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Influence of Mycorrhiza and Different Yeast Strains on Physical, Chemical and Organoleptic Properties of Graševina (*Vitis vnifera* L.) Wine

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The object of the mycorrhizal association between vine and fungus is to increase the absorption of water and/or nutrients from the soil. The aim of this research was to investigate the influence of mycorrhiza to the final product-wine. Besides, for the fermentation process authentic yeasts and four different commercial strains of yeasts were used. Grapes were grown in the wine growing region of Kutjevo, Croatia, in 2013. A typical variety from this region is Graševina. Obtained samples of wines were tested for alcohol, total acids, total polyphenol content (Folin-ciocalteu method), antioxidant activity (ABTS and DPPH method) and color hue. Sensory evaluation was done by professional tasters. Results showed that the usage of the different starter cultures of yeasts had different influences on wine depending on the mycorrhiza. Samples with mycorrhiza had a good extraction of polyphenols with the usage of authentic yeasts, while without micorrhiza starter cultures showed better results, which was evident in the physical and sensory properties as well.

Keywords: *Graševina, mycorrhiza, polyphenols, yeasts*

Introduction

Graševina is the most widely spread white grape variety in Croatia. The most important synonyms are: Italien Riesling (Romania), Olasz Rizling (Hungary) Laški Rizling (Slovenia, Serbia), Welschriesling (Austria, Germany and Switzerland) (Robinson et al., 2012). It gives fine wines of average and outstanding quality, pleasant varietal aroma and flavor, with a medium content of acid (Mirošević and Turković, 2003). In years in which the grape ripens normally, the wine is a clear greenish-yellow, or yellow with green tinges. It is delicate, moderately to well pronounced, with a complex fruity, floral aroma with a bouquet of almonds (Mirošević et al., 2011).

The study was conducted in the Kutjevo vineyards, which have over the years become synonymous of high quality Graševina wines. The Kutjevo vineyards spread on the southern slopes of Papuk and Krndija at altitudes from

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200 to 400 meters (Mirošević et al., 2011). The average annual air temperature is around 10.5 °C, and the mean annual temperature is characteristic for a continental climate. The vegetation flow temperature and rainfall allow proper course stages of the annual biological cycle of the vine (Maletić et al., 2008).

Mycorrhiza is a symbiosis of plants and fungi in this case the fungi are at the root of the vine. An important factor influencing mineral uptake is the symbiotic association formed between the root and mycorrhiza fungus (Jackson, 2014). Many researches confirmed the favourable impact of mycorrhiza on vegetative growth of the vine primarily because it enhances nutrients (mostly P and N) uptake and increases tolerance of vine to abiotic stresses (water stress, soil salinity, heavy metals) (Karoglan et al., 2015, Trouvelot et al., 2015). From that point of view P is particularly interesting because it is very important for plant growth, but it is often poorly available to plants due to its low solubility and mobility (Smith et al., 2011). Previous research (Karoglan et al. 2015) has shown mycorrhiza influence on yield and composition of grapes, but to the best of our knowledge there are no data about the influence on the chemical and sensory quality of wine as a final product.

The principal yeast specie involved in the grape must fermentation, particularly *Saccharomyces cerevisiae*, comprise a very large number of strains with varied technological properties (Ribereau-Gayon et al., 2007). The yeast strains involved in the winemaking process, influence the fermentation speed, the nature and quantity of the secondary products formed during alcoholic fermentation, and the aromatic characters of the wine (Robinson et. al., 2012).

The aim of this research was to determine the effect of mycorrhiza and different yeast strains to physicochemical parameters and the sensory quality of the Graševina wine from 2013.

Materials and Methods

Materials

This research is conducted in the vineyards and the cellar of Polytechnic in Požega. Grapes of the Graševina (*Vitis vinifera L.*) variety were used for the experiment. The vineyard is situated on southern slopes of Papuk mouneteen at an altitude 250 m. It belongs to the Eastern Slavonia region, the Kutjevo sub region.

The mycorrhization of vine is done on June the 7th 2013. The application of micorrhizal preparation was done in a way that the Mycoflor solution is injected in the root zone in the depth 30-40 cm.

Grapes were manually harvested on September the 24th of 2013. The average yield per vine was 1.0-1.5 kg. The amount of grapes used for the research was 50 kg/1 repetition (25L of must/1 repetition). The sugar level in the grape must from vine without micorrhiza treatment was between 106 and 113 °Oe, and in the must from vine with micorrhiza was between 106 and 116

°Oe. The total acidity of the must was 8.5-9.5 g/L. After the crushing and pressing of the grapes (mechanical press), must is put in tanks for precipitation. After 24 hours, clear must is decanted into fermentation barrels and starter cultures of yeasts were added. Besides commercial starter cultures, fermentation of both musts (with and without micorrhizal treatment) is also done by authentic yeasts.

By the combination of treatments the following samples were obtained:

GRKI-control sample, vine is not treated with micorrhiza and fermentation is done by authentic yeasts.

GRMI-vine is treated with micorrhiza and fermentation is done by authentic yeasts.

GRKII-control sample, vine is not treated with micorrhiza and fermentation is done by starter culture of yeast strain Siha active 7.

GRMII- vine is treated with micorrhiza and fermentation is done by starter culture of yeast strain Siha active 7.

GRKIII- control sample, vine is not treated with micorrhiza and fermentation is done by starter culture of yeast strain Siha White arome.

GRMIII- vine is treated with micorrhiza and fermentation is done by starter culture of yeast strain Siha White arome.

GRKIV- control sample, vine is not treated with micorrhiza and fermentation is done by starter culture of yeast strain Uvaferm CEG.

GRMIV- vine is treated with micorrhiza and fermentation is done by starter culture of yeast strain Uvaferm CEG.

GREX- control sample, vine is not treated with micorrhiza and fermentation is done by starter culture of yeast strain Anchor Exotics.

Total Polyphenols Determination

Polyphenols were determined according to the Folin-Ciocalteu method (Sharma et al., 2012, with modifications). An aliquot of wine (200 μ L) was mixed with 2 mL of water and of 100 μ L Folin-Ciocalteu reagent (Kemika, Croatia). The mixture was allowed to equilibrate for 5 minutes, and then 300 μ L of sodium carbonate solution (20 %) was added. After incubation at room temperature in the dark for 30 min, the absorbance of the mixture was read at 725 nm (Camspec M501, UK). Acidified methanol was used as a blank. The total polyphenols were determined with 3 replications. Gallic acid (Carlo Erba reagents, Italy) was used as a standard (calibration curve $y = 0.1602x - 0.0008$, $R^2 = 0.9998$), and results were expressed in mg of gallic acid equivalents per liter.)

Antioxidant Activity Determination (ABTS)

ABTS^{·+} radical was obtained by mixing 7.4 mM ABTS (Fluka, Switzerland) solution and 2.6 mM solution of ammonium persulfate in 1:1 ratio. The solution was left in the dark through the night in order to develop

stable radical, and then the radical solution was diluted with ethanol in 2:70 ratio to obtain an absorbance of approximately 1.100 (A_{ABTS}). An aliquot of wine, was mixed with 3.2 mL of diluted $ABTS^{\cdot+}$ radical. After incubation at room temperature in the dark for 95 min, the absorbance of the mixture was read at 734 nm (A_{EXTR}), and ΔA was calculated as $A_{ABTS} - A_{EXTR}$. Trolox (Sigma Aldrich, USA) was used as a standard. The decrease in absorbance caused by trolox was done in the same way as for the samples, and standard curve ΔA /trolox concentration was created ($y = 496.11x - 18.506$, $R^2 = 0.9962$). Determination of antioxidant activity was done in 3 replications. The results were expressed in μmol of the trolox equivalents per liter.

Antioxidant Activity Determination (DPPH)

An aliquot of wine (50 μL) is mixed with 2 mL DPPH of radical solution (0.1mM in ethanol). The absorbance of the mixture is read at 517 nm during a period of 30 min, the results were expressed as the mean of 3 replications. Pure ethanol is used as a blank.

$$\% \text{ inhibition} = [(A_0 - A_t) / A_0] \times 100$$

A_0 - absorbance of DPPH radical solution.

A_t - absorbance after 30 minutes.

Colour Hue Determination

The colour hue was determined by Hanna Instruments HI 83742 instrument according to the instruction manual. The results were expressed as the mean of three repetitions.

Alcohol, Total Acidity, SO_2 Determination

Alcohol strength by volume and total acidity were determined according to the official OIV methods (OIV-MA-AS312-01A, OIV-MA-AS313-01). SO_2 was determined according to the Ripper procedure (Wahl and Converse, 1980).

Sensory Evaluation

Wines were evaluated according to the 100 points OIV official method (O.I.V., 2001) by the panel of 11 professional tasters. The results were expressed as the mean value.

Data Analysis

The chemical composition data were analysed by Statistica 12 software, using *post hoc* LSD at 95% level.

Results and Discussion

Wine phenolics belong to two main groups, non-flavonoid (namely, hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) and flavonoid (namely, anthocyanins, flavan-3-ol monomers and polymers, flavonols and dihydroflavonols) (Castillo-Sanchez et al., 2008). Wine phenolics are considered to scavenge reactive oxygen species, to inhibit oxidation of oil systems, and to inhibit human low-density lipoprotein (LDL) oxidation (Roussis et al., 2008). When using the Folin-Ciocalteu method for polyphenols determination, it is very important to have a low value of free SO₂ (as shown in Table 1), because Folin-Ciocalteu reagent reacts not only with polyphenols, but also with other compounds with high antioxidant activity, especially ascorbic acid. Inorganic ions Fe²⁺, Mn²⁺, I⁻, and SO₃²⁻ also showed reactivity toward this reagent (Everette et al., 2010).

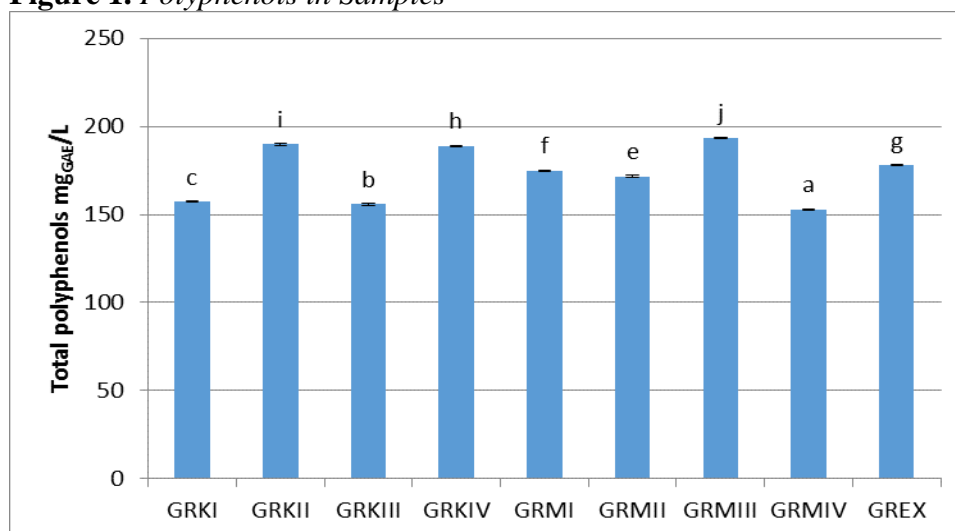
Table 1. Alcohol, Total Acids and the Amount of SO₂ in Samples ^{A,B}

Sample	Alcohol (vol%)	Total acid (g/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)
GRKI	14.30±0.03 ^b	7.65±0.14 ^{ab}	3.46±0.06	60.42±0.20
GRKII	13.03±0.04 ^a	7.48±0.11 ^a	5.76±0.08	73.60±0.23
GRKIII	14.60±0.03 ^d	7.85±0.28 ^{bc}	4.86±0.11	65.79±0.18
GRKIV	14.31±0.01 ^b	7.40±0.00 ^a	5.76±0.08	70.40±0.06
GRMI	14.32±0.00 ^b	7.95±0.14 ^{cd}	3.97±0.12	51.20±0.12
GRMII	14.50±0.00 ^c	8.05±0.07 ^{cd}	4.22±0.06	45.31±0.08
GRMIII	14.51±0.01 ^c	7.93±0.04 ^{bc}	5.50±0.04	50.43±0.23
GRMIV	14.79±0.01 ^e	7.85±0.07 ^{bc}	6.40±0.11	62.72±0.28
GREX	14.31±0.01 ^b	8.00±0.14 ^{cd}	6.66±0.10	56.96±0.16

^A Results were expressed as the mean of three repetitions ± standard deviation.

^B Means followed by the same letter in the columns are not statistically different at 5% probability.

Figure 1. Polyphenols in Samples ^{A,B}



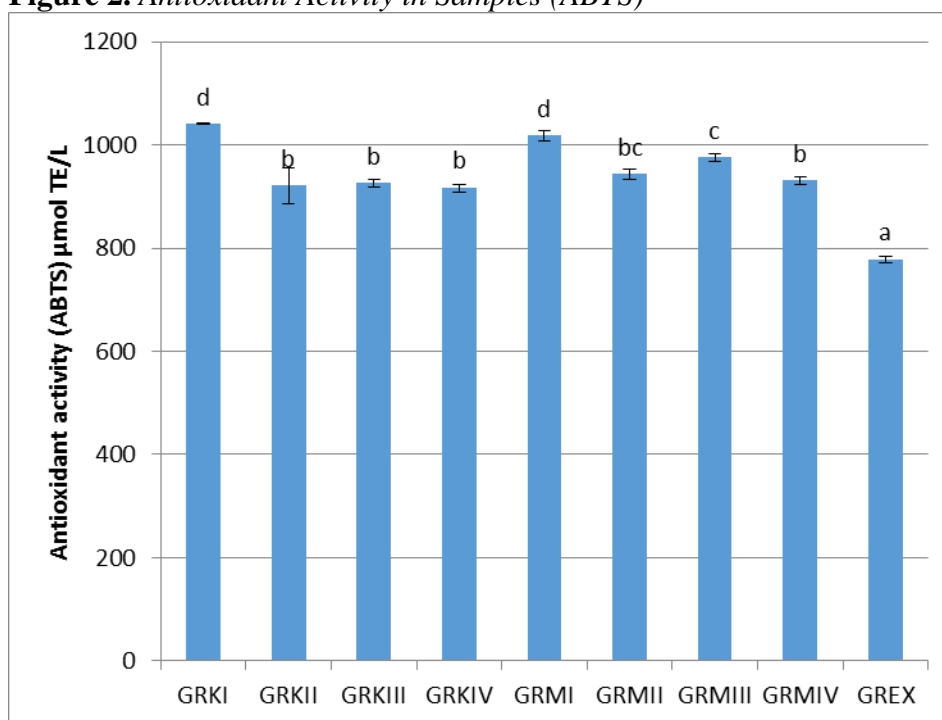
^A Results were expressed as the mean of three repetitions ± standard deviation.

^B Means followed by the same letter are not statistically different at 5% probability.

Winemaking techniques and oenological practices strongly affect the phenolic composition of red (Baiano et al., 2009), and white wines (Hernanz et al., 2007; Hernanz et al., 2009) As presented in Figure 1 the amount of total polyphenols in all samples is between 152.67 mg_{GAE}/L (GRMIV) and 188.80 mg_{GAE}/L (GRKIV), which is the typical value for the white wines (Roussis et al., 2008; Kallithraka, 2009). It can be seen that the extraction of polyphenols is under the significant influence of the yeast strain used for fermentation. Easy adaptability of yeast strains Siha 7 and Uvaferm CEG resulted in better extraction of the polyphenols to wines, but only in samples without mycorrhiza. On the other hand, in wines with mycorrhiza authentic yeasts also provided relatively similar results compared to Siha 7 yeast strain (174.62 mg_{GAE}/L and 171.50 mg_{GAE}/L, respectively), but the highest amount of polyphenols was determined in sample GRMIII.

Although polyphenols are the most important and the most popular antioxidants in wines there are also other molecules with antioxidant properties like carotenoids or products of Maillard reactions (Moreno at al., 2007). The correlation of antioxidant activity and polyphenolic content also depends on the method. ABTS and DPPH methods have gained popularity for the study of the antioxidant activity of wines due to their speed and simplicity, and both of them are based on free-radical scavenging activity.

Figure 2. Antioxidant Activity in Samples (ABTS)^{A,B}



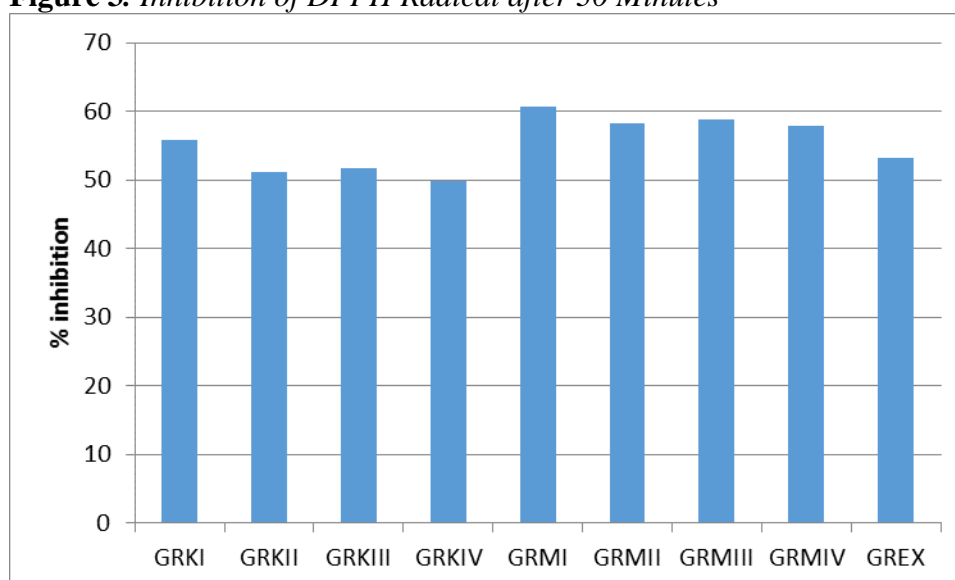
^A Results were expressed as the mean of three repetitions \pm standard deviation.

^B Means followed by the same letter are not statistically different at 5% probability.

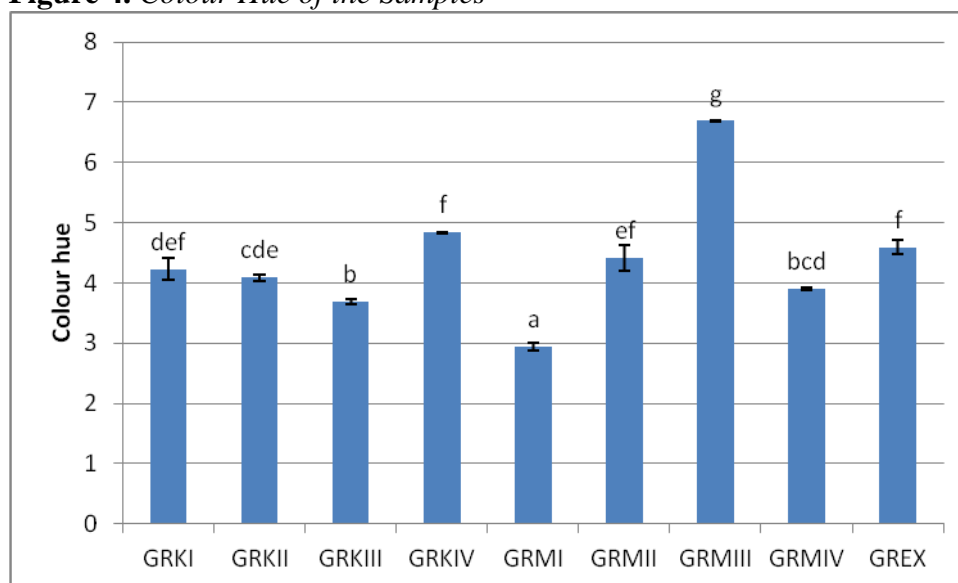
Baiano et al. (2009) showed low correlation between the amount of polyphenols in wines and antioxidant activity. They also concluded that

besides the previously mentioned antioxidants, antioxidant activity depends not only on the phenolic concentration, but also on the specific chemical structure of each phenolic compound. Previous researches showed good a correlation between the total polyphenols content and antioxidant activity in red wines (Büyüktuncel et al., 2014, Piljac-Žegarac et al., 2007), while the correlation in white wines varieties was low (Piljac-Žegarac et al., 2007). As it can be seen (Figures 2 and 3) antioxidant activity shows very bad correlation to polyphenol content regardless of the method used for antioxidant activity determination. The highest antioxidant activity (ABTS method) showed wines produced by authentic yeasts, but exotic yeast strains resulted in very low antioxidant activity. The results obtained by the DPPH and ABTS method have a bad linear correlation ($R^2 = 0.256$). When expressed as a percentage of DPPH radical inhibition, samples with mycorrhiza showed higher values of inhibition in period of 30 minutes than samples without micorrhiza.

Figure 3. Inhibition of DPPH Radical after 30 Minutes



The highest value for the colour hue from the samples without micorrhiza showed sample GRKIV (Figure 4), the same as for the polyphenols. In samples with micorrhiza the highest polyphenolic content and the highest value for colour hue had the sample GRMIII. Although the colour depends on other compounds besides polyphenols (carotenoids, browning reactions products), in this case it can be seen that is directly connected to the level of polyphenols.

Figure 4. Colour Hue of the Samples^{A,B}

^A Results were expressed as the mean of three repetitions \pm standard deviation.

^B Means followed by the same letter are not statistically different at 5% probability.

Table 2 shows the average value of properties: appearance (clarity, color), odor (cleanliness, intensity, quality), flavour (cleanliness, intensity, durability, quality), harmony and overall rate for Graševina wine, harvest 2013.

Table 2. The Appearance (Clarity, Color), Odor (Cleanliness, Intensity, Quality), Flavour (Cleanliness, Intensity, Durability, Quality), Harmony and Overall Rate, Graševina Wine, 2013

Graphics	GR KI	GR MI	GR KII	GR MII	GR KIII	GR MIII	GR KIV	GR MIV	GR EX
Clarity	5	5	5	5	5	5	5	5	5
Colour	10	10	10	10	10	10	10	10	10
Appearance	15	15	15	15	15	15	15	15	15
Cleanliness	4.3	4.5	3.9	4.2	4.3	4.1	4	4	3.9
Intensity	5.6	5.9	5.1	5.6	5.5	5.5	5.4	5.6	5.4
Quality	12.2	12.5	11.3	11.8	11.6	11.8	11.8	11.5	11.3
Odour	22.1	22.9	20.3	21.6	21.4	21.4	21.2	21.1	20.6
Cleanliness	4.3	4.6	4.4	4.4	4.8	4.5	4.3	4.1	4.5
Intensity	6.5	6.5	5.6	6.2	6.3	6.4	6.5	6.2	6.4
Durability	6.4	6.6	5.8	5.8	6.4	6.5	6.3	6.3	6.2
Quality	16.5	16.5	16.5	16.3	16.8	17.1	16.3	14.9	16
Flavour	33.7	34.2	32.3	32.7	34.3	34.5	33.4	31.5	33.1
Harmony	9.3	9.3	9.2	9.2	9.6	9.6	9.2	9.1	9.6
Overall rate	80.1	81.4	76.8	78.5	80.3	80.5	78.8	76.7	78.3

Considering that wines have not been filtered and there were no colour defects, clarity and colour of all samples were graded with maximum points. Wines fermented with authentic yeasts had better scores for odour (22.1 and 22.9 out of a maximum 30) than the wines fermented with other yeast strains, regardless of micorrhizal treatment. The minimum score for odour is assigned to wine fermented by exotic yeast strains. Out of the maximum 44 points, the highest score for flavour was assigned to the sample GRMIII, followed by GRKIII. As previously mentioned the same sample had the highest amount of polyphenols and the highest colour hue. Usage of yeast strain Siha White arome results with very good chemical characteristics and fruity taste of white wines, which was recognised by panellists and confirmed by chemical analysis. Samples obtained by fermentation with authentic yeasts also had very good scores for flavour. In general, samples with micorrhizal treatment had better scores for flavour than control samples fermented by the same yeast. The overall rate was the highest for wines produced by authentic yeasts, followed by the Siha White arome yeasts. In three of four cases wines with micorrhizal treatment had a higher overall rate.

Conclusions

Both treatments used in this study, mycorrhizal treatment and usage of different yeast strains had a significant influence on all analysed parameters. Siha White arome yeast strain showed the best results considering polyphenols, colour hue and sensory evaluation. Authentic yeast strain showed very good results in sensory evaluation and antioxidant activity. Polyphenols content and antioxidant activity showed a low correlation regardless the method used for antioxidant activity determination. Wines obtained from grapes with micorrhizal treatment compared to the control wines showed better results for all tested parameters. Additional researches are needed in the field of mineral content in wines as well as the repetition of research in the following year.

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