INFLUENCE OF AUTHOCHTONOUS YEASTS ON THE QUALITY OF WINES FROM VRANEC AND CABERNET SAUVIGNON VARIETIES

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Summary

In this study 80 autochthonous yeast strains have been isolated from Vranec and Cabernet Sauvignon grape varieties grown in Tikveš wine region in the Republic of Macedonia. After the yeasts insulation, 10 yeast strains were selected and then used for fermentation and production of Vranec and Cabernet Sauvignon wines in order to test their influence on the wine quality. For that purpose, some basic parameters, including alcohol content, total acids, volatile acids, reducing sugars and pH were determined. Moreover, an analysis of total anthocyanins and polyphenols, as well as the color intensity and hue have been performed applying spectrophotometric methods. The results showed that the highest content of polyphenols and anthocyanins were measured for the wine fermented with F-20 yeast for Cabernet Sauvignon (CS-4 wine), and wine fermented with F-8 yeast, for Vranec (V-2 wine). An overall, the best wine quality was obtained with these two yeasts, showing highest phenolic profile as well as the best sensorial properties.

Key words: *autochthonous yeasts, wine, Vranec, Cabernet Sauvignon, polyphenols.*

INTRODUCTION

Polyphenols are large and complex group of compounds responsible for the characteristics, quality and colour of the grape and wines. Polyphenolic constituents are classified as flavonoids and non-flavonoids, which contribute to wine sensory characteristics, especially to colour, flavour and astringency and therefore, to the differences between the red and white wines. Wine flavonoids are divided to anthocyanins, flavan-3-ols, flavonols and dihydroflavonols, whereas the non-flavonoids include phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives) and stilbenes (resveratrol). In particular, flavan-3-ols confer the astringency and structure of the wine, and anthocyanins, as red pigments, are responsible for the colour of the wines. Anthocyanins are characterized as 3-monoglycosides, 3-acetylglycosides, 3-*p*-coumaroylglycosides, and 3-caffeoylglycosides of cyanidin, delphinidin, peonidin, petunidin, and the dominant, malvidin. The ratio of acetylated/*p*-coumaroylated anthocyanins and the sum of acylated anthocyanins is significant and characteristic for certain grape varieties (Ivanova et al. 2011).

Different factors, such as grape variety, temperature, soil and climate, ripening stage, influence the regional character of the grapes and thus the corresponding wine. In addition, the wine-making technologies and enological practices, such as intensity of pressing, fermentation temperature, maceration time, yeast strains, enzymes, SO_2-doses, affect the extraction of grape polyphenolic compounds into the juice (Ivanova et al., 2009, Ivanova et al., 2011). Furthermore, during the wine aging, polyphenolics could be modified by the temperature of storage or presence of oxygen, which could promote different chemical and enzymatic reactions affecting the wine quality.

The potential of yeast selection to affect the extraction of phenolic compounds in red wines has already attracted the scientists' attention. Yeast interacts with phenolic compounds and could influence the wine colour. At the beginning of fermentation, when yeasts are present in a variable amount, yeast cell wall can absorb anthocyanins and other polyphenolic compounds, influencing the colour. The absorption intensity depends on the yeast strain and cell wall affinity for water. Furthermore, yeast could enhance the phenolics extraction during maceration, influencing and improving the wine colour. In addition, it is very well known that yeast cell can release polysaccharides which are mainly mannoproteins and glucans. Their extraction occurs during the extended contact between the lees and wine due to the yeast autolysis. The extracted polysaccharides increase

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the wine mouthfeel, release pleasant aroma compounds, protect the tartaric precipitation and stimulate the malolactic fermentation. In a previous study (Vasserot et al., 1997), differences in polyphenolic concentration in wines fermented with two yeasts were found, which can be attributed to their interaction with the yeast cell walls during the fermentation followed with adsorption of anthocyanins and tannins (Mazauric and Salmon, 2005). The effect of yeasts on the content of polyphenolic components is related to yeast metabolism (e.g. pyruvic acid, acetaldehyde, tyrosol, glycerol) and use of ethanol as a source of carbon and energy.

Wine possess a variety of recognized beneficial effects on human health, such as anti-inflammatory, antimicrobial and anti-aging effects, and also plays a part in preventing of cardiovascular diseases. All these effects are due to the high content of stilbenes, anthocyanins, flavonols, flavan-3-ols and phenolic acids in the wine. In addition, polyphenols are confirmed to be the key compounds responsible for the antioxidant potential of wine, and therefore, it is very important to analyze these compounds and follow their changes during the wine fermentation, storage conditions and aging. Various assay methods for polyphenolic compounds have been developed and among them spectrophotometry (Ivanova et al., 2010) as very fast and simple method, as well as liquid chromatography (HPLC-DAD-MS) (Ivanova et al., 2011a, Ivanova et al., 2011b) are one of the mostly used techniques.

"Vranec" is the most widely cultivated and the most important red variety in Republic of Macedonia for production of high quality wines. The purpose of this study was to provide data for the phenolics content of Vranec wiens, as well as Cabernet Sauvignon wines produced with different yeast strains, isolated for the first time from local grapes, in order to study their influence on wine quality.

MATERIALS AND METHODS

Yeast strain isolation

Yeast strains were isolated from 10 Vranec and 5 Cabernet Sauvignon grape samples (15 kg of each sample), grown in different regions in the Tikveš area, Republic of Macedonia. Isolation of the yeasts was performed according to the method of Koh (Bambalov et al., 1996). For that purpose, after the grape crushing, SO_2 was added in a dose of 20 mg/kg SO_2, followed by a spontaneous fermentation carried out on a temperature of 25 – 28 °C. Maceration time of 6 days was applied. During fermentation, the grape mash was mixed 2-3 times per day, and after maceration period, the wines were separated from pomace. The material for isolation of yeasts was taken at the fourth day of the fermentation, and the isolation was performed from unitary colonies grown into a firm nutritious environment (sterile grape juice) in test-tubes cultivated on temperature of 25°C in a thermostat. The isolated strains were subjected to a gradual selection. At the beginning, 80 new strains were isolated during the primary selection, whose fermentable activity was being followed in a sterile grape juice (22.25% dry substances) on a temperature of 25 °C with refractometer. Forty sorts of yeasts from both varieties were isolated, which was the first step of the selection. The 40 sorts of yeasts selected on the first level were sowed into glass bottles with a volume of 330 mL in which 150 mL sterile grape juice was put with a sugar concentration of 222 g/dm^3, dry substances 22.45%, TK 4.14 g/dm^3 and pH 3.25. In each bottle the sowing was done with a quantity of 2cm^3 of fluid sowing culture. The bottles were enclosed with filtration locks.

Wine-making

Grapes from Vranec and Cabernet Sauvignon varieties were harvested at optimal maturity and transported to the winery. Then, grapes were crushed, followed with addition of SO_2 (20 mg/L) and different isolated yeasts for fermentation. 10 isolated yeasts from Vranec grapes and 10 isolated yeasts from Cabernet Sauvignon grapes were used for fermentation, obtaining 20 wines in total. 10 Vranec and 10 Cabernet Sauvignon wines were produced in order to study the influence of different yeasts on the content of polyphenols as well as some basic parameters, including alcohol content, total acids, total SO_2 and reducing sugars.

During the fermentation, wines were be "pumped over" two-three times a day. After the mechanical pressing, obtained wines were transferred to tanks for stabilization and aging.

Polyphenolic characterization

Determination of total polyphenols, anthocyanins, colour intensity and hue was performed applying spectrophotometric methods. Thus, for determination of total phenolics, the Folin-Chiocalteu method was used, measuring the absorbance at wavelength of 765 nm (Ivanova et al. 2010). For anthocyanin analysis, wine was

dilluted with solution of ethanol/water/HCl = 70/30/1 (*v/v/v*) and absorbance was measured at 540 (Ivanova et al. 2010). Colour and hue analysis were performed by direct measurement of wine samples at wavelengths of 420, 520 and 620 nm (Ivanova et al. 2010).

Statistical analysis

Different statistical treatments, including means, standard deviations, and Principal Component Analysis (PCA) were performed applying STATISTICA 6.0 (StatSoft Inc., USA) software package in order to ascertain possible significant differences between the studied wines. Significant difference was considered statistically at the level of $p < 0.05$.

RESULTS AND DISCUSSION

Content of total phenolics (TP), total anthocyanins (TA), colour intensity (CI) and hue (H) (Table 1) were determined applying spectrophotometric methods for analysis of Vranec and Cabernet Sauvignon wines, in order to check the influence of autochthonous yeasts on the extraction of phenolic components from grapes from both varieties. Furthermore, some basic parameters, such as alcohol, sugars, total acids, volatile acids and pH (Table 1) were determined applying international methods of analysis of wines and musts (OIV methods). A subset of ten yeast strains *Saccharomyces cerevisiae* were selected from the original 80 yeast screened, used to ferment Vranec and Cabernet Sauvignon grapes sourced from Tikveš wine region in Republic of Macedonia.

Different yeast strains (*Saccharomyces cerevisiae* species) could have influence on the extraction of grape polyphenols into the wine. In this study all Cabernet Sauvignon wines fermented with different yeast strains presented higher content of total polyphenolics and anthocyanins compared to the control wine fermented with a commercial yeast (Siha, Begerow), observing that wine CS-4 contained highest amount of these compounds and wine CS-8 had lowest amount. For Vranec wines, yeast strains presented different influence on the content of polyphenols and anthocyanins, observing highest content in wines V-2 and V-7. The effect of yeasts on the content of polyphenolic components is related to yeast metabolism (e.g. pyruvic acid, acetaldehyde, tyrosol, glycerol) and use of ethanol as a source of carbon and energy. Wines from both varieties with a highest content of polyphenols and anthocyanins exhibited highest colour intensity values, followed by lowest hue values. Hue values indicate that analyzed wines are young wines, since values between 0.5-0.7 are characterized for young wines.

Tab. 1. Content of total phenolics (TP), total anthocyanins (TA), colour intensity (CI) and hue (H) in Vranec and Cabernet Sauvignon wines

Wines	TP (mg/L)	TA (mg/L)	CI	H	Alcohol (%)	Sugars (g/L)	Total acids (g/L)	Volatile acids (g/L)	pH
Vranec									
Control	2413±9.8	421±8	1.65±0.02	0.56±0.004	14.2±0.01	2.6±0.01	4.76±0.5	0.24±0.01	3.61±0.01
V-1	2526±6.5	551±2.3	2.09±0.02	0.53±0.001	14.6±0.02	1.5±0.02	6.97±0.3	0.3±0.03	3.41±0.03
V-2	2847±6.4	619±2.3	3.30±0.02	0.46±0.001	14.2±001	2.4±0.9	7.12±0.3	0.3±0.03	3.37±0.03
V-3	1944±3.3	425±2.3	1.43±0.01	0.55±0.001	14.4±0.01	1.0±0.01	5.88±0.1	0.3±0.2	3.48±0.02
V-4	2032±9.8	453±4.6	1.69±0.01	0.56±0.002	14.6±0.02	1.6±0.01	6.07±0.2	0.39±0.03	3.48±0.01
V-5	2326±3.3	483±3.4	2.32±0.02	0.52±0.001	14.4±0.02	1.5±0.02	7.87±0.2	0.36±0.04	3.34±0.01
V-6	2227±3.5	467±2.3	1.86±0.00	0.54±0.002	14.5±0.01	1.9±0.8	6.3±0.2	0.36±0.03	3.38±0.02
V-7	2789±6.2	579±2.3	3.14±0.01	0.47±0.001	14.4±0.01	2.9±0.9	7.95±0.5	0.3±0.01	3.21±0.01
V-8	2401±6.4	509±2.3	2.55±0.02	0.49±0.001	14.0±0.01	0.8±0.05	6.41±0.4	0.36±0.02	3.27±0.01
V-9	2174±6.4	460±1.1	1.76 ±0.01	0.52±0.000	14.0±0.02	1.3±0.03	6.67±0.6	0.3±0.02	3.23±0.02
V-10	2473±16.4	497±1.1	2.58±0.01	0.50±0.001	14.5±0.01	3.2±0.04	6.86±0.4	0.3±0.01	3.17±0.01
Cabernet Sauvignon									
Control	1726±3.28	356.5±5.7	1.24±0.00	0.58±0.03	13.5±0.02	2.6±0.03	7.16±0.4	0.24±0.03	3.45±0.01
CS-1	2227±3.28	469.7±3.4	2.01±0.01	0.53±0.001	13.9±0.02	2.9±0.02	6.37±0.5	0.3±0.02	3.63±0.03
CS-2	2464±3.28	422.0±64	1.92±0.02	0.58±0.001	13.8±0.01	2.6±0.03	6.3±0.2	0.3±0.02	3.66±0.02
CS-3	2399±36.1	391.3±2.3	1.37±0.01	0.65±0.001	14.1±0.01	4.2±0.05	6.77±0.2	0.3±0.01	3.5±0.01

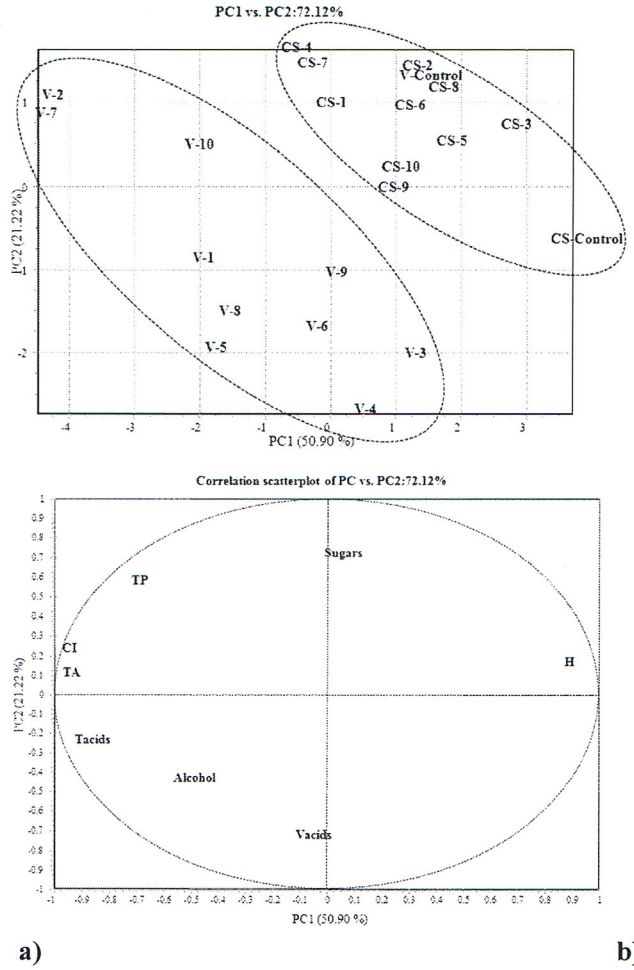
CS-4	2679±6.56	495.6±5.7	2.24 ±0.00	0.54±0.001	13.8±0.01	3.8±0.01	6.97±0.5	0.27±0.01	3.53±0.01
CS-5	2448±26.3	387.3±10	1.64±0.00	0.59±0.002	13.8±0.01	3.2±0.03	6.26±0.5	0.24±0.01	3.58±0.02
CS-6	2422±3.29	472.2±4.6	1.85±0.01	0.59±0.001	13.7±0.02	4.5±0.06	7.57±0.7	0.21±0.01	3.46±0.03
CS-7	2397±6.53	465.7±2.3	2.18±0.01	0.55±0.001	14.1±0.01	2.9±0.04	5.66±0.2	0.27±0.03	3.49±0.02
CS-8	2176±22.9	469.7±15	1.70±0.01	0.58±0.001	13.9±0.03	4.5±0.02	7.05±0.6	0.3±0.02	3.44±0.04
CS-9	2336±7.4	419.6±19	1.70±0.02	0.56±0.004	14.1±0.01	4.5±0.03	6.56±0.4	0.33±0.02	3.28±0.02
CS-10	2352±3.6	448.7±8	1.92±0.00	0.59±0.001	14.1±0.01	3.8±0.03	6.0±0.3	0.37±0.01	3.41±0.02

V: Vranec, Cs: Cabernet Sauvignon; TP: total polyphenols; TA: total anthocyanins, CI: colour intensity; H: hue
Results are average values of three replicates ± SD (standard deviation)

In general, the content of polyphenolics and anthocyanins was not statistically different between most of the wines which was in accordance to a previous study of Vranec wines (Ivanova et al. 2012). In fact, interaction of yeasts with the pigmented phenolic compounds in not well understood. Several works studied the influence of yeast selection on the phenolic profile of wines. Thus, the first study performed on Burgundy wine (Cuinier 1988) presented small effects on colour intensity and total phenolics. Another study of the effect of two yeast strains used for vinification of Pinot noir wines showed that there was no difference in total phenolics, anthocyanins and colour intensity observed in the wines fermented with two yeasts (Girard et al. 2001). Similarly, in other research whereas Vranec wines with same amount of SO_2, but fermented with different yeasts were analyzed, it was found that the influence of yeast on TP, TA, TF, $TF_{3\text{-}ols}$, CI and H was not statistically significant ($p > 0.05$), probably because the used yeasts for fermentation were from the same *Saccharomyces cerevisiae* species (Ivanova et al. 2012). In addition, recent studies showed that anthocyanins can be adsorbed at the yeast cell walls (Morata et al. 2003, Mazauric et al. 2005) leading to a reduction of their contents.

Concerning the alcohol which content was ranged from 13.5 to 16.6 % in the analyzed Vranec and Cabernet Sauvignon wines, there was not statistical difference between the wines fermented with different yeast species. The content of sugar ranged between 1 to 4.2 g/L indicates that fermentation was finished in all wine sets and sugar was converted into alcohol by the selected yeast species. Wines from both varieties did not present significant difference between the total and volatile acids, except control Vranec wine that contained lower amount of total acids. In fact, the content of total acids was relatively high, which is characteristic for young wines, as well as typical for Vranec wines that usually present high amount of these compounds. And, the volatile acids were present in a very low amount, ranged between 0.2 to 0.4 g/L indicating low amount of acetic acid, but also lactic, formic, butyric and propionic acids. All wines presented stable pH values, characteristic for wine.

Principal component analysis (PCA). Principal component analysis was performed on the following parameters: TP, TA, CI, H, alcohol, sugars, total and volatile acids in order to show which parameters are responsible for distinction of wines. Projection of Vranec and Cabernet Sauvignon wines on the first two principal components (72.12 % of the total variability) showed a clear separation according to the variety (Fig. 1a). In fact, Vranec wines were located in the negative part of PC1 (accounting for 50.90 % of the total variability) and Cabernet Sauvignon wines in the positive part of PC1.



a) **b)**

Fig. 1. Component score plot (a) and correlation scatterplot (b) of the variables with PC1 and PC2 based on total polyphenols (TP), total anthocyanins (TA), colour intensity (CI), hue (H), alcohols, sugars, total acids (Tacids) and volatile acids (Vacids) for the analyzed Vranec and Cabernet Sauvignon wines

PCA results of the variables used for characterization of the wine samples displayed into the first two principal components are presented in Fig. 1b. It could be noticed that hue values and sugars prevail in PC1 responsible for the separation of Cabernet Sauvignon wines, which showed higher amounts of these two parameters compared to Vranec wines. Total anthocyanins, colour intensity, total polyphenols and total acids were dominant in the second principal component and characteristic for Vranec wine, probably related to varietal character as well as influence of yeast species.

CONCLUSIONS

Results from this study provide valuable information about the content of total phenolics, anthocyanins and colour parameters, as well as basic chemical parameters of Vranec and Cabernet Sauvignon wines fermented with different autochthonous yeasts isolated for the first time from local grapes grown in Republic of Macedonia. It was shown that isolated yeasts species influenced the phenolic content, providing higher amount of extracted polyphenols in the wines during the maceration and fermentation. This was especially evident for the Cabernet Sauvignon wines, whereas control wine fermented with commercial yeast had lowest amount of total phenols and anthocyanins compared to the other wines fermented with isolated yeast strains.

REFERENCES

Bambalov, G., Spasov, H., Cvetanov, O., Bambalov, K. (1996): New strains of yeast for the production of red wines, Viticulture and winemaking, 5, 22-26 (on Bulgarian).

Cuinier, C. (1998): Influence des levures sur les composés phénoliques du vin. Bull. OIV, Vol. 689-690; pp. 596-60.

Ivanova, V., Dörnyei, Á., Márk, L., Vojnoski, B., Stafilov, T., Stefova, M., Kilár, F. (2011a): Polyphenolic content of Vranec wines produced by different vinification conditions, Food Chem., Vol. 124 No, 1; pp. 316-325.

Ivanova, V., Stefova, M., Chinnici, F. (2010): Determination of polyphenol contents in Macedonian grapes and wines assessed by standardized spectrophotometric methods. J. Serb. Chem. Soc., Vol. 75; pp. 45-59. (2010).

Ivanova, V., Stefova, M., Vojnoski, B., Dörnyei, Á., Márk, L., Dimovska, V., Stafilov, T., Kilár, F. (2011b): Identification of polyphenolic compounds in red and white grape varieties grown in R. Macedonia and changes of their content during ripening, Food Research Int., Vol. 44; pp. 2851-2869.

Ivanova, V., Stefova, M., Vojnoski, B. (2009): Assay of the phenolic profile of Merlot wines from Macedonia: effect of maceration time, storage, SO_2 and temperature of storage. Maced. J. Chem. Chem. En., Vol. 28; pp. 141-149.

Ivanova, V., Vojnoski, B., Stefova, M. (2012): Effect of winemaking treatment and wine aging on phenolic content in Vranec wines, J. Food Sci Tech. Mys., Vol. 49, No. 2; pp. 161-172.

Mazauric, J.-P., Salmon, J.-M. (2005): Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the Resulting wines, J. Agric. Food Chem., Vol. 53, No.14; pp. 5647-5653.

Morata, A., Gómez-Corovés, M. C., Suberviola, J., Bartolomé, B. (2003): Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines, J. Agric. Food Chem. Vol. 51; 4084-4088.

Vasserot, Y., Caillet, S., Maujean, A. (1997): Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters, Am. J. Enol. Viticult. Vol. 48; pp. 433-437.