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Study of the chemical composition, the antioxidant activity and the organoleptic profile of Bulgarian wines from hybrid grape varieties

Проучване на химичния състав, антиоксидантната активност и органолептичния профил на български вина от хибридни сортове

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ABSTRACT

The chemical composition, the antioxidant activity and the organoleptic profile of Bulgarian wines from the hybrid varieties Misket Kaylashki, Rubin, Kaylashki Rubin and Trapezitsa was studied. The properties of the red wines were compared to those of Cabernet Sauvignon wine. The varieties were grown in the region of Pleven, Central Northern Bulgaria. The results had shown that the wine composition was mainly determined by the variety and its peculiarities. Misket Kaylashki white wine had the lowest rate of sugar-free extract and total acidity. From the red wines, Rubin contained the highest sugar-free extract and glycerol, Kaylashki Rubin had the lowest concentration of glycerol and the highest total acidity, Trapezitsa had the lowest sugar-free extract. In the red wines, the rate of total phenolic compounds and anthocyanins was highest in Rubin and the lowest in Trapezitsa. Misket Kaylashki, as a typical muscat wine, had the highest rates of esters. From the red wines Rubin contained the highest rate of total esters and aldehydes and the lowest higher alcohols. According to the ABTS and MRAP methods, Misket Kaylashki had the lowest antioxidant capacity, while from the red samples Rubin was reported to have the highest and Kaylashki Rubin the lowest.

Keywords: antioxidant activity, chemical composition, hybrid varieties, organoleptic profile, wine

АБСТРАКТ

Проучен е химичният състав, антиоскидантната активност и органолептичният профил на български вина от хибридните сортове Мискет Кайлъшки, Рубин, Кайлъшки Рубин и Трапезица. Характеристиките на червените вина са сравнени с тези на вино Каберне совиньон. Сортовете са отглеждани в района на гр. Плевен, Централна Северна България. Резултатите показват, че съставът на вината се обуславя от сорта и неговите особености. Бялото вино Мискет Кайлъшки има най-нисък беззахарен екстракт и обща киселинност. От червените вина Рубин съдържа най-много беззахарен екстракт и глицерол, Кайлъшки рубин е с най-ниска концентрация на глицерол и най-висока обща киселинност, Трапезица е с най-нисък беззахарен екстракт. При червените вина количеството на общи фенолни съединения и антоциани е най-голямо в пробата Рубин и най-малко в Трапезица. Мискет Кайлъшки, като типично мискетово вино е с най-висока концентрация на естери. От червените вина Рубин съдържа най-много общи естери и алдехиди и най-малко висши алкохоли. Според методите ABTS и MRAP, Мискет Кайлъшки е с най-ниска антиоксидантна способност, докато при червените проби най-висока е отчетена при Рубин, а най-ниска при Кайлъшки рубин.

Ключови думи: антиоксидантна активност, химичен състав, хибридни сортове, органолептичен профил, вино

INTRODUCTION

In modern vine and wine growing, the variety is one of the most important factors determining the quality of grapes and wine. In recent years, the increasing consumer demands have imposed new and higher criteria on the variety and its derivatives. New varieties with the desirable features, traits and qualities of the products are obtained through hybridization and clonal selection (Ivanov and Nakov, 2002).

Grapes and wine have a complex chemical composition made up of a number of volatile and non-volatile components as their rates depending on the degree of maturity, the soils and the climate in the region of cultivation, the agricultural practices in the vineyards, etc. (Fang and Qian, 2004). The varietal stock imposed in the modern Bulgarian vine-growing corresponds in full with the ecological and agro-technical conditions available in the country. Under the proper technological practices, the varietal nature of wine is directly dependent on the specific features of the different varieties.

The content and the ratio of the various components of the wine composition have an impact on its organoleptic profile, aromatic and taste qualities. Sugars (carbohydrates) are mainly represented by the monosaccharides glucose and fructose, disaccharide sucrose and minimal rates of rhamnose, arabinose, xylose and pentosans. In the grape juice, glucose accounts for almost 50% of the total amount of sugars and easily ferments. Fructose ferments harder. Sucrose can not ferment before its decomposition by the enzyme invertase synthesized by the yeasts (Yankov, 1992).

During the alcoholic fermentation, yeasts form a significant amount of glycerol from sugars, which affects the wine taste. Its content in wine is determined by a number of factors such as the composition of the medium (fermentable sugars, ammonium salts), sulphitation, yeast type and strain, amount of cultured media, aeration, fermentation temperature (Belenik, 1999; Minarik, 2002).

The titratable acidity is an indicator varying within wide ranges for the separate varieties. From the organic acids, the tartaric and malic acids are prevailing, which in a certain ratio with the other components form the flavour. After the malolactic fermentation, the malic acid decreases merely to traces and wine acquires a mild and pleasant taste. Small amounts of citric, succinic, glycolic, oxalic and other acids are also found (Dimov and Getov, 2003).

The aromatic wine composition is a combination of grape varietal aromas and those formed in the winemaking process. Their composition and quantity depend on the variety. Some varieties have a strong muscat aroma, mainly due to the terpenes (Blagoeva et al., 2016). Most of the esters, aldehydes, and higher alcohols in the wine are synthesized during alcoholic and malolactic fermentation as a product of the yeast metabolism and bacteria. In maturation and aging of wines as a result of the process of esterification and hydrolysis processes, some aromatic components are transformed and new ones are formed (Fischer, 2007; Callejon et al., 2012).

The phenolic compounds also significantly affect the organoleptic features of red wines. Their amount in grapes is specific for the variety. They are found mostly in the pulp, and the anthocyanins - in the skin of these varieties. Their rate in wine depends on the grapes (variety, area of cultivation) and the technology used (the conditions of the alcoholic fermentation and maceration) (Getov, 2002; Stoyanov et al., 2004). Their ability to bind with free radicals in the body determines their antioxidant properties. The antioxidant activity of the different wines is closely related to the total and individual content of the phenolic compounds (Burns et al., 2003). In addition, a number of other compounds exhibiting antioxidant properties are also present in wine (Kerchev et al., 2005). Gugulyas et al. (2002a, 2002b) have investigate the antioxidant strength of standard antioxidants (Vitamin C, Trolox) and found that they are weaker compared to the wine polyphenols (quercetin, tannin, gallic and caffeic acid, catechin, ferulic acid). Their study has also shown that the Bulgarian red wines have lower antioxidant activity compared to the Greek ones, while for the white wines it is just opposite. The antioxidants rate is one of the most important factors determining the healthy food and drinks (Joubert and Beer, 2006; Polovka, 2006).

The objective of the study was to determine and compare the chemical composition, the antioxidant activity and the organoleptic profile of five Bulgarian wines produced from the hybrid varieties Misket Kaylashki, Rubin, Kaylashki Rubin, Trapezitsa and from the cosmopolitan variety Cabernet Sauvignon.

MATERIAL AND METHODS

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria and at the Slovak University of Agriculture (SUA) - Nitra, Slovakia. The study was focused on wines, vintage 2015, made from the hybrid varieties Misket Kaylashki, Rubin, Kaylashki Rubin, Trapezitsa and Cabernet Sauvignon - variety grown in all viticultural regions in the world. The hybrid varieties were selected at IVE – Pleven through intraspecific and interspecific hybridization and distinguished for their increased resistance to diseases and low winter temperatures.

Misket Kaylashki is white late-ripening variety, obtained through interspecific hybridization by crossing of Misket Hamburgski x Vilar blanc. Grapes are characterized by high sugar accumulation and titratable acidity. The wines have a gold-yellowish colour, fine and lasting muskat aroma and fresh, harmonious taste (Ivanov et. al., 2010).

Rubin is red middle-ripening variety, obtained through intraspecific hybridization by crossing of Nebiolo x Shiraz. Grapes are distinguished by high sugar accumulation and low titratable acidity. The wines are dark coloured, with high extract, suitable for aging (Radulov et al., 1992; Simeonov et al., 2009).

Kaylashki Rubin – red, middle-ripening variety, obtained through interspecific hybridization by crossing of (Pamid x Hybrid VI 2/15) x (Gamay Noir x *Vitis amurensis*). It has good sugar accumulation. Wine is ruby-red in colour with a high content of anthocyanins, pleasant fruity flavour, full, harmonious taste, suitable for aging (Ivanov et al., 2011).

Trapezitsa - red, early to middle-ripening variety, obtained through interspecific hybridization by crossing of Dunavska Gamza x Marsilsko ranno. The grapes have good sugar accumulation. The wines have ruby red colour, fruity aroma, and harmonious soft flavour with pleasant freshness (Ivanov et al., 2012).

Cabernet Sauvignon is red middle-ripening variety with high sugar accumulation, relatively high titratable acidity and high content of colouring substance. The wine has a dark ruby colour, aroma of berries, extractive, full-bodied taste with medium level of acidity. They are suitable for aging in bottles or in oak barrels (Radulov et al., 1992).

The varieties were grown at the Experimental vineyards of IVE – Pleven (Central Northern Bulgaria). Upon reaching technological maturity, grapes were picked up and processed at the Experimental winery under the conditions of micro-vinification. The classical methods for making red and white dry wines were applied (Yankov, 1992). The alcoholic fermentation was induced by pure culture lyophilized wine yeast *Saccharomyces cerevisiae*, in the amount of 20 g/hl, at temperature 18 °C (white wine) and 25 °C (red wines). After the completion of the process the wines were decanted and further sulfated to 30 mg/L free SO_2.

The must and wine chemical composition of the samples were analyzed in the laboratories of IVE – Pleven and SUA – Nitra. The following methods were used:

Chemical composition

Density; Alcohol, vol. %; Saccharose, g/L; Glucose, g/L; Fructose, g/L; Glycerol, g/L; Total acids, g/L; Tartaric acid, g/L; Malic acid, g/L; Lactic acid, g/L; Citric acid, g/L; Acetic acid, g/L - fourier-transform infrared spectrometry, Bruker ALPHA FT-IR analyser for wine (Bruker, USA). FT-IR analysis is based on the use of infra-red light properties and each chemical has its own infra-red fingerprint.

Total extract (TE), g/L - distillation apparatus with densitometry (DEE Destillation Unit with Densimat and Alcomat, Gibertini, Italy).

Sugar-free extract (SFE), g/L - calculation method, the difference between TE and residual sugars in wine.

pH – pH meter (FEP20-ATC-KIT, Mettler Toledo, Switzerland).

Total phenolic compounds (TPC), g/L galic acid – method of Singleton and Rossi with a Folin-Ciocalteu reagent; Anthocyanins, mg/L - method of Gayon and Stonestreet by pH changing; Colour intensity I [abs. units] - method of Somers (UV-Vis spectrophotometer Cary 50, Varian) (Ivanov et al., 1979; Chobanova, 2007).

Total esters, mg/L - method of saponification with NaOH (Ivanov et al., 1979).

Total aldehydes, mg/L - bisulphate method (Ivanov et al., 1979).

Total higher alcohols, mg/L - modified method of Komarovsky – Felenber (Ivanov et al., 1979).

Antioxidant activity

ABTS radical cation decolorization assay - this assay was determined by the method of Re et al. (1999) with slight modifications. ABTS (2,2´-azinobis [3ethylbenzthiazoline]-6-sulfonic acid) was dissolved in distilled water to 7 mM concentration, and potassium persulphate added to achieve a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-16 h) in the dark before use. The resultant intensely-coloured $ABTS^{\bullet+}$ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.00 to give an absorbance value of ~0.70 at 734 nm. A 2 mL of ABTS solution was mixed with 0.98 mL of PBS and 0.02 mL of sample. Absorbance was measured spectrophotometrically (Jenway 6405 UV/Vis, England) 6 min after the addition of sample. Trolox (100 – 100 mg/L; R^2 = 0.9991) was used as a standard, and the results were expressed in mg/L of Trolox equivalents.

Molybdenum reducing antioxidant power (MRAP), mg TEAC/L – this assay was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled and the absorbance at 700 nm was detected using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10-1000 mg/L; R^2=0.998) was used as a standard and the results were expressed in mg/L of Trolox equivalents.

The value of each analyzed indicator of the chemical composition of the experimental wines was average of the measurement of two parallel samples. If a significant difference was found in the values, a third sample was measured and the two closest values were taken into account.

Organoleptic profile

The organoleptic characteristics of the experimental samples were determined according to 100-score scale for the indicators: colour (clarity, tint, intensity), aroma (purity, intensity, finesse, harmony), taste (purity, intensity, body, harmony, durability, aftertaste) and general impression (Prodanova, 2008) by a nine-member tasting committee at the IVE – Pleven.

RESULTS AND DISCUSSION

The chemical composition, the antioxidant activity and the organoleptic profile of one white and three red Bulgarian wines, obtained from the hybrid varieties Misket Kaylashki, Rubin, Kaylashki Rubin and Trapezitsa have been investigated. The properties of the red wines were compared to those of Cabernet Sauvignon wine.

The chemical composition of the experimental samples is presented in Table 1. The results did not show deviations from the normal rates of the tested wine indicators. They were within the typical ranges of each variety, according to its specifics and varietal potential.

The alcoholic fermentation had occurred completely, as evidenced by the rates of the analyzed sugars in the samples – the disaccharide sucrose and the monosaccharides glucose and fructose. All studied wines contained sucrose in an amount of 0.60 to 1.20 g/L. Glucose fermented easily, thus it was found in minimal rates in only two of the samples – Kaylashki Rubin and Cabernet Sauvignon. Fructose fermented harder and it was found in all wines except Trapezitsa.

The experimental wines had different alcohol content. The highest being recorded in Misket Kaylashki and Kaylashki Rubin samples. The red samples from the hybrid varieties exceeded Cabernet Sauvignon control in alcohol concentration. The reason was the different sugar accumulation of the varieties and the amount of fermentable sugars in the grape juice.

The amount of sugar-free extract (SFE), glycerol and the composition of the organic acids, determining wine density and freshness that depended mainly on the variety potential, were of great importance for its taste. The amount of glycerol in the tested samples ranged from 6.80 (Kaylashki Rubin) to 8.30 (Rubin) g/L. From the red wines, Rubin and Trapezitsa exceeded the Cabernet Sauvignon control for this indicator (7.80 g/L). Although glycerol is part of the wine sugar-free extract, the data from the analysis did not show a unidirectional difference in the rates of both indicators. Typically, Misket Kaylashki white wine had the lowest SFE (19.86 g/L), but not the lowest glycerol rate (Table 1). From the analyzed red wine samples, the amount of SFE (26.78 g/L) and glycerol was the highest, in Rubin wine, whereas in Trapezitsa sample despite the high glycerol content, SFE was the lowest (23.63 g/L). That was explained by the specifics and the potential of the respective variety, as well as the involvement of other components from the wine composition, at different concentrations, in the formation of the SFE. Accordingly, the results from the organoleptic analysis (Figure 1a) of the red wines revealed that Rubin was evaluated the highest (83.00) and Trapezitsa – the lowest (80.14).

Referring the acid composition, some basic organic acids – tartaric, malic, lactic and citric, were found in the experimental samples. Misket Kaylashki white wine had the lowest total acidity – 4.80 g/L, as it was also rated the lowest in the organoleptic analysis (79.12), due to the lack of freshness in its taste. From the red samples, the wines from the studied hybrid varieties exceeded Cabernet Sauvignon control in the total acid content. The highest rates were reported for Kaylashki Rubin (6.70 g/L), due to the higher tartaric acid content (2.30 g/L). However, during the tasting, this wine was not rated the highest (Figure 1a). Reducing the malic acid content and the increase of the lactic acid in the red wine samples was a result of the characteristic malolactic fermentation, leading to softening of wine taste and a reduction in the total amount of titratable acids.

From the analyzed organic acids in the wines, the citric acid was in the lowest rates as in Rubin and Trapezitsa samples it was practically not found (Table 1).

The total phenolic compounds content (TPC) was also analyzed in the experimental samples, as the rates of this indicator corresponded to the type of wine and its varietal availability. Their content was normally the lowest in the white wine and increasing in the order Misket Kaylashki < Trapezitsa < Kaylashki Rubin < Cabernet Sauvignon < Rubin. In red wines, it was observed a proportional correlation between the amount of TPC, the anthocyanins and the colour intensity. Their rates were the highest in Rubin sample and the lowest in Trapezitsa. These indicators of the wine composition affected the colour and taste from its organoleptic profile. The higher rates had determined its denser colour, taste and tasting score, respectively (Table 1, Figure 1a, b).

With regard to the wine aromatic profile, the content of total esters, total aldehydes and total higher alcohols was determined that were mainly synthesized during the alcoholic fermentation and were derived from the yeast metabolism. Cui et al. (2012) found that muskat wines contained relatively high levels of terpenes and esters which contributed to the rich floral, fruity aromas and sweet musky flavour and lower contents of higher alcohols. Misket Kaylashki white wine, as a typical muscat wine, had the highest rates of esters (299.20 mg/L). Manolache et al. (2018) noted that higher alcohols and esters were the main aromatic contributor for red wines – 17-76% and 16-23% of the total volatiles, respectively.

The ester profiles of red wines were strongly influenced by grape composition and in particular grape

Table 1. Chemical composition of the experimental wines from the studied varieties

Wines Indicators	Misket Kaylashki	Rubin	Kaylashki Rubin	Trapezitsa	Cabernet Sauvignon
Density	0.9907	0.9942	0.9932	0.9931	0.9939
Alcohol, vol %	13.00	12.00	13.00	12.40	11.90
Saccharose, g/L	0.80	1.20	0.60	1.00	0.80
Glucose, g/L	0.00	0.00	0.40	0.00	0.20
Fructose, g/L	0.60	0.10	0.80	0.00	0.40
Glycerol, g/L	7.80	8.30	6.80	7.90	7.80
SFE, g/L	19.86	26.78	24.17	23.63	25.86
Total acids, g/L	4.80	5.10	6.70	5.10	5.00
Tartaric acid, g/L	1.17	1.54	2.30	1.09	1.58
Malic acid, g/L	0.70	0.90	1.10	1.80	0.20
Lactic acid, g/L	1.23	1.26	0.97	1.26	1.25
Citric acid, g/L	0.41	0.00	0.25	0.00	0.23
pH	3.21	3.49	3.11	3.48	3.31
TPC, g/L g. a.	0.44	3.18	2.28	1.77	2.60
Anthocyanins, mg/L	-	410.25	288.25	256.74	367.60
Colour intensity I, [abs. units]	-	12.15	10.15	9.88	11.80
Total esters, mg/L	299.20	246.40	228.80	193.60	228.00
Total aldehydes, mg/L	44.00	52.80	46.20	26.40	46.20
Total higher alcohols, mg/L	304.00	300.00	314.00	354.00	324.00

nitrogen and lipid metabolism. The concentration of the ethyl esters was significantly affected by grape maturity and Shiraz wine exhibited much higher concentration of C6-alcohols and C6-aldehydes than Cabernet Sauvignon (Antalick et al., 2015).

For the red wine samples in our study, the analysis data showed that Rubin wine contained the highest rate of total esters and aldehydes and the lowest rate of higher alcohols, while for Trapezitsa wine just the opposite trend was observed. Just because of the less pronounced aroma indicators, Trapezitsa sample was evaluated to be the lowest among the red wines – 80.14 (Table 1, Figure 1a).

Almost all groups of phenolic compounds had the ability to bind with free radicals and to disable the active oxygen particles in the human body. Due to the richer and more versatile phenolic composition, red wines belonged to beverages containing more natural antioxidants. Valkova et al. (2004) pointed that the higher antioxidant activity of red wines was due to the higher phenolic content as well as on the different degree of polymerization of procyanidins in white and red wines and the different ratio of individual catechins in the polymer phenols molecule. Two analytical tests (ABTS, MRAP) were applied to determine the antioxidant activity of the studied wines, but the results were not unidirectional. The obtained data showed that the higher rates of the phenolic compounds in the samples did not always determine their higher antioxidant activity. This was observed only for Rubin. Misket Kaylashki white wine had the lowest antioxidant capacity from the studied wines, while from the red samples the highest rates were reported for Rubin and the lowest for Kaylashki Rubin (Table 2).

The antioxidant activity of the experimental samples, according to the ABTS method, increased in the order Misket Kaylashki <Kaylashki Rubin <Cabernet Sauvignon <Trapezitsa <Rubin. According to the MRAP test, the gradation in the ascending order was as follows: Misket Kaylashki<Kaylashki Rubin <Trapezitsa <Cabernet Sauvignon <Rubin. That was probably due to the involvement of other components of the wine composition exhibiting antioxidant properties (Kerchev et al., 2005).

The scores of the studied wines from their tasting and their organoleptic profile are presented in Figure 1. The red wine samples had better properties as the most prominent variety aroma, harmony and balance of the tasting indicators and respectively the highest tasting score had Rubin (83.00) and Kaylashki Rubin (82.00).

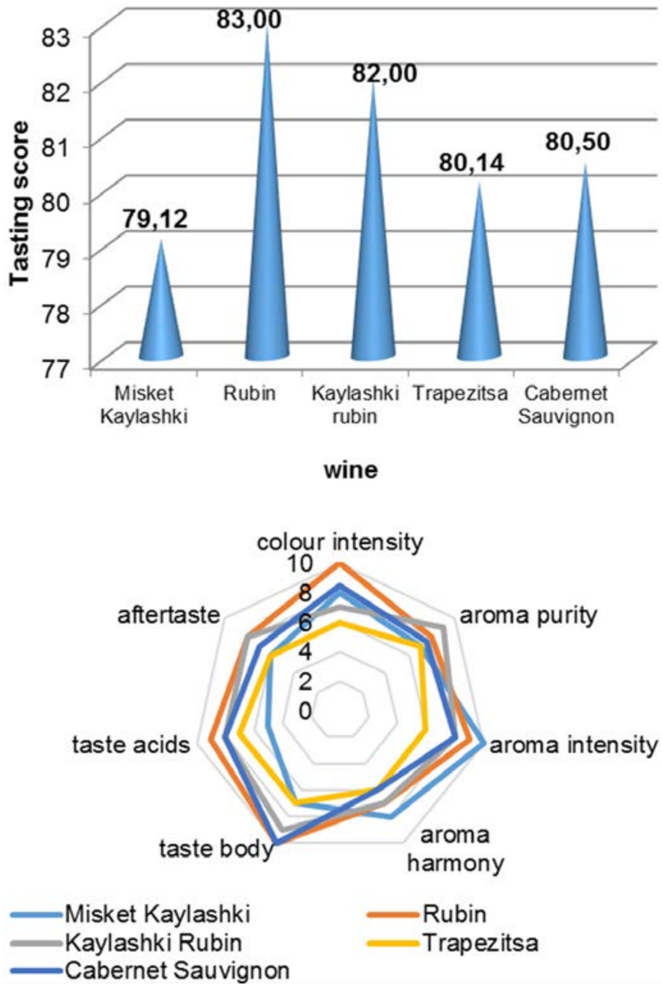

Figure 1. Tasting score (a) and organoleptic profile (b) of the experimental wines from the studied varieties

Table 2. Antioxidant activity of the experimental wines from the studied varieties

Wines	Misket Kaylashki	Rubin	Kaylashki rubin	Trapezitsa	Cabernet Sauvignon
Radical Scavenging Activity					
ABTS, mg TEAC/L	179.318	583.375	207.495	547.872	445.872
Molybdenum reducing antioxidant power, mg TEAC/L	176.437	951.705	481.979	504.405	864.808

*DPPH – 1,1-diphenyl-2-picrylhydrazil; ABTS – (2,2´-azinobis[3ethylbenzthiazoline]-6-sulfonic acid); MRAP – Molybdenum reducing antioxidant power

CONCLUSIONS

Based on the results of the study it could be summarized:

- The wine composition was mainly determined by the variety and its peculiarities. Misket Kaylashki white wine had the lowest rate of SFE (19.86 g/L) and total acidity (4.80 g/L). From the red samples, Rubin wine contained the highest rates of SFE (26.78 g/L) and glycerol (8.30 g/L), Kaylashki Rubin had the lowest concentration of glycerol (6.80 g/L) and the highest total acidity (6.70 g/L), Trapezitsa had the lowest SFE (23.63 g/L).
- TPC and anthocyanin rates are determined by the varietal availability and their content increased in the order Misket Kaylashki < Trapezitsa < Kaylashki Rubin < Cabernet Sauvignon < Rubin. In red wines their rates were the highest in Rubin wine samples and the lowest in Trapezitsa. The higher rates had determined its denser colour, taste and tasting score, respectively.
- Misket Kaylashki white wine had the highest rates of esters (299.20 mg/L). From the red wine samples Rubin wine contained the highest rate of total esters and aldehydes and the lowest higher alcohols, while the opposite trend was observed for Trapezitsa wine. Trapezitsa sample was rated the lowest from the red wines.
- The higher content of phenolic compounds in the samples did not always determine their higher antioxidant activity. According to the ABTS and MRAP methods, Misket Kaylashki white wine had the lowest antioxidant ability from the studied wines, while from the red wine samples the highest rates were reported for Rubin and the lowest for Kaylashki Rubin.

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