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INFLUENCE OF VARIETY AND VINTAGE ON THE BASIC PHUSICO-CHEMICAL COMPOSITION OF SMEDEREVKA AND VRANEC WINES

Violeta Ivanova-Petropulos[1*], Ile Ilievski[1], Štefan Ailer[2]

[1]*Faculty of Agriculture, Goce Delcev University, Stip, Krste Misirkov 10-A, 2000, Stip, Republic of N. Macedonia*
[2]*Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

**Corresponding author: violeta.ivanova@ugd.edu.mk*

Abstract

In this study, Smederevka and Vranec wines from *V. vinifera* L. *cv*., the most important and dominant varieties in Macedonia, have been produced in 6 (six) consecutive years applying traditional fermentation methods, in order to study the influence of variety and vintage on the general basic wine quality. The physico-chemical parameters that confirm the basic wine quality have been determined, including alcohol, specific gravity at 20°C, dry extract, reducing sugars, total and volatile acidity, pH, free and total SO_2. The results showed that variety significantly affected the chemical composition of wines, presenting higher contents of alcohol, density and dry extract for Vranec wines, while Smederevka wines were richer with total acids and contained higher amount of total SO_2. In addition, slight influence of vintage was noticed for both varieties, especially on the alcohol, specific gravity and dry extract contents, for which highest values were noticed in vintage 2017 in Vranec wines, while for Smederevka wines, values were highest in vintage 2020. Principal component analysis showed a clear separation of the wines according to the variety.

Key words: *variety, vintage, Smederevka, Vranec, basic physico-chemical parameters.*

INTRODUCTION

Wine production in the Republic of N. Macedonia has a long and significant history. Macedonian wines are characterized by intense aromas that are the result of the combined influence of two climates, mediterranean and continental. According to the climatic characteristics and classification of the European Union, the Republic of N. Macedonia is categorized as III-C-b zone for growing vines. In Macedonia, the wine-growing region is divided into three regions, as follows (Official Gazette of the Republic of Macedonia, No. 12, 1980; Official Gazette of the Republic of Macedonia, No. 74, 2024):

1) Povardarie Region (Vardar valley or Central Wine Region), which includes about 87% of the total grape production in the country;
2) Pelagonija-Polog Region (Western Region), which covers about 7% of the total grape production in the country, and
3) Pčinja-Osogovo Region (Eastern region), which covers about 6% of the total grape production in the country.

These three regions are divided into 16 sub-regions (vineyards) characterized by different production conditions and production intensity.

Wine production is a complex technological process which includes several procedures, such as: harvesting the grapes, crushing the grapes (separating the stems from the berries), adding SO_2, pressing, fermentation, maceration (mostly applied to red wines), clarification, stabilization, ripening and wine bottling. Determining the right time to harvest grapes, the structure, grapes condition and quality is performed based on the appearance, taste and analyses (sugar content, acidity, total polyphenols) of the

grapes and bunches. In order to preserve the freshness, fruity aroma and taste of the grapes, it is best to harvest early in the morning, when the temperatures are low.

After *addition of* SO_2, the grape mash is inoculated with yeast (*Saccaromyces cerevisie*) to start the *alcoholic fermentation*, during which the carbohydrates are converted into ethyl alcohol and carbon dioxide, releasing energy. The choice of the type of yeast depends on the grape variety as well as the characteristics of the wine that the producer wants to obtain (Lin et al. 2012). During the alcoholic fermentation, suitable mineralized nutrients necessary for the metabolism of the yeast are added into the mash in order to support the reproduction of the yeast (mostly in the form of diammonium phosphate and nitrogen). The alcoholic fermentation is a complex biochemical process which depends on many factors such as: temperature, content of sugars, pH, acidity, presence of phenolic compounds as well as the content of the produced alcohol (Fleet, 2003, Divol et al., 2012, Mamolar-Domenech et al. 2023, Vion et al. 2023).

Maceration is the period of contact between the solid parts of the grape (skins and seeds) and the grape juice. During maceration, the grape components are released, and between them, the phenolic compounds, including anthocyanins (in red grapes), phenolic acids, flavonoids and flavan-3-ols are the most important components for the wine quality, responsible for the colour and for the structure of the wine (Ivanova et al. 2011a; Ivanova et al. 2011b; Ivanova-Petropulos et al., 2014; Raičević et al. 2017). The quantities and ratios of these compounds is variable, depending on the composition of the grapes and their maturity. According to the grapes' quality, their maturity, the origin, as well as the type of wine that will be produced, the development of the maceration processes can be described as: short, long, carbonic, thermal, pre-fermentation cold and long maceration combined with fermentation. After finishing of the alcoholic fermentation, the second microbiological transformation or malolactic fermentation takes place in the wines. During malolactic fermentation in a presence of lactic acid bacteria, the content of malic acid decreases due to its conversion to lactic acid, which concentration increases in the wine.

The next step after the fermentation, is *racking off* the wine, which removes the coarse particles that can lead to cloudiness. The first racking off is carried out after the end of the quiet fermentation, usually in November or December, while the second racking off is carried out in February or March, with the previous addition of sulphur dioxide, as an additional protection against oxidation. *Fining* of the wines is performed by substances (fining agents) in order to bind suspended particles into larger molecules that precipitate out more rapidly. The common fining agents include (a) organic agents: egg whites, casein (milk), gelatine and isinglass (fish bladders) and (b) mineral materials: bentonite clay, activated carbon, silica and kaolin. Before *bottling*, for stabilization, wines are *filtered* in order to eliminate microbiological phenomena caused by residual active yeasts and lactic acid bacteria. Also, wine aging is necessary to be performed in a period of few days (for young wine, e.g. Beaujolais wine) to nine months, eighteen months or even longer period of time, depending of the wine style and quality that is expected to be obtained. Maturation is usually performed in stainless steel tanks, concrete tanks or oak tanks.

Since the wine quality depends on climate conditions of the years, the vintage year has a statistically significant influence on the all parameters in wine (Hosu et al. 2016, Raičević, et al. 2017, Jovanović-Cvetković et al. 2023). The aim of this study was to examine the effect of variety and vintage on the basic physico-chemical composition of Smederevka and Vranec wines (the dominant white and red grape varieties in Republic of N. Macedonia, with the highest impact on the wine production and financial benefits of the country), produced in six consecutive years, produced from the grapes grown in Ovce Pole sub-region, which is also important wine subregion, but not very well studied. Therefore, the results will ensure valuable data for the influence of the variety and vintage, especially focused on one su-bregion, important for the vine growers and wine producers from Ovce Pole and from the whole country, in general.

MATERIAL AND METHODS

Chemicals and reagents

For performing the chemical analysis, the following chemicals and reagents, with analytical grade of purity, have been used: NaOH, concentrated H_2SO_4, $Na_2S_2O_3$, phenolphthalein, bromothymol blue, starch, KI, Feling I, Feling II and buffers (pH: 4, 7 and 7), all of them purchased from Alkaloid (Skopje). Ultra-pure deionized water with 0.0005 μS conductivity was obtained with a membrane filtration unit (Millipore, Molsheim France).

Winemaking

White winemaking. Grapes from Smederevka (*Vitis vinifera* L.) variety grown in Ovce Pole sub-region, have been harvested at optimal technological maturity (average value: 19.8° Brix) and used for wine production (vintages 2018-2023). Ovce Pole wine sub-region is located around the cities of Sveti Nikole, Stip and Probistip, north from the river Zletovo and covers area of 2456 hectares vineyards, at attitude of 300 to 560 m.

Harvested grapes (about 1 million kg) were transported to Vineks winery (with capacity of 1.8 million litres, located in Sveti Nikole, Ovce Pole wine sub-region), for wine production. Grapes were processed using electrical inox crusher/ destemmer, and the grape juice was immediately separated from the pomace and placed in a tank. Then, SO_2 was added into the grape juice (ca. 10 g/100L), followed with inoculation with *Saccharomyces cerevisiae* yeast strain (Fermactive Blanc Aromatique, Sodinal, Bulgaria, in a dose of 20 g/100 L) and addition of balanced nutrients composed of organic nitrogen, mineral nitrogen and vitamins (Fermactive Activateur Complexe, Sodina, Bulgaria, in a dose of 10 g/100 L) for yeast nutrition. In addition, enzyme (Speed up Blanc, Sodinal, Bulgaria, in a dose of 10 g/100L) and tannin (Tanivin Blanc, Sodinal, Bulgaria, in a dose of of 10 g/100L) were added into the juice in order to ensure fast and efficient clarification and stabilization of redox potential of wine. After finishing the alcoholic fermentation, wine was stabilized with bentonite (2 g/L) and followed with addition of a product consisting of metatartaric acid and gum Arabic (10 g/100 L) (MetaGum, Erbslöh, Geisenheim, Germany) to ensure long-term crystal wine stabilization.

After 5-6 months of storage, wine was bottled. Before bottling, wine was three times filtered, at first passing thought a filter with pore sizes of 1.3 microns, followed with passing of the wine thought a filter with pore sizes of 0.9 microns and 0.6 microns and additionally protected with appropriate amount of SO_2. A total of 700 000 litres of Smederevka wine was produced.

Red winemaking. Grapes from Vranec (*Vitis vinifera* L.) variety grown in Ovce Pole wine sub-region, have been harvested at optimal technological maturity (average value: 22.9° Brix) (vintages 2017-2022). Harvested grapes (about 1.5 million kilograms) were transported to Vineks winery for wine production. Grapes were processed using electrical inox crusher/ destemmer, then added with SO_2 (ca. 10 g/100L) before inoculation with *Saccharomyces cerevisiae* yeast strain (Fermactive aroma varietale, Sodinal, Bulgaria, in a dose of 20 g/100 L), followed with addition of nutrients (Fermactive Activateur Complexe, Sodina, Bulgaria, in a dose of 10g/100 L) for the yeast nutrition. The grape mash was macerated for 5-6 days at 25-30 °C, with pumping over and delastage performed twice per day, followed by separation of the wine from the sediment and treatment with bentonite (1 g/L) and with a product consisting of metatartaric acid and gum Arabic (10 g/100 L) (MetaGum, Erbslöh, Geisenheim, Germany) to ensure long-term crystal stabilization in wine. After 5-6 months of storage, wine was bottled. Before bottling, wine was two times filtered, at first passing thought a filter with pore sizes of 1.3 microns, followed with passing of the wine thought a filter with pore sizes of 0.9 microns and additionally protected from oxidation by adding appropriate amount of SO_2. A total of 1 million litres of Vranec wine was produced.

Principal chemical composition

The principal chemical composition of wines was determined using the official methods of analysis of wines (OIV 2022). The following parameters have been determined: alcohol (OIVMA-AS312-01 A), dry extract (OIV-MA-AS2-03B), specific density (OIV-MA-AS2-01 A), total acidity (OIV-MAAS313–01), volatile acidity (OIV-MA-AS313–02), pH (OIV-MA-AS313-15), reducing sugars, free SO_2 and total SO_2 (Ivanova-Petropulos and Mitrev 2014).

Statistical analysis

Each wine was analysed in three replicates. Results were statistically treated including determination of means, minimum, maximum, standard deviation (SD) and relative standard deviation (RSD), calculated using Microsoft Excel (2013). Principal component analysis was performed on the results for both wine varieties produced in 6 various years, using the XLStat software (Addinsoft, Version 2015.5.01.22537), in order to study possible groupings of wines as an influence of the vintage and variety.

RESULTS AND DISCUSSION

The basic physico-chemical parameters that are important for wine quality are: alcohol, dry extract, specific density, total acidity, volatile acidity, pH, reducing sugars, free SO2 and total SO2. These parameters were determined for wine samples from Smederevka and Vranec varieties produced during 6 consecutive years (period of 2018 to 2023 for Smederevka and 2017 to 2022 for Vranec). The results for the basic parameters of the wines are shown in Tables 1 and 2.

Influence of variety

The alcohol content, which is considered as one of the most important factors for wine quality and stability, ranged from 10.7 to 11.26% in the wine samples from both varieties in accordance to the literature data (Neceva et al. 2016). Vranec wines contained slightly higher content of alcohol (on average: 11.52%) compared to Smederevka wines (on average: 10.8%), regardless of the year of production, probably because of the higher content of reducing sugars in Vranec grapes compared to Smederevka grapes.

The values for specific gravity in Smederevka wines ranged from 0.9913 to 0.9944 (on average: 0.9931), which were lower than the specific gravity values of Vranec, ranged from 0.9936 to 0.9961 (on average: 0.9953). The obtained results were expected and comparable to the specific gravity for white and red wines published in the literature, which usually range from 0.9912 to 1.0038 (Piperevski, et al. 2023; Budziak-Wieczorek et al., 2023). In fact, the specific gravity is important parameter that is necessary to determine the dry extract of wine. Wine contains a mixture of dissolved nonvolatile solids, such as carbohydrates, acids, phenols, glycerol etc., which increase the specific gravity. Since red wine usually contains higher content of phenols, especially anthocyanins which are not present in white wine, it is expected red wines to have higher density compared to white wines.

In accordance to the specific gravity and in accordance to the literature (Ivanova-Petropulos et al., 2015, Pajović-Šćepanović et al., 2016, Piperevski et al., 2023), the wine dry extract values were lower in white wines, compared to red wines (regardless the vintage), ranged from 20.8 g/L (on average for Smederevka wines) to 28.3 g/L (on average for Vranec wines). Higher values of dry extract mean higher amount of extractive components from the grapes, fuller and stronger wine. Moreover, the values for density in the Macedonian Vranec wines examined in this study, were much higher compared to the Montenegrin Vranec (Vranac) wines (Pajović-Šćepanović et al., 2016). Since the climate conditions, such as temperature, influence on the grape and wine composition and quality, the higher temperatures in Macedonia and different geographic region are the main factors influencing on higher non-volatile compounds, such as polyphenols in Vranec grapes, and then in the corresponding wines, resulting with higher density of the wines.

Table 1. Basic physic-chemical composition of Smederevka wines produced in various vintages.

Vintage/ Parameters	Specific gravity at 20°C	Alcohol (%, *v/v*)	Total dry extract (g/L)	Total acidity (g/L)	Volatile acidity (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	pH	Reducing sugars (g/L)
2018	0.9939	10.7	21.3	5.6	0.56	23.1	75.5	3.53	1.0
2019	0.9919	10.76	18.3	5.3	0.57	22.3	62.0	3.18	1.6
2020	0.9944	10.8	23.7	5.8	0.55	24.3	71.7	3.21	1.9
2021	0.9913	11	19.1	7.2	0.3	18.6	70.9	3.19	1.3
2022	0.9938	10.86	21.6	4.8	0.31	16.4	68.4	3.17	2.2
2023	0.9928	10.21	17	6.2	0.53	23.2	63.4	3.33	0.6
Average	**0.99306**	**10.8**	**20.8**	**5.74**	**0.46**	**20.9**	**69.7**	**3.25**	**1.6**
Min	**0.9913**	**10.7**	**18.3**	**4.8**	**0.3**	**16.4**	**62.0**	**3.17**	**1.0**
Max	**0.9944**	**11**	**23.7**	**7.2**	**0.57**	**24.3**	**75.5**	**3.53**	**2.2**
SD	**0.001**	**0.11**	**2.15**	**0.90**	**0.14**	**3.31**	**5.00**	**0.15**	**0.47**
RSD (%)	**0.14**	**1.06**	**10.3**	**15.7**	**30.5**	**15.8**	**7.18**	**4.73**	**29.6**

Abbreviations: Min - minimum; Max - maximum; SD - standard deviation; RSD - relative standard deviation.

Table 2. Basic physic-chemical composition of Vranec wines produced in various vintages.

Vintage/ Parameters	Specific gravity at 20°C	Alcohol (%, *v/v*)	Total dry extract (g/L)	Total acidity (g/L)	Volatile acidity (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	pH	Reducing sugars (g/L)
2017	0.9961	11.62	29.8	5.4	0.68	16.6	44.8	3.71	1.0
2018	0.9953	11.38	27.0	5.5	0.71	23.1	43.5	3.51	1.3
2019	0.9959	11.42	28.8	5.7	0.49	19.2	33.3	3.40	2.2
2020	0.9955	11.56	28.2	4.6	0.48	29.4	39.4	3.53	1.0
2021	0.9936	11.61	27.8	5.0	0.42	23.2	40.4	3.45	1.9
2022	0.9955	11.53	28.0	4.6	0.70	19.4	44.8	3.50	1.9
Average	**0.9953**	**11.52**	**28.3**	**5.13**	**0.58**	**21.8**	**41.0**	**3.51**	**1.6**
Min	**0.9936**	**11.38**	**27.0**	**4.6**	**0.42**	**16.6**	**33.3**	**3.40**	**1.0**
Max	**0.9961**	**11.62**	**29.8**	**5.7**	**0.71**	**29.4**	**44.8**	**3.71**	**2.2**
SD	**0.001**	**0.10**	**0.95**	**0.47**	**0.13**	**4.49**	**4.41**	**0.11**	**0.52**
RSD (%)	**0.09**	**0.86**	**3.37**	**9.19**	**22.5**	**20.6**	**10.7**	**3.01**	**33.3**

Abbreviations: Min - minimum; Max - maximum; SD - standard deviation; RSD - relative standard deviation.

In addition, the concentration of reducing sugars, composed by the main carbohydrates in grapes, glucose and fructose, was very low in the analysed wines from both varieties. The content ranged from 1 to 2.2 g/L in both wines. All wines were dry (< 5 g/L) and in all of them the fermentation ended successfully, which was in accordance to the previous published data (Piperevski et al., 2023).

Total wine acidity is a sum of non-volatile and volatile acidity, and includes all types of acids, such as formic acid, organic acids (tartaric, malic and citric), as well as amino acids. The total acidity is expressed in tartaric acid equivalents since the tartaric acid is the predominant acid in must and wine (Piperevski et al., 2023). In this study, the average concentration of total acidity in Smederevka wines was 5.74 g/L, which was slightly higher compared to acidity in Vranec wines (5.13 g/L). These values are relatively high and sufficient to ensure satisfactory chemical and microbiological stability of wines as well as sufficiently optimal freshness. Red wines are stable even at lower acidity due to the presence of phenols that enable stability of the wines (Piperevski et al., 2023).

pH is another factor that influence the wine stability and freshness. In this study, the average pH values were: 3.25 for Smederevka wines and 3.51 for Vranec wines, which was in accordance to the total acidity in the wines (higher total acidity leads to lower pH values). In fact, the values in range between 3.14 to 3.6 are considered as normal and typical values for wines (Piperevski et al., 2023).

The volatile acidity showed an overall average value of 0.46 g/L for Smederevka wines and 0.58 g/L for Vranec wines with no influence

on the quality of wines that were protected from further oxidation and microbial contamination by the free SO_2 present in a sufficient level in the wines (16.4 to 29.4 mg/L) and sufficient total SO_2 (41 to 69.7 mg/L) (Ivanova-Petropulos et al., 2015). According to the stated regulations, the maximum allowed content of volatile acidity is 1.2 g/L acetic acid for red wines (Official Gazette of the Republic of Macedonia, No 16, 2012).

Influence of vintage

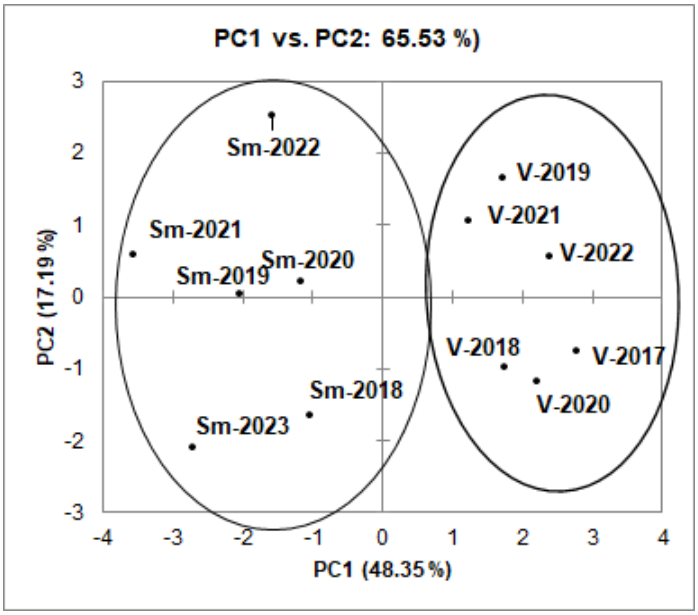
By observing the influence of the vintage during six consecutive years, slight differences have been noticed in both varieties. The main parameters influenced by the vintages have been alcohol, specific gravity and total dry extract, in accordance to the literature (Raičević et al., 2017). Free SO_2, total SO_2 and volatile acidity are factors that mainly are not influenced by the vintage and no significant differences have been noticed between wines from both varieties. Thus, the highest values of alcohol, specific gravity and total dry extracts were noticed in vintage 2020 for Vranec wines and in vintage 2017 for Smederevka wines. The contents of reducing sugars were very similar between all wines meaning that the conversion of glucose and fructose during the alcoholic fermentation was completed in all wines. Since we do not have data for the air temperature and precipitation, we cannot make any specific conclusion about influence of climate conditions of the grape composition.

Principal component analysis (PCA)

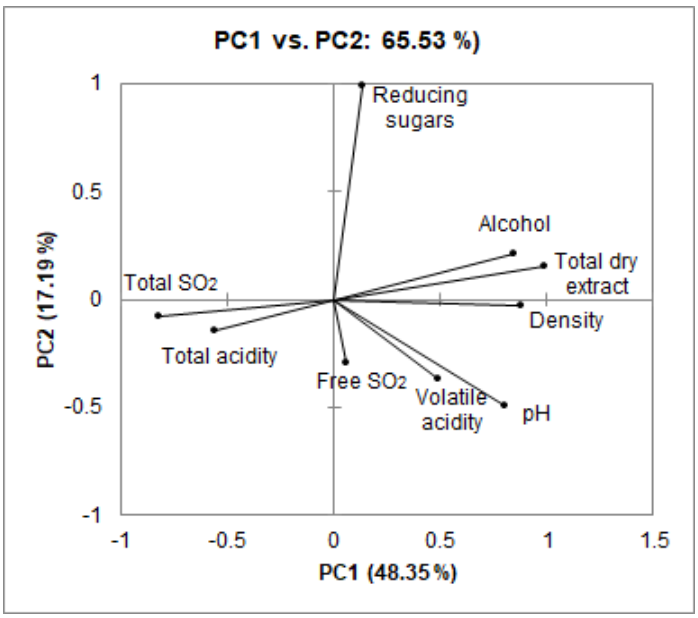
Principal component analysis (PCA) was applied to explore the contribution of each physico-chemical parameter on possible grouping among the analysed wines. PCA was performed in order to evaluate the effect of each factor (variety and vintage) on the physico-chemical profile of the analyzed Smederevka and Vranec wines and to identify the parameters that best discriminate the wines. The first two principal components, PC1 and PC2, accounted for 65.53% of the total variance (48.35% for PC1 and 17.19% for PC2). Projection of the wines on the first two principal components showed a separation mainly according to the variety (Fig. 1a). Thus, the wines from Smederevka variety, located at the negative part of PC1, were clearly separated from the wines from Vranec variety, located in the positive part of PC1. Further, wines were grouped and divided into subgroups according to the vintage. Thus, Vranec wines from vintages 2017, 2018 and 2002 were located at the positive part of PC2, while Vranec wines from 2019, 2021 and 2022 vintage were separated and located in the positive part of PC2. Concerning Smederevka variety, wines from vintages 2018 and 2023 were located in the negative part of PC2, while all other wines (2019-2022) were grouped in the positive part of PC2. In this respect, PC1 was mostly related to the variety, while PC2 to the vintage.

PCA results of the variables used for physic-chemical characterization of the wine samples displayed into the first two principal components are presented in the scatter plot in Fig. 1b. It could be noticed that only total acidity and total SO_2 prevail in the negative part of PC1, while all other parameters prevailed in the positive part of PC1. In fact, total acidity and total SO_2 were discriminant factors for the white wines and their content was higher in Smederevka wines, since white wines have to be protected with higher dose of SO_2 due to the absence of anthocyanins and lower dose of phenolic compounds in general, compared to red wines. For better stability, higher acidity in white wines is typical.

In general, the stronger correlation between alcohol, density and dry extract was notices, since all these parameters depends on each other and are considered as very important factors defining the wine quality in general.



(a)



(b)

Fig. 1. Principal component score plot (a) and correlation scatterplots (b) of the variables with PC1 and PC2 based on physico-chemical parameters for Smderevka and Vranec wines produced in six consecutive vintages.

CONCLUDING REMARKS

Vranec and Smerevska wines (*V. Vinifera* L. *cv*.) have been produced in 6 (six) consecutive years in order to study the influence of variety and vintage on the general physico-chemical parameters. It was noticed that variety significantly affected the chemical composition of wines, presenting higher contents of alcohol, specific gravity and dry extract for Vranec wines, while Smederevka wines contained higher concentration of total acids and higher amount of total SO_2. Influence of vintage was noticed for both varieties, observing highest values of alcohol, specific gravity and dry extract in vintages 2017 and 2020 for Vranec and Smederevka wines, respectively. Principal component analysis presented grouping of the wines mainly according to the variety.

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ВЛИЈАНИЕ НА СОРТАТА И ГОДИНАТА НА БЕРБА ВРЗ ОСНОВНИОТ ФИЗИЧКО- ВЛИЈАНИЕ НА СОРТАТА И ГОДИНАТА НА БЕРБА ВРЗ ОСНОВНИОТ ФИЗИЧКО-ХЕМИСКИ СОСТАВ НА ВИНА ОД СОРТИТЕ СМЕДЕРЕВКА И ВРАНЕЦ

Виолета Иванова Петропулос[1], Иле Илиевски[1], Стефан Аилер[2]

[1]Земјоделски факултет, Универзитет „Гоце Делчев", Штип, „Крсте Мисирков" 10 А, 2000 Штип, Република Северна Македонија

[2]Институт за хортикултура, Факултет за хортикултура и пејзажна архитектура, Словачки универзитет за земјоделие, „А. Хлинку" 2, 949 76 Нитра, Словачка

**Контакт автор: violeta.ivanova@ugd.edu.mk*

Резиме

Во ова истражување произведени се вина од сортите Смедеревка и Вранец (*V. Vinifera* L. *cv*.) во текот на шест последователни години, со примена на традиционални ферментациони методи, со цел да се проучи влијанието на сортата и годината на берба врз основниот квалитет на вината. Беа определени физичко-хемиски параметри со кои се потврдува основниот квалитет на виното, вклучувајќи алкохол, специфична тежина на 20°C, редуцирачки шеќери, вкупна и испарлива киселост, pH, слободен и вкупен SO_2. Резултатите покажаа дека сортата значително влијае на хемискиот состав на вината, при што највисоки содржини на алкохол, специфична тежина и вкупен сув екстракт се забележани за вината од сортата Вранец, додека вината од сортата Смедеревка содржеа најмногу вкупни киселини и вкупен SO_2. Дополнително, беше забележано мало влијание на годината на берба врз хемискиот состав на вината од двете сорти, особено на параметрите алкохол, специфична тежина и вкупен сув екстркат. Притоа, највисоки вредности за алкохол, специфична тежина и вкупен сув екстркат беа забележани во 2020 година за вината од сортата Вранец, додека за вината од сортата Смедеревка највисоки вредности на овие параметри се забележани во 2017 година. Анализата на главните компоненти покажа јасно сепарирање на вината според сортата.

Клучни зборови: *сорта, година на берба, Смедеревка, Вранец, основни физичко-хемиски параметри.*