“Developing Identity ON Yield, SOil and Site”
“DIONYSOS”
MIS Code: 5016090

Deliverable: 3.5.2 “Selecting main local wine varieties and identifying their characteristics”

3.5.2 (a) Grapes Analysis Results

Prepared by Oenolysis- Vassiliki Troianou

The Project is co-funded by the European Regional Development Fund and by national funds of the countries participating in the Interreg V-A “Greece-Bulgaria 2014-2020” Cooperation Programme.

Contents

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).

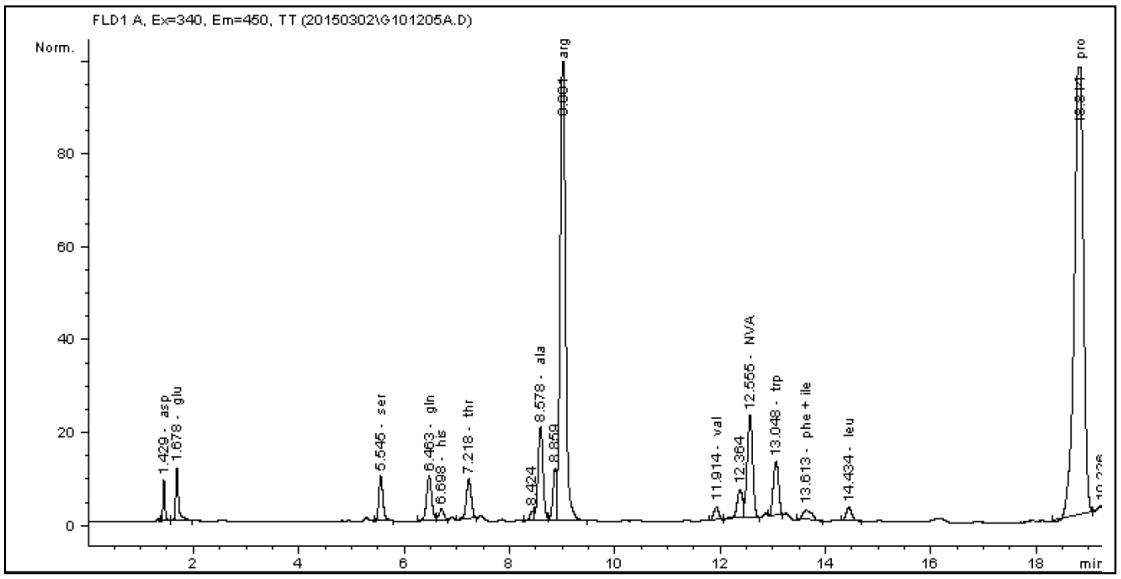


Figure 1. : Chromatogram presenting HPLC analysis results of amino acid analysis.

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.

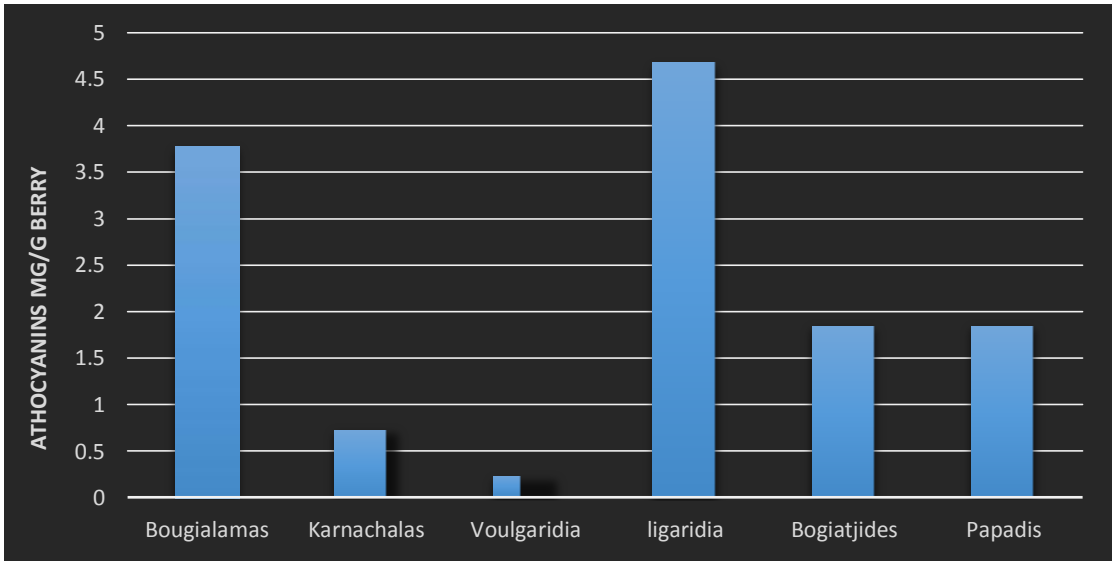


Fig.2. Anthocyanes levels of red grape Soufli rare varieties

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.

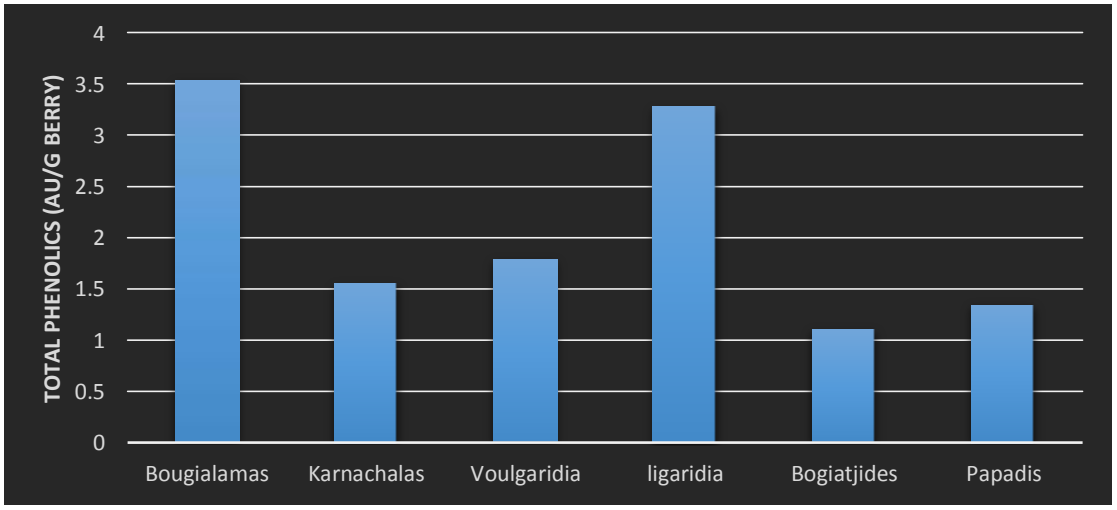


Fig.3. Total phenolics levels of red grape Soufli rare varieties

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.

ΟΜΟΣΠΟΝΔΙΑ ΕΠΑΓΓΕΛΜΑΤΙΚΩΝ ΒΙΟΤΕΧΝΙΚΩΝ & ΕΜΠΟΡΙΚΩΝ ΣΩΜΑΤΕΙΩΝ ΕΒΡΟΥ

FEDERATION OF PROFESSIONAL, CRAFTS AND TRADE ASSOCIATIONS OF EVROS, GREECE

Interreg Greece-Bulgaria DIONYSOS European Regional Development Fund

WP3

The Wine ID of the Regional Area

Deliverable: D3.5.2

Selecting main local wine varieties and identifying their characteristics

Description: "Winemaking procedures of the rare varieties from Soufli"

- **Federation of Professional Crafts and Trade Associations of Evros**
- **InnoVino**

Project entitled: "Developing Identity ON Yield, SOil and Site"

Acronym: 'DIONYSOS' / MIS CODE: 5016090

Project co-funded by European Union, European Regional Development Funds (E.R.D.F.) and by National Funds of Greece and Bulgaria

Content

1. Project ‘DIONYSOS’

Co-funded Projects enable EU to demonstrate in a practical way how the EU impacts on the everyday lives of its citizens. The Project entitled "Developing Identity ON Yield SOil and Site" (MIS CODE: 5016090) using as acronym the name of the ancient God of Wine: “DIONYSOS”, is funded by the Cooperation Program INTERREG V-A “GREECE-BULGARIA 2014-2020” (co-funded by the ERDF and national funds of the participating countries)by 735.778,70 € (ERDF: 625.411,90 € and National Funds: 110.366,81 €). The project officially started on November 1st, 2017 and is expected to end by October the 31st, 2019.

DIONYSOS Project is implemented under the Priority axis [2] - A Sustainable and Climate adaptable Cross-Border area, under the Investment priority: [6c] - Conserving, protecting, promoting and developing natural and cultural heritage and the Intervention fields: [091]: Development and promotion of the tourism potential of natural areas and [094]: Protection, development and promotion of public cultural and heritage assets. It was submitted under the 2nd Call for proposals under priority axes 2 & 4.

The Project’s main objective is to preserve the local biodiversity (more specifically the indigenous wine varieties) and at the same time to valorize the area's aspect as the oldest wine-producing area in Europe for the development of oenotourism. The Partnership of the Project consists of the following beneficiaries:

1. LB: Agricultural University of Athens, Department of Food Science & Human Nutrition/Special Account for Research Funds

2. Institute of Viticulture and Enology

3. University of Plovdiv "Paisii Hilendarski" , Faculty of Economics and Social Sciences

4. Kavala Development Agency - Local Authorities Enterprise

5. Federation of Professional, Crafts and Trade Associations of Evros

6. Agency for Transnational Training and Development

7. Municipality of Kirkovo

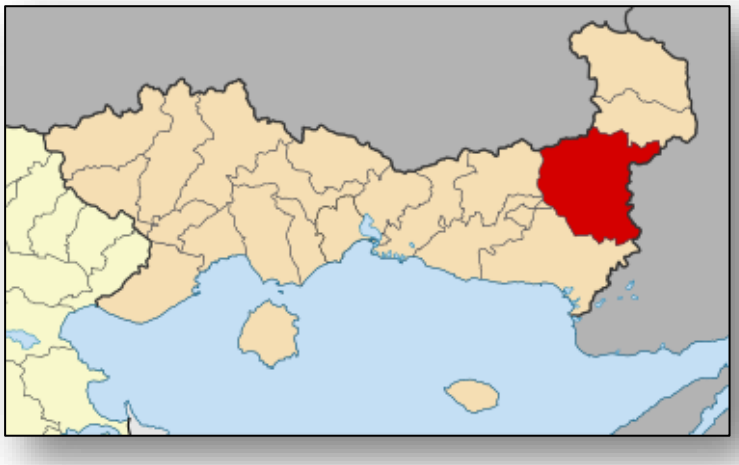
8. Haskovo Chamber of Commerce and Industry

Target area of the project is the wine-producing areas of East Macedonia and Thrace in Greece and of Haskovo-Kardjali areas respectively in Bulgaria which directly falls intothe Thracian Valley, historically connected with the Dionysos Cult.Given that, DIONYSOS project aims at valorizing the specific aspect as a natural and cultural asset for developing oenotourism, so as to increasing area's attractiveness while enhancing preservation of the local landscape and biodiversity.As such, the project enforces the cultivation of the old local vine varieties, highlights the local productive identity and applies integrated approaches to promoting the area as an oenotourism destination by redesigning the current conventional vinicultural and wine producing model by enhancing cultivation of the old authentic grapes as the area's differentiating element and its comparative advantage.

The local terroir, the wider micro flora and fauna, the main factor providing the special characteristics of topology and origin on the organoleptic and the other significant parameters of wine, is emphasized. Scientific researches in both countries, under a common methodology, are carried out for the identification-phylogenetic analysis of local varieties, for the isolation and identification of yeasts. A digital map for the area's vineyard, a plan for wine roads, placement of identification signposts with QR Codes, pilot interventions to improving the aesthetic image of settlements, an internet platform for promoting the vineyard are implemented to raising area's local identity. Awareness raising actions contribute for local communities to become conscious of their local natural asset along with the area's oenotourism value, while other actions support its placement on the market. Pilot actions are planned in the wider areas of Paggaion (Kavala), Soufli (Evros), Kirkovo and Haskovo.

2. Introduction

Grapes of seven varieties all planted in Soufli region were harvested at technological maturity and then the Vinification process is described below.



3. **White varieties**: White Patiki

The grapes from the white variety Patiki, was hand harvested during early hours and they were then transferred to the experimental winery of the Oenolysis Athens firstly in the fridge overnight. In addition, 200 berries were frozen at -20° C for further analyses. Next morning, they were destemmed, crashed and pressed. Potassium metabisulfite (7g/hL) was added to the juice and two hours later pectolytic enzymes (3 g/hl) were added as well. The juice was then chilled down to 10° C and 24 hours later it was racked to 5L capacity plastic bottles. At this stage, the sugar content (Brix) of the juice was determined and also classical analyses were performed (pH and total acidity) and YAN (OIV), results were shown at Deliverable 1 . The must was then inoculated with yeast strain UCLM 325 and the alcoholic fermentation started. The next day the must was supplemented with 20g/hl springferm as a nitrogen supplement.. Brix measurements were performed daily.

When the alcoholic fermentation was successfully completed, the wine was racked, chilled to 4° C and 8g/hL of Potassium Metabisulfite was added. The wines were bottled and kept to a constant temperature of 10° C. Then the wine was stabilized for

proteins and tartrates . The wines were analysed one month later (results in Deliverable 2).

4. **Red Varieties:** Karnachalas, Bougialamas, Voulgaridia, Ligaridia, Bogiatzides, Papadies

The grapes from each red variety were hand harvested during early hours and they were then transferred to the experimental winery Oenolysis Pallini, Athens and placed in the fridge overnight in separate bins. In addition, 200 berries were frozen at -20^{o} C for further analyses (Iland, *et al*., 2004), results presented in Deliverable 1. The following morning, they were destemmed, crashed and cold pre-fermentation maceration for 24hours was performed at 5^{o} C. An addition of Potassium metabisulfite (7g/hL) was done. At this stage, the sugar content (Brix) was determined and classical analyses were performed (pH and total acidity) as also chromatic characteristics and YAN analysis was done (OIV). They were then inoculated with yeast strain UCLM 377 (20 g/hl) and the alcoholic fermentation was initiated a few hours later. Three times a day the cap was punched down in order to submerge the skins and extract anthocyanes and tannins. Additions of yeast nutrients (Springferm, 20 g/hl) was also made 24 hours after inoculation. Brix measurement was performed daily. Six days after the end of alcoholic fermentation and when the color intensity was stabilized, juice/wine was racked and the skins were separated from and then pressed. After the completion of the alcoholic fermentation, the malolactic fermentation occurred by inoculating with CH16 lactic bacteria strain and as soon as it was completed, the wines were racked and treated with 8 g/hL of Potassium Metabisulfite. The wines were kept at room temperature, then cold stabilized, filtered and then bottled.

Classical analyses (Brix, pH, Total Acidity, Volatile Acidity, % Alcoholic Content, Reducing Sugars), the determination of Malic acid Total YAN and the chromatic characteristics (according the OIV guidelines) were carried upon the completion of the alcoholic and malolactic fermentation.

Figure 1 : Baskets of Bougialamas grapevine berries

Figure 2: Destemming berries by hand

Figure 3: Crushing process of Bougialamas grape berries

Figure 4: Pressing process of the skins after separating the skins from the must by the end of alcoholic fermentation

5. Fermentation Kinetics

All the fermentations were achieved by the 10th day however for Voulgaridi, Ligaridia, Bougialamas the fermentation kinetics were slower and fermentations were completed by the 14th day. By the of fermentation the reducing sugars for all the other varieties it was less than 1 g/L. In any case all the fermentations were achieved and the wines produced are characterized as dry which is important for the sensory evaluation which will be the next step.

Table 1. Fermentation Kinetics of all the 7 musts of the study.

Dates		Day 0	Day 1	Day 2	Day 3	Day 5 -	Day 6	Day 7 -	Day 8-	Day 9	Day 10	Day12	Day 14	
No.	Codes	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	P 20°c	G/F
1	KARNACHALAS	1.0923	1.0907	1.0849	1.0785	1.0404	1.0220	1.0155	1.0049	0.9956	0.9921	0.9921	0.9921	0.14
1'	KARNACHALAS B	1.0923	1.0907	1.0825	1.0729	1.0351	1.0198	1.0133	1.0035	0.9962	0.9927	0.9927	0.9927	0.51
2	MPOUGIALAMAS	1.0915	1.0874	1.0858	1.0793	1.0456	1.0337	1.0183	1.0063	0.9993	0.9965	0.9945	0.9945	0.25
2'	MPOUGIALAMAS B	1.0915	1.0874	1.0833	1.0729	1.0396	1.0292	1.0119	1.0028	0.9996	0.9968	0.9948	0.9948	0.14
3	VOULGARIDIA	1.0890	1.0874	1.0874	1.0866	1.0697	1.0610	1.0456	1.0322	1.0123	1.0053	0.9955	0.9923	0.05
3'	VOULGARIDIA B	1.0899	1.0874	1.0862	1.0841	1.0713	1.0602	1.0411	1.0292	1.0124	1.0044	0.9958	0.9924	0.08
4	LIGARIDIA	1.0990	1.0932	1.0866	1.0858	1.0761	1.0657	1.0479	1.0300	1.0154	1.0084	0.9975	0.9954	0,02
4'	LIGARIDIA B	1.0990	1.0932	1.0858	1.0817	1.0673	1.0587	1.0419	1.0270	1.0157	1.0077	0.9979	0.9957	0,03
5	BOJIATJIDES	1.0948	1.0957	1.0833	1.0744	1.0426	1.0205	1.0126	1.0028	0.9951	0.9921	0.9921	0.9921	0.11
5'	BOJIATJIDES B	1.0948	1.0907	1.0809	1.0673	1.0337	1.0176	1.0119	1.0021	0.9952	0.9922	0.9922	0.9922	0.15
6	PAPADIS	1.0990	1.0890	1.0874	1.0866	1.0689	1.0571	1.0411	1.0183	0.9921	0.9913	0.9913	0.9913	0.08
6'	PAPADIS B	1.0990	1.0899	1.0866	1.0866	1.0641	1.0525	1.0374	1.0162	0.9923	0.9915	0.9915	0.9915	0.09
7	White PATIKI	1.0907	1.0866	1.0793	1.0618	1.0278	1.0126	1.0042	1.0000	0.9919	0.9911	0.9911	0.9911	0.09
7'	White PATIKI B	1.0907	1.0874	1.0721	1.0571	1.0198	1.0070	1.0007	0.9987	0.9923	0.9915	0.9915	0.9915	0.17

6. Genetic Analysis

PCR results

Implantation control of the inoculated yeasts UCLM 325 and UCLM 377 was applied. It was found by using PCR analysis that the cells of these yeasts dominated in the fermentation, as we found around 90% of cells among the colonies tested at 2/3 of fermentation.

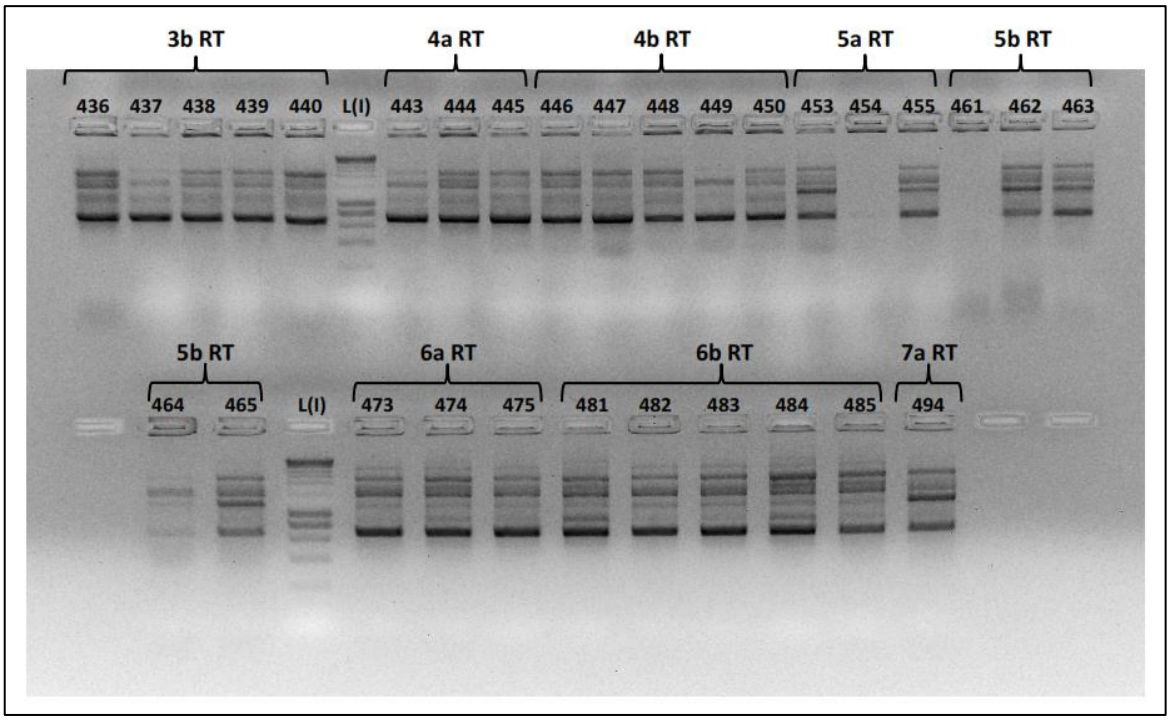


Figure1. Example of PCR analysis 6a and 6b 473-485 represents fermentations that achieved by uclmS377.

7. Conclusion

Winemaking of rare indigenous varieties from Soufli was realized during this study. Classical winemaking steps were applied in order to produce white and red wines. Fermentation kinetics were normal while a slower fermentation rate was observed for Voulgaridi, Ligaridia and Bougialamas. Implantations control at 2/3 of the fermentation showed that all fermentations were achieved by the selected yeast. By the end, all the wines seemed to be of a good quality and the following step of the study will be the chemical and sensory evaluation of the wines.

Interreg
Greece-Bulgaria
DIONYSOS
European Regional Development Fund

EUROPEAN UNION

Ο.Ε.Β.Ε.Σ ΕΒΡΟΥ

ΟΜΟΣΠΟΝΔΙΑ ΕΠΑΓΓΕΛΜΑΤΙΚΩΝ
ΒΙΟΤΕΧΝΙΚΩΝ & ΕΜΠΟΡΙΚΩΝ
ΣΩΜΑΤΕΙΩΝ ΕΒΡΟΥ

FEDERATION OF PROFESSIONAL,
CRAFTS AND TRADE ASSOCIATIONS
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INNOVINO

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Deliverable: 3.5.2 “Selecting main local wine varieties and identifying their characteristics”

3.5.2 (c) Results of the produced wines originating from the Rare Varieties from Soufli

Prepared by Oenolysis- Vassiliki Troianou

The Project is co-funded by the European Regional Development Fund and by national funds of the countries participating in the Interreg V-A “Greece-Bulgaria 2014-2020” Cooperation Programme.

Grapes of seven varieties, all planted in Soufli region, were harvested at technological maturity and then the vinification process adopted was described in 3.5.2 b. The wines were stabilized, treated with sulfites, filtered, and analyzed two months after bottling. The names of the grapes were the following: Red grapes – Ligaridia, Voulgaroudi, Papadies, Bougialamas, Bogiatides, Karnachalas, White grapes – White Mpatiki. Besides the red varietal wines, one wine produced as a blend of all the grapes, called Dionysus was analyzed during these experimentations.

Must and Wine Classical Analyses

Main compositional parameters of grape juice and wines, such as reducing sugars, titratable acidity, volatile acidity, alcohol and sulfur dioxide, were determined by the official methods of analysis (Compendium of international methods of wine and must analysis, OIV 2019).

Measurements of wine phenolics compounds

One or more aromatic rings with one or more hydroxyl groups are contained by phenolic compounds. Two main groups are generally distinguished in Flavonoids, compounds that contain a common C6−C3−C6 structure, and to the non-flavonoids compounds. Within the flavonoid group three main families, namely, flavonols, flavan-3-ols, and anthocyanins are found. On the other hand, in the family of non-flavonoid representative compounds are the hydroxycinnamic acids and their tartaric acid esters, whereas stilbenes are also important due to their potential health benefits. Additional information on grape and winephenolic compounds could be found in a number of relevant research reviews (Castelarin et al., 2012; Cheynier et al., 2010; Cheynier et al., 2006). The parameters analyzed during these experimentations were the following:

Total Phenolics Index.

The measurement of phenolic compounds using UV spectrophotometry was first proposed in the late 1950s by Ribereau-Gayon (Flanzy and Poux, 1958). The absorbance at 280 nm was proposed as the best predictor for the phenolic content in wines because of the characteristic sharp absorbance peak at this wavelength. One ml of wine was diluted to 100 ml and then measured at 280 nm by the spectrophotometer.

Folin−Ciocalteu index

The Folin−Ciocalteu index (FCI) relies on the redox reaction of phenolic compounds with a mixture of phosphotungstic (H3PW12O40) and phosphomolybdic (H3PMO12O40) acids in an alkaline medium to create a blue-colored complex that can be quantified at 750 nm (Singleton and Rossi, 1965).

Wine Color, Color Density

Phenolic compounds such as anthocyanins are responsible for the red wine color. During winemaking and aging the expression of color due to the interactions and reactions of anthocyanins with other phenolic compounds.

According to Sudraud (Sudraud, 1958) the wine absorbances at 420 and 520 nm, corresponds to the yellow and red colorations, in order describe wine color (color intensity or color density). The ratio between these two colorations (A420 nm/A520 nm) corresponded to the tonality or hue, which gives an estimation of the color change toward the orange tones observed in wines during aging. The A620 nm, which accounts for the wine blue coloration, was included (Glories, 2016). The color intensity gives thus an estimation of the total color of a sample (Ribereau-Gayon et al., 2006).

Total Anthocyanins

The method for the determination of total anthocyanins was performed according Ribereau-Gayon and Stonestreet (1965) and is named Bisulfite Bleaching Method. This method provides a measurement of the total red pigments of a wine sample.

Results

The results recorded based on the analysis of the wines are presented in Table 1. The basic wine parameters studied were the following: density (P), alcohol (Alc), residual sugars, total acidity (Tot. Acid.), pH, Malic acid, Lactic acid and Acetic acid.

Table 1: Basic wine parameters of red wines

Codes	P (20°C)	Alc (20°C)	Residual Sugars (g/L Gluc-Fruc)	Tot.Acid. (tartaric acid g/L)	pH	Malic Acid (g/L)	Lactic acid (g/L)	Acetic acid (g/L)
Dionysos	0,9917	12,2	0,04	5,20	3,61	0,00	0,84	0,51
Ligaridia	0,9957	12,7	0,00	5,06	3,78	0,00	1,01	0,32
Voulgaroudi	0,9921	12,0	0,08	6,45	3,20	1,35	0,00	0,21
Papadies	0,9913	13,4	0,09	6,34	3,36	1,49	0,00	0,41
Bougialamas	0,9948	12,0	0,00	5,14	3,81	0,00	0,95	0,73
Bogiatzides	0,9921	12,4	0,10	5,10	3,53	1,48	0,02	0,21
Karnahalas	0,9927	11,8	0,00	5,44	3,45	0,00	0,87	0,32

According to the Table 1 it seems that all the wines from the under extinction varieties presented acceptable alcohol levels, close to the commercial wines levels. Karnachalas recorded the lowest value (11,8% alc) while Papadies the highest (13,4% alc). All the other wines presented alcohol levels between these values. In all the cases the wines have completed the alcoholic fermentation as the residual sugars were found to be close to zero. Moreover, the total acidities and pH values were found to be, as expected, different from one variety to another. The lowest Total Acidity level was found for Ligaridia wines. The Total acidities of the other wines was close the level found for Ligaridia in exception to the levels found for Voulgaroudi and Papadies, for which the values was rather high. Consequently their pH values were the lowest, 3,20 and 3,36 respectively. These levels are explained by the non accomplishment of the malolactic fermentation, as both of the wines recorded levels of malic acid 1,35 and 1,49 g/L respectively. Another wine, have not completed the malolactic fermentation, as a level of 1,48 g/L of malic acid was found. This result was found for Bogiatzides variety. Bougialamas presented higher levels of acetic acid, 0.71 and 0.73 g/L of acetic acid respectively, however well below the upper limit of volatile acidity - 1.2 g/L of acetic acid. All the other wines recorded very low levels for this parameter, a result which is rather positive for the organoleptic quality of the wines especially when produced adopting microvinification conditions (working with small volumes of grapes thus more exposed to oxidations).

Color indexes as evaluated by Sommers methodology.

The color of red wines is determined by the concentration of monomeric anthocyanins and polymers pigments. In young wines the color is mainly determined by the monomeric anthocyanins, which decrease during ripening and form the more stable polymers pigments. Since conventional color measurements are largely influenced by changes in pH and SO_2 concentration, Sommer's methodology was used in this study, in order to obtain more objective and comparable measurements.

Color indexes as calculated by applying Sommers methodology are shown in Table 1. The chemical age expresses the wine's ability to mature and is correlated with the transformation of monomeric anthocyanins into polymers. The results found for these parameters were more or less similar for all the varieties.

Table 2: Color indicators by Sommers

Codes	Chemical age 1	Chemical age 2	Ionization degree of anthocyanins (%)	Total anthocyanins (mg/L)	Intensity	Intensity absence SO2	Hue	Total phenolics	colorized compounds presence SO2
Dionysos	0,48	0,11	25	163	3,7	3,7	0,785	27,4	1,66
Ligarida	0,46	0,06	18	1428	19,3	19,4	1,083	89,9	4,31
Voulgaroudi	0,56	0,16	15	35	1	1,1	1,149	27,4	0,28
Papadies	0,4	0,14	26	138	1	4,1	0,705	21,5	0,98
Bougialamas	0,4	0,14	22	544	13,8	15,3	0,614	71,6	3,84
Bogiatzides	0,41	0,12	23	180	4,7	4,7	0,694	21,8	1,12
Karnachalas	0,51	0,22	29	46	1,8	1,9	0,842	18,1	0,51

The ionization degree of the anthocyanins was high for Karnachalas (29%) and low for Voulgaroudi. The amount of anthocyanins was found to be extremely high for Ligaridia, 1428 mg/L which is even higher than the values found for the most deep colored international red varieties such as Shiraz and Cabernet Sauvignon. The second variety recording high anthocyanins levels was Bougialamas, achieving values close to Cabernet Sauvignon and Merlot wines. Bogiatzides recorded relatively high levels also close to Agiorgitiko variety the main Greek red variety. The lowest levels were found for Voulgaroudi and Karnachalas. More specifically, for each parameter analyzed the following results were found :

Intensity of color -Intensity of color in absence of SO_2

The intensity of colour is considered to be sum of the absorbance at 420 (yellow colour), 520 nm (red colour) and at 620 (blue colour). The Intensity of color-absence SO2 is the intensity that the wine would have if it did not contain any colorless anthocyanin-SO2 complexes. It is a more objective measurement because it eliminates the possible color differences that the samples may have due to different concentrations SO2 or a different proportion of it in its various forms. As expected Ligaridia and Bougialamas scored the highest levels, 19,4 and 15,3 respectively.

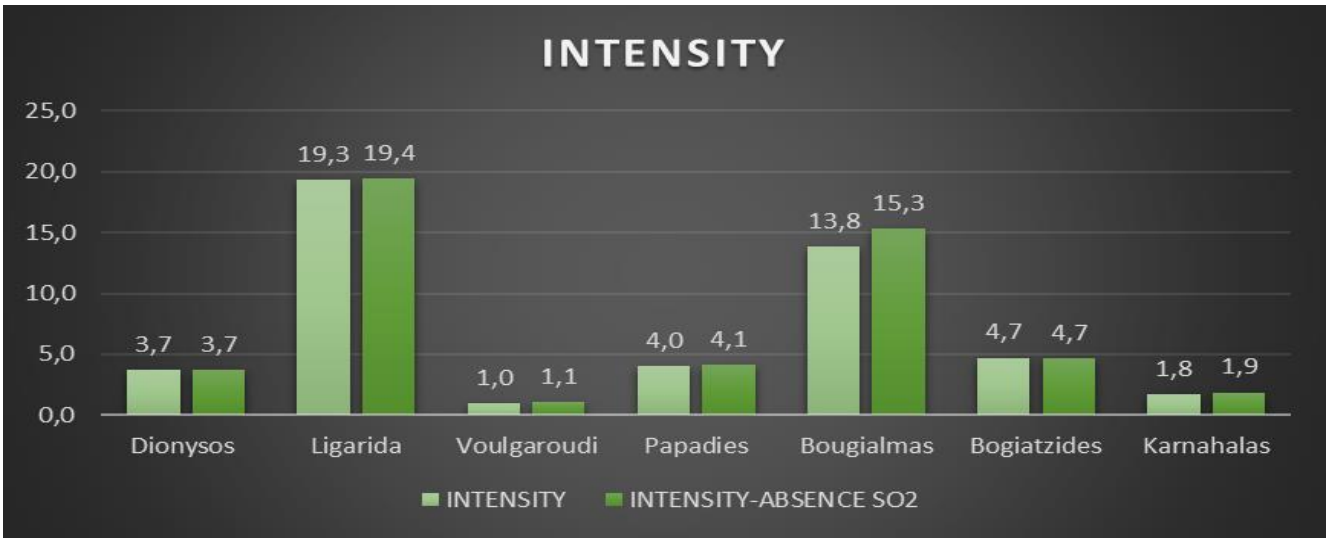

Figure 1: Intensity of color & Intensity of color-absence SO_2

It is clear, as shown in Figure 1, that there are no significant differences between the two intensities. Only in the case of Bougialamas, the intensity-absence SO2 has increased by 1.5 points than the intensity index. The values found for Ligaridia are extremely high, higher than the most known Cabernet sauvignon and Shiraz wines from all over the world. Inversely the values found for intensity index are very low for Voulgaroudi and for Karnachalas. Bogiatzides recorded values quite high however much less than Bougialamas. These findings, besides the anthocyanins levels could show the destination of each variety and for example Voulgaroudi variety could be interesting for rose wines but not interesting for producing red wines.

Hue

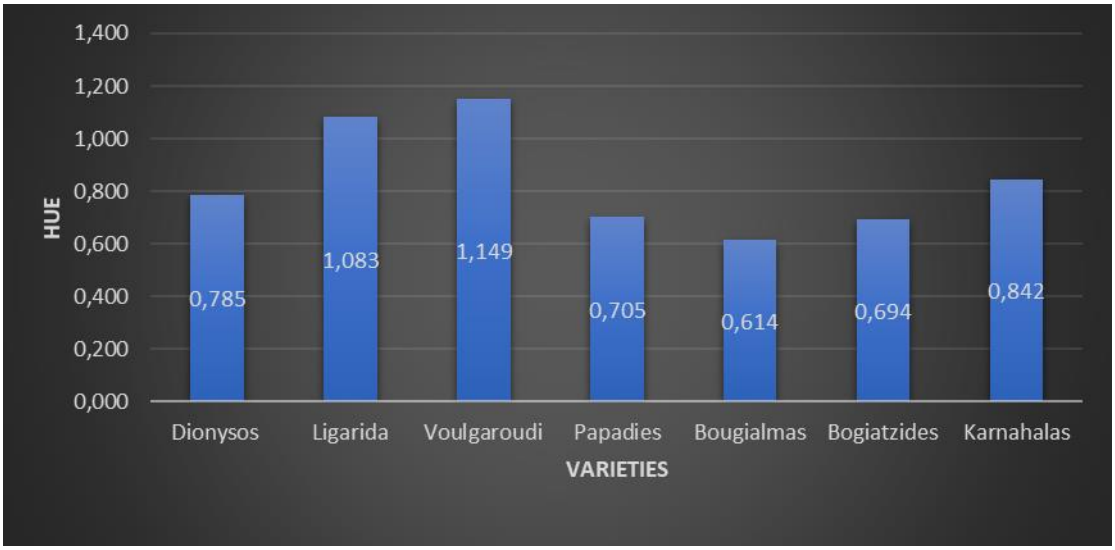

Figure 2: Hue values found for the wines

According to the **Figure 2 ,** Voulgaroudi and Ligaridia recorded the highest hue values, the same was found for Papadies, Dionysos and Karnachalas wines presenting also high hue values, while Bougialmas and Bogiatzides recorded mid-range values. It is rather strange that Ligaridia presented high hue values, as it was the wine presenting the highest Intensity value. Apparently this variety scored also high values at 420 nm as it seems to be a variety with high tannin levels and it is known that tannins absorb at 420nm. In any case presenting such high levels as that found for the intensity the colour of this wine seems to be dark red.

Total anthocyanins

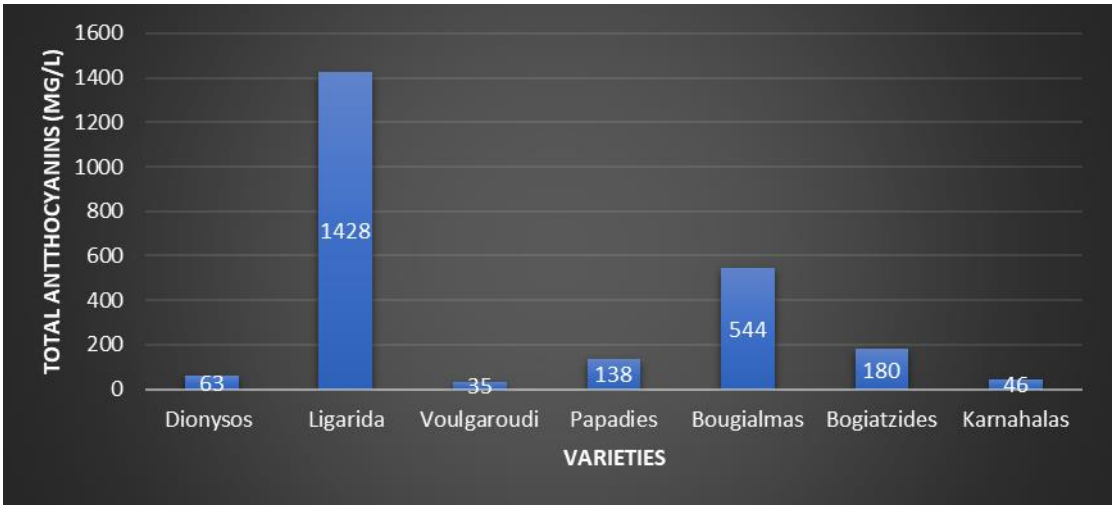

Figure 3: Total anthocyanins levels recorded for the wines

Figure 3 shows better what previously was mentioned, that Ligaridia presented the highest levels of total anthocyanins, followed by Bougialmas however the difference is huge, almost three times less than that found for Ligaridia.

Next variety recording relatively high levels of anthocyanins was found to be Bogiatzides and Papadies. Dionysos, Karnachalas and Voulgaroudi have recorded the lowest levels of anthocyanins. As mentioned previously these wines would be destinated better to produce rose wines than red wines.

2.5 Colorized Compounds presence SO_2

Colorized Compounds in the presence of SO_2 are referred to as polymerized anthocyanin complexes, which are formed during maturation. These polymers are not bound by SO_2 and are more stable than the corresponding monomers. The color of aged wines is mainly determined by these compounds.

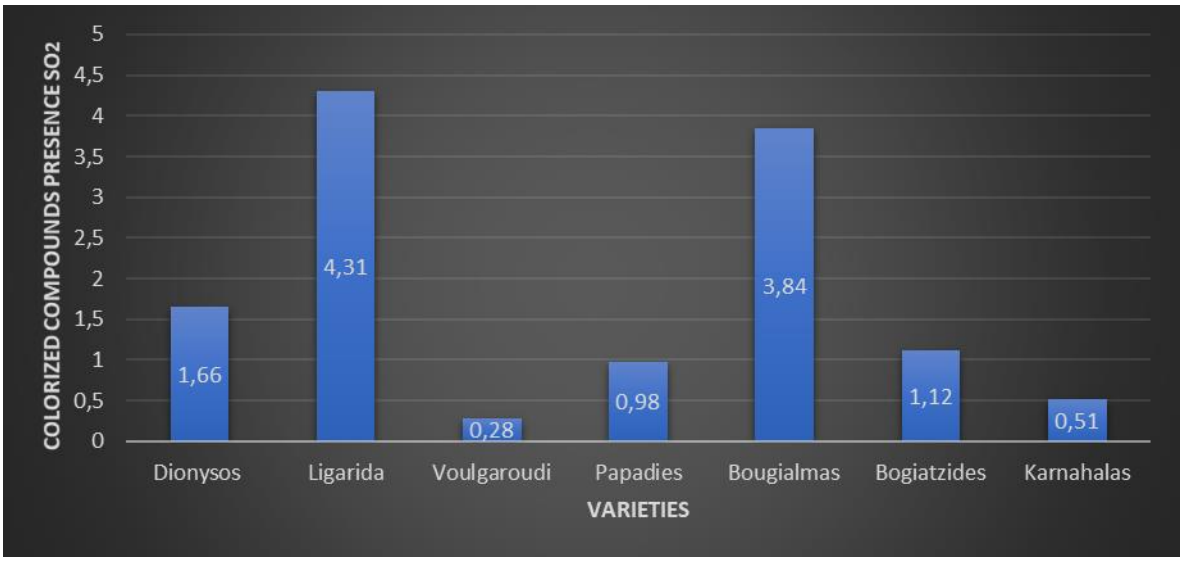

Figure 5: Colorized Compounds presence SO_2

In figure 5, the values vary. For Ligarida was found that the wines of this variety presented the most coloured compounds in the presence of SO_2 (4,31). Next one was found to be Bougialmas wine (3,84) and the last one was Voulgaroudi.

2.6 Total phenolics

In Fig. 6 the levels of phenolic indexes of the under extinction varieties are presented.

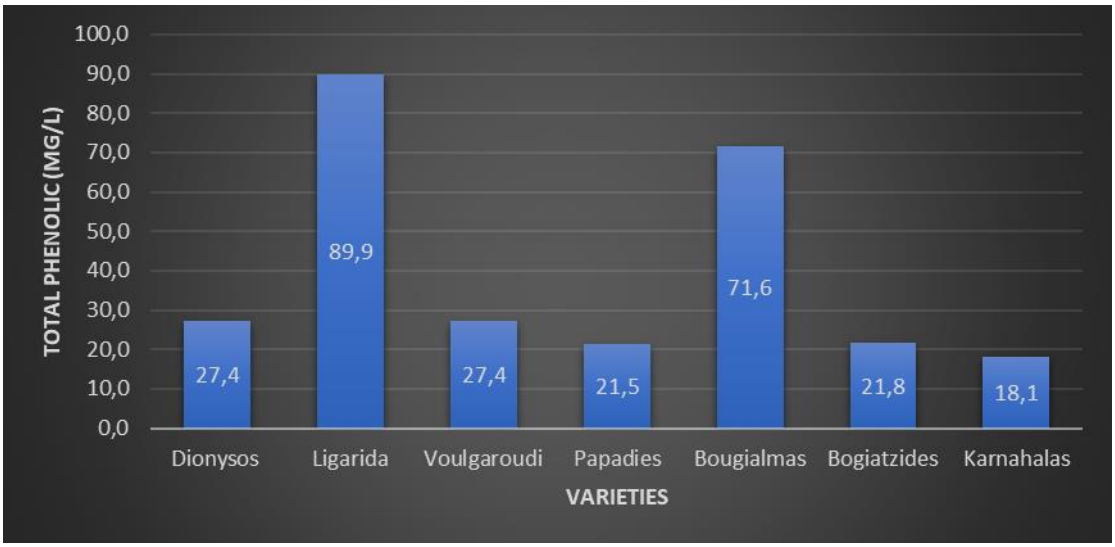

Figure 6: Total phenolic compounds of the wines of the rare varieties (mg/l)

As shown in figure 6, Ligaridia wine scored very high levels of phenolics with an index found to be at 89,9 which is one of the highest values that someone could find in the bibliography. Also, Bougialamas

recorded relatively high total phenolic (71,6) levels, while all the other varieties presented lower phenolic levels. The lowest values were found for Karnachalas variety.

White Mpatiki.

Besides all the red grape varieties, one white variety was also analyzed. The name of this variety is white Mpatiki or Batiki.

Table 3: Basic wine parameters of white wine, variety Mpatiki

p(20°C)	Alc	Res. Sugars (g/L)	Tot. Acid.(H2T g/L)	pH	Total Phenolics
0,9989	12.2	1	5,85	3,15	7,76

As shown in Table 3, this variety produced a wine with commercially welcome values for the main parameters, such as alcohol, acidity and pH. Mpatiki recorded an alcohol value of 12.2 v/v % which is the most common for white varieties and it is important that there is not any need to proceed with any adjustment as it is often the case with many other varieties. But the most important finding was the acidity and the pH values found for this white wine. A value of around 3,15 for pH is highly desired for white wines, just to mention that Asyrtiko made its reputation due to its pH values being around 3.1-3.2. Besides, acidity levels were found to be at around 6 g/L of tartaric acid, and this is a nice passport for exports. Furthermore, the browning tests showed a remarkable resistance to oxidation presenting a k value of 0,014 and absorbance at 420 nm lower than 0,160 after 10 days of treating thermally the wine. This practically means that this variety produces wines with an ageing potential which is rather rare for white varieties.

Conclusion

According to the results found during this study some of the under extinction varieties should be treated carefully and exploited better and further. Ligaridia and Mpougialamas appeared to be varieties with an excellent enological potential thus merit to be planted in the following years and wineries use them for commercial winemaking. Their character is for producing high quality red wines

with ageing potential. These findings will be compared with the results found during the sensory evaluation of the wines. On the other hand, white Mpatiki gave excellent results for producing a wine with high acidity and ageing potential. These three varieties could be of a great potential for the region of Thrace and could be the passport for new markets and exports. Viticulture knowledge and adapted winemaking practices will lead to an international style wines.

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MIS Code: 5016090

Deliverable: 3.5.2 “Selecting main local wine varieties and identifying their characteristics”

3.5.2 (d) Sensory evaluation and flavor profiling of the wines of the Rare Varieties from Soufli

Prepared by Oenolysis- Vassiliki Troianou

The Project is co-funded by the European Regional Development Fund and by national funds of the countries participating in the Interreg V-A “Greece-Bulgaria 2014-2020” Cooperation Programme.

Introduction

The domesticated grapevine (*Vitis vinifera*) is one of the classic fruits of the Old World, providing fresh berries, dried raisins, and juice for wine fermentation. A large body of historical evidence for wide viticulture practice are found in the wider area of Thrace and Macedonia. This testifies to the extent and importance of wine in the diet, religion and culture of the specific area. The wine have gained prominence in recent decades as result of a shift from bulk production towards ultra-premium quality table wines. Grapevines are cultivated in diverse geographical areas of that region due to its high yields and adaptability of the climatic conditions.

Over the last few decades the introduction and spread of world renowned varieties has caused a massive loss of indigenous grapevine varieties traditionally grown in various grape-growing regions. Minority grape varieties are cultivated in the wider area of Thrace, which is characterized by its special climatological conditions (warm summers, cold winters and and low rainfalls) that could influence on the aroma composition.

Wine is a complex matrix containing volatile and non-volatile components that may interact with each other and these interactions can affect the perception of aromas, taste and mouthfeel. Sensory characteristics can be very different in wines. In this context, the importance of studying sensorial characteristics of wines is highlighted. The scientific literature on the sensory aspects of the wines produced from indigenous grapevine varieties is limited to a few Greek Master thesis, and in some instances old references that do not cover modern-day wine. The current study is the first one evaluationg wines produced from indigenous grapevine varieties from the wider area of Soufli by characterizing the flavor profile of them with the assistance of trained panel of assessors.

Grapes of seven varieties, all planted in Soufli region, were harvested at technological maturity and then the vinification process adopted was described in 3.5.2 b. Chemical analysis of the wines are presented in 3.5.2 c. The wines were evaluated sensorially four months after bottling. The names of the grapes were the following : Red grapes – Ligaridia, Voulgaroudi, Papadies, Bougialamas, Bogiatides, Karnachalas, White grapes – White Mpatiki.

Material and Methods

Quantitative descriptive sensory analysis of wines

Quantitative descriptive sensory analysis was performed by a panel of 13 trained panelists, highly experienced in wine sensory analysis.

Figure 1: Sensory booth

Panelists

13 panelists (6 female and 7male; mean age: 35 years (age range: 21 to 43 years), enologist and staff of Oenolysis & Innovino, participated in the study. They had been recruited according to their interest and availability to participate in the study. They were habitual consumers of white and red wines of Greek varieties. All of them had passed through a series of screening tests to ensure their ability of taking part in wine sensory assessments.

Training process

In total, 11 training sessions of 45 minutes took place over a period of two months. Panelists were instructed to avoid eating, drinking, and smoking 1 hour prior to the sessions. Furthermore, they were asked to avoid the use of perfumes or perfumed cosmetics. Panelists attended these sessions over a period of 2

months. Training included smelling of odor reference standards and odor description of wines.

In particular, during the first 3 sessions panelists smelled aroma reference standards from an aroma box in order to get familiarized with odors that can be found in wine. Furthermore, in the first sessions they described 2 wines in each session using the wine aroma wheel of Noble et al. 1987. During the following sessions, a predetermined descriptor list was given to the panelists. This list was compiled after searching the literature for the varieties of this study. A common list of 15 descriptors for all varieties was compiled using the mentioned sources. Furthermore, from the 4th session onwards, different reference standards were presented to the panelists to familiarize them with the vocabulary. In some cases, different recipes for the same attribute were presented in order to conclude to the most appropriate for the panel. Regarding the first sessions, the first part included reference smelling and the second part included description of two wines. At the end of each session, results were discussed and the most cited attributes for each wine were highlighted by the panel leader. In session 12, panelists evaluated 5 wines in booths using Compusense Cloud (Compusense, Guelph, Canada) in order to familiarize them with the evaluation procedure. In the following sessions, panelists smelled reference standards during the first part, while during the second part they described 2 to 3 wines similar to those of the study using up to five descriptors.

Wine Evaluation

The evaluation of the wine samples (7 wines in two replicates, i.e. 14 samples in total) was divided in 2 sessions of 7 samples over a period of two weeks. Wine bottles were opened 30 minutes prior to the test session and were verified free of cork taint by the panel leader. 30 mL were poured into transparent INAO wine glasses covered by plastic Petri dishes in order to allow volatiles move to the headspace. Wine samples were stored, prior to sensory evaluation at room temperature and for the white wine at 4^{o}C. Wines were served in standard wine-tasting glasses at room temperature (20 °C / 8°C for the white) under white light. Tasters were seated in separate purpose-made booths, and the environment was free of interference in terms of noise, visual stimulation and ambient odor (Figure 1). A 10-minute break was mandatory after the first 4 samples in each session. Between samples a 1-minute break was enforced. Samples were presented with 3-digit blinding codes in a monadic sequence according to a Latin Square Design. Data was collected using Compusense Cloud, Academic Consortium (Compusense, Guelph, Canada).

Results

Wines made by Bougialamas, Ligaridia and Karnachalas, recorded high intensities for odours such as red fruits. The highest values were obtained for Karnachalas and Ligaridia varieties. Furthermore, wines of Bougialamas and Ligaridia were characterized by higher expression of spicy flavours. Moreover, Bougialamas and Ligaridia wines recorded the highest values for colour intensity as it was found during the chemical analysis of the corresponding wines. On the other hand, Voulgaroudi variety recorded high values for hue index showing a tendency to colour oxidation (Figure 2). Ligaridia wine was also found to be the wine with the highest aromatic intensity. Generally, the wine of this variety was evaluated as high at most of the categories. It was also evaluated as the best concerning the taste balance while it was not characterized as astringent.

What concerns the white variety, Mpatiki provided a wine which is characterized by a strong aroma of fruits and flowers and with a decreased herbal aroma and an important acidity (Figure 3).

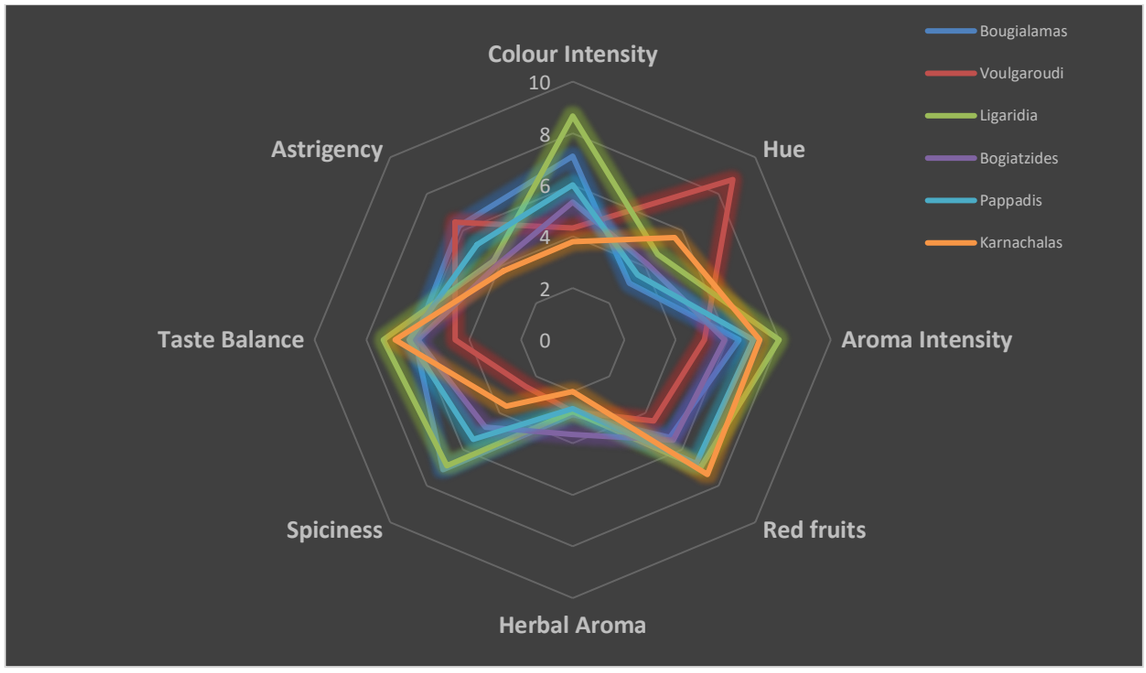

Figure 2: Sensory analysis of the wines of the under extinction red grapevine varieties

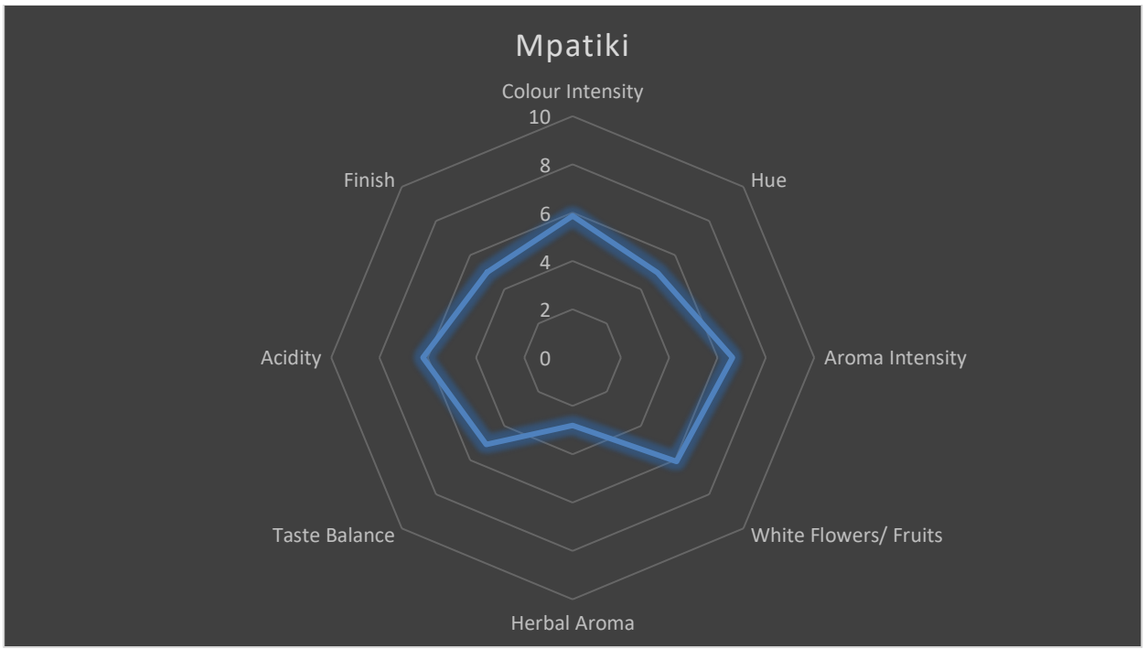

Figure 3: Sensory analysis of the unique white grapevine variety of the experimentation Mpatiki

Conclusions

In recent years, consumers have been showing high interest in various characteristics and quality of wine. Indeed, in addition to the nutritional facts, the choice of modern consumers is continuously influenced by having more knowledge about the environment, culture, history and peculiarity of the territory in which the wine is produced. Especially in the case of wine, consumers take into great consideration geographical origins, grape varieties, vintages and enological practices. As a result, it is well-known that the European Community has adopted specific regulations in order to protect the origin and typicity of local foods.

For the first time, a study of sensory properties was conducted for wines produced from rare and indigenous grapevine varieties from the wider area of Soufli/ Thrace.

Wines produced from six red Bougialamas, Karnachalas, Ligaridia, Pappadis, Bogiatjides, Voulgaroudi and one white, Mpatiki, indigenous grapevine varieties took place at the Laboratory of Oenolysis. Grapes from Soufli originating from these varieties were harvested and transferred to Oenolysis winemaking ateliers for making wines. The wines were treated, bottled, stored and then analyzed chemically and sensorially. The examined wines presented high richness concerning their aromatic intensity and were characterized by red fruits, spicy flavours and floral descriptors. According to the sensory test Bougialamas and Ligaridia were confirmed as promising varieties since these varieties produce

wines with high color intensity and high aromatic intensity, characterized by red fruits and spicy flavours. Besides, Karnachalas provided wines with strong aroma of red fruits. On the other hand, the white variety Mpatiki resulted to wines with strong aroma of white flowers and fruits, and with high acidity values which is very important for a white variety.

These characteristics are corelated with consumer's expectations for wines. Indeed, based on the results, these wines could be totally compared to other most popular high-quality red and white wines produced in other famous Greek important viticultural regions.

Certainly, these findings will be useful to improve viticultural and enological practices and enhance the potential peculiarities of the wines of the rare varieties produced in the area of Thrace.

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