Hewlett Packard 9153C drive, Hewlett Packard ChemStation 9000/300 and Hewlett Packard Think Jet printer. The chromatographic column was a narrow bore C18 HP AminoAcid Analysis (200 x 2.1 mm), protected by a 15 × 2.1 mm guard column. Eluent A was a 20 mM sodium acetate buffer (pH 7.2 adjusted with acetic acid solution, 2% v/v), containing 0.018% v/v triethylamine. Eluent B was a 20% 100 mM sodium acetate buffer (pH 9.10 adjusted with acetic acid diluted 2% v/v), 40% acetonitrile and 40% methanol. Flow gradient conditions are presented into Table 3.8 (Godel et al., 1991).

Table 1: Flow gradient program of primary amino acid determination by HPLC.

Time (min)	Eluent A%	Eluent B%	Flow rate (ml/min)
0.0	100	0	0,45
17.0	40	60	0,45
18.0	0	100	0,45
18,1	0	100	0,45
18,5	0	100	0,80
23,9	0	100	0,80
24,0	0	100	0,45
25,0	0	100	0,45

Excitation/emission wavelengths were respectively 340/450 nm and the free amino acid quantification was performed by the internal standard method with norvaline (primary amino acid internal standard). Peaks were identified by comparison of retention times with commercial standards (L-Amino acids Kit) purchased from Sigma-Aldrich (Germany) (Figure 3.3).

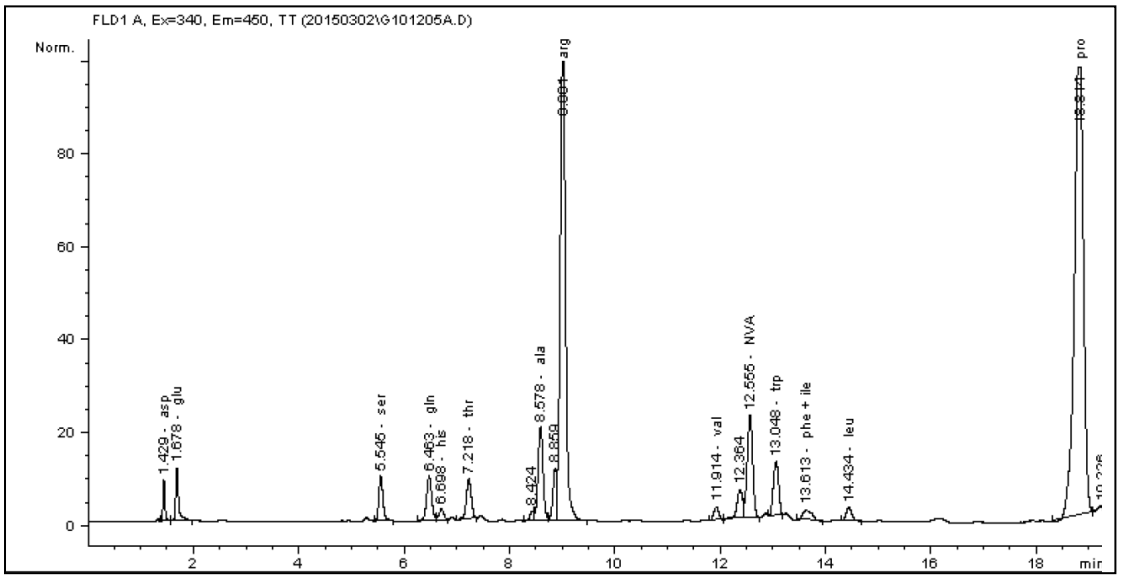

Figure 1. : Chromatogram presenting HPLC analysis results of amino acid analysis.

Free amino acid nitrogen fraction (FAN) or yeast assimilable nitrogen (YAN) was also measured according to the method descripted by Dukes and Butzke (1998). All analyses were performed in duplicate.

Results

Concerning the results found for the grape juices of the varieties shown at table 2 we could highlight the small volume of berries of Ligaridia variety, a result that could be highly interesting. This could show a high potential giving grapes and thus wines rich in anthocyanes and tannins. On the other hand, Ligaridia variety resulted to very low acidities and high pH values which is not positive. In fact concerning the acidities all the red varieties presented low acidities and high pH values indicating the need for different vine cultivation methods. However, the only white variety of the study scored higher acidity values while having relatively high pH value. Papadis and Ligaridia scored the highest Brix levels indicating a potential of producing high alcohol levels.

Table 2. Classical analysis results of the grape juices of Soufli rare varieties

Variety	Berry volume (mL)	Brix	Total Acidity (g/L)	pH
Karnachalas	2,8	21,5	4,1	3,5
Bougialamas	2,4	21,1	3,8	5
Voulgaridia	2,4	20,6	4	3,5
Ligaridia	0,7	22,9	3,7	3,9
Bogiatjides	3,6	21,9	2,8	3,8
Papadis	3,3	23,1	4,1	3,7
White Patiki	3,1	21,5	5,86	3,5

Concerning the Yeast assimilable levels the results are shown at Table 3. Ligaridia and Papadis scored the lowest Inorganic Nitrogen levels showing the nececity of exogenous additions. However Ligaridia presented the highest organic nitrogen levels.

Generally all the samples scored levels lower than the minimum needed for easy going alcoholic fermentation, thus during the fermentations assimilable nitrogen will be added. It is also interesting as all the vines received the same viticultural methodologies and the same fertilization regime, showing probably a different varietal behavior concerning the assimilable nitrogen accumulation in the grapes of each variety.

Table 3. Yeast assimilable nitrogen of the samples of the different Soufli varieties.

Variety	YAN (mg/L)	Amino acid Nitrogen (mg/L)	Inorg. Nitrogen (mg/L)
Karnachalas	157	128	29
Bougialamas	145	130	15
Voulgaridia	88	76	12
Ligaridia	168	164	4
Bogiatjides	154	129	25
Papadis	80	71	9
White Patiki	97	87	10

Extraction from red grapes resulted to a coloured juice and measurements of colour intensity and colour hue was realized to this juice. The extremely high colour intensity firstly of Ligaridia and secondly of Bougialamas was impressive. These grapes will probably result to deep red coloured wines and probably to full body wines. As expected we have not any results for White Patiki as it is a white variety not having any anthocyanes.

Table 4. Colour intensity and Hue of grape juices

Variety	Color Intensity	Hue
Karnachalas	0,35	2,2
Bougialamas	3,14	2,04
Voulgaridia	0,46	1,96
Ligaridia	11,5	1,02
Bogiatjides	0,6	1,52
Papadis	0,39	1,74
White Patiki	-	-

After extracting the juice using a blender the previous results were confirmed, and Ligaridia and Bougialamas grapes presented very high antocyanes levels. Then Papadis and Bogiatzides scored high anthocyanes levels however less than the half concentration of Ligaridia.

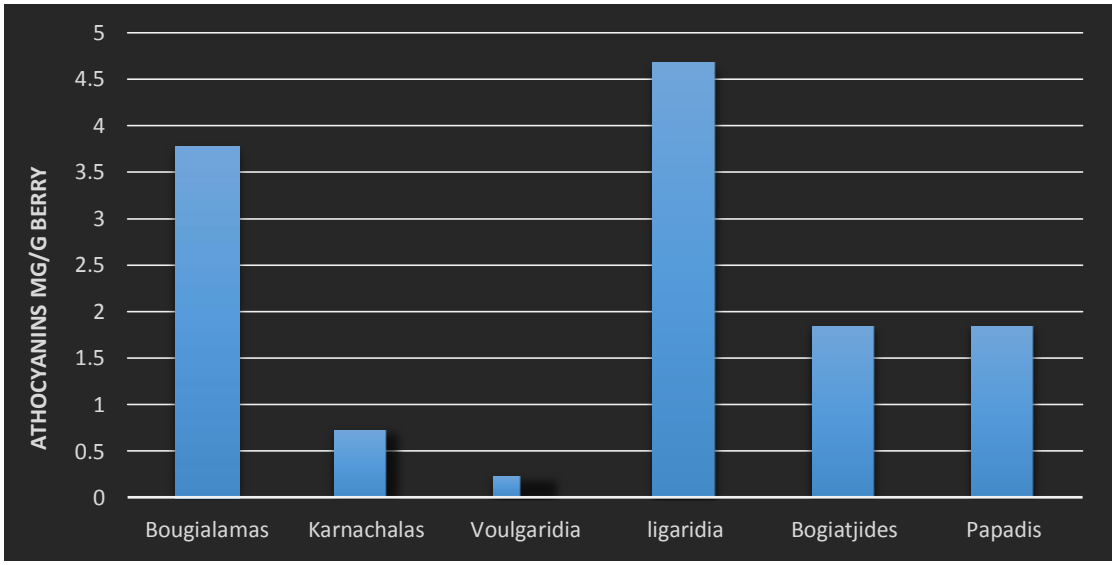

Fig.2. Anthocyanes levels of red grape Soufli rare varieties

The pattern changes when total phenolics were analysed. Bougialams grapes scored higher levels of total phenolics. Besides Karnachalas and Voulgaridia scored relatively high total phenolics, while these verities were the poorest concerning their anthocyanes levels.

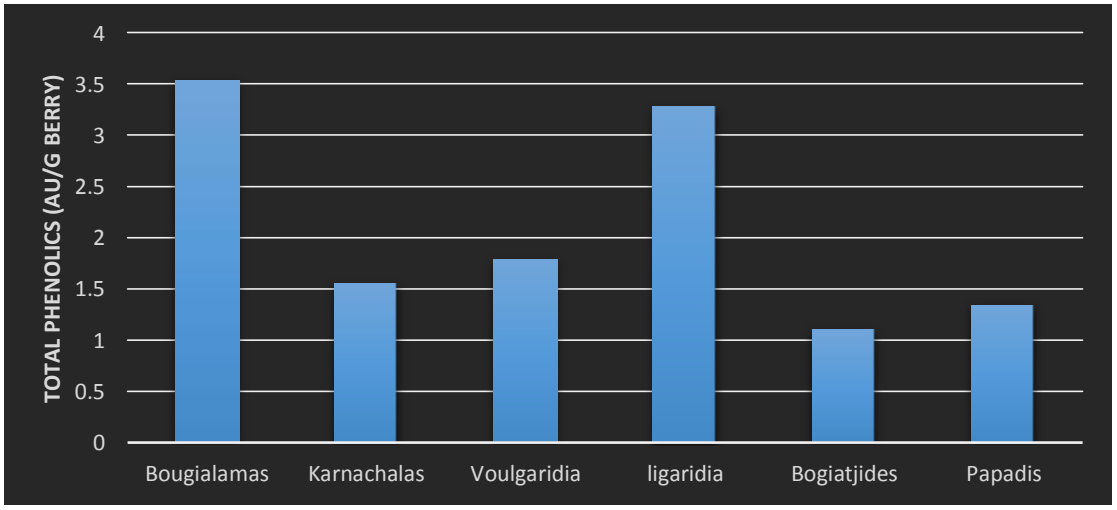

Fig.3. Total phenolics levels of red grape Soufli rare varieties

Conclusions

Concerning the potential of the grapes to produce high quality wines it seems that Bougialamas and Ligaridia could be used for full body red wines. Their high antocyanes levels, higher even than the anthocyanes levels of Cabernet Sauvignon and Merlot grapes as found in the literature could guide us for the ongoing research. The only drawback of Ligaridia could be the low acidities and high pH levels which should be improved by using the adequate cultivation methods or by acitidy adjustments during winemaking or by blending with other varieties. Papadis also showed promising results and further research should be done. These results will be compared to the wine results.

Concerning the only white variety a promising ratio sugar/acidity shows that this variety could produce interesting wines with a promising market career. High malic acid levels versus relatively low tartaric acid levels could explain the relatively high pH levels.