

Comparative oenological characteristics of wines from seedless coloured hybrid forms and vine varieties (*Vitis vinifera* L.)

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Abstract

Roychev, V., Spasov, H., Tzanova, M., Keranova, N. & Dimitrov, D. (2024). Comparative oenological characteristics of wines from seedless coloured hybrid forms and vine varieties (*Vitis vinifera* L.). *Bulg. J. Agric. Sci.*, 30(1), 151–162

A comparative technological study of wines from four seedless coloured hybrid vine forms – 30/2, 30/3, 30/10, 31/9 and the varieties – Syrah clone 174, Sangiovese, Marselan and Regent. It was found that on the content of trans-resveratrol, quercetin and antioxidant activity of wine, all studied hybrid forms are superior to Sangiovese and inferior to Syrah-clone 174. With a high degree of balance and stability of environmental conditions of the varieties are Syrah-clone 174, Marselan and Regent, and of the hybrid forms – 30/10 and 30/2. Various representatives of the volatile aromatic composition have been identified in the studied wines, belonging to the groups of higher alcohols, esters and terpenes, acetaldehyde and methanol. The total quantitative presence of volatile compounds is higher in the wines of the hybrid forms compared to those of the control varieties. The content of sugars and acids in grapes, and alcohol and sugars in wine in hybrid forms and varieties does not differ significantly. The amounts of extract, titratable acids, anthocyanins are higher in hybrid forms. The highest amount of total phenols is observed in the wine of 30/10 – 3295.66 mg/dm³, and the lowest in Sangiovese – 2060.91 mg/dm³. With the highest organoleptic evaluation is the wine from Syrah clone 174 – 86.0, and from the seedless coloured hybrid forms are 30/10 – 83.0 and 30/3 – 80.0. It is possible through staged sexual hybridization to create seedless coloured varieties suitable for the production of quality wines, characterized by high taste characteristics, nutritional and medicinal value.

Keywords: seedless coloured hybrid forms; vine varieties; wine; comparative technological; ecological and tasting characteristics

Introduction

There are no seedless coloured varieties in the literature that are suitable for making quality wines. The colored juice of their berries is an important economic feature that significantly increases their nutritional and medicinal value. It is known that the content of phenolic compounds directly affects the aging, physicochemical stability and antioxidant properties of wine. The proven positive effect of resveratrol

and quercetin on the antioxidant activity of grapes, wine and raisins implies priority creation and cultivation of wine and dessert grape varieties with maximum content of these compounds (Kopp, 1998; Kennedy et al., 2000, 2000; Bhat et al., 2001, 2001; Sato et al., 2002; Cai et al., 2003; Karioti et al., 2004; Anli et al., 2006; Puja et al., 2007; Kennedy, 2008; Sharma & Bhat, 2009; Roychev et al., 2020; Naiker et al., 2020; Fia et al., 2021). Due to its great economic importance, seedlessness in vines continues to be the sub-

ject of various studies (Mejía et al., 2011; Karaagac et al., 2012; Nosulchak et al., 2021). In the selection of the vine there are results of hybridological analysis and evaluation of seedlings of seedless coloured vine varieties (Roychev, 2014; 2014; 2015). The working hypothesis is that within the species *Vitis vinifera* L. ssp. *sativa* DC. through staged sexual hybridization, genotypes can be created to combine seedlessness with coloured grape juice and musket taste, and at the next stage, after crosses with interspecific hybrids, and with resistance to low winter temperatures and the most economically important grape diseases. The varieties Alicante Bouschet and Grand Noir transmit up to 50% of the colour of the berry juice in the F_1 generation, regardless of whether the other parent variety has white, red or black coloured berries (Valchev, 1990). Milutinovic et al. (2000) determined monohybrid inheritance of skin colour and berry juice in F_1 generation in the cross Evita x Beogradska Besemena, but the expected full combination of the desired traits – seedlessness, coloured juice, high yield and quality of grapes – was not obtained in any of the seedlings. The aim of this study is to analyze the technological characteristics of wines obtained from grapes of newly created seedless hybrid forms with coloured grape juice.

Material and Methods

The experimental work includes wines from three vintages of four selected elite seedless hybrid forms with coloured red grape juice – 30/2, 30/3, 30/10 of the F_1 generation of crosses between seed coloured and seedless grape variety (Alicante Bouschet x Beauty Seedless), 31/9 (Alicante Bouschet x Kondarev 10) (Figure 1) and for comparison – control samples, the famous varieties Syrah clone 174, Sangiovese, Marselan and Regent (interspecific hybrid). Seedlings, in the grains of which the seed buds (rudiments) are not felt during tasting, are considered seedless. The two groups of vines are grafted on the rootstock of Berlandieri x Riparia SO 4 and are grown on the Moser arrangement in the ampelographic assortment of the Department of Viticulture, Agricultural University-Plovdiv. Experimental wines of all hybrid forms and varieties are prepared according to the classic technology for red wines under micro-winemaking conditions in the Training and Experimental Wine Cellar.

For the quantification of the phytoalexins trans-resveratrol (t-RVT) and quercetin (QU) in wine, the method of Tzanova & Peeva (2018) was applied: immediately before the analysis, the samples were filtered through a membrane

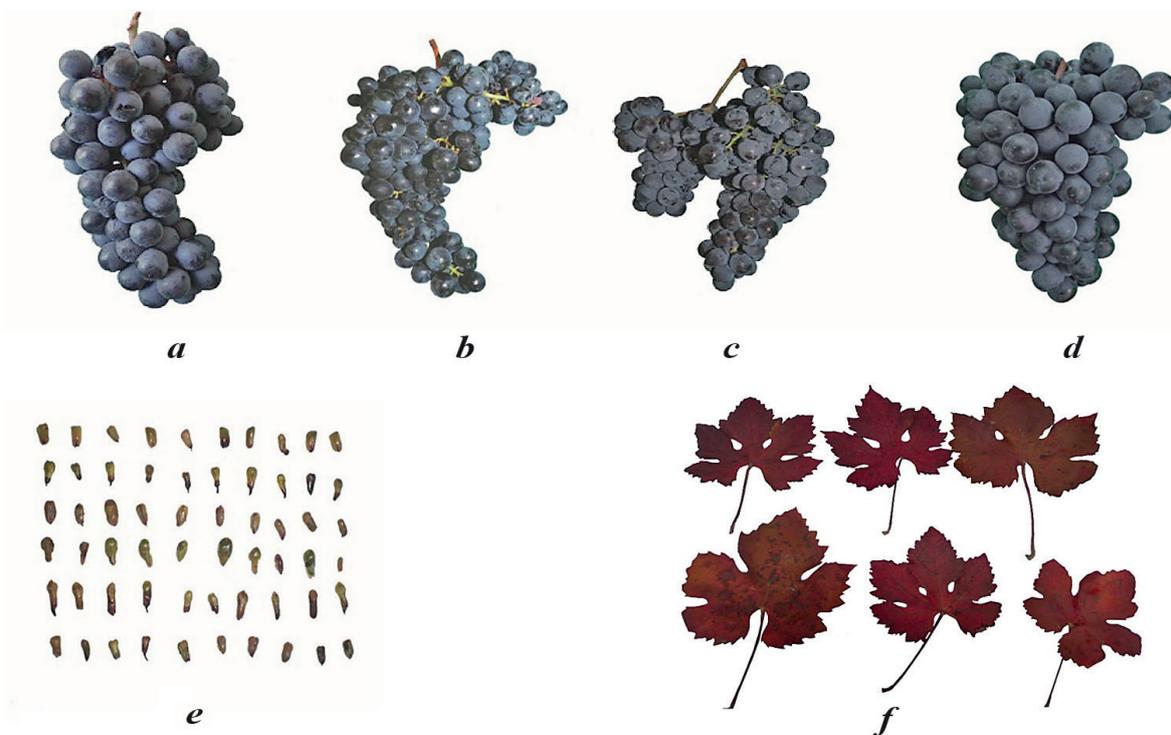


Fig. 1. Cluster (a – 30/2; b – 30/3; c – 30/10; d – 31/9); e – seed buds (rudiments); f – leaves of the studied seedless coloured hybrid vine forms

filter with a pore size of 45 μm . A small amount of each sample was placed in the autosampler of the HPLC system. Calibration graphs were constructed in advance at five points (0.05; 0.50; 1.0; 2.0 and 5.0 mg/l) using reference materials – trans-resveratrol with purity of HPLC – min. 99% and quercetin with purity of HPLC – min. 98%. An excellent linear dependence was achieved with a regression coefficient, $r^2 = 0.9992$ for RVT and $r^2 = 0.9998$ for QU. HPLC is a Thermo system that includes a Hypersil Gold C18 column (5 μm , 150 mm \times 4.6 mm); pump – Surveyor LC Pump Plus; autosampler – Surveyor Autosampler Plus; detector – Surveyor PDA Plus. Chromatograms were recorded at 306 nm in a 7-min single cycle, achieving retention times of approximately 4.7 minutes for trans-resveratrol and 5.6 minutes for quercetin. The results are expressed in mg/l of wine.

Antioxidant activity (TE) in wine was established by determining the radical scavenging capacity by the DPPH (1,1'-diphenyl-2-picrylhydrazyl-radical) method. A methodology for measuring the potential described by Tzanova et al. (2019; 2020) was applied. To 3.9 ml of a 100 μM solution of DPPH in methanol, 0.1 ml of a sample pre-filtered through a 45 μm membrane filter was added. Three parallel samples were analyzed. The results of their radical scavenging capacity were compared and calculated by regression analysis of the linear relationship between the concentration of Trolox (water-soluble vitamin E analogue) and the measured absorbance at 517 nm on a Thermo Scientific Evolution 300 spectrophotometer. The results are expressed as mmol of Trolox equivalent (TE) in 1 l of wine.

The comparative analysis between the content of trans-resveratrol (t-RVT), quercetin (QU) and antioxidant activity (TE) in wine of coloured hybrid forms and varieties was performed by a one-way analysis of variance and LSD-test at a statistical confidence level of 0.05. Correlation and Path analysis were used to establish the relationships between these three components. The coefficient of determination was also calculated. Trend lines were built, visualizing the change of the respective indicators. Regression equations were compiled, presenting the studied dependencies in analytical form. The statistical software IBM SPSS 23 and MS Excel were used for mathematical data processing. The ecological stability of all hybrid forms and varieties in the conditions of the experiment was established by determining the eco coefficient, conditionally expressing their reaction to the genotype-environment interaction (Wricke, 1962; 1966).

The content of the main volatile aromatic compounds in the wine of each hybrid form and variety was determined by GC-FID based on stock standard solution – IS 3752:2005. The

standard solution in the present study included the following compounds (purity > 99.0%): acetaldehyde, ethyl acetate, methanol, isopropyl acetate, 1-propanol, 2-butanol, propyl acetate, 2-methyl-propanol, isobutanol, 1-butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl isovalerate, 1-pentanol, pentyl acetate, 1-hexanol, ethylhexanoate, hexyl acetate, 1-heptanol, linalool oxide, phenylacetate, ethyl caprylate, α -terpineol, nerol, β -citronellol, geraniol. 1-octanol was used as an internal standard. The resulting standard solution containing all compounds was injected in an amount of 2 μl into a Varian 3900 gas chromatograph (Varian Analytical Instruments, Walnut Creek, California, USA) with a VF max MS capillary column (30m, 0.25mm ID, DF = 0.25 μm), equipped with flame ionization detector (FID). The carrier gas was He. The parameters of the gas chromatographic determination were: temperature of the injector – 220°C, temperature of the detector – 250°C, initial temperature of the furnace – 35°C/ret. 1 min, increase to 55°C with a step of 2°C/min for 11 min, rise to 230°C with a step of 15°C/min for 3 min. Total chromatography time – 25.67 min. The retention time of the individual compounds in the standard solution was determined. The identification and quantification of volatile compounds was based on the injection of 2 μl of wine distillate into the gas chromatograph. The LSD Post Hoc Test was applied to evaluate the differences in comparative evaluation between the studied seedless coloured hybrid forms and the varieties Syrah clone 174 and Sangiovese, according to the content of higher alcohols, esters and terpenes in wines at statistical reliability of $\alpha = 0.5$. A correlation analysis was performed to prove the existence of relationships between the studied indicators using Pearson's correlation coefficients. Mathematical data processing was performed using the statistical software product IBM SPSS 23 (Aldrich & Conningham, 2016).

During the period 2018–2020, physico-chemical analyzes of grapes and wines in the conditions of micro-wine-making were performed, obtained from the studied seedless coloured hybrid forms and vine varieties. A commission of seven oenologists from the Department of Wine and Beer Technology at the University of Food Technology-Plovdiv tasted wines from the hybrid forms and varieties Syrah clone 174 and Sangiovese, 2020 vintage. Descriptive methods for organoleptic analysis were applied – scoring scale method (100-point scale) and the method of the main characteristics, as the evaluation was based on positivism. A terminological description of the sensory profile of the experimental wines was also made. Scores were set on a 100-point scale and ranked by preference. The aromatic and taste characteristics of the wines were presented separately.

Results and Discussion

The content of trans-resveratrol (t-RVT) in the wine of all studied seedless coloured hybrid forms varies within a narrow range of 2.490 mg/l (30/3) – 2.862 mg/l (30/2) and differs significantly from Sangiovese, in which in the conditions of the experiment it is many times lower – 0.649 mg/l and from Syrah clone 174 – 6.491 mg/l, characterized by the highest value of this indicator (Table 1).

Table 1. Comparative evaluation of the values of the studied indicators in wine from seedless coloured hybrid forms and vine varieties

Variety Hybrid form	t-RVT, mg/l	Syrah	San-giovese	Marselan	Regent
		6.491	0.649	2.465	1.977
30/2	2.862	*	*	n.s.	n.s.
30/3	2.490	*	*	n.s.	n.s.
30/10	2.686	*	*	n.s.	n.s.
31/9	2.507	*	*	n.s.	n.s.
SS	72.362				
F-Test	8.992				
Sign.	0.000				
	QU, mg/l	7.634	0.739	2.065	2.263
30/2	2,357	*	*	n.s.	n.s.
30/3	4,300	*	*	*	*
30/10	2,956	*	*	*	*
31/9	3,137	*	*	*	*
SS	107,818				
F-Test	11,245				
Sign.	0,000				
	TE, mmol/l	0.840	0.333	0.656	0.594
30/2	0,625	n.s.	*	n.s.	n.s.
30/3	0,657	n.s.	*	n.s.	n.s.
30/10	0,668	n.s.	*	n.s.	n.s.
31/9	0,665	n.s.	*	n.s.	n.s.
SS	0,701				
F-Test	3,277				
Sign.	0,023				

There are no statistically proven differences in the levels of this phytoalexin with the Marselan and Regent varieties. Sangiovese stands out as a variety with a very low content of quercetin (QU) in wine – 0.739 mg/l, and Syrah clone 174 – 7.634 mg/l, statistically proven to exceed twice its amount compared to all studied hybrid forms. Marselan and Regent have no proven differences with only 30/2, which has a minimum QU content of 2.357 mg/l. All other hybrid forms are statistically superior to these two varieties in the amount of quercetin. The weakest antioxidant activity (TE) of the wine is in Sangiovese

– 0.333 mmol/l, as a result of which statistical differences were proved with all hybrid forms, in which it is up to twice as high from 0.625 mmol/l – 30/2 to 0.668 mmol/l – 30/10. The other varieties are characterized by higher antioxidant activity: Syrah clone 174 – 0.840 mmol/l, Marselan – 0.656 mmol/l and Regent – 0.594 mmol/l, which determines the lack of statistically proven differences between them and the hybrid forms. There is a strong correlative positive relationship between the content of t-RVT and TE in the studied two groups of vines ($R = 0.844^{**}$) and a significant positive relationship between QU and TE ($R = 0.777^{**}$). Depending on the values of the coefficients of determination, 71% of the change in antioxidant activity in wines is explained by changes in the content of trans-resveratrol and 60% – of quercetin. These data reflect the independent effect of each factor on antioxidant activity, which is why the amount exceeds 100%. The existing relationship between the studied indicators is expressed by the composite regression equations (Figure 2, Figure 3).

The positive sign of the coefficient in front of the independent variable proves the existence of a direct one-way relationship between them and their positive effect on the level of antioxidant activity. Both mathematical models can be used to predict the antioxidant activity of wines under conditions close to

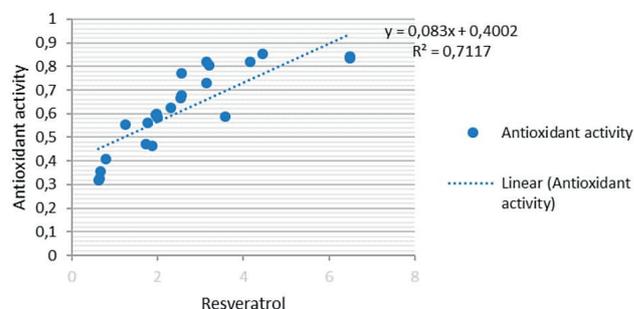


Fig. 2. Influence of resveratrol (RVT) content on the antioxidant activity (TE) of the wine in the studied seedless coloured hybrid forms and vine varieties

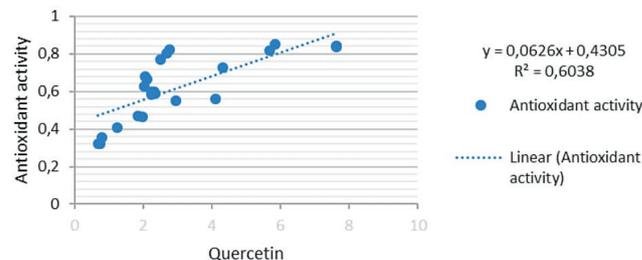


Fig. 3 Influence of quercetin (QU) content on the antioxidant activity (TE) of the wine from the studied seedless coloured hybrid forms and vine varieties

those in this study. According to the visualized complex effect of the amount of trans-resveratrol (x_1) and quercetin (x_2) on the antioxidant activity (y) of the experimental wines, expressed by the calculated coefficients of direct and indirect impact, the

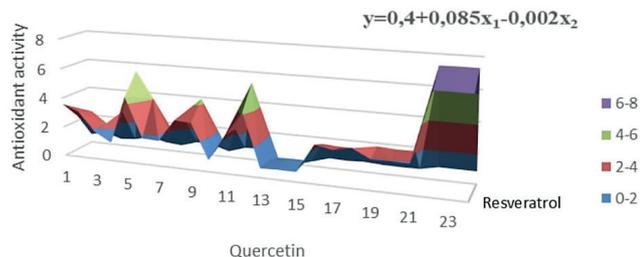


Fig. 4 Complex influence of the content of trans-resveratrol (t-RVT) and quercetin (QU) on the antioxidant activity (TE) of the wine from the studied seedless coloured hybrid forms and vine varieties

Table 2. Comparative evaluation of the studied indicators in wine from seedless coloured hybrid forms and vine varieties according to the resistance to environmental condition

Variety Hybrid form	W-Eco Coeff. t-RVT, mg/l	Syrah	San-giovese	Marselan	Regent
		0.091	0.082	0.153	0.090
30/2	0.777	n.s.	n.s.	n.s.	n.s.
30/3	1.331	*	*	*	*
30/10	0.170	n.s.	n.s.	n.s.	n.s.
31/9	1.462	*	*	*	*
SS	14.173				
F-Test	2.420				
Sign.	0.018				
W-Eco Coeff. QU, mg/l		0.157	0.141	0.183	0.185
30/2	0.333	n.s.	n.s.	n.s.	n.s.
30/3	0.998	n.s.	n.s.	n.s.	n.s.
30/10	0.468	n.s.	n.s.	n.s.	n.s.
31/9	2.317	*	*	*	*
SS	22.052				
F-Test	2.651				
Sign.	0.050				
W-Eco Coeff. TE, mmol/l		0.124	0.112	0.168	0.138
30/2	0.555	n.s.	n.s.	n.s.	n.s.
30/3	1.164	n.s.	n.s.	n.s.	n.s.
30/10	0.319	n.s.	n.s.	n.s.	n.s.
31/9	1.889	*	*	n.s.	*
SS	0.002				
F-Test	1.369				
Sign.	0.028				

first can be considered to have a stronger direct effect and the second – a stronger indirect effect (Figure 4).

The hybrid forms 30/2 and 30/10 have no statistically proven differences with any of the control varieties in terms of the change in trans-resveratrol content in the wine as a result of environmental influences, while in 30/3 and 31/9 the opposite is observed (Table 2).

In 30/2, 30/3 and 30/10 there are no differences with the varieties included in the study in terms of the amount of quercetin in the wine and the antioxidant activity. The most dependent on external influences is 31/9, which is characterized by high instability in the level of quercetin and antioxidant activity (except Marselan) and should not be grown in micro-regions with characteristic frequent climate change. According to the ecological assessment, the hybrid form 30/10 is relatively resistant to environmental factors and the change in the amount of trans-resveratrol, and of the varieties – Syrah clone 174 (Figure 5). In terms of quercetin content, the most stable is 30/2, and all varieties show resistance, with the highest level of quercetin being Syrah clone 174 (Figure 6). Sangiovese has the lowest stability of antioxidant activity compared to all hybrid forms and varieties (Figure 7). The hybrid form 31/9 is the most dependent on external conditions. With a high degree of balance in terms of antioxidant activity-environmental resistance of the varieties are Syrah clone 174, Marselan and Regent, and of the hybrid forms – 30/10 and 30/2.

In the wine of the hybrid form 30/2 3 higher alcohols, 4 esters, 2 terpenes, 1 aldehyde and methanol were identified; in 30/3 – the volatile fraction is represented by 3 higher alcohols, 3 esters, 1 terpene, 1 aldehyde and methanol – in this wine the lowest individual variety of volatile compounds was found, compared to all other studied variants; in 30/10 –

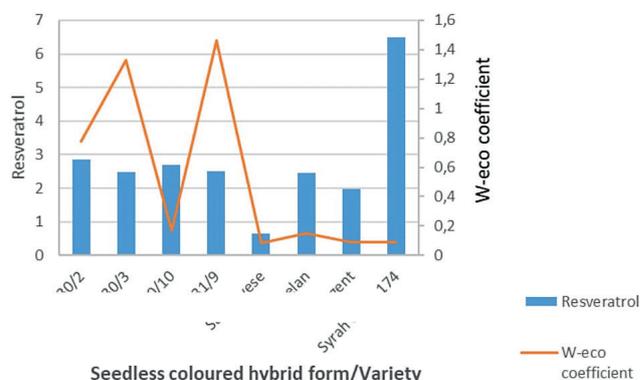


Fig. 5. Stability of the resveratrol content in the wine of the studied seedless coloured hybrid forms and vine varieties

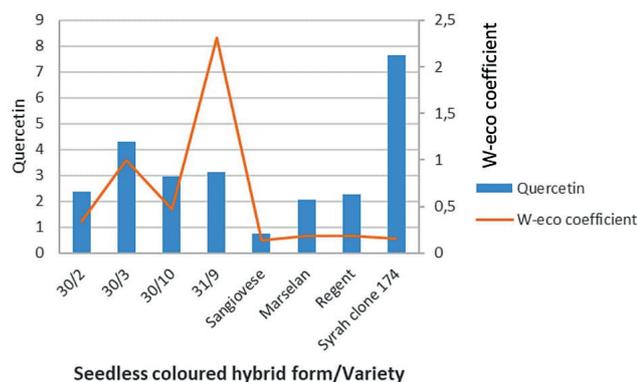


Fig. 6. Stability of the quercetin content in the wine of the studied seedless coloured hybrid forms and vine varieties

3 higher alcohols, 5 esters, 3 terpenes, 1 aldehyde and methanol; in 31/9 – 3 higher alcohols, 4 esters, 3 terpenes, 1 aldehyde and methanol (Table 3). A different number of volatile compounds were found in the control varieties, and in the wine from Syrah clone 174 they are a total of 9 – 2 higher alcohols, 5 esters, 2 terpenes, 1 aldehyde and methanol; in

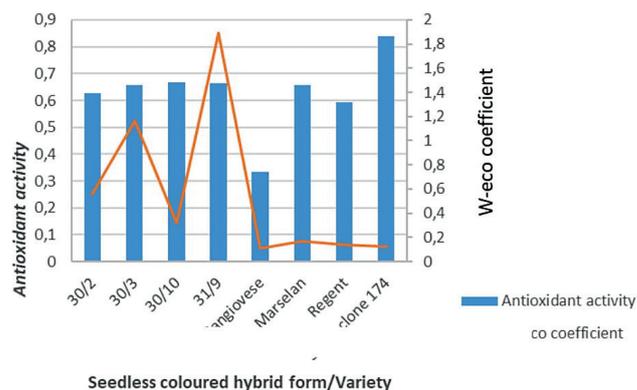


Fig. 7. Stability of antioxidant activity in the wine of the studied seedless coloured hybrid forms and vine varieties

Sangiovese, a higher total number of volatile compounds was identified – 11, of which 3 higher alcohols, 5 esters, 3 terpenes, 1 aldehyde and methanol. The average total content of the found volatile aromatic compounds in the wines of all seedless coloured hybrid forms is higher than that of the two control varieties. It should be noted that in 30/3 the

Table 3. Comparative evaluation of the content of the main volatile aromatic compounds in the wine of the studied seedless coloured hybrid forms and vine varieties (mg/dm³)

Name	30/2			30/3			30/10			31/9			Syrah	Sangiovese
	Average value	Syrah	Sangiovese											
Higher alcohols														
2-methyl-1-butanol	38.71	*	n.s.	55.45	*	*	56.27	*	*	41.53	*	n.s.	27.53	40.14
3-methyl-1-butanol	85.21	*	n.s.	110.26	*	*	108.58	*	*	94.08	*	n.s.	62.10	83.33
1-propanol	10.36	*	*	2.62	*	*	5.12	*	*	2.34	*	n.s.	0.00	0.05
Total higher alcohols	134.28			168.33			169.97			137.95			89.63	123.52
Esters														
Ethyl acetate	33.08	*	n.s.	56.15	n.s.	*	25.03	*	n.s.	36.40	*	n.s.	60.77	27.99
Propyl acetate	31.82	*	n.s.	29.81	*	n.s.	33.38	*	*	33.10	*	*	15.27	26.61
Pentyl acetate	0.00	*	*	0.00	*	*	0.05	n.s.	n.s.	0.00	*	*	0.05	0.05
Isopropyl acetate	0.05	*	n.s.	2.73	0.05									
Isopentyl acetate	1.82	*	*	0.00	*	n.s.	0.05	*	n.s.	2.27	*	*	5.57	0.05
Total esters	66.77			86.01			58.56			71.82			84.39	54.75
Terpenes														
Nerol	0.00	*	*	0.00	*	*	0.05	n.s.	n.s.	0.05	n.s.	n.s.	0.05	0.05
β - citronellol	0.05	*	n.s.	0.00	n.s.	*	0.05	*	n.s.	0.05	*	n.s.	0.00	0.05
Geraniol	0.40	*	*	0.27	*	*	0.24	*	*	0.47	*	*	0.15	0.18
Total terpenes	0.45			0.27			0.34			0.57			0.20	0.28
Acetaldehyde	10.34	n.s.	*	25.02	*	n.s.	22.59	*	n.s.	14.35	n.s.	n.s.	12.34	20.55
Methanol	145.87	n.s.	*	176.20	*	*	111.28	n.s.	n.s.	159.90	*	*	130.05	99.28
Total	357.71			455.83			362.74			384.59			316.61	298.33

result obtained on this indicator – 455.83 mg/dm³ is significantly higher not only than Syrah clone 174 – 316.61 mg/dm³ and Sangiovese – 298.33 mg/dm³, but also than other hybrid forms.

In terms of the total amount of higher alcohols, it is the lowest in the two control samples – Syrah clone 174 (89.63 mg/dm³) and Sangiovese (123.52 mg/dm³). Higher quantitative presence of higher alcohols was found in the hybrid forms, which ranged from 134.28 mg/dm³ (30/2) to 169.97 mg/dm³ (30/10). The total content of esters found in the analyzed wines is lower than that of higher alcohols, which is due to the fact that in young wines their initial accumulation at lower levels is based on yeast metabolism. Unlike esters, the main amount of higher alcohols is due to the amino acid metabolism of yeast cultures. The total ester content in the wine from Syrah clone 174 – 84.39 mg/dm³ is higher than the established levels for this group of aromatic compounds in 30/10, 30/2 and 31/9. Their quantity is relatively the largest in the wine from 30/3 – 86.01 mg/dm³. In the second control sample – Sangiovese, fewer esters were secreted – 54.75 mg/dm³, compared to all experimental variants.

Terpenes are an aroma-determining component in wines derived from musket varieties (Rapp, 1998; Mateo & Jimenez, 2000). No high total terpene concentrations were found in this study. The lowest is the total content of terpenes in wine from Syrah clone 174 – 0.20 mg/dm³, followed by 30/3 – 0.27 and Sangiovese – 0.28 mg/dm³. Impressively significantly higher content in 30/2 – 0.45 mg/dm³ and 31/9 – 0.57 mg/dm³ compared to control varieties.

The fraction of higher alcohols is mainly represented by 3-methyl-1-butanol (isoamyl alcohol), which has the highest amount in wines of all variants: from 85.21 mg/dm³ – 30/2 to 110.26 mg/dm³ – 30/3 and 62.10 mg/dm³ – Syrah clone 174, and 83.33 mg/dm³ – Sangiovese. Its characteristic aroma of malt and whiskey has been found in other studies that confirm its importance as a volatile component in the studied wines (Francis & Newton, 2005; Meng et al., 2011; Gurbuz et al., 2006). In the wines of all hybrid forms and varieties, 2-methyl-1-butanol (active amyl alcohol) has been identified, which is second in quantitative presence from the fraction of higher alcohols. The lowest amount of this alcohol was found in Syrah clone 174 – 27.53 mg/dm³, 30/10 and 30/3 show slightly higher amounts of this alcohol compared to the two control samples, and 30/2 and 31/9 have close concentration levels to those of Sangiovese. The third higher alcohol – 1-propanol has the lowest quantitative presence. It is not identified in the wine of Syrah clone 174, and in Sangiovese it is in a minimum concentration – 0.05 mg/dm³. In all wines of the hybrid forms its content is higher than the two control samples. It was found in the highest amount in

30/2 – 10.36 mg/dm³. According to the results of the comparative evaluation of the studied wine indicators, all seedless coloured hybrid forms have statistically proven differences in the content of higher alcohols with Syrah. Compared to Sangiovese, 30/3 and 30/10 are proven to differ in the three indicators, 30/2 – only in the content of 1-propanol, and 31/9 – no significant differences.

The ester fraction is represented by ethyl acetate, propyl acetate, isopropyl acetate, pentyl acetate and isopentyl acetate. The last three are identified at lower levels. The main quantitative presence is observed in ethyl acetate and propyl acetate. Ethyl acetate is an ester which, depending on its concentration level, can have a positive or negative effect on the quality of the wine. At concentrations up to 50.00 – 80.00 mg/dm³, it imparts fruity nuances to the aroma, but above them, it begins to affect the aromatic profile with a manifestation of acetic acid taste (Tao & Li, 2009). The highest quantitative presence of this ester was found in Syrah clone 174 – 60.77 mg/dm³, and in Sangiovese – 27.99 mg/dm³. In the wine from hybrid forms, its content ranges from 25.03 mg/dm³ – 30/10 to 56.15 mg/dm³ – 30/3. In all wines, its quantities are indicative of a positive effect on their aromatic profile. The lowest amount of propyl acetate is in the wine of Syrah clone 174 – 15.27 mg/dm³, and in Sangiovese it is 26.61 mg/dm³. In the wines from hybrid forms, it is in larger quantities, ranging from 29.81 mg/dm³ – 30/3 to 33.38 mg/dm³ – 30/10. This ester has been found to be a component of the aromatic matrix of Australian Cabernet Sauvignon and Syrah wines (Antalick et al., 2015). All observed differences in the amounts of the individual components of the esters in the hybrid forms, compared to Syrah clone 174, were statistically proven with the exception of 30/3, which did not differ in the content of ethyl acetate, and 30/10 – of pentyl acetate. With Sangiovese only 30/3 has a proven difference in the content of ethyl acetate, and 30/10 and 31/9 – in the amount of propyl acetate. Pentyl acetate was found only in the wine from 30/10, and the content of isopropyl acetate in all hybrid forms and this variety is the same. Regarding isopentyl acetate, only 30/2 and 31/9 have proven differences with Sangiovese.

The study identified 3 terpene alcohols. Nerol is absent only in 30/2 and 30/3, and in all other wines it is in a concentration of 0.5 mg/dm³, which is within its normal quantitative presence. The wine from Syrah clone 174 and 30/3 has no β -citronellol, and in the others it is in a concentration identical to that of nerol. The main terpene found in the largest amount is geraniol, which gives the wine a characteristic rose aroma. In the two control samples this terpene was found in the lowest amounts: 0.15 mg/dm³ – Syrah clone 174 and 0.17 mg/dm³ – Sangiovese. There is a tendency of in-

creasing its content in wines from hybrid forms: 0.24 mg/dm³ – 30/10, 0.27 mg/dm³ – 30/3, 0.40 mg/dm³ – 30/2, 0.47 mg/dm³ – 31/9. Due to its largest quantitative presence, it occupies a major percentage of the total terpene content of the studied wines. In the wine from 30/2 less nerol was found compared to Syrah-clone 174 and more β -citronellol and geraniol, which determines the existence of significant differences with it. All hybrid forms are proven to be superior to this variety in the amount of geraniol. In terms of nerol content, 30/10 and 31/9 do not differ from it, but have a proven higher amount of other terpenes. The content of β -citronellol in almost all hybrid forms and Sangiovese is minimal and they do not differ, as only in 30/3 this terpene was not found. The wines of all hybrid forms have more geraniol than Sangiovese, which determines the existence of statistically proven differences with it.

The aldehyde fraction is mainly represented by acetaldehyde in the range from 10.34 mg/dm³ – 30/2 to 25.01 mg/dm³ – 30/3.

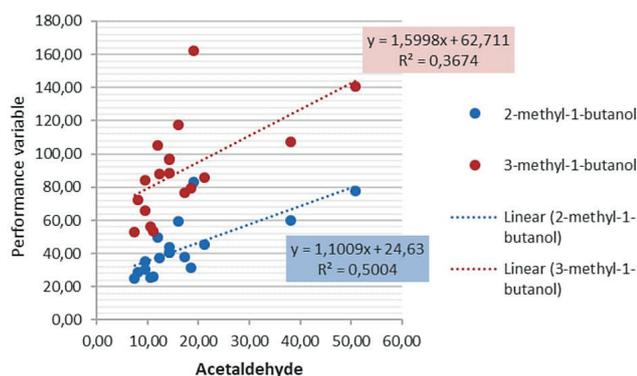


Fig. 8. Influence of acetaldehyde on 2-methyl-1-butanol and 3-methyl-1-butanol in the wine of the studied seedless hybrid forms and vine varieties

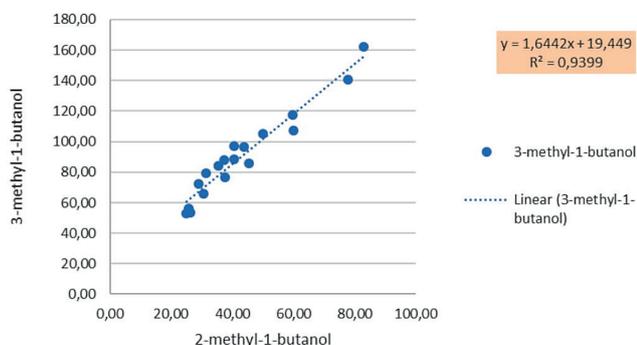


Fig. 9. Influence of acetaldehyde on 2-methyl-1-butanol in the wine of the studied seedless hybrid forms and vine varieties

Its established levels indicate a properly conducted fermentation process and the absence of presulfitation of grape must. Methanol has also been identified, which is a normal component of the volatile wine composition resulting from the degradation of fruit pectin by the pectolytic enzyme complex of grapes. In the two control samples it is in the amount of 130.05 mg/dm³ – Syrah clone 174 and 99.28 mg/dm³ – Sangiovese, and in the hybrid forms it is in the range from 111.28 mg/dm³ – 30/10 to 176.209 mg/dm³ – 30/3. All established methanol concentrations correspond to its normal quantitative presence in dry red wines. In the established differences between the amounts of acetaldehyde and methanol in the two groups of wines, there is no tendency in the manifestation of statistical proof.

After application of correlation analysis and calculation of Pearson's correlation coefficients, a strong, positive relationship was found between the content of acetaldehyde and 2-methyl-1-butanol (0.709) and significant with 3-methyl-1-butanol (0.600) (Figure 8).

A very strong positive relationship exists between 2-methyl-1-butanol and 3-methyl-1-butanol (0.969) (Figure 9).

Physicochemical analysis shows that the content of sugars and acids in grapes have similar values in hybrid forms and varieties (Table 4). The alcohol and sugars in the wines are also within the normal condition for dry red wines. The amount of extract is the lowest in Sangiovese: total – 26.60 g/dm³ and sugar-free – 24.86 g/dm³, and the highest in Regent – 34.60 g/dm³/31.98 g/dm³. In the case of hybrid forms, this indicator is in the range from 27.60 – 34.60 g/dm³/26.31 – 30.76 g/dm³. The titratable acids in the wine vary from 6.14 – 8.10 g/dm³ for the hybrid forms to 5.52 – 6.37 g/dm³ for the varieties. There are differences in the two compared groups in the content of anthocyanins, which are more in the wines of the hybrid forms – from 650.13 mg/dm³ – 30/3 to 1332.63 mg/dm³ – 30/10, compared to control varieties – from 469.88 mg/dm³ – Sangiovese to 992.50 mg/dm³ – Regent. The highest amount of total phenols is in the wine from 30/10 – 3295.66 mg/dm³, and the lowest in Sangiovese – 2060.91 mg/dm³. The content of total phenols is high in Syrah – 2936.45 mg/dm³ and Regent – 2892.50 mg/dm³. The colour intensity of the wine from Sangiovese – 11.36 is several times lower than that of all hybrid forms, reaching 37.59 in 30/10. The wine colour shade is strongest in Regent – 0.578, and in hybrid forms it is in the range of 0.408-0.458. The predominant red colour in the wine is higher in hybrid forms than in the control varieties.

The wines from the studied seedless colour hybrid forms and vine varieties differ in a number of aspects of the sensory characteristic (Figure 10). Their average tasting scores vary widely from 74.0 to 86.0 points. The colour shades of all

Table 4. Physico-chemical analysis of grapes and wines from the studied seedless coloured hybrid forms and vine varieties, average of 2018-2020 vintages

Hybrid form-variety Indicators		30/2	30/3	30/10	31/9	Syrah	Sangiovese	Marselan	Regent
GRAPE	Sugars, %	21.10	22.05	22.67	23.40	24.02	23.06	22.65	23.10
	Titrate acids, g/dm ³	5.98	6.39	5.44	6.34	6.84	6.84	6.09	4.73
Relative density		0.9959	0.9968	0.9968	0.9946	0.9938	0.9942	0.9952	0.9954
Alcohol, tot.%		11.09	12.36	12.62	13.5	14.48	12.19	12.02	13.85
Sugars, g/dm ³		1.29	3.14	4.58	2.49	3.53	1.74	1.51	2.62
Extract, g/dm ³	total	27.60	33.9	34.60	31.50	32.30	26.60	28.70	34.6
	sugar-free	26.31	30.76	30.02	29.01	28.77	24.86	27.19	31.98
pH		3.42	3.07	3.36	3.12	3.46	3.46	3.48	3.80
Titrate acids, g/dm ³		6.14	8.10	6.86	7.80	6.37	5.98	6.50	5.52
Volative acids, g/dm ³		0.52	0.50	0.42	0.40	0.44	0.52	0.48	0.90
Sulfur dioxide, mg/dm ³	free	26.28	11.68	29.20	23.36	16.09	16.79	15.50	16.80
	total	114.61	89.79	110.23	94.90	63.88	97.78	64.80	74.00
Anthcyanins, mg/dm ³		889.88	650.13	1332.63	1055.25	847.88	469.88	747.88	992.50
Total phenols, mg/dm ³	as gallic acid	2438.07	2352.76	3295.66	2687.20	2936.46	2060.91	2571.5	2892.50
Colour intensity		21.38	22.57	37.59	28.00	25.04	11.36	12.27	15.14
Colour shading		0.458	0.408	0.442	0.415	0.495	0.563	0.557	0.578
Colour, %	yellow-brown	28.17	26.48	27.56	26.46	29.35	31.78	32.51	31.63
	red	61.47	64.85	62.33	63.74	59.35	56.43	58.37	54.73
	blue	10.36	8.67	10.11	9.80	11.30	11.79	9.12	13.64

wines are bright ruby. With the lowest colour intensity is the wine from Sangiovese, followed by 30/3 – with a slightly more saturated colour, 31/9, 30/2 and Syrah clone 174 are practically identical, and with the strongest intensity is 30/10.

The wine from 30/2 has sweet vanilla notes at the beginning, but after aeration an irritating atypical nuance of rotten

fruit, of something dirty and stagnant appears. The body is weakly to moderately structured, with a relatively balanced taste, with the presence of irritating green acids and a pronounced dryness and sandiness on the finish.

The wine from 30/3 has an oxidized, sweet and heavier aroma, with hints of boiled fruit and marmalade. The body is empty, poorly built, there are early appearing, persistent and aggressive acids. The taste is oxidized and rough. The wine is short, the aftertaste is sour-drying and irritating, which may be the result of vinification, which took place in micro-winemaking conditions and may not be typical of the hybrid.

The wine from 30/10 has a clean, slightly open sweet aroma, dominated by nuances of dry plant mass, sweetness and vanilla notes. The body of the sample is better structured, the length is about average. The dynamics of the taste is more pronounced and positive, and the taste aroma is plenty and fruity. Acids that are softer and more built-in can be felt, and the aftertaste is spicy and dry.

The wine from 31/9 has a clean, medium-intense aroma, dominated by ripe fruit notes of dried black berries, and there are also nuances of dry plant mass. Tastefully, it has a poorly built and very short body and there is a feeling of wateriness. The plant aroma, unlike the nasal one, is green planty, raw, and the acids are dominant, especially persistent and aggressive

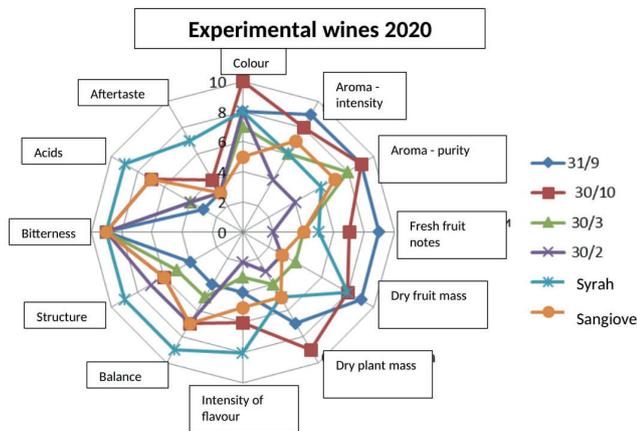


Fig. 10. Sensory profile of the experimental wines from the studied seedless coloured hybrid forms and vine varieties

sive on the finish, which is without individuality and is dry.

The wine from Syrah clone 174 has a relatively strong aroma, initially with fusty and coarse nuances, which after aeration are cleared and predominant ripe and dry fruit notes appear. It tastes clean, with excellent balance, the structure is above average, without being rough and heavy, the acids are soft and built-in, the length is medium, the aftertaste is sweet and spicy dry.

The wine from Sangiovese has a typical winous, medium-intensity aroma, dominated by plant notes, nuances of powder, earthy notes and almost no fruity nuances. As for taste, it has a medium body, the aroma is of medium intensity, with nuances of dry plant mass and earthiness. Aggressive and dominant acids, dryness, granular phenolic compounds and aggressive aftertaste are felt.

Conclusions

The content of trans-resveratrol in the wine of all studied seedless coloured hybrid forms varies from 2.490 mg/l to 2.862 mg/l and differs significantly from Sangiovese – 0.649 mg/l and Syrah clone 174 – 6.491 mg/l, and with Marselan and Regent – no statistically proven differences. With a very low content of quercetin in the wine is Sangiovese – 0.739 mg/l, and Syrah clone 174 exceeds twice its amount compared to all studied hybrid forms – 7.634 mg/l. Most hybrid forms are statistically superior to Marselan and Regent in quercetin.

The antioxidant activity of wine is the lowest in Sangiovese – 0.333 mmol/l, and the highest in Syrah clone 174 – 0.840 mmol/l. In the case of hybrid forms, the values of this indicator are from 0.625 mmol/l – 30/2 to 0.668 mmol/l – 30/10. There is a strong positive relationship between trans-resveratrol content and the antioxidant activity of wine in the two groups of vines studied ($R = 0.844^{**}$) and a significant positive relationship between quercetin and antioxidant activity ($R = 0.777^{**}$). The change in antioxidant activity in the studied wines is explained by 71% changes in the content of trans-resveratrol – direct effect and 60% of quercetin – indirect effect.

Relatively resistant to environmental factors and changes in the amount of trans-resveratrol in wine is the 30/10 hybrid form, and of the varieties – Syrah clone 174. All varieties are characterized by stability of the quercetin content, with the highest level being Syrah clone 174, and of the hybrid forms – 30/2. Sangiovese has the lowest stability of antioxidant activity compared to all hybrid forms and varieties in the study. With a high degree of balance and resistance to environmental conditions of the varieties are Syrah clone 174, Marselan and Regent, and of the hybrid forms – 30/10 and 30/2.

Various representatives of the volatile aromatic composition belonging to the groups of higher alcohols have been identified in the studied wines – 3-methyl-1-butanol, 2-methyl-1-butanol and 1-propanol; esters – ethyl acetate, propyl acetate, pentyl acetate, isopropyl acetate and isopentyl acetate; terpenes – nerol, β -citronellol, geraniol; aldehydes – acetaldehyde; methanol. Their diversity is greatest in Sangiovese and 30/10. The total quantitative presence of volatile compounds is higher in the wines of the hybrid forms compared to those of the control varieties.

There are specifics in the individual and total content of volatile components in wines in individual hybrid forms and varieties. The highest amount of higher alcohols is found in hybrid forms and 3-methyl-1-butanol predominates. The wines from Syrah clone 174 and 30/3 are characterized by a higher total ester accumulation, and Sangiovese – with the lowest. Of this group, ethyl acetate and propyl acetate have the relatively largest amount in all variants. The hybrid forms are superior to the two control varieties in total terpene content, where the main component is geraniol. The aldehyde fraction is represented by acetaldehyde at acceptable levels for a controlled fermentation process. Methanol was identified in all analyzed wines, with a concentration presence in the norm for dry red wines.

The content of sugars and acids in grapes in hybrid forms and varieties does not differ significantly. The alcohol and sugars in the wines are also within the normal condition for dry wines. The amounts of extract, titratable acids, anthocyanins are higher in hybrid forms. The highest amount of total phenols is in the wine from 30/10 – 3295.66 mg/dm³, and the lowest is in Sangiovese – 2060.91 mg/dm³. The content of total phenols is high in Syrah – 2936.45 mg/dm³ and Regent – 2892.50 mg/dm³. The colour intensity of the wine from Sangiovese is significantly lower than that of all hybrid forms, and the colour shade is strongest in Regent. The red colour in the wine prevails, which is more in the hybrid forms than in the control varieties.

With the highest organoleptic evaluation is the wine from Syrah clone 174 – 86.0, characterized by typical aroma, which to a lesser extent applies to Sangiovese – 75.0. Of the seedless coloured hybrid forms, the wines from 30/10 – 83.0 and 30/3 – 80.0 were rated the highest, and the lowest from 31/9 – 78.0 and 30/2 – 74.0. It is possible through staged sexual hybridization to create seedless coloured varieties suitable for the production of quality wines, characterized by high taste characteristics, nutritional and medicinal value.

Acknowledgments

The authors wish to express their most sincere gratitude to Tech. Eng. Yovka Radkova for the preparation of all ex-

perimental wines in this study. The authors thank the financial support of Agricultural University – Plovdiv.

References

- Aldrich, J. & Conningham, J.** (2016). Using IBM SPSS Statistics: An interactive hands-on approach. *SAGE Publications, Inc.*, United States of America, 286.
- Anli, E., Vural, N., Demirey, A. & Ozkan, M.** (2006). Trans-resveratrol and other Phenolic compounds in Turkish red wines with HPLC. *Journal of Wine Research*, 17(2), 117-125. <https://doi.org/10.1080/09571260601004328>.
- Antalick, G., Šuklje, K., Blackman, J., Meeks, S., Deloire, A. & Schmidtke, M.** (2015). Influence of grape composition on red wine ester profile: comparison between Cabernet Sauvignon and Shiraz cultivars from Australian warm climate. *Agricultural and Food Chemistry*, 63, 4664-4672. <https://doi.org/10.1021/acs.jafc.5b00966>.
- Bhat, K. P., Lantvit, D., Chistov, K., Mehta, R., Moon, R. & Pezzuto, J.** (2001). Estrogenic and antiestrogenic properties of resveratrol in mammary tumor models. *Cancer Res.*, 61, 7456-7463.
- Bhat, K. P., Kosmeder, J. & Pezzuto, J.** (2001). Biological effects of resveratrol. *Antioxid. Redox. Signal*, 3(6), 1041-1064. doi: 10.1089/152308601317203567.
- Cai, Y. J., Fang, J., Ma, L., Yang, L. & Liu, Z.** (2003). Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. *Biochim. Biophys. Acta*, 1637, 31-38. [https://doi.org/10.1016/S0925-4439\(02\)00174-6](https://doi.org/10.1016/S0925-4439(02)00174-6).
- Fia, G., Bucalossi, G., Proserpio, C. & Vincenzi, S.** (2021). Unripe grapes: an overview of the composition, traditional and innovative applications, and extraction methods of a promising waste of viticulture. *Australian Journal of Grape and Wine Research*, on-line version, <https://onlinelibrary.wiley.com/doi/full/10.1111/ajgw.12522>.
- Francis, I. & Newton, J.** (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*, 11, 114-126. <https://doi.org/10.1111/j.1755-0238.2005.tb00283.x>.
- Gurbuz, O., Rouseff, J. & Rouseff, R.** (2006). Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 54, 3990-3996. DOI: 10.1021/jf053278p.
- Karaagac, E., Vargas, A., Andres, A., Carreno, I., Ibanez, J., Carreno, J., Martinez-Zapater, J. & Cabezas, J.** (2012). Marker assisted selection for seedlessness in table grape breeding. *Tree Genetics, & Genomes*, 8, 1003-1015, <https://doi.org/10.1007/s11295-012-0480-0>.
- Karioti, A., Hadjipavlou-Litina, D., Mensah, M., Fleischer, T. & Skaltsa, H.** (2004). Composition and antioxidant activity of the essential oils of *Xylopiya aethiopica* (Dun) A. Rich. (Annonaceae) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. *Journal of Agricultural and Food Chemistry*, 52, 8094-8098. doi: 10.1021/jf040150j.
- Kennedy, J. A.** (2008). Grape and wine phenolics: Observations and recent finding, *Ciencia e Investigacion Agraria*, 35(2), 107-120. <http://dx.doi.org/10.4067/S0718-16202008000200001>.
- Kennedy, J. A., Troup, D. J., Pilbrow, J. R., Hutton, D. R., Hewitt, D., Hunter, C., Ristic, R., Iland, P. & Jones, P.** (2000). Development of seed polyphenols in behries from *Vitis vinifera* L. cv. Shiraz. *Australian Journal of Grape and Wine Research*, 6, 244-254. <https://doi.org/10.1111/j.1755-0238.2000.tb00185.x>.
- Kennedy, J. A., Matthews, M. A. & Waterhouse, A. L.** (2000). Changes in grape seed polyphenols during fruit ripening. *Phytochemistry*, 55, 77-85. DOI: 10.1016/s0031-9422(00)00196-5.
- Kopp, P.** (1998). Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the "French paradox", *European Journal of Endocrinology*, 138, 619-620. DOI: 10.1530/eje.0.1380619.
- Mateo, J. J. & Jimenez, M.** (2000). Monoterpenes in grape juice and wines. *Journal of Chromatography*, 881(1-2), 557-567. DOI: 10.1016/s0021-9673(99)01342-4.
- Mejía, N., Soto, B., Guerrero, M., Casanueva, X., Houel, C., Miccono, M., Ramos, R., Cunff, L., Boursiquot, J., Hinrichsen, P. & Adam-Blondon, A.** (2011). Molecular, genetic and transcriptional evidence for a role of VvAGL11 in stenospermocarpic seedlessness in grapevine, *BMC Plant Biology*, 1471-2229/11:57. doi: 10.1186/1471-2229-11-57.
- Meng, J., Fang, Y., Gao, Y., Zhang, A., Liu, J., Guo, L., Zhang, Z. & Li, H.** (2011). Changes in aromatic compounds of Cabernet Sauvignon wines during ageing in stainless steel tanks. *African Journal of Biotechnology*, 10(55), 11640-11647. eISSN: 1684-5315.
- Milutinovic, M., Nikolic, D., Avramov, L. & Rakonjac, V.** (2000). Recombination of some characteristics in F1 generation of grapevine. *Acta – Horticulturae*, 528, 641-644. DOI: 10.17660/ActaHortic.2000.528.95.
- Naiker, M., Anderson, S., Johnson, J., Mani, J., Wakeling, L. & Bowry, V.** (2020). Loss of trans-resveratrol during storage and ageing of red wines, *Australian Journal of Grape and Wine Research*, 26(4), 385-387. <https://doi.org/10.1111/ajgw.12449>.
- Nosulchak, V. A.** (2021). On the inheritance of parthenocarpy in grapes. *Winemaking and Viticulture*, 2, 4-11.
- Puja, A., Fthi, H. & Chandradhar, D.** (2007). Antioxidant effects and drug interactions of resveratrol present in wine. *Journal of Wine Research*, 18(2), 59-71. <https://doi.org/10.1080/09571260701660839>.
- Rapp, A.** (1998). Volatile flavor of wine: correlation between instrumental analysis and sensory perception. *Nahrung*, 42(6), 351-363. doi: 10.1002/(sici)1521-3803(199812)42:06<351::aid-food351>3.3.co;2-u.
- Roychev, V.** (2014). Individual and complex selection evaluation of seedless hybrid vine forms with coloured berry juice. Collection of Scientific Articles. Proceedings of the International Scientific-Practical Conference, August 15.2014, Novochoerkassk, VNIIViV, Russia, 143-152.
- Roychev, V.** (2014). Selection characterization and selection of seedless hybrid vine forms with coloured berry juice. *Winemaking and Viticulture*, 3, 38-42.
- Roychev, V.** (2015). Selection of elite plants according to cluster and berry size in F1 progeny of hybrid combinations between the vine cultivars Alicante Bouschet x Russalka 1 and x Beau-

- ty seedless. Russian grapes. Collection of Scientific Papers, 1, Novocherkassk, VNIIV, Russia, 42-50.
- Roychev, V., Tzanova, M., Keranova, N. & Peeva, P.** (2020). Antioxidant content and antioxidant activity in raisins from seedless hybrid vine varieties with coloured grape juice. *Czech Journal of Food Sciences*, 38(6), 410–416. DOI: 10.17221/160/2020-CJFS.
- Sato, M., Maulik, N. & Das, D.** (2002). Cardioprotection with alcohol: role of both alcohol and polyphenolic antioxidants. *Ann. N.Y. Acad. Sci.*, 957, 122-135. doi: 10.1111/j.1749-6632.2002.tb02911.x.
- Sharma, P. & Bhat, K.** (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202-1205. <https://doi.org/10.1016/j.foodchem.2008.08.008>.
- Standard 3752:2005.** Alcohol Drinks – Methods of Test (Second Revision).
- Ta, Y. & Li, H.** (2009). Active volatiles of Cabernet Sauvignon wine from Changli County. *Natural Science*, 1, 176–182. DOI: 10.4236/health.2009.13029.
- Tzanova, M. & Peeva, P.** (2018). Rapid HPLC Method for Simultaneous Quantification of trans-Resveratrol and Quercetin in the Skin of Red Grapes. *Food Analytical Methods*, 11(2), 514-521. DOI 10.1007/s12161-017-1022-z.
- Tzanova, M., Atanasov, V., Ivanov, M., Iliev, A., Atanassova, S., Peeva, P., Grozeva, N., Gerdzhikova, M. & Dinev, T.** (2019). Antioxidant constituents and antioxidant activity of some red wine and red table grape varieties, cultivated in different regions of Bulgaria. *Bulg. J. Agric. Sci.*, 25(Suppl. 3), 3-11.
- Tzanova, M., Atanassova, S., Atanasov, V. & Grozeva, N.** (2020). Content of Poly-phenolic compounds and antioxidant potential of some Bulgarian red grape varieties and red wines, determined by HPLC, UV, and NIR spectroscopy. *Agriculture*, 10, 193, doi:10.3390/agriculture10060193.
- Valchev, V.** (1990). Intraspecific and Interspecific Hybridization of the Vine. Habilitation thesis, Pleven, 272 (Bg).
- Wricke, G.** (1962). Über eine Methode zur Erfassung der ökologische Streubreite in Feldversuchen. *Z. Pflanzenzuchtg*, 47(1), 92-96.
- Wrick, G.** (1966). Über eine Biometrische Methode zur Erfassung der ökologischen Anpassung. *Acta Agric. Scand. Suppl.*, 16(1), 98-101.

Received: March, 14, 2023; Approved: October, 19, 2023; Published: February, 2024