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Production of Red Wines

Implementation of Anutochtonous Yeast Strains

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Abstract

Microbiology is a science that studies the morphology, physiology, genetics and ecology of small organisms - microorganisms, as well as their role and meaning in human and environmental life. Microorganisms participate in the transformation of matter, and the processes that cause them are known for a long time. The role of almonds in the process of transformation of grape juice into wine has been elaborated in the second half of the 19th century, when the research carried out by Louis Pasteur suggests that alchohol fermentation takes place with the help of small, living organisms, rather than actually taking place only "lifeless" chemical reactions. As an ancient tradition, over the years, wine production has spontaneous fermentation of grape juice with the participation of wild yeasts, which belong to different genera and species. The number, species and their presence in the fermentation process depends on several factors: most of the quality of grapes, the specific region, climatic conditions in the harvest year, which leads to unpredictability of the results in spontaneous fermentation. Often this fermentation leads to deterioration of the organoleptic of the wine - the result of unwanted processes, malolactic or acetic acid fermentation, etc., which indicates the need to use pure yeast cultures in the production of wine. In the mid-60s of the 20th century on the market appear the first products active selective yeasts, which are well accepted by wine producers. Nowadays wine production uses mostly pure cultures of selected wines, which has led to significant technological progress, due to a number of advantages: ability for quick and efficient fermentation of grape pulp or must with high concentration of sugars, resistance to high concentration of ethanol and SO_2, resistance to high temperatures during fermentation. Increasing use of commercial products - selected types of yeasts in wine production leads to the loss of autochthonous, local for a given region of yeast populations. In recent years, there has been a growing interest among scientists and wine producers for autochthonous strains of yeasts. After the selection of autochthonous strains of yeasts with good characteristics, they can be successfully used in the production of wine. This would improve biodiversity in a

given area, enrich the biological heritage, which is essential for the production of wines of controlled origin, with typical taste and aroma.

Two autochthonous yeast strains, called F-8 and F-78 (isolated and selected from the Tikveš wine-producing region) were inoculated in wine musts from Vranec and Cabernet Sauvignon grape varieties. The fermentation process and quality of the produced wines were compared to the wines produced from the same grape varieties, but with a commercial yeast strain (D-80). The fermentation was undertaken at 23-25ºC for 16 days. The highest alcohol content was detected in Vranec wine fermented with autochthonous F-8 yeast strain (14.96%), while Cabernet Sauvignon wine produced by autochthonous yeast strain F-78 contained the highest amount of alcohol (14.68%) for this specific grape variety. Unlike to the alcohol content, Vranec wine produced by commercial yeast strain D-80 indicated the highest concentration of total phenolic compounds (1450 mg/L) and total anthocyanins (572.1 mg/L), while the lowest concentrations were observed in wine fermented by autochthonous yeast strain F-78 (1612 mg/L and 470 mg/L, respectively).

Introduction

Grapes (*Vitis* spp L.) are one of the most important horticultural crops across the world and have widely received considerable attention because of their polyphenols. Polyphenols are a major source of phytochemicals in grapes, mainly stilbenes (resveratrol), flavanols, proanthocyanidins, anthocyanins, and phenolic acids in varying amounts in the skin, flesh, and seed. They show a wide spectrum of health promoting properties, including antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial activities [1–4]. The annual world grape production for 2017 exceeded 77 million tons. Turkey is one of the world's three largest grape producers, at nine million tons [5]. The *Vitis* genus is represented by 80 species, composed of two sub-genera, Muscadinia Planch. and Euvitis Planch. Most cultivated grapevines belong to the Euvitis sub-genus, including two particularly economically important cultivated grapevines, V. vinifera (Eurasian group), which accounts for most of the world's Vitis varieties, and V. labrusca (American group). However, species belonging to a third group (East Asian group) are of limited importance in viticulture [5]. It has been reported that V. labrusca including its hybrids and V. vinifera grapes (Isabel, Concord, Bordô, Niagara, etc.) are the main cultivars used in the grape processing industry (for use in wine, juice, raisins, table grapes, grappa, etc.) in the world's 10 largest producers (more than 85% in the USA and Brazil).

The most abundant polyphenols in grapes and wines are anthocianins. Anthocyanins are natural pigments responsible for orange, pink, red, violet and blue colours in the flowers and fruits of many plants which are using as colorants in food and beverages industry. Anthocyanins (of the Greek anthos=flower and kianos=blue) are under huge investigation due to the growing interest of substitution the potential cancerogenic synthetic colors which are still using in food industry [6]. Anthocyanins exhibit a huge range of biological activities as antioxidant activity and prevention of neuronal and cardiovascular diseaseses, cancer and diabetes. The role of natural pigments in the coloring of different plants is under current investigations of many studies [7]. The structure of anthocyanins and their properties as colorants in plants

were subject of investigations of Brouillard et al. [8]. Radical scavenger activity of anthocyanins and their glycols were also studied in the work of Khknen and Heinonen [9]. Grape skin contains a great number of anthocyanins, the concentration varies greatly according to the variety and is influenced by cultivar, season and environmental factors. The most abundant anthocyanins in red grapes are 3-glycosides, 3-acetylglycosides and 3-p-coumaroyl-glycosides of malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy) [10, 11]. Changes of anthocyanins profile in Vitis vinifera grapes grown in the Douro Valley was studied in the work of Mateus, as well as their concentration in produced wines [12, 13].

In present days, mainly pure selected cultures of wine yeasts are used in winemaking, which led to a significant improvement of the wine technology, because of the multiple benefits: ability to a quick and effective fermentation of grape juice or must with high sugar concentration; tolerance to high levels of ethanol and SO_2; resistibility to high temperatures during fermentation [14]. The increasing usage of trade produced selected yeasts for winemaking really lead to a lost of the natural and regional populations of yeasts. The relationship between growing region, cultivar, climate and microbial biogeography of 273 grape must samples from the region of California in two separated vintages (2010 and 2012) has been the focus of study of Bokulich et al. The group of Bokulich confirmed nonrandom regional distribution of grape microbiota across growing regions. The growing regions included in this study were distinguished by several key groups of grapevine fungi and bacteria. It demonstrated that microbiological community patterns across the regions and vintages depended from grape variety and local environmental conditions [15]. The most used trade produced yeast belongs to Saccharomyces species but, other species of yeasts such Hanseniaspora, Kluyveromyces, Metchnikowia, Candida, Zygosaccharomyces, Brettanomyces can be active at different stages during fermentation and destructive for wine favor. Therefore, the group of Bokulich, developed terminal restriction fragment length polymorphism (TRFLP) approach for

profiling the yeast community of wine [16]. Last few years, an increasing interest among scientist and winemakers in the local yeast cultures was observed. After selection, yeast cultures with good characteristics could be applied successfully in winemaking [17]. The quality markers of red wines from Vranec variety were published by few researchers in the last few years. The effect of enological practices on the stilbene levels in Vranec wines was published by Kostadinovic´ et al. (2012). According to their findings, the most important factor for the level of trans-resveratrol and its piceid in Vranec wines was maceration time. Although both yeasts, i.e. ‘‘Levuline CHP” and ‘‘Vinalco” belong to the same group of Saccharomyces cerevisiae, higher concentrations of trans-resveratrol and piceid have been obtained with French yeast ‘‘Levuline CHP” in comparison to Macedonian yeast ‘‘Vinalco”. Total phenolic acid and anthocyanins were higher in Vranec wines produced with locally isolated yeasts in comparison to wines fermented with commercial yeasts [18].

Nowadays, mainly selected pure cultures of wine yeasts are used in winemaking, which led to a significant improvement of the wine technology and respective final quality, because of multiple benefits, viz.: the ability to undertake a quick and effective fermentation of grape juice or must with high sugar concentration; resistibility to high levels of ethanol (C_2H_6O) and sulfur dioxide (SO_2); and resistance to high temperatures during fermentation.

Indeed, the increasing usage of traded selected yeasts for winemaking leads inevitably to a loss of the autochthonous yeast populations naturally present in regional grapes and, consequently, drives to the potential loss of genetic diversity and heritage. Largely, the specificities, authenticity, uniqueness and, mostly important, the quality characteristics of the wine are dependent on the natural microbiota found in the grapes of each viticulture region and, of course, the quality of the grapes by itself – which deeply depends on soil and climate conditions of the geographic region and the employed viticulture techniques, which also determines per si the type of microbiota present therein.

Over the last years, an increasing interest among scientists and winemakers was observed in exploiting autochthonous yeast strains. After isolation and selection of yeast strains with good phenotype and technological characteristics, they can be further employed in winemaking processes with high potential to be successful. Such a trend can be very important towards ameliorating the microbial biodiversity in the selected region, and contributing to enrich the biological heritage – which is of great importance, in a highly competitive market, for the winery industry chasing for wine appellation and production of wines with unique/differentiated flavors and aromas.

Comparison of the main communities of cultivable yeast species and strains between two different neighboring Vitis ecosystems suggested that specific (yet unknown) characteristics of different symbiotic Vitis species may contribute to the assembly of specific communities of biologically compatible yeast strains from a given number of species. In turn, the growth dynamics of these yeast populations during spontaneous fermentation can be translated into specific organoleptic and sensory traits of the final wines, depending on each variety of grape. In addition, the microbial dynamics of alcoholic fermentation significantly affect the extraction of anthocyanins from grape skins and tannins from grape seeds, which will further influence in cascade the overall bitterness and astringency of the red wines [14]. Moreover, different maceration techniques – namely enzyme treatment, cold soaking, post-maceration, as well as the combination of cold with post-maceration – influences the extraction process and the subsequent level of anthocyanins and tannins in wines [15]. The environment of a wine fermentation has a great impact on the extraction and retention of tannins and other phenolic compounds from the red grape skins and seeds [16, 17]. An adequate selection of the indigenous and commercial yeast strains combined with the use of enological tannins containing a mixture of phenolic compounds was employed to drive the transformation of the grape anthocyanins into certain derivatives, which, some of them, are more stable from a chemical standpoint [18,19]. On the other hand, anthocyanins are the main compounds present in young red wines, being responsible for their intense red color.

These pigments are mainly located in the grape skins and their extractability during winemaking depends on many factors, such as their concentration in cell vacuoles and their interaction with the cell-wall polysaccharides, thus further affecting their stability and concentration in the must [20]. The biochemical pathways of formation, in red wine, of a great variety of pyranoanthocyanin structures, namely carboxypyranoanthocyanins, methylpyranoanthocyanins, pyranoanthocyanin-flavanols, pyranoanthocyanin-phenols, portisins, oxovitisins and pyranoanthocyanin dimers, can significantly influence the color and the overall taste of red wines as well as the monomeric anthocyanins and their color expression [21-23].

The main object of the current research effort was to attain the estimation of the best winemaking procedure and the most favorable yeast strains towards the production of high-quality premium wines from Vranec and Cabernet Sauvignon grape variety from the "Tikveš" wine-growing region. The wines from both grape varieties were fermented by two autochthonous yeast strains, F-8 and F-78, and a commercial yeast strain, D-80, all strains belonging to Saccharomyces cerevisiae species. The microbial dynamics of alcoholic fermentations were ascertained and the quality of the produced wines estimated. The enological parameters under scrutiny were the percentage of alcohol, sugar content, total and volatile organic acids, and pH of the wines. Furthermore, total phenolic content, total anthocyanins and color intensity (IC) were also determined in order to estimate the best winemaking procedures to yield in such a high-quality premium wines from Vranec and Cabernet Sauvignon grape variety from the "Tikveš" wine-growing region of the Republic of North Macedonia (MK).

Chapter 1

Materials and Methods

1.1. Grapes from Vranec and Cabernet Sauvignon variety for wine-making

Grapes were provided by local farmers from Kavadarci wine-growing region. Grapes from Vranec and Cabernet Sauvignon varieties for this study were harvested in September 2016 and 2017 in the area of "Crveni Bregovi", located at the "Tikveš" wine-growing region (Republic of North Macedonia). The grapes were harvested at their optimum time of maturation. Samples of undamaged grapes were taken at random for further analyses, placed in sterile bags and kept in the refrigerator at 5ºC.

Prior to the analyses, the grapes were selected manually by tossing out rotten or peculiar-looking grapes. After washing and removing the steams, the grapes were crushed for releasing the must. The total sugar content was determined according to the methodology described by the International Standard Method OIV-AS2-02-SUCREF (2007) 15, while titratable acidity and pH were determined by the International Standard Method OIV-AS-313-01-ACITOT (2009) 16. These assays in grapes were performed in two replicates of samples and the chemical parameters were determined in duplicate. All chemicals for these grape analyses were purchased from Sigma-Aldrich (Seelze, Germany) and Merck (Darmstadt, Germany), and were of analytical-reagent grade.

1.2. Isolation, selection and identification of indigenous yeast strains F-8 and F-78 from the Tikveš wine-growing region

The procedure of isolation and selection of indigenous yeast strains F-8 and F-78 was previously described by research group of Ilieva [12-14]. In brief, a spontaneous fermentations of 10 different lots Vranec and 5 different lots of Cabernet sauvignon from different micro-regions were held. After destemming, crushing and

sulfiting (dosage 20 mg/kg, 5% sulphureous acid), the spontaneous fermentation took place at 25-28 ºC in polyethylene terephthalate (PET) vessels. From the experimental trials, pure yeast cultures were isolated following the Koch method (Bambalov and al., 2000) 20. The microbial isolation was executed from single colonies, cultivated in test tubes with nutrient agar (NA) culture medium with sterilized grape juice, in a thermostat in water bath at 25ºC. Subsequently, the isolated and purified strains were subjected to a three-stage selection based on their fermentation ability.

Moreover, the fermentation activity of the selected pure yeast strains F-8 and F-78 was evaluated in sterilized liquid grape juice. In sterilized sample tubes with 10 mL of the aforementioned juice was inoculated each yeast culture, previously activated during 72-hours, using a sterile inoculating loop. The assays were thermostated in water bath at 25°C, and the evolution of the alcoholic fermentation was checked with a refractometer (Krüss, Hamburg, Germany). The F-8 and F-78 autochthonous yeast strains were selected based on their superior technological characteristics. Afterwards, they were inoculated in the prepared sterile tap glass bottles in the amount of 2 mL of liquid inoculum for evaluation of their fermentation activity. The bottles were closed with fermentation caps and the course of the fermentation was monitored by evaluation of the levels of residual sugar. The first determination of sugar fermentation capacity of the selected yeasts was determined in Petri dishes by pouring each strain in a mixture of 0.6% (w/v) yeast extract solution with the addition of 0.2% (w/v) of each of the following carbohydrates: glucose, fructose, galactose, maltose, saccharose and raffinose (in this last case in a concentration of 0.4%, w/v).

For the second, and final confirmation of the ability of the strains to assimilate carbon sources the kit BioMerieux API 20C AUX (Merck, Darmstadt, Germany) was used (Ilieva et al., 2019) 19. Biomass from pure fresh colonies were suspended in a saline solution (0.1%, w/v). After first homogenization, 0.1 mL of the previous suspension was added to the control mixture (C) from the kit, which contains nitrogen derivatives, vitamins, growth factors but in the absence of carbohydrate

sources. After a second homogenization, the mixture was pipetted into the wells of the API 20C strip and incubated at 25ºC. At the incubation times of 24, 48 and 72 h, the turbidity of the liquid in the wells was compared against to the control (C) to verify the capacity to metabolize the different carbon sources. Based on these results, the identification to the species level of the yeast strains was possible to be achieved.

1.3. Inoculation of indigenous and commercial yeast strains in the wine-making of Vranec and Cabernet Sauvignon wines

Vranec and Cabernet Sauvignon grapes were equally allocated to the lots, in order to make similar fermentation conditions. About 20 kg destemmed grape grains were crushed with manual crusher and 20 mg/kg SO2 was added. Two hours after crushing, the two indigenous yeast strains (F-8 and F-78) and one commercial yeast strain D-80 (Lallemand) for each lot and each variety were inoculated by dosage of 0.2 g/L. The period of maceration for all trials was 16 days. Alcoholic fermentation was controlled on the temperature range of 23-25°C.

1.4. Determination of oenological parameters in trial wines

Determination of the amount of alcohol was performed eudiometrically by Dujardin-Salleron ebuliometer (GW Kent, Ypsilanti, USA) method (Zoecklein et al. 1995) , and for the determination of total reducing sugars the Luff-Schoorl method (ISI 28-1e: Determination of Reducing Sugar, DE by Luff-Schoorl's method) was used and the results expressed as g/L. Quantification of titratable (TA) and volatile (VA) organic acidity in wines was performed according to the methodologies described by Ilieva et al. (2017) 19, and both expressed as g/L. Determination of color intensity (IC) (as absorbance units, a.u.). was performed spectrophotometrically by measuring the absorbance of the wines at 420 nm (yellow color), 520 nm (red color) and 620 nm (blue color) with an UV spectrophotometer (Shimadzu 1800, Shimadzu Corporation, Kyoto, Japan). Determination of total monomeric anthocyanins was performed by the AOAC International Method (Official Method 2005.02: Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages,

Natural Colorants, and Winesand) and expressed as mg/L. Determination of total phenolic content was performed by the AOAC International Method (AOAC SMPR 2015.009: Estimation of Total Phenolic Content Using the Folin-Ciocalteu Assay, 2015) and expressed as mg/L. The pH values of the wines were determined by the International Standard Method according to OIV-MA-AS313-01.

The oenological parameters were determined in two replicates and the analytical measurements were performed in duplicate (2 x 2 = 4). All chemicals in these experiments were purchased from Millipore Sigma (Burlington, USA) and Merck (Darmstadt, Germany), and were of analytical-reagent grade.

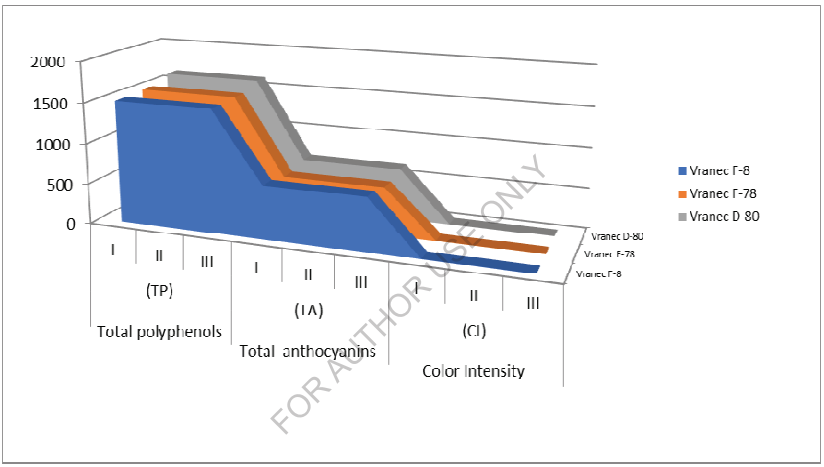
1.5. Statistical analysis

A one-way ANOVA was used to examine the impact of every selected yeast strains on the level of total anthocyanins, total phenolic compounds, reducing sugars, pH, volatile and titratable acids. The level of significance in differences between anthocyanin content and total phenolic content was determined by 5% by a one-way ANOVA using Tukey's test. SPSS v.16.0 software (IBM corporation, USA) was used for the creation of the treatments and further data analysis.

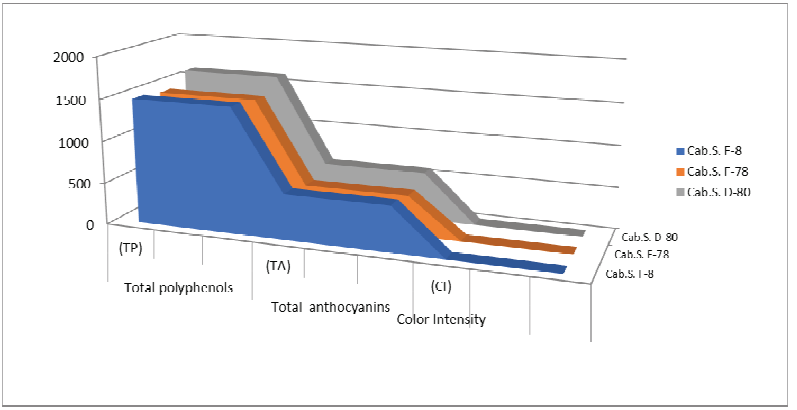
Chapter 2

Results and discussion

The six experimental wines were produced from Vranec and Cabernet Sauvignon grape varieties by application of indigenous yeast strain (F-7 and F-78) and commercial yeast strain (D-80) (Graph 1 and 2).



Graph 1. The graph shows the content of total phenolics (TP), total anthocyanins (TA), color intensity (CI) in wine Vranec produced by indigenous strains of yeasts F-8, F-78 and commercial D-80 of three replicates.



Graph 2. The graph shows the content of total phenolics (TP), total anthocyanins (TA), color intensity (CI) in wine Cabernet Sauvignon produced by indigenous strains of yeasts F-8, F-78 and commercial D-80 of three replicates.

2.1. Dynamics of alcoholic fermentation of produced wine from Vranec and Cabernet Sauvignon grape varieties

The indigenous and commercial yeast strains demonstrated different dynamics of alcoholic fermentation and different content of fry matter for Vranec wines. As we can see from Table 1, application of indigenous yeast strain F-78 indicated fast and sharp decrease of sugar in the wines from the Vranec grape variety while application of commercial yeast D-80 implicated slow decrease of sugar in the wines from the same grape variety. The difference of the shape of the curves for three Vranec wines is especially notable from 5th till 8th day of fermentation (Table 1).

Table 1. Chemical characteristic of wine Vranec and Cabernet Sauvignon at 2017 year

Wines	**Yeast**	**Alcohol (% v/v)**	**Sugar (g/L)**	**Total scids (g/L)**	**Volatile acids (g/L)**	**pH**	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**
Vranec	F-8	15,14	1,9	6,7	0,69	3,31	20	68
Vranec	F-78	14,45	1,7	7,01	0,41	3,29	21	58
Vranec	D-80	14,68	1,5	7,19	0,5	3,22	23	62
Cabernet Sauvignon	F-8	14,78	1,7	5,21	0,45	3,71	21	95
Cabernet Sauvignon	F-78	14,95	1,5	6,17	0,61	3,75	25	74
Cabernet Sauvignon	D-80	14,05	1,8	5,15	0,5	3,72	32	98

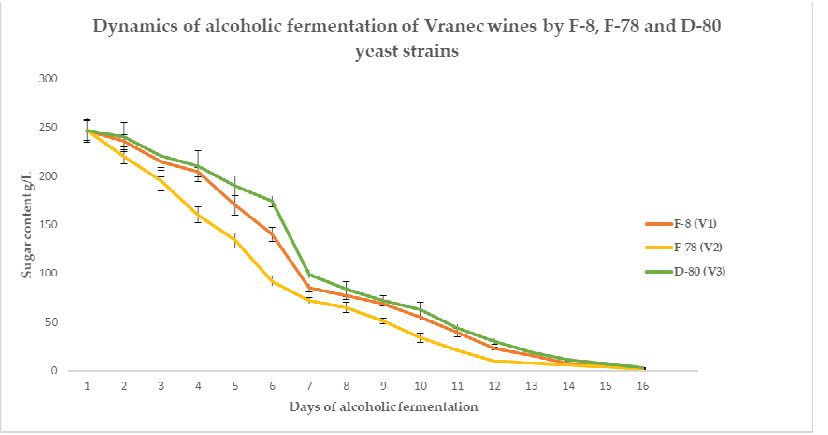


Figure 1. Dynamics of alcoholic fermentation for Vranec wines produced by indigenous and commercial yeast strains

At the end of alcoholic fermentation (16th day of fermentation), the amount of residual sugar was in the range of 2 to 3 g/L which implicated production of dry wines while the percentage of alcohol was in the range from 13.97-14.68. Results from Figure 1, indicated that the other two yeasts (F-8 and F78) also finished the alcoholic fermentation with similar amounts of residual sugars.

A dynamics of alcoholic fermentation of wines produced from Cabernet Sauvignon grape variety by application of the same yeast strains (F-8, F-78 and D-80) is presented in Figure 2.

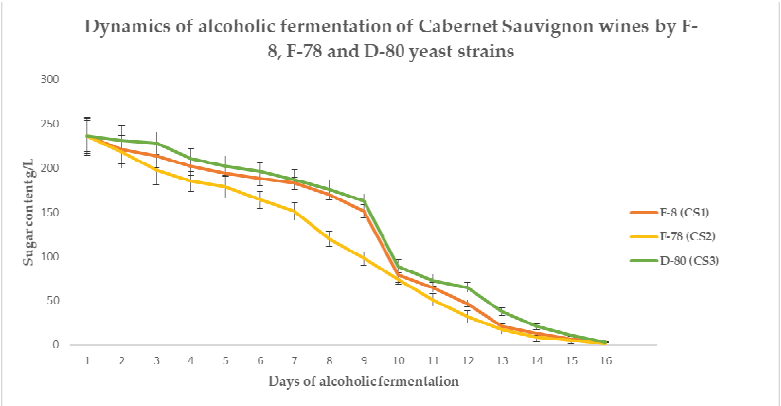


Figure 2. Dynamics of alcoholic fermentation of Cabernet Sauvignon wines produced by indigenous and commercial yeast strains

As we can see from Figure 2, the shapes of the curves are slightly different in comparison to Vranec wines. The decease of sugar in Cabernet Sauvignon wine fermented by F-78 (CS2) is smooth while CS1 (F-8) and CS3 (D-80) wines had similar shape of curves with sharp decrease of sugar content from 9th till 11th day of fermentation. The percentage of alcohol for Cabernet Sauvignon wines were in the range from 14.11-14.96%-vol. The amount of residual sugar was less than 3 g/L.

2.2. Oenochemical parameters of Vranec and Cabernet Sauvignin wines produced by indigenous and commercial yeast strains

The results from oenochemical parameters of Vranec and Cabernet Sauvignon wines produced by application of indigenous yeast strains F-8 and F-78 and commercial yeast strain D-80 are presented in Graph 1.

The results from Graph 2 indicated significant difference for Cabernet Sauvignon wine CS3 produced by application of commercial yeast D-80. The percentage of alcohol in other produced Vranec wines (V1-V3) and Cabernet

Sauvignon wines (CS1 and CS2) was above 14%. The percentage of sugar for all six wines was in the range from 2.0-2.3 g/L. However, the lowest amount of total acids was measured for wines from Cabernet Sauvignon grape variety fermented by indigenous yeast strain (F-8) and commercial yeasts strain (D-80), 5.21 g/L and 5.92 g/L, respectively. The smallest concentration of volatile acids for wines from both variety was observed in wines fermented by indigenous yeast strains. Namely, the amount of volatile acids in Vranec wine V2 fermented by indigenous yeast strains F-78 was 0.43 g/L, while the amount of volatile acids in wine CS1 fermented by indigenous yeast strain F-8 was 0.42 g/L. Generally speaking, the pH values for Vranec wines produced by indigenous and commercial yeast strains was lower in comparison to the Cabernet Sauvignon wines produced by the same yeast strains.

Opposite to the oenochemical parameters presented in Graph 2, the influence of yeast strains on the values of polyphenolic compounds, total anthocyanins and color intensity was significantly higher. Results presented in Graph 3, showed the highest amount of total phenolic compounds for wines of both varieties by application of the commercial yeast strain (D-80). On the other hand, the highest levels of anthocyanins was detected for Vranec wine V1 fermented by indigenous yeast strain F-8 (653 mg/L) and Vranec wine V3 fermented by commercial yeast strain (D-80) (637 mg/L). The highest color intensity for Vranec wines was observed for Vranec wine V1 while Cabernet Sauvignon wines CS1 and CS3 had higher values for intensity color in comparison to wine CS2 fermented by indigenous yeast F-78 (Figure 3).

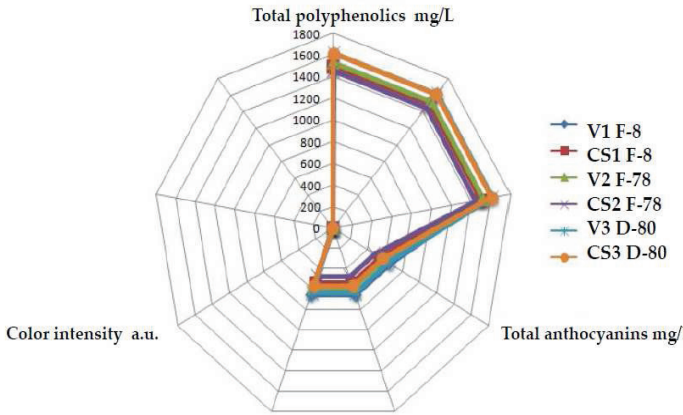


Figure 3. Total phenolic content, total anthocyanins and color intensity of wines fermented by indigenous and commercial yeast strains

Results from our studies showed significant influence of type of yeast strains on the fermentation, the oenochemical parameters, amount of total phenolic compounds, total anthocyanins and color intensity of produced wines. Furthermore, the influence of yeast type during wine-making depends from the grape variety. The influence of the same indigenous and commercial yeast strains is different for wines from Vranec and Cabernet Sauvignon grape variety produced by the same conditions.

Although indigenous yeast strains (F-8 and F-78) and the commercial yeast strain D-80 belongs to the same type Saccharomyces cerevisiae yeast strains, the dynamics of alcoholic fermentation presented in Figures 2 and 3, confirmed the statement of research group of Barrajón, that indigenous yeast strains had the ability for better adaptation in grape must in comparison to must conditions [15]. In addition, dynamics of alcoholic fermentation depends of tannins and fermentation activators [16]. This can be explained by the fact that the same fermentation yeast under the same wine-making conditions resulted by different shapes of fermentation curves (Figure 1 and Figure 2). As we can notice, for Vranec wines the difference of the

shape of the curves was from 5th till 8th days of fermentation while, for Cabernet Sauvignon wines it was occurred in the late stage of fermentation from 9th till 11th day of fermentation. According to the findings of Vigentini et al. 2014, the evolution of yeast populations during controlled fermentation of Chardonnay musts in two Italian wineries depended from the presence of indigenous yeast strains. More precisely, in the first winery, where the oenologist carefully managed only one starter culture and did not make any spontaneous fermentation, the commercial strain always mastered the process; conversely, in the second winery, where the oenologist performed also spontaneous fermentation, the starter culture did not even take over the dominance and a continuous succession of indigenous strains overcame without one prevailed on the others [17]. Sensitivity of yeast strains on the toxicity of ethanol is also condition which can affect the dynamics of fermentation. The tolerance of yeast strains on the toxicity of ethanol is strongly dependent of temperature and leads to conclusion that some yeast strains are able to finish fermentation on lower temperature [18-20].

Results from our study showed the stronger influence of yeast strains on the phenolic profile of wines in comparison to oenochemical parameters. Generally speaking, higher amount of total phenolic compounds and total anthocyanins for wines produced from both grape varieties was observed by application of indigenous yeast strain F-8 and commercial yeast strain D-80. The dynamics of specific yeast population during controlled fermentation could translate into specific organoleptic and sensory characteristics on final wines dependent of each grape variety [21]. Bitterness and astringency of produced wines depends from the amount of anthocyanins from the grape skin and tannins from the grape seeds [22]. The grapes from Vranec variety had higher amount of anthocyanins in the skins in comparison to Cabernet Sauvignon grapes. That is the reason for higher amount of total anthocyanins for all Vranec wines in comparison to the wines from Cabernet Sauvignon grapes produced under the same wine-making conditions. However, the highest amount of total phenolic compounds for wines from both varieties were in

favor of fermentation by commercial yeast strain D-80. The commercial yeast strain ICV D80 was isolated from the Côte Rôtie area of the Rhône Valley for its ability to ferment red musts rich in polyphenols and it is one of the preferred strains for contributing high tannin content. The importance of tannin is its contribution to organoleptic characteristics such as astringency [23-24]. In general, tannin activity is primarily driven by molecular size. Based upon maceration time, many studies indicated that observed increases in perceived astringency quality, if related to tannin chemistry, are driven by tannin molecular mass as opposed to pigmented tannin formation or oxidation [25-26].

Moreover, intermolecular and intramolecular copigmentation of anthocyanin and colourless copigments can significantly changed the overall color and intensity of red wines [27-28]. Results from our study confirm this statement. Although Vranec wines V1 and V3 contained the highest amount of total anthocyanins, the intensity of the color was remarkable for Cabernet Sauvignon wines CS1 and CS3. The anthocyanin composition in red wines depends not only on the original anthocyanin profile in grape berries, but also on the enological techniques applied [29-31]. The yeast behavior can modified the phenolic content of red wines by their ability to absorb the phenolic compounds on the yeast cell walls [32-38]. Moreover, absorption of the phenolic compounds on the yeast cell during fermentation can results with losing the color of wine [39-41]. Results from our study implicated the highest value for color intensity of Vranec wines V1 and V3 fermented by yeast strains F-8 and D-80 respectively, as well as wine Cabernet Sauvignon wine CS3 fermented by yeast strain D-80. This behavior lead us to conclusion that naturally isolated strains do not meet all the desired traits for winemaking, they generate a biodiversity background, which is very useful for successive improvements [42-45]. The genetic improvement of yeast strains can be achieved regarding variety of grapes and fermentation conditions. Although indigenous yeast strain F-78 selected from Tkiveš wine-growing region was superior for fermentation of Vranec wines, changes or fermentation conditions can be in favor of indigenous F-8 of commercial D-80 yeast

strains [46-48]. The comparison of results from our study and results from the research of Ilieva et al. 2016, Ilieva et al. 2017, unequivocally showed that fermentation of wines from the same grape varieties from different wine-making locations, different vintage years and the same yeast strains showed different behavior [25, 26]. This can be linked to the conclusion for presence of different yeasts during fermentation, specially winery-specific strains, contribute to increased wine complexity and differentiation [49]. Moreover, the fact that wines were produced in different vineries lead us to conclusion that S. cerevisiae strains that occur at higher percentages in spontaneous alcoholic fermentations are more competitive, possibly because of their higher capability to fit the progressively changing environmental conditions in terms of ethanol concentrations and temperature [50-54]. Some predominant S. *cerevisiae* strains persisted in different fermentations in the same winery from one year to another and they seemed to be representative of a single winery rather than of an oenological area [55-61].

2.3. Descriptive analysis and correlations

The Pearson's coefficient of correlation (ρ) received between two variables: chemical parameters of wines from Vranec grape variety and type of yeast which was applicated during the production of wines D-80, F-78 and F-8. The Pearson's coefficient of correlation (ρ) indicated which parameters of wines had higher correlations. If ρ=1 means that we had perfect correlation, which means increased value of one variable means increased value of another variable. If coefficient of correlation is between 0.5 and 0.9 means that we had significant correlation. Finally, If coefficient of correlation is between 0.5 and 0.2 means that we had correlation which is not significant. Negative values of coefficient of correlation means that we had inverse correlation.

As we can see from table 1, we had significant correlation between wines produced from Vranec grape variety by application of three yeasts: two autohtenous and one commercial yeast strain.

The Pearson's coefficient between Vranec wines produced by application of yeast strains F-8 and D-80 is 0.996 while Pearson's coefficient between Vranec wines produced by application of yeast strains F-78 and D-80 is 0.999 (Table 1).

Table 1. Display of Pearson's coefficient of correlation (ρ) received from corelation between wines Vranec produced from indigenous (F-8/F-78) and commercial (D-80) strains of yeasts.

Correlations

	D80	**F78**	**F8**
Pearson Correlation	1	**,999****	**,996****
Sig. (2-tailed)		,000	,000
N	7	7	7
Pearson Correlation	***,999****	1	,993**
Sig. (2-tailed)	,000		,000
N	7	7	7
Pearson Correlation	***,996****	,993**	1
Sig. (2-tailed)	,000	,000	
N	7	7	7

Correlations

	D80	F78	F8
Pearson Correlation	1	**,999****	**,996****
Sig. (2-tailed)		,000	,000
N	7	7	7
Pearson Correlation	***,999****	1	,993**
Sig. (2-tailed)	,000		,000
N	7	7	7
Pearson Correlation	***,996****	,993**	1
Sig. (2-tailed)	,000	,000	
N	7	7	7

**. Correlation is significant at the 0.01 level (2-tailed).

The Pearson's coefficient between Cabernet Sauvignon wines produced by application of yeast strains F-8 and D-80 is 0.999 while Pearson's coefficient between Cabernet Sauvignon wines produced by application of yeast strains F-78 and D-80 is 0.997 (Table 2).

Table 2. Display of Pearson's coefficient of correlation (ρ) received from corelation between wines Cabernet Sauvignon produced from indigenous (F-8/F-78) and commercial (D-80) strains of yeasts.

Correlations

		D80	F78	F8
D80	**Pearson Correlation**	1	**,997****	**,999****
	Sig. (2-tailed)		,000	,000
	N	7	7	7
F78	**Pearson Correlation**	***,997****	1	,993**
	Sig. (2-tailed)	,000		,000
	N	7	7	7
F8	**Pearson Correlation**	***,999****	,993**	1
	Sig. (2-tailed)	,000	,000	
	N	7	7	7

**. Correlation is significant at the 0.01 level (2-tailed).

Descriptive statistics are brief descriptive coefficients that summarize a given data set, which can be either a representation of the entire or a sample of a population. Descriptive statistics are broken down into measures of central tendency and measures of variability (spread). Measures of central tendency include the mean, median and mode, while measures of variability include standard deviation, variance, minimum and maximum variables, and kurtosis and skewness. Descriptive statistics, in short, help describe and understand the features of a specific data set by giving short summaries about the sample and measures of the data. The most recognized

types of descriptive statistics are measures of center: the mean, median and mode, which are used at almost all levels of math and statistics. The mean, or the average, is calculated by adding all the figures within the data set and then dividing by the number of figures within the set. The mode of a data set is the value appearing most often, and the median is the figure situated in the middle of the data set. It is the figure separating the higher figures from the lower figures within a data set. However, there are less common types of descriptive statistics that are still very important.

All descriptive statistics are either measures of central tendency or measures of variability, also known as measures of dispersion. Measures of central tendency focus on the average or middle values of data sets, whereas measures of variability focus on the dispersion of data. These two measures use graphs, tables and general discussions to help people understand the meaning of the analyzed data.

Measures of central tendency describe the center position of a distribution for a data set. A person analyzes the frequency of each data point in the distribution and describes it using the mean, median or mode, which measures the most common patterns of the analyzed data set.

Measures of variability, or the measures of spread, aid in analyzing how spread out the distribution is for a set of data. For example, while the measures of central tendency may give a person the average of a data set, it does not describe how the data is distributed within the set. So while the average of the data may be 65 out of 100, there can still be data points at both 1 and 100. Measures of variability help communicate this by describing the shape and spread of the data set. Range, quartiles, absolute deviation and variance are all examples of measures of variability.

Results presented in Table 3 implicated descriptive analysis by including the percenatage of alcohol in Vranec wines, total polyphenols (TP) and Color intensity (CI).

Table 3. Descriptive statistics for the wine from the grape variety Vranec

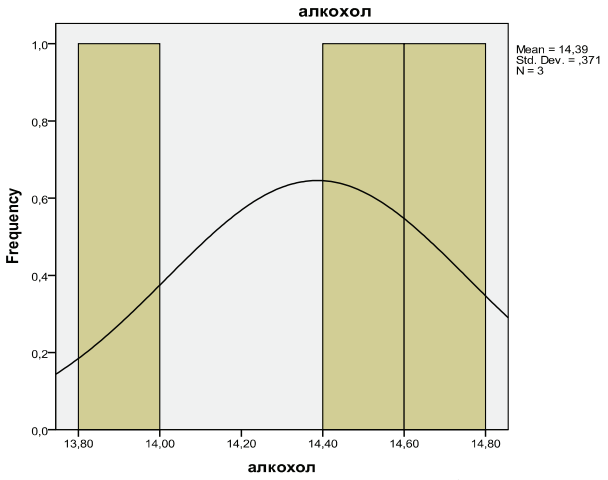
	N	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Median	Mode	Statistic	Statistic
Alcohol	3	14,11	14,96	14,53	14,11[a]	,42501	,181
Total polyphenols (TP)	3	1513,00	1618,60	1528,3000	1513,00[a]	57,06654	3256,590
Total anthocyanins (TA)	3	583,20	652,70	636,2000	583,20[a]	36,31230	1318,583
Color Intensity (IC)	3	2,11	3,13	2,3798	2,11[a]	,52962	,280

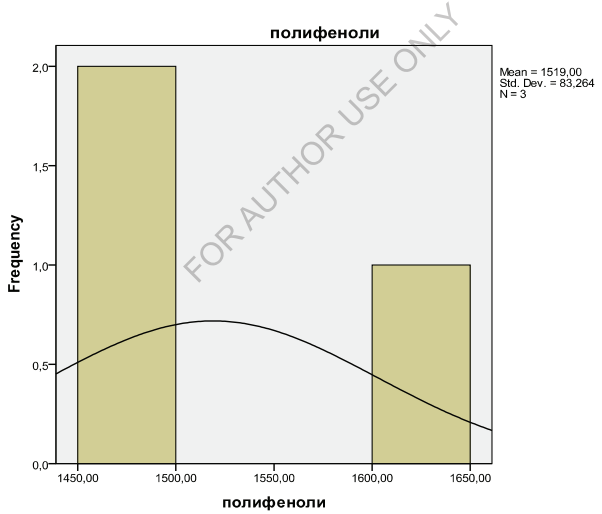
As we can see from Table 3, the standard deviation for alcohol in Vranec wines is 0.42501, while the same parameter for total phenolic, toatal anthocyanins and color intensity was 57.06654, 36.31230, 0.52962 respectively.

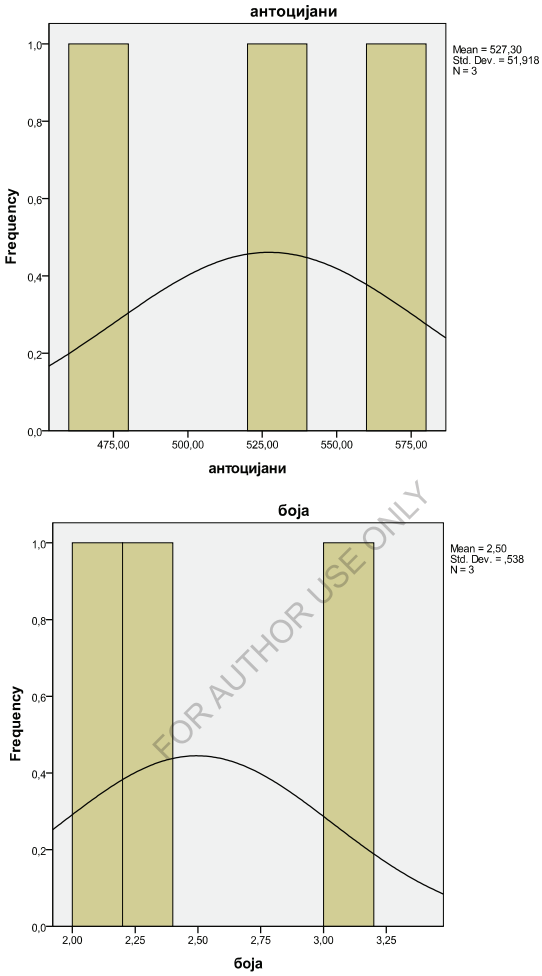
Table 4. Descriptive statistics for the wine from the grape variety Cabernet Sauvignon

Descriptive Statistics

	N	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Median	Mode	Statistic	Statistic
Alcohol	3	13,97	14,68	14,5100	13,97[a]	,37072	,137
Total polyphenols (TP)	3	1450,30	1611,60	1495,1000	1450,30[a]	83,26362	6932,830
Total anthocyanins (TA)	3	470,40	572,10	539,4000	470,40[a]	51,91849	2695,530
Color Intensity (IC)	3	2,10	3,11	2,2756	2,10[a]	,53823	,290







Graph 3. Histogram for descriptive statistics for the wine from the grape variety Cabernet Sauvignon

Conclusion

To conclude, the wines from Vranec grape variety had a higher amount of anthocyanins which is related to grape variety, but overall intensity of the color was not significantly differ in comparison to wines produced from Cabernet Sauvignon grape variety. Total phenolic content of wines produced from Cabernet Sauvignon grape variety was lower in comparison to Vranec wines. The dynamics of alcoholic fermentation for Vranec wine V2 fermented by indigenous yeast strain F-78 showed sharper decrease of the sugar content. The changes of color intensity can be resulted to the individual ability of yeast strain to absorb the anthocyanins and other phenolic compounds as well as copigmentation during wine-making.

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