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“GLOBAL CHALLENGES THROUGH THE PRISM OF RURAL
DEVELOPMENT IN THE SECTOR OF AGRICULTURE AND
TOURISM“



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Agriculture

CHEMICAL COMPOSITION OF SMEDEREVKA AND VRANEC WINES

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ABSTRACT

In this study, white Smederevka wine and red Vranec wine (vintage 2021) were produced from grapes grown at Ovce Pole wine district, Republic of N. Macedonia. Production of wines have been performed in a large-scale winery. In order to determine the quality of wines, following principal chemical parameters have been determined: alcohol, specific gravity, dry extract, total and volatile acidity, SO₂ (free and total), reducing sugars and pH. In addition, citric acid and toxic elements such as Cd, Cu, Fe and Pb have been analyzed in the wines. Wines presented satisfactory quality, containing alcohol content of 11 % and 11.61 % in Smederevka and Vranec, respectively. Total acidity was relatively high to ensure chemical and microbiological stability of wines, while volatile acidity was low in both wines influencing good balance and sensory quality. The wine stability was confirmed by pH (3.19 in Smederevka and 3.45 in Vranec). Citric acid is another mandatory parameter that should be determined according to regulations in order to ensure wine quality. Smederevka and Vranec wines contained low concentration of citric acid (32 mg/L in Smederevka wine and 122 mg/L in Vranec wine), meaning that citric acid was not added to the wines. Both wines were dry wines, with reducing sugar content lower than 4 g/L and they were well protected from oxidation with appropriate concentration of added SO₂. In addition, Smederevka and Vranec wines presented very low levels of the toxic and harmful elements Cd, Cu, Fe and Pb, lower than maximal allowed concentrations according to the safety regulations, which confirmed that the wines were safe for consumption with satisfactory chemical quality and stability.

Keywords: chemical parameters, citric acid, toxic elements, Smederevka, Vranec.

INTRODUCTION

Wine is a widely consumed beverage in the world, obtained by alcoholic fermentation of sugars from the grape in the presence of yeast (*Saccharomyces cerevisiae*), and followed by an appropriate period of aging. The chemical composition of wine is very complex since it contains a mixture of a large number of compounds including carbohydrates, organic acids, polyphenols, esters, alcohols, aldehydes, proteins, macro and micro elements, biogenic amines, amino acids, vitamins, etc. (Ivanova-Petropulos, et al. 2013, 2014, 2015). However, the two main components in wine are water, which consist of about 80%, and ethanol, present at about 12%. Ethanol is the most important alcohol in wine which is produced by the yeast fermentation and it is a significant factor for wine quality. The alcohol content mainly depends on the ripeness of the grapes and it can be accumulated at up to about 14-15% under standard fermentation conditions. Wines have a high alcohol content in years when the weather conditions are significantly good, followed by sunny days. Reducing sugars, glucose and fructose, are the main carbohydrates in grapes, which are converted into ethanol during the yeast fermentation (Ivanova Petropulos & Mitrev, 2014b).

Organic acids are important components affecting the composition, stability and organoleptic characteristics of wines, especially white wines (Ivanova-Petropulos et al., 2020). Young white wines with high acidity have more potential for aging. Red wines are stable at lower acidity due to the presence of phenols that increase acidity and enable stability of the wines during maturation. Citric acid is an organic acid present in grapes and also widely distributed compound in nature (e.g. in lemons). It is an important component in biochemical and metabolic processes (Krebs cycle). Citric acid slows the growth of yeasts, but it does not block their activity. Its concentration in must and wine, before malolactic fermentation, ranges from 0.2 to 1 g/L.

Volatile acidity is another very important parameter which determines the wine quality. Acetic acid is the main volatile acid, which is a product of the yeast and bacteria metabolism and its concentration should be monitored during the winemaking and wine aging. Increased volatile acidity in wine negatively affects its organoleptic characteristics, as well as its overall quality. Usually, volatile acidity is determined by wine distillation and is expressed as acetic acid equivalents (in g/L) (Piperevski et al., 2023).

Trace elements are considered as contaminants and information about the content of metals in wine is of great importance for producers and consumers for their health protection against toxic elements, as well as for the government authorities in order to guarantee the quality of the own traditional product. Trace elements concentration in wine is a significant parameter with a positive or negative effect on the quality of the final product (Pohl, 2007, Ivanova-Petropulos et al., 2013). From nutritional and toxicological points of view, elements are classified into essential and non-essential. Elements such as Ca, Cr, Co, K, Mg, Mn, Na Se and Zn are considered as essential elements for the human organism, while As, Cd and Pb are harmful and toxic elements because they are not chemically or biologically degradable (Ivanova-Petropulos et al., 2013).

In order to protect wine from oxidation, the mostly used agent is SO₂. Sulfur dioxide is efficient antioxidant which reduces the enzymatic and non-enzymatic oxidation, inhibits reactions of browning especially visible for white wines, as well as acts as antimicrobial agent slowing down the development of microorganisms, thus influencing the wine stability (Ivanova Petropulos & Mitrev, 2014b). SO₂ can be added into wine in several forms: as liquid gas, SO₂ solution and in a form of power or

effervescent tablets of salt potassium metabisulphate ($K_2S_2O_5$). In addition, $K_2S_2O_5$ is a white crystalline salt, which contains 57.6 % sulfur dioxide (SO_2) and it should be dissolved in a warm water before being used. When is added into the must, it reacts with the natural acids to release sulfur dioxide, which protects wines from unwanted microorganisms and oxidation.

Republic of N. Macedonia has a very long tradition of wine production. Wine is one of the key products related to agriculture and very important for the economy in the country, as well as for development of the rural tourism in the country. In total, 81 grape varieties are permitted for planting, in which Smederevka is most dominated white grape variety in R. Macedonia, planted in all vineyard areas, followed by Riesling and Chardonnay. The most widely cultivated and the most important variety for production of quality red wines is Vranec, followed by Merlo and Cabernet Sauvignon. This study is focused on production of wines from Smederevka and Vranec grapes grown in Ovce pole wine-growing district and determination of the principal chemical parameters, such as alcohol, specific gravity, dry extract, total and volatile acidity, SO_2 (free and total), reducing sugars, pH, citric acid as well as toxic elements Cd, Cu, Fe and Pb.

MATERIAL AND METHODS OF WORK

Vinification protocol

White winemaking. Grapes from Smederevka (*Vitis vinifera* L.) variety grown in Ovce Pole wine district, have been harvested at optimal technological maturity (19.2° Brix) at vintage 2021 vintage and used for wine production. Ovce Pole wine district is located around the cities of Sveti Nikole, Štip and Probištip, north from the river Zletovo and covers area of 2456 hectares vineyards, at attitude of 300 to 560 m.

Grapes (about 1 million kg) were transported to Vineks winery (with capacity of 1.8 million liters, located in Sveti Nikole, Ovce Pole wine district), 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. 75 mg/L total concentration), 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 10g/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 10g/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 through a filter with pore sizes of 1.3 microns, followed with passing of the wine through 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 liters of Smederevka wine was produced.

Red winemaking. Grapes from Vranec (*Vitis vinifera* L.) variety grown in Ovce Pole wine district, have been harvested at optimal technological maturity (22.5° Brix), at vintage 2021 vintage. Grapes (1.5 million kilograms) were transported to Vineks winery

for wine production. Grapes were processed using electrical inox crusher/destemmer, then added with SO₂ (ca. 50 mg/L total concentration) 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 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 (1g/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 through a filter with pore sizes of 1.3 microns, followed with passing of the wine through a filter with pore sizes of 0.9 microns and additionally protected from oxidation by adding appropriate amount of SO₂. A total of 1 million liters of Vranec wine was produced.

Chemical composition

The principal chemical parameters have been determined using the official methods of analysis of wines (OIV, 2022): alcohol (OIVMA-AS312-01 A), specific density (OIV-MA-AS2-01 A), dry extract (OIVMA-AS2-03B), total acidity (OIV-MAAS313-01), volatile acidity (OIV-MA-AS313-02) and pH (OIV-MAAS313-15). Determination of reducing sugars, free SO₂ and total SO₂ was performed according to the methods published by Ivanova-Petropulos & Mitrev (2014b).

HPLC-DAD analysis of citric acid was performed according to the method published by Ivanova-Petropulos et al. (2020), and analysis of heavy metals Cd, Cu, Fe and Pb was performed according to official OIV method (OIV, 2022): (OIV-MA-AS323-07). All analyses have been performed after three to four months of storage.

Statistical analysis

Statistical analysis included determination of means and standard deviation, calculated using Microsoft Excel (2013).

RESULTS AND DISCUSSION

The basic chemical parameters that are important for wine quality are alcohol, specific density, dry extract, total acidity, volatile acidity, free SO₂, total SO₂, pH, reducing sugars and citric acid. These parameters have been determined in white Smederevka and red Vranec wines, vintage 2021 (Table 1).

Table 1. Basic chemical composition of Smederevka and Vranec wines

Parameters	Smederevka	Vranec
Alcohol, %, v\v	11.0±0.012	11.61±0.011
Specific density at 20°C	0.9913±0.0001	0.9936±0.0001
Total dry extract (g\L)	19.1±0.12	27.8±0.16
Total acids (g\L)	7.2±0.014	5.0±0.11
Volatile acids (g\L)	0.3±0.001	0.42±0.002
Free SO ₂ (mg\L)	75.5±1.42	23.04±1.35
Total SO ₂ (mg\L)	140±2.71	70.4±2.68
pH	3.19±0.01	3.45±0.01
Reducing sugars (g\L)	1.3±0.38	1.9±0.45
Citric acid (mg\L)	32±1.53	122±3.56

Results are average values of three repetitions ± standard deviation

The alcohol content determined in Smederevka and Vranec wines was 11 % and 11.61 %, respectively. Smederevka wine contained a slightly lower content of alcohol compared to the Vranec wine, because Smederevka grapes had a slightly lower content of reducing sugars than Vranec grapes (19.2° Brix in Smederevka grapes and 22.5° in Vranec grapes). It is already known that the alcohol content mainly depends on the sugar content in the grapes/must, since the alcohol is produced during the alcoholic fermentation by the yeasts, which transforms the carbohydrates (glucose and fructose) into ethanol and carbon dioxide.

The values for the specific gravity were 0.9913 for Smederevka wine and 0.9936 for Vranec wine, as expected and in accordance to the determined alcohol content and dry extract. Regarding the dry extract which consists of nonvolatile soluble components, including sugars, non-volatile acids, glycerol and phenols, wines presented relatively high values appropriate for white and red wine. Thus, the dry extract for Smederevka was 19.1 g/L and for Vranec was 27.8 g/L.

Total acidity is defined as a sum of “non-volatile and volatile acidity”, including the all types of acids, such as formic acid, organic acids (tartaric, malic and citric), as well as amino acids. Tartaric acid is the predominant organic acid in the must and wine, and therefore, the total acidity is expressed in tartaric acid equivalents. In this study, the measured content of total acidity for Smederevka and Vranec was 7.2 g/L and 5.0, respectively. Both wines presented relatively high values of total acids which are sufficient to ensure the chemical and microbiological stability of wines.

pH is another factor important for the wine stability and acidity. In this study, Smederevka and Vranec wines presented pH 3.19 and 3.45, respectively, which was in a correlation to the total acidity. Usually, the pH values of wine range between 3.1 to 3.6. As the pH is lower, the more stable is the wine since it is the more difficult for microorganisms to survive at lower pH.

Citric acid also influences the acidity of wines and it is one of the main organic acids which is found in grapes. The content of citric acid in both wines was lower than 1 g/L (32 mg/L in Smederevka wine and 122 mg/L in Vranec wine) which is in accordance with regulations (Official Gazette of Republic of N. Macedonia 2012).

Usually, this acid is added to wine in order to correct the wine acidity, and therefore, its content should be controlled. In our study, citric acid was not added to the wines and its concentration was in normal ranges.

Volatile acidity of wine represents a significant physico-chemical parameter, which should be monitored during the winemaking process. According to the wine law, the maximal allowed content of acetic acid in wine is 1.08 g/L for white and rose wine, and 1.2 g/L for red wine (Official Gazette of Republic of N. Macedonia 2012). In this study, Smederevka and Vranec wines presented low values of volatile acidity (0.3 and 0.42 g/L, respectively), which means that wines had satisfactory quality.

SO₂ is a valuable component which is used in winemaking due to its ability to act as an effective antioxidant and antimicrobial agent. Moreover, SO₂ has the ability to eliminate unpleasant odours in wine and selectively act against wild yeasts which originate from grape skins, blocking their activity. Considering the content of SO₂ in the analyzed wines in this study, they were regularly protected from oxidation and microbial contamination by the free SO₂ (75.5 mg/L in Smederevka and 23.04 mg/L in Vranec). The total SO₂ ranged from 70.4 (Vranec) to 140 mg/L (Smederevka) (Table 1). These results were in a correlation to the legal regulations according to which the maximum allowed amount of SO₂ in red wines is 150 mg/L and 200 mg/L in white and rose wines (Official Gazette of Republic of N. Macedonia 2012).

Reducing sugars are glucose and fructose, the main carbohydrates in grapes. Their content in grapes depends on the variety, ripeness, health and growing conditions. During alcoholic fermentation, the content of reducing sugars decreases as a result of their conversion to ethyl alcohol (Neceva & Ivanova Petropulos, 2016). In this study, the analyzed wines had a low content of reducing sugars (1.3 g/L Smederevka and 1.9 g/L Vranec), which means that wines were dry and in all of them the alcoholic fermentation ended successfully. The results for basic chemical parameters are in good agreement with previously published data for Smederevka and Vranec wines, as well as for other wine varieties (Neceva & Ivanova Petropulos, 2016, Pajović-Šćepanović et al., 2016, Piperevski et al., 2023).

The concentrations of some heavy metals (Cd, Cu, Fe and Pb) have been determined in Smederevka and Vranec wines, and presented in Table 2.

Table 2. Content of heavy metals (mg/L) in Smederevka and Vranec wines

Heavy metals (mg/L)	Smederevka	Vranec
Cd	0.0064±0.001	0.032±0.002
Cu	0.15±0.01	0.21±0.02
Fe	0.98±0.11	1.25±0.4
Pb	0.016±0.02	0.028±0.004
Σ	1.15	1.52

Results are average values of three repetitions ± standard deviation

The application of fining agents, such as bentonite, copper sulfate (Al, Ca, Cd, Cr, Cu, Fe, Zn) and other environmental pollution (Cd, Co, Cr, Hg, Ni, Pb, V) may contribute to wine contamination. Most of the heavy metals in the grapes precipitate during the alcoholic fermentation causing significant reduction of their content in the wine. The limit for the heavy metals and harmful elements is set by the International Organization of Vine and Wine (OIV). Maximal acceptable limits for these toxic elements are as follows: Cd 0.01 mg/L, Cu 1 mg/L, Fe 10 mg/L and Pb 0.2 mg/L (OIV). From the obtained results (Table 2) it can be seen that wine samples presented very low levels of Cd, Cu, Fe and Pb, lower than maximal allowed concentrations in wine according to the Macedonian food safety regulation (Official Gazette of Republic of N. Macedonia 2012). as well as according to the maximal allowed threshold recommended by the International Organization of Vine and Wine.

The presence of Cd, which is a highly toxic element, depends on both natural and exogenous factors. The main sources of cadmium pollution are the nonferrous metal production and mining, waste incineration, phosphate fertilizer manufacture, wood, coal, oil and gasoline combustion, as well as iron and steel production (Cvetković et al., 2006). In addition, natural factors of Cd in wine are soil type and composition, grape variety, climate, amounts of products used in a particular vineyard, the vintage period, the amount of rain, etc. Exogenous factors, such as fermentation process, maceration time, addition of sulfur dioxide and yeasts, could affect the Cd concentration in wine. Generally, the cadmium content in the analyzed wines has been found to be very low (0.0064 mg/L in Smederevka wine and 0.032 in Vranec wine).

Copper is also one of the most frequently occurring heavy metals in wine. The main sources of Cu in wine are use of copper-containing fungicides to control powdery mildew, treatments of vine plants with copper sulphate, brass fittings, or use of copper sulphate for treatment of reductive off-flavors in wine. High residual copper can contribute to an enhanced rate of oxidative spoilage, which results in wine browning, particularly white ones. The copper concentration in analyzed wines was low (0.15 mg/L in Smederevka and 0.21 mg/L in Vranec), even lower than generally recommended concentration (0.3 – 0.5 mg/L) (Pyrzynska, 2007), meaning that Smederevka and Vranec wines in relation to the copper content did not pose a risk to the wine stability and human health as well.

Iron exist in two forms in wine, maintaining oxidation-reduction balance, according to the equation: $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + e^-$. The $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio in wine depends on the storage conditions, especially on the concentration of free SO_2 . Therefore, in wine subjected to aeration, turbidity and instability from iron (ferric casse) appears because the level of Fe^{3+} , the ion responsible for that phenomenon, increases. At low concentrations, Fe is an enzyme activator, stabilizer and functional component of proteins. At higher concentrations, it participates in oxidation-reduction systems in wines, affects the sensory properties of wine and participates in formation of complexes with tannins and phosphates. Therefore, its concentration should be controlled. The content of Fe in Smedervka and Vranec wines was low (0.98 and 1.25 mg/L, respectively), which means that the wines were stable.

The determined content of lead (Pb) in wines was 0.016 mg/L in Smederevka and 0.028 mg/L in Vranec wines, which indicates that the wines were safe for consumption. Lead contamination of wine arises from numerous sources, natural and anthropogenic. Atmospheric lead from industrial pollution and leaded gasoline can contaminate wine through deposition on vine plants. Moreover, use of lead-based pesticides can be a significant source of lead in grapes (Handson 1984). Lead contamination of wine can arise from winemaking practices as well as use of brass components in winemaking, such as pumps, valves, faucets and piping (Tariba et al. 2011).

In general, white wine contained lower amount of heavy metals (1.15 mg/L), compared to the red wine (1.52 mg/L) as it was expected since red wines are produced with maceration which prolongs the extraction of elements from the grapes into the wine. The results are in good agreement with previously published data in the literature (Cvetković et al., 2006, Tariba et al., 2011, Ivanova-Petropulos et al., 2013, 2015, Mitic et al., 2014).

CONCLUSIONS

The chemical composition of Smederevka and Vranec wines produced in a large-scale winery have been determined. Wine presented relatively high values of total acidity and low values of volatile acidity, while the alcohol content was 11 and 11.6 % for Smederevka and Vranec, respectively, confirming that the wines were chemically and microbiologically stable, with satisfactory quality. Moreover, wines were well protected from oxidation containing enough content of SO₂ and contained small amount of reducing sugars (1.3 and 1.9 g/L). The natural concentration of citric acid in wines was determined (32 mg/L in Smederevka wine and 122 mg/L in Vranec wine). In addition, the content of toxic elements Cd, Cu, Fe and Pb was lower than the maximal allowed concentrations according to the safety regulations, confirming their quality and safety for consumption.

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