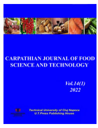

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INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE QUALITY OF GRAPES AND AROMA-RELEASING ENZYME ADDITION ON THE CHEMICAL COMPOSITION, AROMATIC COMPLEX AND ORGANOLEPTIC PROFILE OF RED WINES

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ABSTRACT

In the period 2013 – 2015 the influence of the meteorological conditions of the year on the harvest quality and aroma-releasing enzyme addition before the alcoholic fermentation on the chemical composition, aromatic profile and organoleptic features of red wines were investigated. The objects of the study were the clone Pamid 5/76, candidate–clones Gamza 52-9-4 and Gamza 52-9-5 and the varieties Kaylashki Rubin and Trapezitsa (obtained by interspecific hybridization). The sugar accumulation dynamics in the grapes was monitored during the ripening phase, in order to determine the time of technological maturity. The latest ripening variety was Kaylashki Rubin, and it demonstrated the most gradual sugar accumulation and acidity reduction. The experimental wines had different composition and organoleptic characteristics, depending on the potential and specifics of the variety and the harvest. Kaylashki Rubin wines had the highest alcohol content and sugar-free extract and the lowest - Pamid 5/76. The amount of total phenolic compounds and anthocyanins in wines was increasing in the order Pamid 5/76 < Trapezitsa < Gamza 52-9-4 < Gamza 52-9-5 < Kaylashki Rubin. The positive effect of the added aroma-releasing enzyme on the content of esters in wines and their aromatic characteristics was confirmed. No effect of the enzyme addition on the amount of total aldehydes and higher alcohols was observed. No correlation was found between the studied components of the aromatic composition of the wines and their tasting evaluation.

1. Introduction

Wine is a beverage containing a large number of organic compounds in different quantitative ratios. It consists of substances coming from grapes as they are contained in it; coming from grapes but undergoing a change in the course of the alcoholic fermentation; formed during alcoholic fermentation; formed during the wine aging (Chobanova, 2012).

The content of the various components is a function of many factors – variety, soil and weather conditions, region and method of cultivation, conditions of vinification, processing and storage of wine.

The substances contained in wine, depending on their organoleptic influence and technological significance, could be divided into volatile and non-volatile (extractive) ones. The group of volatile compounds includes ethyl alcohol, volatile acids, esters, aldehydes, higher alcohols, terpenes, etc., which form the wine aroma (bouquet). Their amount in wine is about 10 times higher than in grapes because they are formed mainly during the alcoholic fermentation. The extractive ones are non-volatile components of organic and inorganic origin, including carbohydrates, organic acids, phenolic compounds, nitrogen and mineral substances, etc. (Chobanova, 2012).

The wine titratable acidity is an indicator varying within wide ranges for the individual varieties. From the organic acids, the tartaric and malic acids are prevailing, which in a certain ratio with the other components form the taste. 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; Kučerová and Široký, 2011; Wilkes, 2016).

Phenolic compounds have a direct or indirect effect on wine quality, especially red wines. Its main characteristics (colour, taste, clarity, stability) are largely related to the presence of phenolic substances and their transformations. The phenolic compounds content in wine depends mainly on the phenolic reserve of the variety, the method of cultivation and the conditions in the growing area, the applied technology of winemaking, ect. (Getov, 2002; Stoyanov *et al.*, 2004; Bai *et al.*, 2013; Kekelidze *et al.*, 2014; Gardin and Altindisli, 2015). Grapes are rich in phenols, which are localized in solids (skins, seeds, rachis) and are a source of flavonoid phenolic substances (including anthocyanins), while the fleshy part is rich in non-flavonoid compounds. Few substances of the phenolic complex appear during the alcoholic fermentation, as well as from the transformation of other products, resulting from the evolution of the natural phenols of the grapes (Chobanova, 2012; Bai *et al.*, 2013).

The aroma is one of the important factors determining wine nature and quality. The wine contains more than 800 volatile components, but only a few dozen of them have an effect on the aroma. The varietal aroma of wines is determined by the content of monoterpenes, norisoprenoids, methoxypyrazines and thiols. However, the main aroma-determining compounds are derived from yeast metabolism. About 90% of the free volatile compounds are esters and higher alcohols, which are formed during the alcoholic fermentation and depend on the conditions under which it occurs – temperature, aeration, yeast strain (Selli *et al.*, 2004; Antalick *et al.*, 2015; Mina and Tsaltas, 2017; Manolache *et al.*, 2018). Ethyl esters are among the key components responsible for the fruity aroma of wines, having a positive effect on their quality. In red wines, after the malolactic fermentation and as a result of the metabolism of the lactic acid bacteria, diethyl succinate is in the highest rates and is determinant of aroma formation (Gil *et al.*, 2006; Manolache *et al.*, 2018).

The climate changes and weather conditions of the year also affect the chemical composition of wines, their aromatic complex, organoleptic profile and aging potential. At higher temperatures during the grape ripening period, methoxypyrazine levels decrease that leads to fading of the herbaceous and herbal nuances of the wine. Hints of unripe green peppers and peas appear. The content of norisoprenoids and monoterpenes that cause the fruity and floral nuances in the aroma is higher when the grapes are exposed to more light and ripen at higher temperatures (Pons *et al.*, 2017). Severe water deficiency during maturation decreases the levels of the volatile thiol precursors (Peyrot des Gachons *et al.*, 2005). Under the conditions of water stress, that delay the grapes ripening, the levels of methoxypyrazines reach rates that adversely affect the wine aroma. The combined effect of the temperature and limited water status causes changes in the vine metabolism that sometimes delay the ripening process (Pons *et al.*, 2017).

The objective of the study was to investigate the influence of the meteorological conditions of the year on the grapes composition and the addition of aroma-releasing enzyme before the alcoholic fermentation on the chemical composition, aromatic profile and organoleptic features of red wines from clones and varieties, cultivated under the soil and climate conditions of the region of Pleven, Central Northern Bulgaria.

2. Material and methods

2.1. Materials

2.1.1. Plant material

The study was carried out at the Institute of Viticulture and Enology (IVE) - Pleven, during the period 2013 – 2015. The objects of the study were the clone Pamid 5/76, candidate–clones Gamza 52-9-4 and Gamza 52-9-5 and the varieties Kaylashki Rubin and Trapezitsa that were selected at the IVE by means of intraspecific hybridization and were characterized by higher practical resistance to diseases and low winter temperatures (Nakov *et al.*, 2011; Ivanov *et al.*, 2011; Ivanov *et al.*, 2012).

The town of Pleven is located in the Northern wine-growing region (the Danube Plain, Central Northern Bulgaria) and is characterized by a typical continental climate, and the soils include all types of black soils.

The vineyards were fruit-bearing, grown at the Experimental Base of the Institute. The experimental vine plots covered areas of 0.2 ha of each studied clone and variety. Pamid clone and Gamza candidate-clones were cultivated, at the ground, on improved Guyot training and planting distance 2.2 m between the rows and 1.2 m in the row. Kaylashki Rubin and Trapezitsa varieties were cultivated on stem Moser training, with stem height of 1.2 m. at planting distance 3.00 m/1.20 m. All varieties were grafted to Berlandieri x Riparia SO4 rootstock.

The sugar accumulation dynamics in the grapes of the selected clones and varieties was monitored during the ripening phase, in order to determine the time of technological maturity and grapes harvest. The change in the sugar concentration was determined refractometrically (under field conditions) and by hydrometer of Dujardin (under laboratory conditions). The titratable acids content was determined by titration with NaOH.

In 2013, Trapezitsa grapes were not harvested because of the poor sanitary status and not enough yield, as a result of a hail storm in the spring that affected the trial plantation.

2.1.2. Grapes processing and vinification

Every year the grapes from the studied clones and varieties were processed in the Experimental Winery of IVE – Pleven. The classical technology for production of dry red wines was applied under the conditions of micro-vinification (Yankov, 1992) – removing the berries, crushing, sulfating (50 mg/kg SO_2), adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure CSM* in the amount of 20 g/hl, fermentation temperature 28°C, separation of solid particles, further sulfating, storage. During the study period, when the must from the samples was insufficient in sugar content, to produce wines with the optimum alcohol concentration, a proportional adjustment was made for sugars with pure sucrose.

The grapes of the studied clones and varieties was divided in equal quantities into two technological variants, 30 kg each: V1 – control; V2 - with the addition of aroma-releasing enzyme *Zymovarietal Aroma G* in the amount of 3 g/100 kg before the alcoholic fermentation.

After the completion of the process, determined by chemical analysis of sugars, the young wines were decanted and further sulfated to 30 mg/l free SO_2.

2.2. Methods

2.2.1. Grapes chemical composition

The chemical composition of grape juice was determined according to the following methods (Ivanov *et al.*, 1979): sugars, g/l - hydrometer of Dujardin; glucose and fructose, g/l – iodometric method; titratable acids (TA), g/l – titration with NaOH; pH - pH-meter; glucoacidimetric index (GAI) – calculation method as the ratio of sugars (%) and TA (g/l).

2.2.2. Wine chemical composition

The main indicators of wines chemical composition were analyzed by conventional methods used in the winemaking practice (Ivanov et al., 1979; Chobanova, 2007): sugars, g/l – Schoorl's method; alcohol, vol. % - distillation method, Gibertini apparatus with densitometry of the distillate density; total extract (TE), g/l - Gibertini apparatus with densitometry, density of alcohol-free sample; sugar-free extract (SFE), g/l - calculation method (the difference between TE and sugars); titratable acids (TA), g/l - titration with NaOH; pH – pH-meter; total phenolic compounds (TPC), g/l – method of Singleton et Rossi; monomeric anthocyanins (mg/l) – method of Singleton et Rossi by pH changing. The colour spectral characteristics were also determined: intensity I [abs. units] – method of Somers; tint T [abs. units] – method of Glories.

The aromatic profile of wines included the following indicators and methods of analysis (Ivanov *et al.* 1979): total aldehydes (mg/l) - bisulphite method; total esters (mg/l) - a method of saponification with NaOH; total higher alcohols (mg/l) - modified method of Komarovsky - Felenber.

The annual trial results presented were the arithmetic mean of two simultaneous samples. In cases where a significant difference in the amount of the analyzed indicator was found, a third sample was prepared and the two closest rates were taken into account.

2.2.3. Wine organoleptic analysis

The organoleptic characteristics of the experimental wines were determined by a 9-member tasting committee using a 100-score scale (Tsvetanov, 2001), by the indicators: colour (clarity, hue, intensity); aroma (purity, intensity, finesse, harmony); taste (purity, intensity, flesh, harmony, durability, aftertaste); general impressions. The tasting results represented an average of the committee members' scores, as the highest and lowest were discarded.

2.2.4. Statistical analysis

Statistical processing of the results of the analyzes was performed including mean and standard deviation (±SD) using the Excel 2007 program from the Microsoft Office package.

The weather characteristics of the years from the study period (Table 1) were determined by the methods of mathematical statistics (Sirakov, 1981).

Table 1. Precipitation (P%) and average daily air temperature for the experimental years.

Year		2013	2014	2015
N	P%	43 (moderate wet)	14 (very wet)	17 (very wet)
T°		94 (very cool)	97 (very cool)	29 (moderately hot)

P – probability; N – precipitations; T°- average air temperature

3. Results and discussion

3.1. Sugar accumulation dynamics in the grapes

The sugar accumulation dynamics in the grapes of the studied red clones and varieties, during the ripening phase, for the study period, is shown in Figures 1, 2, 3. The influence of the meteorological conditions of the year on the process, composition and quality of the grapes has been defined.

The data on the changes in the sugar and acid content of grapes, 2013 harvest are presented in Figure 1 and show normal course of ripening. That was due to the favorable weather conditions during this period (July-August-September) – hot summer, without precipitation. The high temperatures in August had determined the good sugar accumulation and the rapid acidity reduction. In 2014, all varieties showed a delay in ripening due to the adverse climatic factors during the period – cool and rainy summer (Figure 2). The preconditions for the deterioration of the sanitary condition of the grapes created by the meteorological conditions necessitated an earlier grape harvest. The sugar accumulation dynamics in 2015 is presented in Figure 3. The graphic data revealed normal ripening of all varieties. The change in the weather conditions in September, manifested by the lower temperatures, rainfall and hail, created prerequisites for the deterioration of the sanitary condition of the grapes.

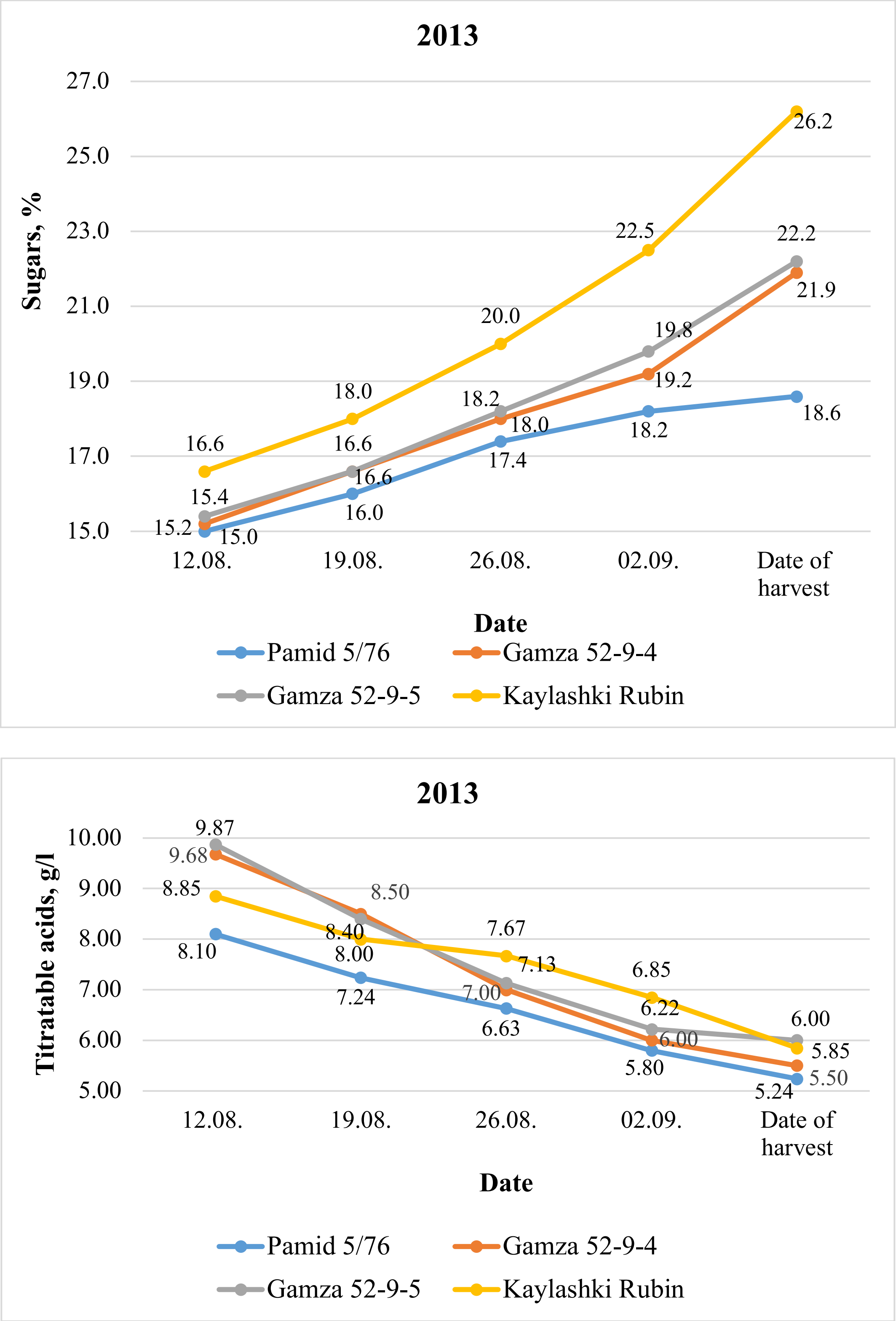

Figure 1. Changes in sugars and titratable acids during the grape ripening period of the studied red clones and varieties, 2013.

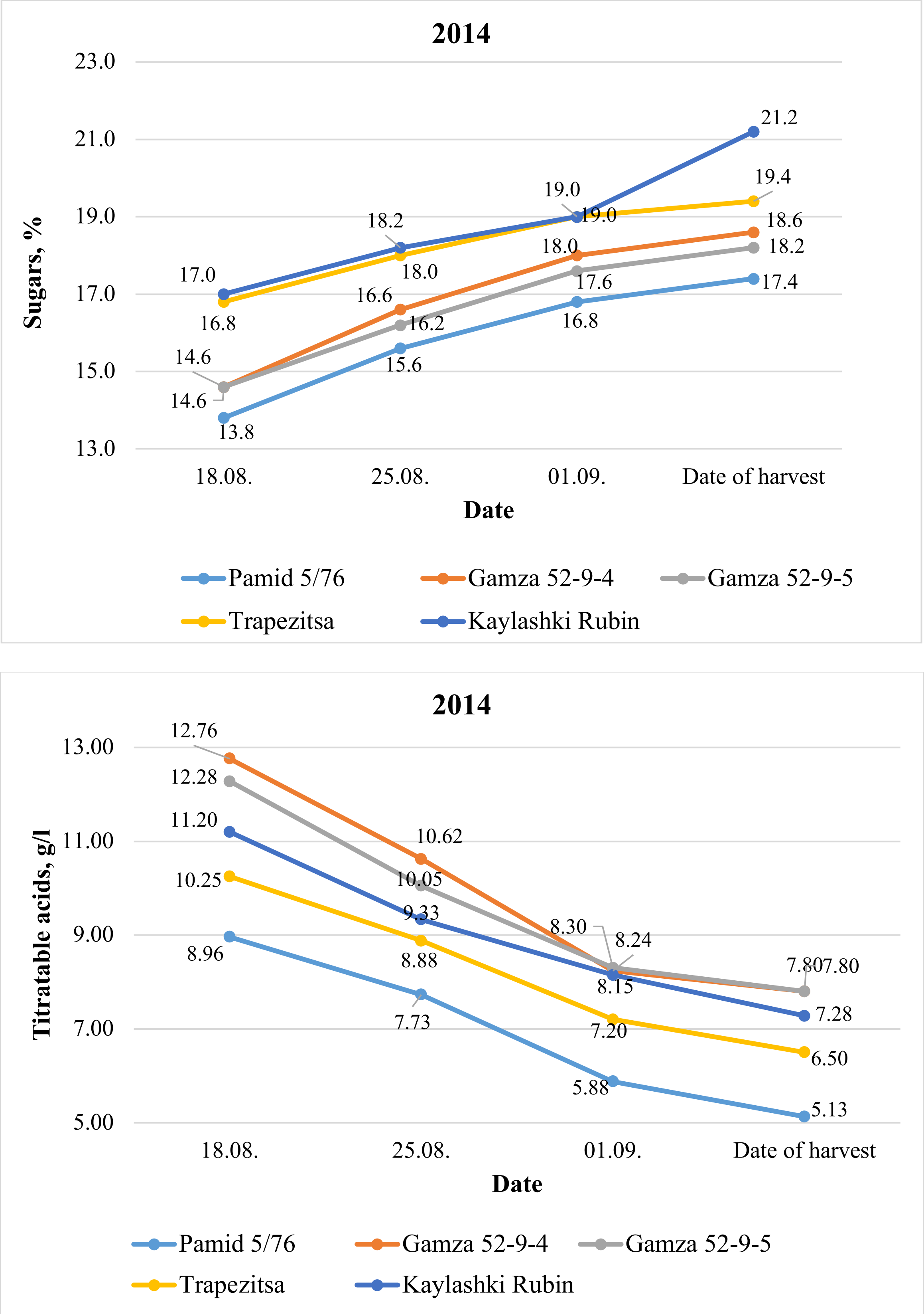

Figure 2. Changes in sugars and titratable acids during the grape ripening period of the studied red clones and varieties, 2014.

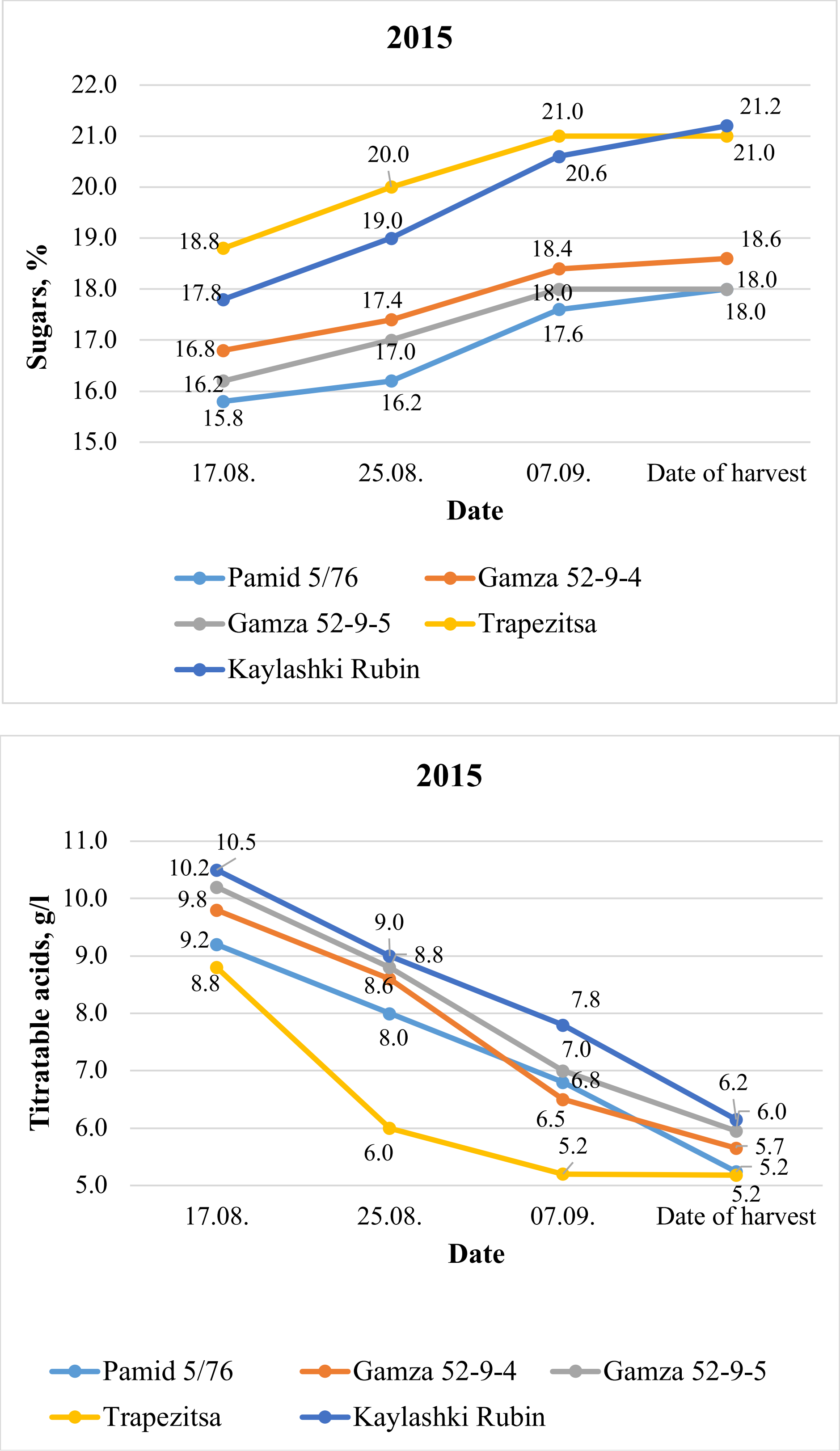

Figure 3. Changes in sugars and titratable acids during the grape ripening period of the studied red clones and varieties, 2015.

The studied red clones and varieties had different ripening times, therefore their harvest took place at different intervals. Usually, all of them reached their technological maturity in the second half of September. The grapes of Pamid 5/76 was characterized by the lowest sugar content and acidity, which was considered a varietal feature. Kaylashki Rubin was the latest ripening, as it had the most gradual sugar accumulation and titratable acids reduction. Both Gamza candidate-clones were with similar rates in terms of the studied parameters. In 2015, Trapezitsa variety was distinguished as the one with the highest rates of sugar accumulation and simultaneous decrease of titratable acids.

3.2. Grapes chemical composition

The data on the grapes composition are presented in Table 2. The general trend for the period was that the lowest sugar accumulation was reported for Pamid 5/76 (mean 180.00±6.00 g/l), the highest for Kaylashki Rubin (mean 228.67±23.69 g/l). Grapes from 2013 harvest, had high sugar content, ranging from 219.00 to 262.00 g/l, except Pamid 5/76 (186.00 g/l). The highest sugar rates were reported for Kaylashki Rubin. In 2014, the sugar concentrations in grapes varied from 174.00 (Pamid 5/76) to 212.00 g/l (Kaylashki Rubin). No significant difference was observed in the sugar amounts in both Gamza candidate-clones. In grapes from 2015 harvest, the sugar concentration ranged from 180.00 (Pamid 5/76, Gamza 52-9-5) to 212.00 g/l (Kaylashki Rubin). The content of monosaccharides glucose and fructose was also determined in the grape juice of all varieties. Their ratio was less than 1, with quantitative predominance of fructose.

The data revealed lower titratable acids in grapes of Pamid 5/76 (mean 5.22±0.08 g/l) and Trapezitsa (mean 5.84±0.93 g/l), which was their varietal feature (Table 2). Higher acidity was reported in Kaylashki Rubin variety (mean 6.43±0.75 g/l) and both Gamza candidate-clones – Gamza 52-9-4 (mean 6.32±1.29 g/l), Gamza 52-9-5 (mean 6.58±1.05 g/l). Characteristic of 2013 harvest was that, despite the higher sugar content of Gamza 52-9-5 grapes and Kaylashki Rubin, higher acids, respectively, 6.00 g/l and 5.85 g/l, were also analyzed. In 2014, the rates ranged from 5.13 (Pamid 5/76) to 7.80 g/l (Gamza 52-9-4 and 52-9-5), and in 2015 from 5.18 (Trapezitsa) to 6.15 g/l (Kaylashki Rubin).

On the basis of the found sugars and titratable acids content in the grapes, the glucoacidimetric index (GAI) was determined for each of the clones and varieties studied. Its values were indicative of the grapes quality and its purpose in wine production. With the highest value of the indicator is Kaylashki Rubin (mean 3.61±0.80). The calculated values for 2013 harvest were higher than 3, with Kaylashki Rubin reaching 4.48. It was a proof that the grapes were suitable for the production of high quality wines in terms of chemical composition and tasting characteristics. In 2014 harvest, GAI ranged from 2.33 (Gamza 52-9-5) to 3.39 (Pamid 5/76), i.e. the grapes not from all varieties had good enough indicators for the production of quality wines. The GAI for 2015 harvest varied from 3.02 (Gamza 52-9-5) to 4.05 (Trapezitsa), indicating the good characteristics of grapes for producing wines with optimal composition and organoleptic profile (Table 2, Table 3).

The data from the chemical composition of the grapes during the study period show that for Pamid 5/76, Gamza 52-9-5 and Kaylashki Rubin, the conditions in 2013 were the most favorable for obtaining the harvest with the highest sugar accumulation, optimal acid content and highest GAI values. These indicators were a prerequisite for the production of wines with normal chemical composition and characteristics (Table 3). For Gamza 52-9-4 and Trapezitsa the most favorable in terms of composition and quality of grapes was 2015. Poor weather conditions during ripening in 2014, were the reason for the deteriorating chemical parameters of the grapes from the studied clones and varieties - insufficient sugar accumulation and reported lower GAI values (Table 2).

Table 2. Chemical composition of grapes from the studied clones and varieties

Variety, clone	Vintage	Date of harvest	Sugars, g/l	Glucose , g/l	Fructose, g/l	Titratable acids, g/l	GAI	pH
Pamid 5/76	2013	13/09/	186.00	75.00	111.30	5.24	3.55	3.19
	2014	12/09/	174.00	77.00	87.00	5.13	3.39	3.35
	2015	10/09/	180.00	80.35	99.65	5.30	3.40	3.31
	mean± SD		*180.00 ±6.00*	*77.45 ± 2.70*	*99.32 ±12.15*	*5.22 ±0.08*	*3.45 ±0.09*	*3.28 ±0.08*
Gamza 52-9-4	2013	19/09/	219.00	92.10	126.90	5.50	3.98	3.24
	2014	12/09/	186.00	81.00	105.00	7.80	2.38	3.30
	2015	10/09/	186.00	87.08	98.92	5.65	3.29	3.22
	mean± SD		*197.00 ±19.05*	*86.73 ±5.56*	*110.27 ±14.72*	*6.32 ±1.29*	*3.22 ±0.80*	*3.25 ±0.04*
Gamza 52-9-5	2013	19/09/	222.00	82.54	139.46	6.00	3.70	3.21
	2014	12/09/	182.00	77.40	104.60	7.80	2.33	3.28
	2015	10/09/	180.00	81.68	98.32	5.95	3.02	3.08
	mean± SD		*194.67 ±23.69*	*80.54 ±2.75*	*114.13 ±22.16*	*6.58 ±1.05*	*3.02 ±0.68*	*3.19 ±0.10*
Kaylashki Rubin	2013	25/09/	262.00	121.35	140.65	5.85	4.48	3.27
	2014	19/09/	212.00	94.00	118,00	7.28	2.91	3.10
	2015	16/09/	212.00	80.00	132.00	6.15	3.45	3.23
	mean ±SD		*228.67 ±23.69*	*98.45 ±21.03*	*130.22 ±11.43*	*6.43 ±0.75*	*3.61 ±0.80*	*3.20 ±0.09*
Trapezitsa	2013	-	-	-	-	-	-	-
	2014	12/09/	194.00	81.00	113,00	6.50	2.98	3.26
	2015	02/09/	210.00	89.10	120.90	5.18	4.05	3.29
	mean± SD		*201.67 ±28.87*	*85.05 ±5.73*	*116.95 ±5.58*	*5.84 ±0.93*	*3.51 ±0.75*	*3.27 ±0.02*

3.3. Wine chemical composition

After the completion of the alcoholic fermentation, the wines obtained from the experimental variants were subjected to chemical and organoleptic analysis. Data on their composition and tasting scores are presented in Table 3 a, b.

The alcohol content of the samples varied over a wide range. During the study period, it was observed the trend for the highest ratio in Kaylashki Rubin wines - from 12.66 to 14.24 vol.% (mean 13.19±0.91 vol.%, V1 and mean 13.15±0.73 vol.%, V2). It was followed by the wines of both Gamza candidate-clones. With the lowest alcohol were the Pamid 5/76 samples - from 12.12 to 12.47 vol.% (mean 12.22±0.15 vol.%, V1 and mean 12.29±0.15 vol.%, V2). The differences in the alcohol ratios were not significant between the different variants of the studied clones and varieties. All experimental wines showed a gradual increase of the rate in V2, except Gamza 52-9-5 (2014, 2015) and Kaylashki Rubin (2013).

The complete alcoholic fermentation was confirmed by the residual sugars rates in wines, that for 2013 vintage was within the range from 1.98 (Gamza 52-9-4, V1) to 3.54 g/l (Kaylashki Rubin, V1), for 2014 vintage - from 0.97 (Pamid 5/76, V1) to 2.05 g/l (Kaylashki Rubin, V2) while for 2015 vintage – from 1.10 (Pamid 5/76, V1, V2) to 2.05 g/l (Gamza 52-9-4, V2).

The sugar-free extract (SFE) had been an important indicator of the wine composition, which was also relevant to their tasting qualities. Its rates were within the typical range for red wines of the respective varieties. The differences found in the SFE content of the samples were due to the varietal specifics and potential. That explained the lower rates for Pamid 5/76 (mean 20.14±0.51 g/l, V1 and mean 20.49±0.44 g/l, V2) and the higher ones for Kaylashki Rubin (mean 23.97±0.11 g/l, V1 and mean 23.97±0.73 g/l, V2). The differences between the variants of one variety were minimal, however, in all experimental wines V2 was characterized by a higher extract. That was also reflected in the tasting score from the organoleptic analysis of the wines. These variants, with the exception of Pamid 5/76 (2013, 2015), Gamza 52-9-4 (2014), Gamza 52-9-5 (2015) and Trapezitsa (2015) received more scores because of their better density and harmony in the taste (Table 3 a, b). The data showed that the samples from 2015 had the highest SFE rate. An exception was observed for Pamid 5/76 and Kaylashki Rubin, where higher rates were reported respectively in the variants from 2014 and 2013 vintage. During the study period it was found a relatively constant trend in the content of the indicator in the experimental wines. From the inter-varietal variants (2013, 2014), the highest SFE rates had Kaylashki Rubin samples, followed by both Gamza candidate-clones. From 2015 the highest SFE rates were found for Kaylashki Rubin and Trapezitsa (Table 3 a, b).

The titratable acidity of the experimental samples also varied within the typical range for red wines and corresponded to the specifics of the variety. Usually lower rates were observed in wines from Pamid 5/76 (mean 4.44±0.45 g/l, V1 and mean 4.28±0.20 g/l, V2) and Trapezitsa (mean 4.31±0.26 g/l, V1 and mean 4.76±0.47 g/l, V2). The titratable acids of the samples, 2013 harvest, were from 4.00 to 5.63 g/l, for 2014 - from 4.13 to 6.95 g/l, for 2015 - from 4.43 to 6.08 g/l (Table 3 a, b). The results did not show significant differences between the variants of the clones and varieties. In 2013, except Gamza 52-9-5, the samples with higher acidity were assessed with fewer points during the tasting. In 2014 the variants of Pamid 5/76, Gamza 52-9-5 and Kaylashki Rubin with lower acid content were evaluated with higher scores during the organoleptic analysis. That trend was also observed in all samples in 2015 (Table 3 a, b).

All wines had normal volatile acidity ranging from 0.42 to 0.66 g/l (2013), from 0.62 to 0.66 g/l (2014) and from 0.60 to 0.70 g/l (2015) that did not deteriorate their organoleptic properties (Table 3 a, b).

The content of TPC and anthocyanins were important indicators of the red wine composition influencing their organoleptic characteristics (Chobanova, 2012). Their rates were directly related and determined by the potential and specifics of the variety, thus they increased in the order Pamid 5/76 < Trapezitsa < Gamza 52-9-4 < Gamza 52-9-5 < Kaylashki Rubin (Table 3 a, b). The total phenolic compounds in the experimental samples ranged from 1.00 to 2.73 g/l (2013), from 0.98 to 2.33 g/l (2014) and from 0.94 to 2.28 g/l (2015). The amount of anthocyanins in the variants varied from 134.78 to 466.50 mg/l (2013), from 122.00 to 279.36 mg/l (2014) and from 117.43 to 288.25 mg/l (2015). The samples from 2013 vintage had the highest content of the analyzed indicators. No significant differences were found in their quantity between the variants of each variety. V2 wines contained more TPC and anthocyanins, but they did not always have better tasting qualities.

Table 3a. Chemical composition of the experimental wines from the studied clones.

Indicators / Wines	Variant	Vintage	Alcohol, vol. %	Total extract, g/l	Sugar, g/l	SFE, g/l	Titratable acids g/l	Volatile acids, g/l	pH	TPC, g/l	Anthocyanins, mg/l	Colour intensity, I [abs. units]	Colour tint, T [abs. units]	Tasting score
Pamid 5/76	1	2013	12.12	22.00	2.12	19.88	4.00	0.66	3.17	1.00	134.78	7.20	0.60	79.11
		2014	12.14	21.70	0.97	20.73	4.90	0.62	3.31	0.98	122.00	7.64	0.61	76.43
		2015	12.39	21.10	1.10	19.80	4.43	0.66	3.30	0.94	117.43	7.76	0.66	79.86
		mean ±SD	*12.22* *±0.15*	*21.60* *±0.46*	*1.40* *±0.63*	*20.14* *±0.51*	*4.44* *±0.45*	*0.65* *±0.02*	*3.26* *±0.08*	*0.97* *±0.03*	*124.74* *±8.99*	*7.53* *±0.29*	*0.62* *±0.03*	*78.47* *±1.80*
	2	2013	12.21	22.80	2.15	20.65	4.13	0.54	3.12	1.22	142.77	7.54	0.58	77.78
		2014	12.20	22.00	1.17	20.83	4.20	0.66	3.32	1.05	130.35	7.88	0.66	79.57
		2015	12.47	20.90	1.10	20.00	4.50	0.62	3.32	0.98	124.48	7.81	0.61	79.00
		mean ±SD	*12.29* *±0.15*	*21.90* *±0.95*	*1.47* *±0.59*	*20.49* *±0.44*	*4.28* *±0.20*	*0.61* *±0.06*	*3.25* *±0.11*	*1.08* *±0.12*	*132.53* *±9.34*	*7.74* *±0.18*	*0.62* *±0.04*	*78.78* *±0.91*
Gamza 52-9-4	1	2013	12.84	23.40	1.98	21.42	5.32	0.42	3.36	2.19	339.93	9.63	0.54	80.67
		2014	12.28	23.60	1.45	22.15	5.63	0.62	3.42	1.61	218.10	9.56	0.68	75.00
		2015	12.51	24.50	1.74	22.76	5.55	0.60	3.32	1.55	226.89	9.37	0.60	79.14
		mean ±SD	*12.54* *±0.28*	*23.83* *±0.58*	*1.72* *±0.26*	*22.11* *±0.67*	*5.50* *±0.16*	*0.55* *±0.11*	*3.37* *±0.05*	*1.78* *±0.35*	*261.64* *±67.94*	*9.52* *±0.13*	*0.61* *±0.07*	*78.27* *±2.93*
	2	2013	12.92	24.40	2.42	21.98	5.13	0.54	3.34	2.32	352.05	9.88	0.56	81.78
		2014	12.34	23.80	1.45	22.35	5.40	0.66	3.37	1.72	226.30	9.75	0.69	74.57
		2015	12.56	24.90	2.05	22.85	5.40	0.60	3.34	1.69	237.50	9.42	0.58	79.86
		mean ±SD	*12.60±* *0.29*	*24.37* *±0.55*	*1.97* *±0.49*	*22.39* *±0.44*	*5.31* *±0.15*	*0.60* *±0.06*	*3.35* *±0.02*	*1.91* *±0.35*	*271.95* *±69.59*	*9.68* *±0.24*	*0.61* *±0.07*	*78.74* *±3.73*
Gamza 52-9-5	1	2013	13.08	23.20	2.25	20.95	5.10	0.54	3.33	2.33	358.18	10.05	0.52	76.44
		2014	12.32	23.20	1.00	22.20	5.55	0.64	3.39	1.70	224.00	9.71	0.69	76.14
		2015	12.55	24.64	1.27	23.37	5.18	0.62	3.28	1.68	234.47	9.45	0.60	77.57
		mean ±SD	*12.65* *±0.39*	*23.68* *±0.81*	*1.51* *±0.66*	*22.17* *±1.21*	*5.28* *±0.24*	*0.60* *±0.05*	*3.33* *±0.05*	*1.90* *±0.37*	*272.22* *±74.63*	*9.74* *±0.30*	*0.60* *±0.08*	*76.72* *±0.75*
	2	2013	13.15	24.10	2.15	21.95	5.63	0.42	3.36	2.30	345.17	9.74	0.56	83.00
		2014	12.27	23.40	1.14	22.26	5.33	0.62	3.39	1.76	228.20	9.73	0.68	78.29
		2015	12.48	25.21	1.71	23.50	5.33	0.60	3.33	1.71	248.32	9.72	0.61	77.29
		mean ±SD	*12.63* *±0.46*	*24.23* *±0.91*	*1.67* *±0.51*	*22.57* *±0.82*	*5.43* *±0.17*	*0.51* *±0.10*	*3.36* *±0.03*	*1.92* *±0.33*	*273.90* *±62.54*	*9.73* *±0.01*	*0.62* *±0.06*	*79.53* *±3.05*

Table 3b. Chemical composition of the experimental wines from the studied varieties.

Indicators / Wines	Variant	Vintage	Alcohol, vol. %	Total extract, g/l	Sugar, g/l	SFE, g/l	Titratable acids g/l	Volatile acids, g/l	pH	TPC, g/l	Anthocyanins, mg/l	Colour intensity, I [abs. units]	Colour tint, T [abs. units]	Tasting score
Kaylashki Rubin	1	2013	14.24	27.60	3.54	24.06	5.54	0.66	3.36	2.72	456.52	11.16	0.62	85.00
		2014	12.66	25.80	1.95	23.85	6.95	0.64	3.14	2.28	273.22	10.12	0.60	75.43
		2015	12.68	25.56	1.55	24.01	6.08	0.66	3.11	2.26	288.25	10.15	0.64	82.00
		mean ±SD	*13.19 ±0.91*	*26.32 ±1.11*	*2.35 ±1.05*	*23.97 ±0.11*	*6.19 ±0.71*	*0.65 ±0.01*	*3.20 ±0.14*	*2.42 ±0.26*	*339.33 ±101.77*	*10.48 ±0.59*	*0.62 ±0.02*	*80.81 ±4.89*
	2	2013	14.00	27.60	3.00	24.60	5.00	0.66	3.35	2.73	466.50	11.37	0.60	85.89
		2014	12.72	26.20	2.05	24.15	6.80	0.66	3.15	2.33	279.36	10.17	0.67	76.71
		2015	12.74	25.80	1.63	24.17	6.00	0.68	3.15	2.28	285.93	10.10	0.63	83.14
		mean ±SD	*13.15 ±0.73*	*26.53 ±0.95*	*2.23 ±0.70*	*23.97 ±0.73*	*5.93 ±0.90*	*0.67 ±0.01*	*3.22 ±0.11*	*2.45 ±0.25*	*343.93 ±106.20*	*10.55 ±0.71*	*0.63 ±0.03*	*81.91 ±4.71*
Trapezitsa	1	2013	-	-	-	-	-	-	-	-	-	-	-	-
		2014	12.48	22.40	1.10	21.30	4.13	0.66	3.27	1.68	220.14	9.66	0.65	75.43
		2015	12.60	24.90	1.27	23.63	4.50	0.66	3.23	1.77	250.10	9.88	0.62	80.14
		mean ±SD	*12.54 ±0.08*	*23.65 ±1.77*	*1.18 ±0.12*	*22.46 ±1.65*	*4.31 ±0.26*	*0.65 ±0*	*3.25 ±0.03*	*1.72 ±0.06*	*235.12 ±21.18*	*9.77 ±0.15*	*0.63 ±0.02*	*77.78 ±3.33*
	2	2013	-	-	-	-	-	-	-	-	-	-	-	-
		2014	12.52	23.40	1.61	21.79	4.43	0.62	3.31	1.65	222.10	9.70	0.67	76.71
		2015	12.64	25.40	1.64	23.76	5.10	0.70	3.24	1.85	256.74	9.93	0.60	79.43
		mean ±SD	*12.58 ±0.08*	*25.40 ±1.41*	*1.62 ±0.02*	*22.77 ±1.39*	*4.76 ±0.47*	*0.66 ±0.06*	*3.27 ±0.05*	*1.75 ±0.14*	*239.42 ±24.49*	*9.81 ±0.16*	*0.63 ±0.05*	*78.07 ±1.92*

The TPC content in wine influenced its taste indicators, and the amount of anthocyanins determined the colour characteristics (Chobanova, 2012; Kekelidze *et al.*, 2014). However, the experimental data obtained did not show correlation with the scores from the organoleptic analysis (Table 3 a, b).

The colour properties of red wines were greatly determined by the concentration of TPC and anthocyanins (Chobanova, 2012; Bai *et al.*, 2013). The wines from the variants containing more anthocyanins had higher rates of intensity and accordingly the colour indices were rated higher when tasting. In accordance with the varietal specifics, the variants of Pamid 5/76 had the lowest colour intensity (mean 7.53±0.29 [abs. units], V1 and mean 7.74±0.18 [abs. units], V2) and the highest – those of Kaylashki Rubin (mean 10.48±0.59 [abs. units], V1 and mean 10.55±0.71 [abs. units], V2). The colour tint indicated the level of evolution of the red colour and its values in the experimental samples ranged from 0.52 to 0.62 [abs. units] (2013), from 0.61 to 0.69 [abs. units] (2014) and from 0.58 to 0.66 [abs. units] (2015), which is within the normal range for young red wines (Table 3 a, b).

3.4. Wine aromatic composition

Data on the aromatic composition of the experimental red wines during the study period are presented in Table 4. The content of total esters, total aldehydes and total higher alcohols was analyzed in all samples.

Table 4. Aromatic composition of the experimental wines from the studied clones and varieties.

Indicators / Wines	Variant	Vintage	Total esters, mg/l	Total aldehydes, mg/l	Total higher alcohols, mg/l
Pamid 5/76	1	2013	176.00	93.00	419.50
		2014	140.80	17.60	378.00
		2015	246.40	33.00	499.00
		mean±SD	*187.73 ±53.77*	*47.87 ±39.84*	*432.17 ±61.48*
	2	2013	193.60	114.00	382.50
		2014	158.40	11.00	342.00
		2015	264.00	22.00	520.00
		mean±SD	*205.33 ±53.77*	*49.00 ±56.56*	*414.83 ±93.30*
Gamza 52-9-4	1	2013	88.00	90.82	455.00
		2014	105.60	35.20	329.00
		2015	264.00	26.40	526.00
		mean±SD	*152.53 ±96.93*	*50.81 ±34.93*	*436.67 ±99.77*
	2	2013	105.60	74.00	436.00
		2014	123.20	35.20	353.00
		2015	316.80	35.20	558.00
		mean±SD	*181.86 ±117.18*	*48.13 ±22.40*	*449.00 ±103.12*
Gamza 52-9-5	1	2013	70.40	73.80	508.50
		2014	105.60	33.00	326.00
		2015	264.00	52.80	550.00
		mean±SD	*146.67 ±103.13*	*53.20 ±20.40*	*461.50 ±119.17*
	2	2013	88.00	101.40	448.50
		2014	140.60	74.80	328.00
		2015	299.20	35.20	470.00
		mean±SD	*175.93 ±109.94*	*70.47 ±33.31*	*415.50 ±76.53*

Kaylashki Rubin	1	2013	140.80	81.10	466.50
		2014	123.20	48.40	374.00
		2015	228.80	46.20	494.00
		mean±SD	*164.27 ±56.57*	*58.57 ±19.55*	*444.83 ±62.86*
	2	2013	176.00	80.00	531.00
		2014	158.40	39.60	432.00
		2015	228.80	37.40	556.00
		mean±SD	*187.73 ±36.64*	*52.33 ±23.98*	*506.33 ±65.58*
Trapezitsa	1	2013	-	-	-
		2014	158.00	22.00	345.00
		2015	176.00	26.40	500.00
		mean±SD	*167.00 ±12.73*	*24.20 ±3.11*	*422.50 ±109.60*
	2	2013	-	-	-
		2014	176.00	26.40	376.00
		2015	193.60	17.60	474.00
		mean±SD	*184.80 ±12.45*	*22.00 ±6.22*	*425.00 ±69.30*

The esters in wine were formed by chemical reaction as a result of esterification processes occurring during the grapes ripening and wine aging or biologically from the yeast during the alcoholic fermentation (Chobanova, 2012; Antalick et al., 2015). During the study period, no strict correlation was found in the amount of total esters in the experimental samples per varieties and vintages. In 2013, the ester content varied in the range from 70.40 to 193.60 mg/l, as it was the lowest in Gamza 52-9-5 (V1) and the highest in Pamid 5/76 (V2). In 2014, their rates ranged from 105.60 (Gamza 52-9-4, V1 and Gamza 52-9-5, V1) to 176.00 mg/l (Trapezitsa, V2). The 2015 samples were distinguished for the highest ratio of esters ranging from 176.00 (Trapezitsa, V1) to 316.80 mg/l (Gamza 52-9-4, V2). An increase in their amount in V2 compared to the control (V1<V2) was also observed due to the application of the aroma-releasing enzyme. Of all V2 with the highest ester content were Pamid 5/76 (mean 205.33±53.77 mg/l) and Kaylashki Rubin (mean 187.73±36.64 mg/l). These results had confirmed the positive effect of the enzyme on the ester content of the wines and their aromatic characteristics.

Aldehydes were found in very small quantities in grapes. In wine they appeared (mainly acetaldehyde) along several major pathways – an intermediate reaction and normal product of the alcoholic fermentation, enzymatic oxidation of alcohols by yeast, non-enzymatic oxidation of alcohols by the action of the oxygen from the air, oxidative deamidation of amino acids, and decarboxylation of keto acids (Chobanova, 2012).

The total aldehydes content in the experimental wines was below 100 mg/l and it did not adversely affect their organoleptic characteristics. The concentration ranged from 73.80 to 114.00 mg/l (2013), from 11.00 to 74.80 mg/l (2014) and from 17.60 to 52.80 mg/l (2015). The samples of Trapezitsa variety had the lowest concentrations during the study period (mean 24.20±3.11 mg/l, V1 and mean 20.00±6.22 mg/l, V2). Differences were observed within the variants of the varieties, but from different vintages. The 2013 samples contained the most total aldehydes. In 2013, Pamid 5/76, V2 and Gamza 52-9-5, V2 contained the most aldehydes. In 2014, V1 of Pamid 5/76 and Kaylashki Rubin had higher aldehyde content, while in Gamza 52-9-5 and Trapezitsa it was V2. In Gamza 52-9-4, V1 and V2 had similar rates. In 2015, a greater amount of aldehydes was analyzed in V1 of Pamid 5/76, Gamza 52-9-5, Kaylashki Rubin and Trapezitsa. The data obtained and their analysis did not reveal a correlation between the influence of the applied enzyme on the aldehydes ratio.

The major part of higher alcohols was formed during the alcoholic fermentation by the degradation of sugars or amino acids, and a small part of them during the aging of wines (Chobanova, 2012; Mina and Tsaltas, 2017; Manolache *et al.*, 2018). The samples from the studied harvests were characterized by high content of higher alcohols, ranging from 382.50 to 531.00 mg/l (2013), from 326.00 to 432.00 mg/l (2014) and from 470.00 to 558.00 mg/l (2015). It was the lowest in wines from 2014 vintage and the highest in the wines from 2015. In 2013, the smallest amount was recorded in the samples of Pamid 5/76 variety, and the highest in the samples from Kaylashki Rubin. Their ratios in the wines from both Gamza candidate-clones were close. In the studied red wines, with the exception of Kaylashki Rubin, the content of total higher alcohols was higher in the control. In 2014, the amount of higher alcohols in the samples from both Gamza candidate-clones was similar and the lowest, and the highest was in the variants of Kaylashki Rubin. With the exception of Pamid 5/76, their ratio was lower in the control. The wines of 2015 vintage had similar higher alcohol content, as with Pamid 5/76, Gamza 52-9-4 and Kaylashki Rubin the control V1 had a lower rate.

The results from the analysis of the wine aromatic composition did not show a strict correlation between the studied components and the organoleptic profile, respectively the tasting evaluation (Table 3 a, b, Table 4). With Pamid 5/76, V1 (2015) was the best evaluated. It had high rates of esters and higher alcohols and low of aldehydes. From the wines of both Gamza candidate-clones 52-9-4 and 52-9-5 with the best organoleptic characteristics were determined the samples from V2 (2013), despite the low concentration of esters and the high rates of higher alcohols. From Kaylashki Rubin variants, V2 (2013), which had high rates of esters, aldehydes and higher alcohols, received the most points (85.89 points). From Trapezitsa, V1 (2015), having higher rates of esters and higher alcohols, was rated the highest (80.14 points).

4. Conclusions

On the basis of the obtained results it could be summarized:

- Under the growing conditions in the region of Pleven, the studied red clones and varieties reached their technological maturity in the middle and second half of September. The latest ripening variety was Kaylashki Rubin that had the most gradual sugar accumulation and reduction of acidity.
- The lowest sugar accumulation and titratable acids were reported for Pamid 5/76 and the highest for Kaylashki Rubin. Grapes from 2013 vintage had the highest sugar content due to favorable weather conditions during the ripening phase, and GAI rates, which showed that it was suitable for the production of wines of optimum quality in terms of chemical composition and tasting features.
- The experimental wines had different composition and organoleptic characteristics depending on the potential and the specifics of the variety and the vintage.
- The highest alcohol content had Kaylashki Rubin wines, followed by the wines of both candidate-clones of Gamza, and the lowest – Pamid 5/76 samples.
- Pamid 5/76 samples had the lowest SFE rates and Kaylashki Rubin samples had the highest. The experimental wines from V2 were characterized by a higher extract and better organoleptic taste. The highest SFE rates were found in 2015 harvest samples.
- The amount of TPC and anthocyanins in the experimental wines increased in the order Pamid 5/76 < Trapezitsa < Gamza 52-9-4 < Gamza 52-9-5 < Kaylashki Rubin. The 2013 vintage samples had the highest rates. The wines from V2 of the studied varieties contained more TPC, anthocyanins and colour intensity, but not always better tasting qualities.

- The positive effect of the aroma-releasing enzyme applied on the content of esters in wines and their aromatic characteristics was confirmed. The 2015 samples had the highest ratio of total esters. It was also observed an increase in their amount in V2 compared to the control (V1 <V2).
- No correlation was found on the effect of the applied enzyme on the total aldehydes amount. The lowest concentrations were analyzed in Trapezitsa wines. In all varieties, 2013 vintage samples contained the most aldehydes, with the highest rates found in Pamid 5/76 variants.
- The samples from the studied vintages were characterized by a high concentration of higher alcohols, the lowest being in wines, 2014 harvest, and the highest in the samples, 2015 harvest. No correlation was found between the tested components of the aromatic composition of the wines and their tasting evaluation.
- No strict correlation was observed between the investigated components of the aromatic composition of the experimental wines and their organoleptic profile, respectively, their tasting evaluation.

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