Agrobiodivers Improv Nutr Health Life Qual, 8, 2024(2): 145–156

Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

# Alcoholic Fermentation, Chemical Composition and Organoleptic Profile of Spontaneously Fermented White and Red Wines

**Tatyana Yoncheva*[1], Iliyan Simeonov[1], Nikolay Genov[1], Dimitar Dimitrov[1], Hristo Spasov[2], Nadezhda Blagoeva[2], Polina Zapryanova[2]**

[1] Agricultural Academy, Institute of Viticulture and Enology, Pleven, Bulgaria
[2] University of Food Technologies, Plovdiv, Bulgaria

Tatyana Yoncheva: https://orcid.org/0000-0002-8156-0530
Iliyan Simeonov: https://orcid.org/0000-0003-4330-9051
Nikolay Genov: https://orcid.org/0000-0001-9562-6353
Dimitar Dimitrov: https://orcid.org/0000-0001-7549-7902
Nadezhda Blagoeva: https://orcid.org/0000-0002-0596-3207



Article Details:

| | |
|---|---|
| Received: | 2024-08-10 |
| Accepted: | 2024-11-05 |
| Available online: | 2024-11-30 |

DOI: https://doi.org/10.15414/ainhlq.2024.0016

The development of viticulture and winemaking, including the preservation and cultivation of old local grape varieties, is an opportunity to preserve traditions and diversity of vine genetic resources in Bulgaria. This research was a part of the DIONYSOS project, investigated the biological diversity of local varieties grown in different regions of Southern Bulgaria. The subject of the current study were 10 grapes samples from 3 white (Dimyat, Red Misket, Tamyanka) and 3 red (Pamid, Mavrud, Gamza) autochthonous to Bulgaria wine grape cultivars grown in the region of Haskovo district. The aim was to determine the chemical composition and organoleptic profile of the experimental wines obtained after spontaneous alcoholic fermentation. After crushing, the fermentation of the grape pomace was run under the impact of wine yeast from the natural microflora of the grapes. The dynamics of the process was monitored by daily measurement of dry matter by refractometer. The yeasts showed different fermentation activity. The process onset was between the 48$^{th}$ and 72$^{nd}$ hours and proceeded dynamically and efficiently. The spontaneous fermentation was complete and ensured a high ratio of sugar conversion into alcohol. An exception was observed in both samples of the cv. Tamyanka. The content of residual sugars, alcohol, titratable acids, volatile acids, pH in the experimental wines were analyzed by generally accepted in winemaking practice methods. No deviations were found in the values of the studied indicators and metabolites formed were within acceptable limits for white and red wines. The organoleptic features of the samples were evaluated by a tasting panel of 7 oenologists. The sensory qualities of the variants were represented by radial diagrams using the method of principal characteristics. All samples had specific sensory profile, with a positive rating of the organoleptic criteria, according to the variety and the created experimental conditions for their making.

**Keywords:** wine, yeast, spontaneous alcoholic fermentation, fermentation activity, chemical composition, organoleptic profile

## Introduction

The south-eastern part of the Balkan Peninsula, which also includes Southern Bulgaria, is one of the oldest wine-growing regions in the world, characterized by excellent soil-climatic conditions and a rich varietal diversity of different introduced and local grape varieties, some of which are grown there from ancient

***Corresponding Author:** Tatyana Yoncheva, Institute of Viticulture and Enology , "Kala tepe" str. 1, 5800 Pleven, Bulgaria
t_ion@abv.bg

times (Nachev, 1981; Dimitrov and Dimitrova, 2015; Dzhabaroba and Arseniu, 2018). The development of viticulture and winemaking, including the preservation and cultivation of old local vine varieties in this region, should be perceived as a unique opportunity to preserve local traditions, rich diversity of grapevine genetic resources and the specifics of the terroir (Dimitrova and Dimitrov, 2017).

The peculiar nature of the different terroirs in Bulgaria contribute to the production of different types of white and red wines, having distinctive individual characteristics, directly resulting from the specific soil-climatic conditions in the growing area, as well as from the composition of the microorganisms present. Versatile microflora had been naturally available in the soil, on the leaves, on the clusters and berries, which to varying degrees participated and carried out a number of important processes in the wine making, significantly affecting the wines' chemical composition and features, emphasizing their regional typicality (Çelik et al., 2017; Bougreau et al., 2019; Jankura et al., 2020; Ivkovikj, 2021; Tofalo, 2021). The microbial population composition on grapes had been determined by various factors – variety, degree of ripeness, weather conditions, geographical location of the plantation, damage caused by diseases and enemies, use of pesticides, etc. (Sabate et al., 2002; Baffi et al., 2011; Bezerra-Bussoli et al., 2013; Zabukovec et al., 2020).

The alcoholic fermentation had been a fundamental process in winemaking where sugars in grapes were converted into $C_2H_5OH$, $CO_2$ and other metabolic products. It was of microbiological nature and occurred under the action of enzymes produced by wine yeast (Gonzales et al., 2007; Alba-Lois and Segal-Kischinevzky, 2010; Jankura et al., 2020; Maicas, 2021; Shahrajabian and Wenli, 2023; Chorniak, 2024).

Yeasts had been microorganisms widely spread in nature and part of the natural microflora of grape berries. They were an important factor in determining the wine quality and features. The main part of them belonged to the *Saccharomyces* genus, and the rest were representatives of non-*Saccharomyces* (wild) yeasts belonging to different genera (*Hanseniaspora, Kloeckera, Brettanomyces, Hansenula, Pichia, Candida, Metschnikowia* etc.). *Saccharomyces* yeasts had better fermentation activity and alcohol-forming ability, and higher resistance to environmental stress factors (temperature, oxygen, $C_2H_5OH$, $CO_2$, $SO_2$). Non-*Saccharomyces* yeasts were more susceptible and easier to inactivate, producing more unwanted metabolites (Bambalov, 1981; Pardo et al., 1989; Sabate et al., 2002; Gonzales et al., 2007; Çelik et al., 2017; Bougreau et al., 2019; Zabukovec et al., 2020; Maicas, 2021; Tofalo, 2021; Chorniak, 2024).

The alcoholic fermentation passes through several stages determined by the yeast cells' development – an initial phase (adaptation), an exponential phase (violent fermentation with intensive yeast reproduction), and a stationary phase (quiet fermentation with attenuation and cellular death) (Bambalov, 1981; Yankov, 1992; Miller and Block, 2020). The process speed and duration depended on the running conditions – temperature, aeration, sugar concentration, and nitrogen sources in the must, etc. (Alba-Lois and Segal-Kischinevzky, 2010; David et al., 2010; Maicas, 2021).

The alcoholic fermentation could be spontaneous and guided. The spontaneous fermentation took place under the influence of yeast naturally found in the grapes, while the guided one was characterized by the introduction of ferment from selected strains with higher resistance (Yankov, 1992). The amount of yeast population on the grapes varied depending on the atmospheric conditions and the fungicides used in the vineyard, so the spontaneous fermentation might start with a delay, proceed slowly or stop before the complete breakdown of the sugars. That resulted in wines having higher volatile acidity and other defects (Bambalov, 1981; Abrasheva et al., 2008; Tofalo, 2021). With this type of fermentation, there had been a sequence in the development of the yeast species present during the different stages. Non-*Saccharomyces* representatives started the process and in its course were replaced by *Saccharomyces cerevisiae*, which began to dominate and complete it due to their higher tolerance to alcohol (Sabate et al., 2002; Gonzales et al., 2007; Baffi et al., 2011; Bezerra-Bussoli et al., 2013; Ilieva et al., 2019; Dimopoulou et al., 2020; Zabukovec et al., 2020; Chorniak, 2024). *Saccharomyces cerevisiae* was present at the beginning of fermentation, but at a very low proportion. Its quantity began to increase from the middle to the end of the fermentation, accounting for 60% of the population at the middle and 100% at the end of the process (Lleixa et al., 2016).

By using a pure culture of *Saccharomyces* yeast, in the form of a dry starter culture of a commercial product, to carry out the alcoholic fermentation, the negative effects of the spontaneous process were prevented. Yeasts with their metabolism influenced the chemical composition and characteristics of the final product, thus in most cases that resulted in making single-type, unified wines. With the application of spontaneous fermentation, more and more winemakers have emphasized the effect of native strains, mainly non-*Saccharomyces* species, for the production of regional wines, to preserve and accentuate the terroir specificity of the region and the microregion. Therefore their metabolism and the secondary products they had synthesized, the wines had certain, specific aromatic and taste features and had acquired a pronounced, specific style (Gonzales et al., 2007; Bezerra-Bussoli et al., 2013; Lleixa et al., 2016; Çelik et al., 2017; Spasov et al., 2017; Bougreau et al., 2019; Dimopoulou et al., 2020; Zabukovec et al., 2020; Jankura et al., 2020; Ivkovikj, 2021; Maicas, 2021; Sidari et al., 2021).

This study was aimed to evaluate the presence of active natural yeast microflora on batches of grapes by following the course of a spontaneously occurring alcoholic fermentation process and to determine the chemical composition and organoleptic profile of the resulting white and red wines.

## Materials and methodology

The research in the present study was carried out within the framework of the activities of the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria, under the DIONYSOS project "Developing identity on yield, soil and site", Subsidy contract B2.6c.04/01.11.2017 with the financial support of Cooperation Programme "Interreg V-A Greece-Bulgaria" 2014–2020, Co-funded by the European Regional Development Fund and National funds of Greece and Bulgaria. The main objective of the DIONYSOS project was to turn the specific aspect into a natural and cultural asset for the development of wine tourism in the cross-border regions of southern Bulgaria and northern Greece, so as to increase the attractiveness of the area, while at the same time strengthening the preservation of the local landscape and biological diversity (https://dionysosvine.eu/en/).

The study, including the selection of local cultivars and vineyards in the region of Haskovo (Eastern Rhodopes, Southern Bulgaria), was carried out by a team of scientists from the IVE – Pleven, during an expedition at the end of September 2018. The samples from the selected local vine cultivars , were collected, stored, and processed after establishing the technological maturity of the grapes.

### Processing and chemical composition of grapes from the studied cultivars

From vineyards in different areas of the Haskovo region, 10 samples of grapes from 3 white and 3 red wine grape varieties autochthonous to Bulgaria were selected and delivered to the laboratory of "Biavin" Company – Plovdiv, in an amount of 5–6 kg. The cultivars and the origin of the samples have been presented in Table 1.

The selected experimental samples were processed under the conditions of microvinification. The grapes were first destemmed into containers cleaned with spirit, then crushed and transferred into brand new 5 l PET vessels. After homogenization, each sample was analyzed for the indicators – reducing sugars (the Schoorl chemical method), titratable acidity (titration with NaOH) and pH (pH-meter) (Ivanov et al., 1979). The results are presented in Table 2.

**Table 1** Cultivar and origin of the experimental grape samples from Bulgaria.

| Sample No | Grape cultivar (colour of the berries' skin) | Origin |
|---|---|---|
| **1** | Dimyat (white) | village of Dimitrovche, Svilengrad municipality, Haskovo region |
| **2** | Red Misket (white) | village of Dimitrovche, Svilengrad municipality, Haskovo region |
| **3** | Mavud (red) | town of Lyubimets, Haskovo region |
| **4** | Pamid (rose) | town of Lyubimets, Haskovo region |
| **5** | Pamid (rose) | village of Kolarovo, Harmanli municipality, Haskovo region |
| **6** | Gamza (red) | village of Shishmanovo, Harmanli municipality, Haskovo region |
| **7** | Tamyanka (white) | village of Shishmanovo, Harmanli municipality, Haskovo region |
| **8** | Mavrud (red) | village of Shishmanovo, Harmanli municipality, Haskovo region |
| **9** | Mavrud (red) | village of Susam, Mineralni Bani municipality, Haskovo region |
| **10** | Tamyanka (white) | village of Susam, Mineralni Bani municipality, Haskovo region |

**Table 2** Chemical composition of grapes from the selected experimental samples.

| Sample No | Grape cultivar | Chemical indicators | | |
|---|---|---|---|---|
| | | Sugars, $g.l^{-1}$ | Titratable acids, $g.l^{-1}$ | pH |
| **1** | Dimyat | 161.00 | 4.57 | 3.41 |
| **2** | Red Misket | 198.00 | 3.50 | 3.84 |
| **3** | Mavud | 184.00 | 4.32 | 3.84 |
| **4** | Pamid | 210.00 | 3.71 | 3.99 |
| **5** | Pamid | 205.00 | 2.64 | 3.96 |
| **6** | Gamza | 189.00 | 4.97 | 3.68 |
| **7** | Tamyanka | 222.00 | 4.16 | 3.88 |
| **8** | Mavrud | 190.00 | 5.05 | 3.67 |
| **9** | Mavrud | 181.00 | 6.87 | 3.52 |
| **10** | Tamyanka | 236.00 | 5.49 | 3.90 |

### Alcoholic fermentation

Sulphitation of the fermentation substrate was applied using a 5% solution of $H_2SO_3$ in a 50 mg.l$^{-1}$ $SO_2$ dosage. The spontaneous alcoholic fermentation took place at a temperature of 25 °C. The process progress was daily monitored by refractometer, after the homogenization of the solid parts with the liquid phase by stirring (Figure 1). The change in dry matter (%) was monitored for 13 days until a constant value was maintained.

**Figure 1** Spontaneous alcoholic fermentation of the selected experimental samples

After establishing the alcoholic fermentation end for all experimental samples, the liquid phase was separated from the solid parts without pressing. The wines were placed in polyethylene terephthalate (PET) containers and stored for further investigation and analysis of their chemical composition and organoleptic properties (Figure 2).

**Figure 2** Experimental wines after completion of the alcoholic fermentation

### Chemical composition of the experimental wines

After completion of the alcoholic fermentation, the experimental wines were analyzed for the indicators (Ivanov et al., 1979):

**residual sugars, g.l$^{-1}$** – Schoorl method
**alcohol, vol.%** – by pycnometric method
**titratable acids, g.l$^{-1}$** – titration with 0.1 n NaOH with bromothymol blue indicator
**volatile acids, g.l$^{-1}$** – titration with 0.1 n NaOH with phenolphthalein indicator
**pH** – by pH meter by direct electrode dipping into the wine

### Organoleptic profile of the experimental wines

Upon determining the chemical composition of the obtained wines, an organoleptic analysis was performed. The experimental samples were evaluated by a tasting panel of 7 oenologists. The sensory characteristics of the variants were represented by radial (spider) diagrams using the principal characteristics method (Prodanova, 2007).

## Results and discussions

### Dynamics of the alcoholic fermentation

The fermentation of grape juice into wine is a complex microbiological process in which yeasts play a central role. They derive from the surfaces of grapes, surfaces of winery equipment, and from inoculum culture. Wine fermentation is either carried out naturally without inoculation or by inoculation of grape juice with selected wine yeasts (Nurgel et al., 2002; Howell et al., 2006; Papathanasiou et al., 2006; Varela et al., 2009; Bouloumpasi et al., 2021).

The spontaneous alcoholic fermentation was carried out mainly by non-*Saccharomyces* yeasts, which were part of the natural microflora of grapes. They were related to versatile species and genera and had different technological characteristics – resistance to alcohol, $SO_2$, osmotic pressure, pesticides, etc. (Zhang et al., 2021; Maicas and Mateo, 2023).

Çelik et al. (2017) observed a diversity of *Saccharomyces cerevisiae* and non-*Saccharomyces* spp. during spontaneous fermentation. *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* were dominant at the beginning. In the middle of the process, *Saccharomyces cerevisiae* started to overcome other yeasts and later it was the only species isolated at the end of the fermentation.

Wine fermentation kinetics studied the growth of wine yeast, the consumption of sugars, the production of ethanol, the generation of heat, and the accumulation of primary and secondary metabolites during fermentation. Early in fermentation, the growth rate was proportional to cell concentration as cells double, commonly referred to as "logarithmic growth", "exponential growth" or the "lag phase". As the growth-limiting nutrient was exhausted, growth levels off, and biomass concentration reached a steady state, termed the "stationary" phase. As cells stopped growing due to the lack of nutrients and senesce, active cell concentration dropped, typically known as the "death" or "inactivation" phase (Miller and Block, 2020).

Yeasts were present in fresh must without $SO_2$ at $10^5$ to $10^6$ CFU/ml. Once fermentation began the cell number increased from $10^7$ to $10^8$ CFU/ml during tumultuous fermentation. The maximum cell number was achieved at that stage. The ethanol formed, the exhaustion of sugars, the accumulation of toxic products such as fatty acids, and the depletion of intracellular sterols killed the yeast cells (Padro et al., 1989).

In the conducted experiment, the course of the fermentation process of white and red wines was monitored refractometrically for 13 days. The dynamic of its progress in all experimental samples has been presented in Figure 3.

Until the 24$^{th}$ – 36$^{th}$ hour, there was no indication of a start of the alcoholic fermentation. For most samples, the process started between the 48$^{th}$ and 72$^{nd}$ hours, and for some of them, for example, Red Misket (No. 2), Mavrud (No. 9) and Tamyanka (No. 10), the onset of the fermentation was delayed by a further 24–48 hours. That was probably due to the inhibitory effect of the added $SO_2$ and the time needed for the available microorganisms to adapt to the surrounding conditions. In the case of the Tamyanka sample (No. 10), the osmotic shock on the available yeast probably intervened too. It was noteworthy that samples Pamid (No. 4, No. 5) and Tamyanka (No. 7) had a more dynamic process despite the higher initial sugar content, compared to Dimyat (No. 1). That had justified the assertion that on the grapes of the separate samples, there were yeasts different in quantity, resistance to inhibitors and fermentation activity, which carried out the process. In some strains, adaptation to the surrounding conditions was necessary to start and carry out active fermentation, in the presence of $SO_2$ and higher sugar content, respectively osmotic pressure.

Most samples practically completed the process of alcoholic fermentation on the 8–9$^{th}$ day. The dry substances concentration, measured with a refractometer, was within the range of 5–8% depending on the fermented sugars and the alcohol formed. Delayed and incomplete fermentation was found in samples of the cv. Tamyanka (No. 7, No. 10) which might be due to the lower concentration of wine yeast on the grapes and the weaker activity of those present. The sample monitoring was suspended after the 13$^{th}$ day.

Spasov et al. (2017) also found a delay in the spontaneous process during the first 24 hours of the fermentation of Mavrud grape pomace, which was due to sulfitation and after adaptation to the medium, *Saccharomyces* yeast became predominant during the process.

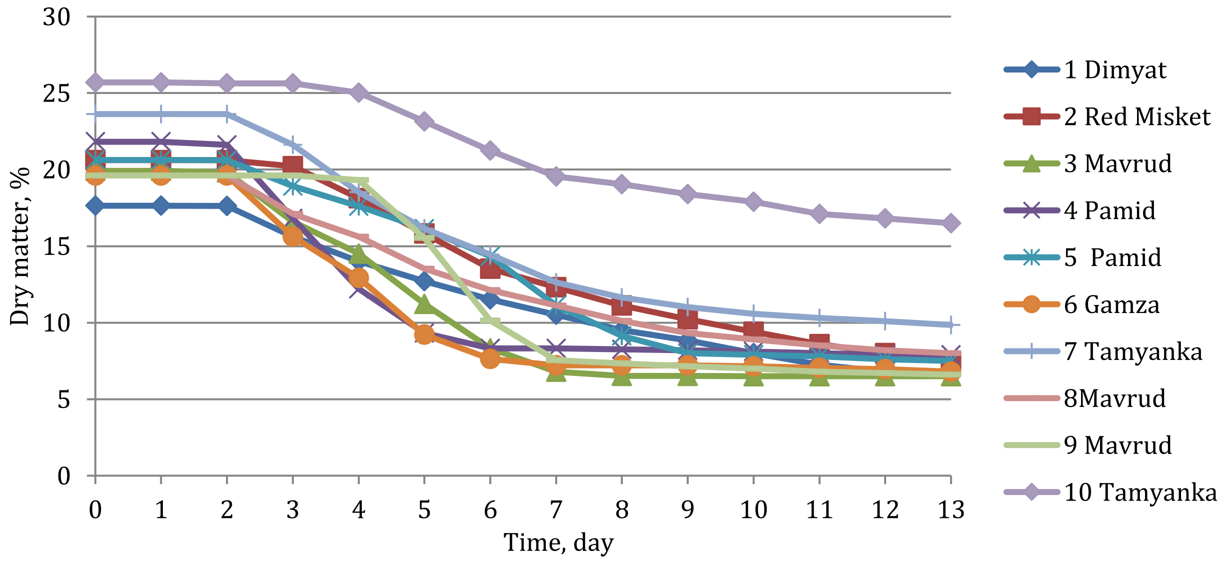


**Figure 3:** Alcoholic fermentation dynamics of the experimental wines.

Lleixa et al. (2016) indicated differences in the rate of fermentation depending on the type of yeast that conducted it, as well as on the variety and composition of the grape pomace. The Macabeo must inoculated with *Hanseniaspora vineae* required a longer fermentation process (19 days) than those inoculated with *Saccharomyces cerevisiae* (14 days) due to slower fermentation kinetics and a longer latency phase. However, Merlot grapes inoculated with *Hanseniaspora vineae* and with *Saccharomyces cerevisiae* showed a similar fermentative process completed in 9 days.

Zabukovec et al. (2020) studied yeast microbiota and dynamics of the spontaneous alcoholic fermentation of grape must of different grape varieties. The kinetics of the process depended on the composition and initial count of the fermentative yeast strains and the must composition, especially the sugar content, assimilable nitrogen, and vitamin concentration. They tested different ratios of the indigenous strains of *Saccharomyces* and non-*Saccharomyces* species for must inoculation, which allowed the positive properties of the strains to be expressed and good quality wines to be produced.

### Chemical composition of the obtained white and red wines

Wine yeast species which drive alcoholic fermentation contribute to the enrichment of wine with different chemical compounds most of them with positive effect improving their sensory characteristics. That is due to their specific metabolism (Vilanova et al., 2005; Dimopoulou et al., 2020; Zhang et al., 2021). Whilst *Saccharomyces cerevisiae* is primarily responsible for the conversion of sugar to alcohol, the activities of various non-*Saccharomyces* species enhance flavor and influence the chemical profile of the wine (Nurgel et al., 2002; Papathanasiou et al., 2006; Varela et al., 2009; Parapouli et al., 2010; Bouloumpasi et al., 2021; Mislata et al., 2021). Some non-*Saccharomyces* yeast are known for lower ethanol yields than *Saccharomyces cerevisiae*. Sugar consumption in those cases produces higher amounts of compounds other than ethanol, such as glycerol or pyruvic acid or to increase yeast biomass (Benito et al., 2015; Karabegovic et al., 2020).

Upon the completion of the spontaneous alcoholic fermentation, determined by monitoring the process dynamics and the change of % dry matter in the medium, the resulting wines were separated from the solid matter. All samples were analyzed to identify the reducing (residual) sugars, alcohol content, titratable acids, volatile acids and pH. The data from the wines' composition analyses have been presented in Table 3.

The residual sugar rates in the wines corresponded to the "dry" category in eight of the ten samples. That was in agreement with the degree of fermentation completion monitored refractometrically. A higher ratio of unfermented sugars was analyzed in the samples of cv. Tamyanka (No. 7, No. 10), as in the second sample they were much more.

The determined alcoholic concentration of the experimental wines corresponded to the ratio of fermented sugars, with the average conversion factor being in the range of 0.59–0.61. The highest alcohol contents were found in the Tamyanka (No. 7) and the Pamid (No. 4) samples. The wines with a lower alcohol ratio were Mavrud (No. 8, No. 9), and the lowest was found in Tamyanka (No. 10), where the process had not been fully completed.

**Table 3** Chemical composition of spontaneously fermented experimental wines.

| Sample No | Cultivar | Residual sugars $g.l^{-1}$ | Alcohol vol. % | Titratable acids $g.l^{-1}$ | Volatile acids $g.l^{-1}$ | pH |
|---|---|---|---|---|---|---|
| **1** | Dimyat | 1.28 | 10.30 | 6.67 | 0.36 | 3.29 |
| **2** | Red Misket | 1.68 | 12.00 | 4.64 | 0.47 | 3.76 |
| **3** | Mavud | 1.68 | 11.20 | 7.93 | 0.27 | 3.56 |
| **4** | Pamid | 1.16 | 12.70 | 4.61 | 0.41 | 3.98 |
| **5** | Pamid | 1.16 | 12.40 | 4.38 | 0.36 | 3.86 |
| **6** | Gamza | 1.31 | 11.40 | 6.17 | 0.35 | 3.63 |
| **7** | Tamyanka | 14.8 | 13.10 | 5.35 | 0.33 | 3.90 |
| **8** | Mavrud | 4.60 | 11.10 | 6.33 | 0.32 | 3.66 |
| **9** | Mavrud | 2.06 | 10.50 | 6.23 | 0.29 | 3.51 |
| **10** | Tamyanka | 87.00 | 7.60 | 11.95 | 3.15 | 3.66 |

The titratable acids ratios varied within relatively wide limits and corresponded to the initial acids in the grapes. There was an increase in acidity in all samples, which was explained by the predominance of synthesis over the metabolism of various organic acids during fermentation. In the Tamyanka sample (No. 10), the increase in titratable acidity was also related to the accumulation of high concentrations of volatile acids. In the remaining samples, the volatile acidity was within the normal ranges for spontaneously fermented wines.

Little changes were found in the pH values compared to those determined in the grape juice. Most wines showed a slight decrease in pH due to increased acid formation.

The resulting values of the investigated main chemical indicators of the wines' composition justified the expectation that a sufficient amount and resistant yeast cells were available on the experimental grapes, which carried out an energetic and in most cases complete alcoholic fermentation, effectively converted sugars in alcohol and formed normal rates of volatile acids. An exception was the Tamyanka sample (No. 10), where the fermentation started hard, proceeded slowly, and did not finish completely. The wine had low rates of alcohol (7.60 vol. %) and more unfermented sugars (87.00 $g.l^{-1}$), and the volatile acids produced were very high in content (3.15 $g.l^{-1}$). The reasons for these deviations in the sample were likely to be the lack of sufficient and active wine yeast in the specific batch of grapes, its high sugar content, and the associated osmotic shock, as well as the presence of unfavorable or harmful microflora.

Spasov et al. (2017) found no deviations in the rates of the main chemical indicators from the composition of experimental samples of the cv. Mavrud, which were within normal ranges for red dry wines. The residual sugar content corresponded to completed fermentation. A more significant difference was observed only in the amount of alcohol formed, due to the difference in the sugar content of the grapes from the individual batches. Relatively higher differences determined the amount of sugars used for the formation of alcohol and those for the accumulation of biomass and secondary metabolites.

Zabukovec et al. (2020) used different initial ratios of indigenous *Saccharomyces* and non-*Saccharomyces* yeasts as inoculum and found different fermentation kinetics and concentrations of volatile and non-volatile compounds in the wine. When the process stopped, different concentrations of unfermented sugars were measured in the samples. They observed the best results in a combination dominated by *Saccharomyces cerevisiae* (80%), *Hanseniaspora uvarum* (10%), and *Starmerella bacillaris* (10%). This combination led to an appropriate volume fraction of alcohol, a lower concentration of acetic acid, and significantly highest quantity of volatile thiols and higher alcohols.

Çelik et al. (2017) identified a total of 30 aroma compounds in Narince spontaneous fermented wines. Isoamyl alcohol and 2-phenylethyl alcohol were the most abundant higher alcohols. Ethyl acetate was the major ester compound and acetaldehyde was the most important carbonyl compound. The most abundant volatile acids were octanoic and hexanoic acids.

Lleixa et al. (2016) in alcoholic fermentation with yeast *Hanseniaspora vineae* and with *Saccharomyces cerevisiae* found that both yeasts produced alcohols, esters, acids, acetates, carbonyl compounds, and terpenes. Of the alcohols phenyl ethanol provided good aromas, described as rose and honey-like. Among the identified esters, ethyl hexanoate reached the threshold of perception and contributed a green apple aroma. Of the identified acids hexanoic, decanoic, and octanoic acid showed the highest concentration. Of the acetates, phenethyl acetate was 50 times more abundant in wines fermented with *Hanseniaspora vineae* than in those fermented with *Saccharomyces cerevisiae.*

**Organoleptic profile of the obtained experimental white and red wines**

Sensory characteristics of wine also strongly depend on the yeast species conducting the alcoholic fermentation. Many substances, especially volatile ones, synthesized by the yeast affect the aroma and taste. Thus they significantly impact the wine's final style (Vilanova et al., 2005; Dimopoulou et al., 2020; Mislata et al., 2021). The indigenous non-*Saccharomyces* and autochthonous *Saccharomyces cerevisiae* strains are highly recommended because they not only improve quality and complexity but also impact the wines with typical sensory characteristics specific to each wine area (Liang et al., 2013; Karabegovic et al., 2020; Bouloumpasi et al., 2021; Zhang et al., 2021).

After determining the chemical composition of the experimental white and red wines, all samples were subjected to an organoleptic analysis by a tasting panel of 7 oenologists. The sensory profiles of the variants have been presented in Figure 4 and Figure 5.

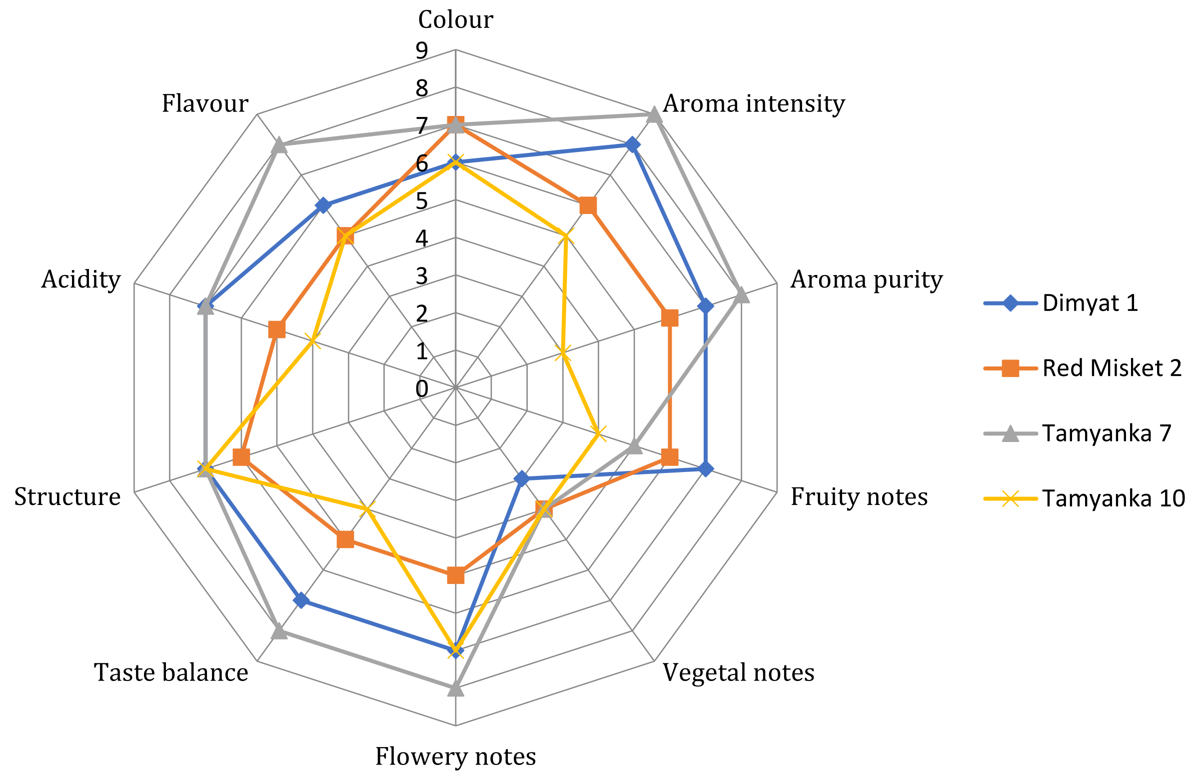


**Figure 4** Sensory profile of white spontaneously fermented wines.

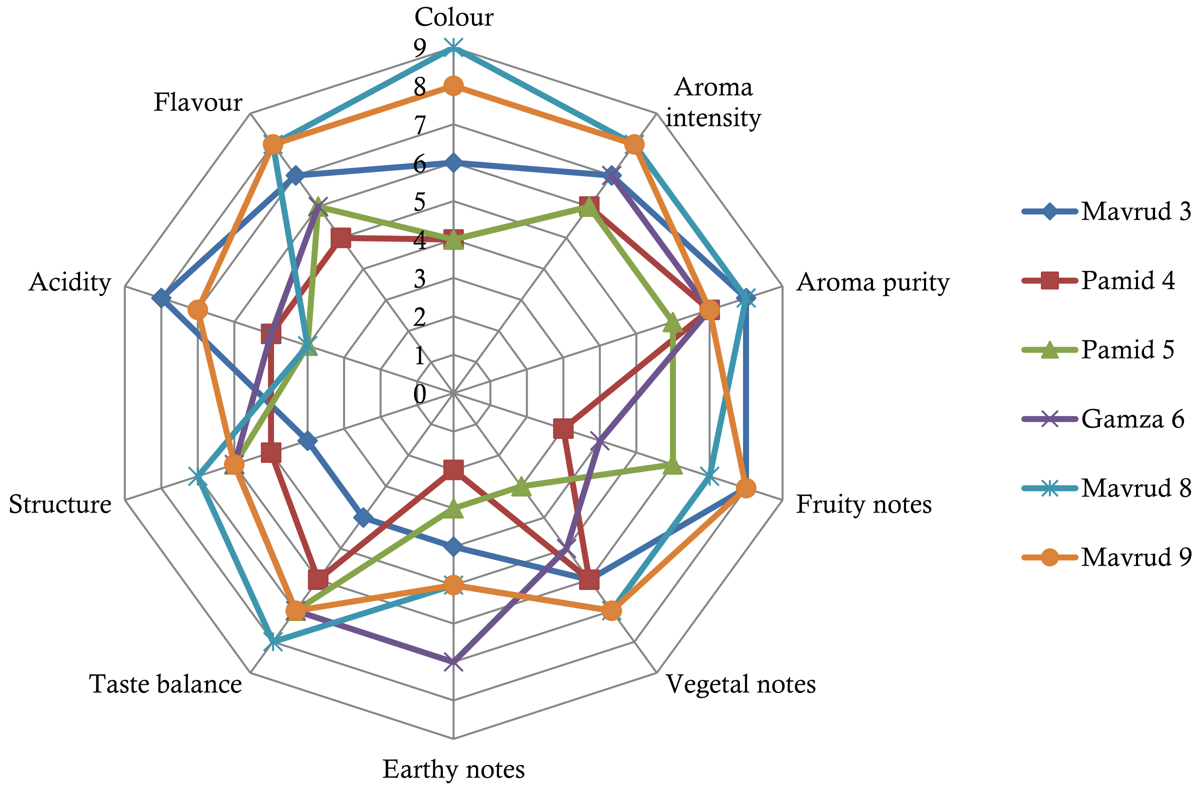


**Figure 5** Sensory profile of red spontaneously fermented wines.

In the case of white wines, the colours were rated low due to the presence of beige-brown nuances, due to the fermentation with the solid parts, and the enrichment of the liquid with phenolic compounds. The intensity and purity of the aromas were relatively well expressed. In the Dimyat sample (No. 1) the fruity nuances dominated, and in the Tamyanka sample (No. 7) the floral notes were more pronounced. In these wines, the more intense taste aroma, fruity-floral, was also highly assessed. The taste of these samples was balanced, and the acids were well incorporated. The Red Misket wine (No. 2) was slightly roughened because the fermentation with the skins had a stronger negative effect on both the aroma and the taste. The problems with the course of the alcoholic fermentation in the sample of the cv. Tamyanka (No. 10) had a negative impact on the wine sensory profile. It had a lot of residual sugars, an impure aroma, and an inharmonious taste. In the case of red wines, the colour intensity was low in all samples, and only in Mavrud (No. 8) it was higher. The aromas were relatively pure, medium-intensive. In Pamid wines, the aromatic nuances were mainly vegetal. In Gamza (No. 6) earthy notes dominated, and in Mavrud (No. 8, No. 9) fruity-vegetal nuances prevailed. In terms of taste, the wine from Mavrud (No. 8) was rated the highest, followed by Mavrud (No. 9) and Gamza (No. 6). All wines had the sensory profile of dry wines that was a prerequisite for the presence of active own strains of wine yeast.

Spasov et al. (2017) established in the spontaneously fermented Mavrud wines a strong intensity, with a deep ruby-red nuance, fruity and vegetal notes in the aroma, and a relatively balanced taste.

Ilieva et al. (2019) found that the organoleptic profile of experimental red wines Vranec and Cabernet Sauvignon, fermented with autochthonous yeast strains *Saccharomyces cerevisiae* revealed superiority in comparison with control wines produced with commercial strain SiHa. The obtained wines were of higher quality. Sensory analyses showed ruby-red, vivid color and enhanced mature fruit nuance in the aroma because of the higher content of esters.

In the descriptive test of Macabeo and Merlot wines, Lleixa et al. (2016) observed that those fermented with *Hanseniaspora vineae* received the best rating. These wines showed a significantly stronger flowery aroma profile than wines produced with *Saccharomyces cerevisiae.*

## Conclusion

The results obtained from the chemical and organoleptic analysis of the experimental spontaneously fermented white and red wines confirmed the presence of active natural microflora on the grapes. The wine yeast strains that carried out the spontaneous alcoholic fermentation in the samples demonstrated high fermentation activity. The process was dynamic, efficient, and fully complete, ensuring a high conversion of sugars into alcohol. The formed metabolites were within acceptable ranges and the trial wines had normal rates of the investigated indicators of their chemical composition. The exception was the Tamyanka sample (No. 10), where the fermentation started hard, proceeded slowly, and did not finish completely, probably due to the lack of sufficiently active natural microflora. All samples had specific sensory profiles, with a positive rating of the organoleptic criteria, according to the variety and the created experimental conditions for their making.

### Conflict of interest

The authors declare no conflict of interest.

### Ethical statement

This article doesn't contain any studies that would require an ethical statement.

### Funding

This research was carried out under the project "Developing identity on yield, soil and site"/DIONYSOS, Subsidy contract B2.6c.04/01.11.2017 with the financial support of the INTERREG V-A GREECE - BULGARIA 2014-2020 COOPERATION Program, Co-funded by the European Regional Development Fund and National funds of Greece and Bulgaria. Total budget: EUR 735 778.70 (https://dionysosvine.eu/en/).

## References

Abrasheva, P., Bambalov K., & Georgiev A. 2008. Лозарство и винарство [Vine growing and winemaking]. Sofia: publ. house Matkom, pp. 261–264, ISBN 978-954-9930-50-4. [in Bulgarian]

Alba-Lois, L., & Segal-Kischinevzky, C. 2010. Yeast fermentation and the making of beer and wine. In *Beer & Wine Makers. Nature Education*, 3(9), 17.

Baffi, M.A., Santos Bezarra, C., Arevalo-Villena, M., Briones-Perez, A.I., Gomes, E., & Da Silva, R. 2011. Isolation and molecular identification of wine yeasts from a Brazilian vineyard. In *Annals of Microbiology*, 61, 75–78, https://doi.org/10.1007/s13213-010-0099-z.

Bambalov, G. 1981. Микробиология на винопроизводството [Microbiology of winemaking]. Plovdiv: publ. house Hristo G. Danov, p. 247. [in Bulgarian]

Benito, S., Hofmann, T., Laier, M., Lochbühler, B., Schüttler, A., Ebert, K., Fritsch, S., Röcker, J., & Rauhut, D. 2015. Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-*Saccharomyces* and *Saccharomyces cerevisiae*. In *European Food Research and Technology*, 241, 707–717, https://doi.org/10.1007/s00217-015-2497-8.

Bezerra-Bussoli, C., Baffi, M. A., Gomes, E., & Da Silva, R. 2013. Yeast diversity isolated from grape musts during spontaneous fermentation from Brazilian winery. In *Current Microbiology*, 67(3), 356–361, https://doi.org/10.1007/s00284-013-0375-9

Bougreau, M., Ascencio, K., Bugarel, M., Nightingale, K., & Loneragan, G. 2019. Yeast species isolated from Texas High Plans vineyards and dynamics during spontaneous fermentations of Tempranillo grapes. In *Plos One*, 14(5), e0216246, https://doi.org/10.1371/journal.pone.0216246.

Bouloumpasi, E., Petraina, A., & Karampetea, A. 2021. Sensory profile of cv. Savvatiano (*Vitis vinifera* L.) wines fermented with the *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* yeasts in individual and mixed fermentation. In *Biology and Life Sciences Forum*, 6(1), 52, https://doi.org/10.3390/Foods2021-11095.

Chorniak, J. 2024. Wild yeast: the pros and cons of spontaneous fermentation. https://winemakermag.com/article/758-wild-yeast-the-pros-and-cons-of-spontaneous-fermentation.

Çelik, Z. D., Erten, H., Darici, M., & Cabaroğlu, T. 2017. Molecular characterization and technological properties of wine yeasts isolated during spontaneous fermentation of *Vitis vinifera* L. cv. Narince grape must grown in ancient wine making area Tokat, Anatolia. *40th World Congress of Vine and Wine,* In *BIO Web of Conference*, 9, 02017, https://doi.org/10.1051/bioconf/20170902017.

David, R., Dochain, D., Mouret, J. R., Vande Wouwer, A., & Sablayrolles, J. M. 2010. Dynamical modeling of alcoholic fermentation and its link with nitrogen consumption. In *IFAC Proceedings Volumes*, 43(6), 496–501, https://doi.org/10.3182/20100707-3-BE-2012.0095.

Dimitrov, V., & Dimitrova, D. 2015. Tangible and intangible cultural heritage of Northwest Planning Region: opportunities for organizing wine routes. In *International Scientific Conference "Cultural corridor Via Adriatica – Cultural Tourism without bounderies*" 15-19.10.2015, Trogir, Croatia, p.120–128.

Dimitrova, D., & Dimitrov, V. 2017. Regional aspects of viticulture and wine production development in Bulgaria. In *Journal of Mountain Agriculture on the Balkans*, 20(3), 162–186.

Dimopoulou, M., Troianou, V., Toumpeki, C., Gosselin, Y., Dorignas, E., & Kotseridis, Y. 2020. Effect of strains from different *Saccharomyces* species used in different inoculation schemes on chemical composition and sensory characteristics of Sauvignon blanc wine. In *OENO One*, 54(4), 745–749, https://doi.org/10.20870/oeno-one.2020.54.4.3240.

Dzhabarova, Y., & Arseniou, S. 2018. Creating wine tourism identity. In *International Scientific Conference "The cultural corridors of South-eastern Europe: Cultural tourism without boundaries"*, Santorini, Greece, 02.10. – 07.10.2018, 58–65.

Gonzales, S.S., Barrio, E., & Querol, A. 2007. Molecular identification and characterization of wine yeasts isolated from Tenerife (Canary Island, Spain). In *Journal of Applied Microbiology*, 102(4), 1018–1025. https://doi.org/10.1111/j.1365-2672.2006.03150.x.

Howell, K.S., Cozzolino, D., Bartowsky, E.J., Fleet, G.H., & Henschke, P.A. 2006. Metabolic profiling as a tool for revealing *Saccharomyces* interactions during wine fermentation. In *FEMS Yeast Res*, 6(1), 91–101. https://doi.org/10.1111/j.1567-1364.2005.00010.x.

Ilieva, F., Kostadinović Veličkovska, S., Dimovska, V., Mirhosseini, H., & Spasov, H. 2019. Isolation of *Saccharomyces cerevisiae* yeast strains from Macedonian "Tikveš" wine-growing region and their impact on the organoleptic characteristics of Vranec and Cabernet Sauvignon wines. In *Research Journal of Biotechnology*, 14(6), 100–110.

Ivanov, T., Gerov S., Yankov A., Bambalov G., Tonchev T., Nachkov D., & Marinov M. 1979. Практикум по винарска технология [Practicum in wine technology]. Plovdiv: publ. house Hristo G. Danov, pp. 530. [in Bulgarian]

Ivkovikj, M.T. 2021. Isolation of indigenous yeasts from different types of red grapes and determination of oenological characteristics of wine. In *KNOWLEDGE – International Jo*urnal, 47(3), 437–442.

Jankura, E., Piknova, L., & Lopašovska, J. 2020. Identification and technological characteristics of yeast strains from vineyards in Slovakia. In *Journal of Food and Nutrition Research*, 59(3), 241–249.

Karabegović, I., Malićanin, M., Danilović, B., Stanojević, J., Stamenković Stojanović, S., Nikolić, N., & Lazić, M. 2021. Potential of non-*Saccharomyces* yeast for improving the aroma and sensory profile of Prokupac red wine. In *OENO One*, 55(2), 181–195. https://doi.org/10.20870/oeno-one.2021.55.2.3859.

Liang, H.Y., Chen, J.Y., Reeves, M., & Han, B.Z. 2013. Aromatic and sensorial profiles of young Cabernet Sauvignon wines fermented by different Chinese autochthonous *Saccharomyces cerevisiae* strains. In *Food Research International*, 51(2), 855–865. https://doi.org/10.1016/j.foodres.2013.01.056.

Lleixa, J., Martin, V., Portillo, M. C., Carrau, F., Beltran, G., & Mas, A. 2016. Comparison of fermentation and wines produced by inoculation of *Hanseniaspora vineae* and *Saccharomyces cerevisiae.* In *Frontiers in Microbiology*, 7, 338, https://doi.org/10.3389/fmicb.2016.00338.

Maicas, S. 2021. Advances in wine fermentation. In *Fermentation*, 7(3), 187. https://doi.org/10.3390/fermentation7030187.

Maicas, S., & Mateo, J.J. 2023. The life of *Saccharomyces* and non-*Saccharomyces* yeasts in drinking wine. In *Microorganisms*, 11(5), 1178, https://doi.org/10.3390/microorganisms11051178.

Miller, K.V., & Block, D.E. 2020. A review of wine fermentation process modeling. In *Journal of Food Engineering*, 273, 109783, https://doi.org/10.1016/j.jfoodeng.2019.109783.

Mislata, A.M., Puxeu, M., Andorra, I., Espligares, N., Lamo, S., Mestres, M., & Ferrer-Gallego, R. 2021. Effect of the addition of non-*Saccharomyces* at first alcoholic fermentation on the enological characteristic of Cava wines. *Fermentation*, 7(2), 64, https://doi.org/10.3390/fermentation7020064.

Nachev, T. 1981. *Лозарството по българските земи от възникването му до началото на 20 век [Viticulture in Bulgarian lands since its occurrence to the beginning of 20th century]*: dissertation theses. Institute of Viticulture and Enology, Pleven, Bulgaria: 260 p. [in Bulgarian]

Nurgel, C., Erten, H., Canbaş, A., Cabaroğlu, T., & Selli, S. 2002. Influence of *Saccharomyces cerevisiae* strains on fermentation and flavor compounds of white wines made from cv. Emir grown in Central Anatolia, Turkey. *Journal of Industrial Microbiology & Biotechnology*, 29(1), 28–33, https://doi.org/10.1038/sj.jim.7000258.

Papathanasiou, I., Selvagini, R., Servili, M., Vaughan-Martini, A., & Roussis, I.G. 2006. Winemaking ability of wild yeast strains and comparative volatile profiles of wines fermented at 12° or 20°C. *Food Science and Technology Research*, 12(3), 194–198, https://doi.org/10.3136/fstr.12.194.

Parapouli, M., Hatziloukas, E., Drainas, C., & Perisynakis, A. 2010. The effect of Debina grapevine indigenous yeast strains of *Metschnikowia* and *Saccharomyces* on wine flavour. *Journal of Industrial Microbiology and Biotechnology*, 37(1), 85–93, https://doi.org/10.1007/s10295-009-0651-7.

Pardo, I., Garcia, M.J., Zuñiga, M., & Uruburu, F. 1989. Dynamics of microbial populations during fermentation of wines from the Utiel-Requena region of Spain. *Applied and Environmental Microbiology*, 55(2), 539–541, https://doi.org/10.1128/aem.55.2.539-541.1989.

Prodanova, N. 2007. Дегустацията или как да опознаем виното [Tasting or how to get to know the wine]. Sofia: publ. house Gourmet, 136 pp. ISBN 978-954-91789-7-5. [in Bulgarian]

Sabate, J., Cano, J., Esteve-Zarzoso, B., & Guillamon, J.M. 2002. Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. *Microbiological Research*, 157(4), 267–274, https://doi.org/10.1078/0944-5013-00163.

Shahrajabian, M.H., & Wenli, S. 2023. Study of different types of fermentation in wine–making process and considering aromatic substances and organic acid. *Current Organic Synthesis*, 20, https://doi.org/10.2174/1570179420666230803102253.

Sidari, R., Ženišova, K., Tobolkova, B., Belajova, E., Cabicarova, T., Bučkova, M., Puškarova, A., Plany, M., Kuchta, T., & Pangallo, D. 2021. Wine yeast selection: laboratory characterization and protocol review. *Microorganisms*, 9(11), 2223, https://doi.org/10.3390/microorganisms9112223.

Spasov, H., Chobanov, Y., Zapryanova, P., Raychevska, Zh., & Dyankova, K. 2017. Isolation, investigation and selection of wine yeast strains for the production of regional wines of the Mavrud variety. *Journal of Mountain Agriculture on the Balkans*, 20(4), 95–106.

Tofalo, R. 2021. Microbial dynamics in wine production. *Microorganisms*, 9(4), 700, https://doi.org/10.3390/microorganisms9040700.

Varela, C., Siebert, T., Cozzolino, D., Rose, L., McLean, H., & Henschke, P.A. 2009. Discovering a chamical basis for differentiating wines made by fermentation with 'wild' indigenous and inoculated yeasts: role of yeast volatile compounds. *Australian Journal of Grape and Wine Research*, 15, 238–248, https://doi.org/10.1111/j.1755-0238.2009.00054.x.

Vilanova, M., Masneuf-Pomarede, I., & Dubourdieu, D. 2005. Influence of *Saccharomyces cerevisiae* strains on general composition and sensorial properties of white wines made from *Vitis vinifera* cv. Albariño. *Food Technology & Biotechnology*, 43(1), 79–83.

Yankov, A. 1992. Технология на винопроизводството [Technology of winemaking]. Sofia: publ. house Zemizdat, pp. 87–102. ISBN 954-05-0167-9. [in Bulgarian]

Zabukovec, P., Čadež, N., & Čuš, F. 2020. Isolation and identification of indigenous wine yeasts and their use in alcoholic fermentation. *Food Technology & Biotechnology*, 58(3), 337–347. https://doi.org/10.17113/ftb.58.03.20.6677.

Zhang, B., Ivanova-Petropulos, V., Duan, C., & Yan, G. 2021. Distinctive chemical and aromatic composition of red wines produced by *Saccharomyces cerevisiae* co-fermentation with indigenous and commercial non-*Saccharomyces* strains. *Food Bioscience*, 41(7), 100925, https://doi.org/10.1016/j.fbio.2021.100925.

https://dionysosvine.eu/en/. DIONYSOS Project 2017–2019. Developing Identity in Yield, Soil and Site, Cooperation Programme V-A, Intetrreg, Greece-Bulgaria.