

PROFESSIONAL PAPER

Chemical, sensory and antioxidant characteristics of Bulgarian wines from native cultivars

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Abstract

A study of the chemical composition, the antioxidant capacity and the sensory characteristics of three Bulgarian wines (vintage 2015) from the native cultivars Misket Vrachanski (white), Pamid (red) and Gamza (red), grown in the region of Pleven, Central Northern Bulgaria was carried out. The results showed that the wine composition was mainly determined by the cultivar and its peculiarities. Misket Vrachanski white wine had the lowest rate of sugar-free extract, glycerol, total phenolic compounds and the highest acid content. From the red wine samples, Pamid had lower rates for the above parameters compared to Gamza. The experimental wines were analyzed for total content of esters, aldehydes and higher alcohols. A correlation was found between the rates of the studied volatile components in the wines and their aromatic sensory characteristics. At the wine tasting, Misket Vrachanski that contained the most of esters and aldehydes, was the best evaluated, followed by Gamza and Pamid. There was no correlation between the amount of total phenolic compounds in the experimental samples and their antioxidant capacity, which according to ABTS and MRAP methods increased in the order of Pamid < Misket Vrachanski < Gamza.

Key words: wine, native cultivars, chemical composition, antioxidant capacity.

Introduction

Grapes and wine have a complex chemical composition made up of a number of volatile and non-volatile components. Their quantity depends on the specifics of the cultivar, the soils and the climate in the region of cultivation, the agricultural practices in the vineyards, etc. Their ratio affects the organoleptic profile of the final product, its 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 (Yankov, 1992). The total acidity is an indicator varying within wide ranges for the separate cultivars. From the organic acids, the tartaric and malic acids are prevailing, which in a certain ratio with the other components form the flavor. 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 substances are concentrated mainly in the grape skin and less in the flesh. Their composition and quantity is a cultivar feature. Some cultivars such as Misket Vrachanski, Muscat Ottonel, Traminer have a strong muscat aroma that passes into wine and is mainly due to terpenes (Katerova et al., 2013). The major part of esters, aldehydes and higher alcohols are a product of yeast metabolism and are synthesized during the alcoholic fermentation (Torrea et al., 2003). They have an impact on wine aroma not only with their quantity but also with the correlation between the separate representatives. The ethyl esters are among the key compounds in the fruity flavors

and they make a general positive contribution to the overall quality of wines.

The tannins and the coloring components represented by the anthocyanins belong to the group of phenolic compounds. Their amount in grapes is specific to the cultivar. The tannins are mainly found in the pulp while the anthocyanins are accumulated in the skin. Their ratio in wine depends on the grapes and the technology used (Getov, 2002; Stoyanov et al., 2004).

The phenolic compounds content in wines is determining for their antioxidant properties. White wines contain a minimal amount, mainly non-flavonoids, while red wines are characterized by high phenolic content, predominantly flavonoid components and anthocyanins (Burns et al., 2003; Otteneder and Marx, 2004). Red wines belong to fermented beverages having high rates of natural antioxidants (Valkova et al., 2004; Monagas et al., 2005). Wine also has a number of other compounds exhibiting antioxidant properties – thiol-containing molecules, some amino acids (proline), glutathione, ascorbic acid, tocopherols, carotenoids, etc. (Kerchev et al., 2005). The antioxidants rate is one of the most important factors determining the healthy food and drinks (Joubert and Beer, 2006; Polovka, 2006).

Due to the favorable geographic, soil and climatic conditions, grapes and wine production in the Bulgarian lands dated back to the ancient times. In the conditions of increasing globalization, overproduction of grapes and wine and growing competition on international markets Bulgaria should maintain its place, reinventing the quality of native cultivars. On the basis of the diverse terroir (extremely varied relief, climate and



soils) in all regions of the country along with the globally distributed, well known vine cultivars, some native ones, characteristic and typical of each region are also grown (Abrashveva et al., 2008). In the region of Pleven from the native vine cultivars are grown Misket Vrachanski, Pamid and Gamza.

Misket Vrachanski is a white middle-ripening cultivar grown in some regions of Northern and Southern Bulgaria. The full maturity occurs in late September. The vines are of good fertility. The cultivar is slightly cold-resistant. Grapes are characterized by good sugar accumulation (21-24%) and a relatively low total acidity (4.5-5.5 g/L). The wines have a fine and lasting muscat aroma (Radulov et al., 1992; Radulov et al., 2004; Nakov et al., 2007).

Pamid is a red middle-ripening cultivar grown mainly in Southern Bulgaria and in some parts of Northern Bulgaria. The maturity of grapes occurs around middle September. The vines have strong growth and excellent fertility. The cultivar is moderately resistant to decay, low temperatures, drought and is not very demanding to soils. Grapes are distinguished by high sugar accumulation (20-24%) and low total acidity (4-5 g/L). The wines are light red and rose with poor acidity and low extract and therefore are not suitable for aging (Radulov et al., 1992; Radulov et al., 2004; Simeonov et al., 2009; Nakov et al., 2013).

Gamza is a red late-ripening cultivar grown in Northern Bulgaria. Grapes ripen in middle September to early October. The vines are of good fertility. The cultivar is not resistant to low winter temperatures but has good recovery capacity. The grapes have good sugar accumulation (20-22%) and higher total acidity (6-8 g/L). The wines have a bright ruby red color with typical resinous flavor and a pleasant freshness. They are suitable for aging (Radulov et al., 1992; Radulov et al., 2004; Nakov et al., 2013).

The objective of the study was to determine the chemical composition, the antioxidant capacity and the sensory characteristics of three Bulgarian wines, vintage 2015, produced from the native cultivars Misket Vrachanski, Pamid and Gamza.

Materials 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 native cultivars Misket Vrachanski, Pamid and Gamza.

The vineyards of the studied cultivars were fruit-bearing, grown at the Experimental Base of IVE – Pleven. The town of Pleven is located in Northern Bulgaria, in the central part of the Danubian plain. The area is characterized by a typical continental climate and leached carbonated black soils.

During the period March – April the necessary agricultural practices were carried out for vine pruning and loading. Misket Vrachanski cultivar were cultivated on stem Moser training while Pamid and Gamza cultivars on improved Guyot training. The loading at mature pruning of Misket Vrachanski was 28 winter eyes per vine, of Pamid and Gamza were 18 winter eyes per vine.

During the period August – September the grapes ripening dynamics was monitored through the changes in sugars

and total acids concentration in the grape juice. Upon reaching technological maturity the grapes were harvested. The chemical composition of grapes of the studied cultivars is given in Table 1. The following methods were applied for determining the grapes composition: sugars (g/L) – areometer of Dujardin; total acids (g/L) – titration with NaOH; pH – pH meter.

Table 1. Chemical composition of grapes of the studied cultivars, vintage 2015.

Indicator \ Cultivar	Misket Vrachanski	Pamid	Gamza
Sugar, g/L	220.00	224.00	226.00
Total acids, g/L	7.05	5.42	6.60
pH	3.05	3.31	3.22

The grapes, in quantity of 30 kg of each cultivar, were processed at the Experimental Winery of IVE – Pleven under the conditions of micro-vinification. The classical methods for making red and white dry wines were applied (Yankov, 1992):

white wine – crushing, destemming, pressing, sulphuring (50 mg/L SO₂), must clarification, adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure B+C* (20 g/hL), fermentation temperature 18°C, duration of alcoholic fermentation 12 days, racking, sulphuring to 30 mg/L of free SO₂, storage.

red wine – destemming, crushing, sulphuring (50 mg/kg SO₂), adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure CSM* (20 g/hL), fermentation temperature 25°C, duration of alcoholic fermentation 7 days (Gamza) 8 days (Pamid), spontaneous malolactic fermentation (10 days), separation of liquid part (young red wine) by pressing and racking, sulphuring to 30 mg/L of free SO₂, storage.

The chemical composition of the wine samples were analyzed by the following methods used:

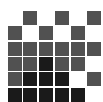
Density; Alcohol, vol. %; Saccharose, g/L; Glucose, g/L; Fructose, g/L; Glycerol, g/L; Total acids, g/L; Tartaric acid, g/L; Malic acid, g/L; Lactic acid, g/L; Citric acid, g/L; Acetic acid, g/L - fourier-transform infrared spectrometry, Bruker ALPHA FT-IR analyser for wine (Bruker, Billerica, Massachusetts, USA). FT-IR analysis is based on the use of infra-red light properties (each chemical has its own infra-red signature similar to a fingerprint). Reflected light energy serves to evaluate the results of the analysis using the calibration equations within the equipment's memory.

Total extract (TE), g/L - distillation apparatus with densitometry (DEE Distillation Unit with Densimat and Alcomat, Gibertini, Milan, Italy). The sample quantity (100 mL) was placed in the distillation chamber and the reagents (anti-froth additive and 12% CaO suspension) were added before the distillation started. The distillate (80 mL) was collected in a volumetric flask, distilled water was added until 100 mL and shook. The densitometry automatically measured the density of the distillate obtained against the density of the wine sample.

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 and



measurement of sample absorption spectrophotometrically at λ 750 nm (UV-Vis spectrophotometer Cary 50, Varian, California, USA).

Anthocyanins, mg/L - method of Gayon and Stonestreet by pH changing and using of buffer solutions with pH 0.6 and pH 3.5. Absorption of both samples was measured spectrophotometrically at λ 520 nm (UV-Vis spectrophotometer Cary 50, Varian, California, USA).

Color intensity I - method of Somers based on the sum of the absorptions measured spectrophotometrically at λ 420 for white wine and at λ 420, 520, 620 nm for red wines (UV-Vis spectrophotometer Cary 50, Varian, California, USA).

Total esters, mg/L - method of saponification (the esters were saponified with NaOH): the distilled wine sample was titrated with NaOH to slightly pink color, boiled 30 min and after cooling was titrated with H_2SO_4 .

Total aldehydes, mg/L - bisulphate method: to the distilled wine sample was added buffer solution with pH 7 and $NaHSO_3$. After 30 min was added HCl, starch and the sample successively was titrated with 0.1N I_2 to slightly blue color, with alkaline borate solution to slightly pink color and with 0.01N I_2 to slightly blue color.

Total higher alcohols, mg/L - modified method of Komarovsky – Felenber (higher alcohols in the sulfuric acid medium with p-dimethylaminobenzaldehyde (PDMA) formed a color compound with a maximum absorption at λ 536 nm): to the distilled wine sample (previously free of esters and aldehydes) was added PDMA. After cooling was added H_2SO_4 . After cooling again and boiling the sample was tempered at room temperature and the absorption was measured spectrophotometrically at λ 536 nm.

The antioxidant capacity of the experimental wines was analyzed by the methods:

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity - evaluation based on colorimetry of free radicals using the reaction by the scheme: $DPPH^* + AH \rightarrow DPPH-H + A$. For this analysis basic and working solution were made. 0.025 g of DPPH and 100 mL of methanol (99.5%) used for basic solution. 10 mL of basic solution and 100 mL of methanol used for working solution. Spectrophotometer GENESYS 20 (USA) used for measurements. Measurements were determined at 515 nm. In compliance with formula $((A_0 - A_{10})/A_0) * 100\%$, where A_0 is 3.9 mL of working solution and A_{10} is 3.9 mL of working solution with 0.1 mL of wine sample (Brand-Williams et al., 1995; Kutlu et al., 2011).

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⁺ 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 (trolox equivalent antioxidant capacity)/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 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.

The sensory characteristics of the experimental samples were determined according to 100-score scale for the properties: color (clarity, tint, intensity), aroma (purity, intensity, fineness, harmony), taste (purity, intensity, body, harmony, durability, aftertaste) and overall quality (Prodanova, 2008) by a nine-member tasting committee. The tasting score of the experimental wines was average value of the committee members' estimates, eliminating the highest and the lowest.

Results and discussion

The chemical composition, the antioxidant capacity and the sensory characteristics of one white and two red Bulgarian wines obtained from the native cultivars Misket Vrachanski, Pamid and Gamza were investigated.

The chemical composition of the experimental samples, vintage 2015, is presented in Table 2. The results did not show deviations from the normal rates of the indicators of the tested wines from this vintage. They were within the typical ranges for each cultivar, according to its specifics and potential.

Table 2. Chemical composition and tasting score of the experimental wines, vintage 2015.

Indicator \ Wine	Misket Vrachanski	Pamid	Gamza
Density	0.9920	0.9911	0.9923
Alcohol, vol %	12.60	13.10	13.50
Saccharose, g/L	0.80	1.20	1.30
Glucose, g/L	0.00	0.00	0.10
Fructose, g/L	1.80	0.00	0.80
Glycerol, g/L	6.90	7.30	8.40
SFE, g/L	19.60	20.40	23.37
Total acids, g/L	6.90	4.80	5.80
Tartaric acid, g/L	2.30	0.78	1.65
Malic acid, g/L	1.90	1.80	0.00
Lactic acid, g/L	0.37	0.92	1.16
Citric acid, g/L	0.57	0.02	0.17
pH	3.00	3.46	3.33



Indicator \ Wine	Misket Vrachanski	Pamid	Gamza
TPC, g/L g. a.	0.35	0.94	1.71
Anthocyanins, mg/L	-	124.48	248.32
Color intensity I	0.32	7.81	9.72
Total esters, mg/L	316.80	246.40	264.00
Total aldehydes, mg/L	57.20	33.00	35.20
Total higher alcohols, mg/L	324.00	299.00	370.00
Tasting score	81.14	79.00	79.86

The alcoholic fermentation had occurred completely, as evidenced by the rates of the analyzed sugars in the samples – sucrose, glucose and fructose. Red wines had higher alcohol content.

The amount of SFE, glycerol and the composition of the organic acids, determining wine density and freshness are of great importance for its taste. Glycerol is part of the SFE and the analysis data revealed similarity in the rates of both parameters. Typically, Misket Vrachanski white wine had the lowest rates of glycerol and SFE. From the red samples, their rates were higher in Gamza wine - 8.40 g/L and 23.37 g/L respectively, which correlated with its higher tasting score (79.86). The rates were lower in Pamid wine as it was considered a specific cultivar feature.

Referring the acid composition, amounts of the basic organic acids (tartaric, malic, lactic and citric) were defined in the experimental samples. Misket Vrachanski white wine had the highest total acidity – 6.90 g/L. That was determined by the predominance of tartaric, malic and citric acid. At the wine tasting the higher acid content of Misket Vrachanski wine had determined the pleasant freshness in its taste and respectively the high tasting score (81.14). 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 (MLF), leading to softening of wine taste and a reduction in the total amount of titratable acids. According to Dimov and Getov (2002) the content of the basic organic acids in the red wines depends mainly on the cultivar and the degree of MLF and their average values were respectively: tartaric acid 1.75 g/L, malic acid 0.36 g/L, lactic acid 2.39 g/L and citric acid 0.073 g/L. The results of our study showed the full completion of the MLF in the Gamza wine and partly in the Pamid wine from vintage 2015.

The total phenolic compounds content was also analyzed in the experimental samples, as the rates of this parameter corresponded to the type of wine and its cultivar availability. Their content was normally the lowest in the white wine and increasing in the order Misket Vrachanski < Pamid < Gamza. In red wines, it was observed a correlation between the amount of TPC, the anthocyanins and the color intensity. Their rates were higher in Gamza sample. These indicators of the wine composition affected its sensory characteristics – color and taste. The higher rates had determined its denser color, taste and tasting score, respectively.

Regarding the wine aromatic profile, the content of total esters, total aldehydes and total higher alcohols was determi-

ned that were mainly synthesized during the alcoholic fermentation and were derived from the yeast metabolism. Volatile composition of wine depends on the grapes cultivar. Climate and soil conditions also could be the key factors of influencing the compounds concentration. Cui et al. (2012) found that muscat wines contained relatively high levels of terpenes and esters which contributed to the rich floral, fruity aromas and sweet musky flavor and lower contents of higher alcohols. Misket Vrachanski white wine, as a typical muscat wine, had the highest rates of esters (316.80 mg/L) and aldehydes (57.20 mg/L). The outstanding wine aroma correlated with its higher tasting score. In red samples, the analysis data showed higher rates of the studied volatile components in Gamza wine. It was characterized with the highest rates of higher alcohols however that did not have a negative influence on its sensory characteristics (Table 2). 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.

Almost all groups of phenolic compounds have the ability to bind the 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 capacity 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. Three analytical tests (DPPH, ABTS and MRAP) were applied to determine the antioxidant capacity of the studied wines, but the results were not unidirectional. According to the data obtained, the higher rates of phenolic compounds in the samples did not always determine their higher antioxidant capacity. Regardless the lower rates of TPC in Misket Vrachanski white wine, it did not show the lowest antioxidant properties, and according to the DPPH method it even had the best antioxidant capacity. The results of the other two ABTS and MRAP methods were unidirectional and revealed that the antioxidant capacity of the studied wines from vintage 2015 increased in the order of Pamid < Misket Vrachanski < Gamza (Table 3). That was probably due to the involvement of other components of the wine composition exhibiting antioxidant properties (Kerchev et al., 2005).

Table 3. *Antioxidant capacity of the experimental wines, vintage 2015.*

Wines \ Radical scavenging capacity	Misket Vrachanski	Pamid	Gamza
DPPH, %	76.52	71.28	67.17
ABTS, mg TEAC/L	205.241	204.113	515.750
MRAP, mg TEAC/L	300.176	190.453	462.758

The sensory characteristics of the studied experimental wines, vintage 2015, is presented in the form of a spider diagram in Figure 1. With the best tasting properties, typical cultivar aroma, harmony and balance of the tasting parameters and respectively the highest tasting score was Misket Vrachanski white wine, followed by Gamza and Pamid.

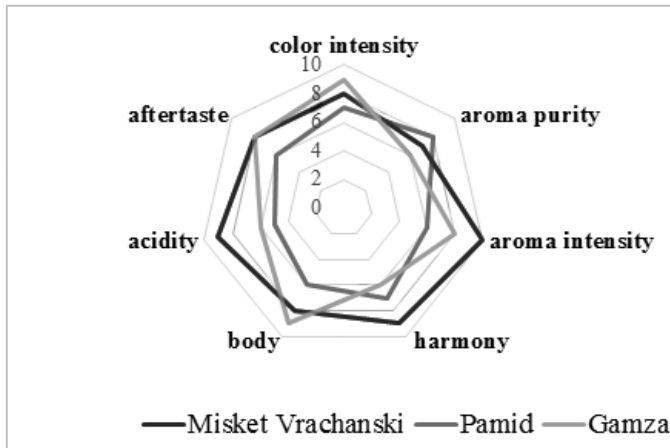


Figure 1. Sensory characteristics of the experimental wines, vintage 2015.

Conclusions

Based on the results of the study about the chemical composition of the experimental wines from vintage 2015 it could be summarized that the white wine Misket Vrachanski had the lowest rate of SFE, glycerol, TPC and the highest acid content. From the red wine samples, Pamid had lower rates for the above indicators compared to Gamza. A correlation was found between the rates of the studied aromatic components in the wines and their aromatic sensory characteristics. Misket Vrachanski wine, that contained the highest concentration of esters and aldehydes, was the best evaluated, followed by Gamza and Pamid. There was no correlation between the amount of TPC in the experimental samples and their antioxidant capacity, which according to ABTS and MRAP methods increased in the order of Pamid < Misket Vrachanski < Gamza.

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