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Red wines from the Mostar area: Physicochemical, antioxidative, and antimicrobial properties

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Abstract:
Wines are complex alcoholic beverages. Apart from alcohol, they also contain other compounds, including those that have a beneficial effect on human health.
This paper features the basic physicochemical properties of four red grape varieties (Blatina, Vranac, Cabernet Sauvignon, Merlot) from the Mostar area, Bosnia and Herzegovina, as well as the antioxidant and antimicrobial properties of wines made of these grape varieties. The wines were produced in a standard way; the results were observed during two consecutive seasons of 2020 and 2021. The physicochemical properties were analyzed by standard methods recommended by the International Organization of Vine and Wine. The study involved tests for total phenolics, flavonoids, and anthocyanins, as well as for antioxidant activity. The methodology included FRAP, DPPH, and ABTS assays. The antimicrobial activity was tested by agar dilution method, which made it possible to determine the minimum inhibitory and bactericidal values. The list of pathogenic and opportunistic bacteria consisted of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*. Pathogenic yeasts were represented by *Candida albicans*. *Lactobacillus plantarum* and *Saccharomyces boulardii* were selected as probiotic cultures.
The physicochemical characteristics of grapes, i.e. must, depended on the harvest year, variety, and their interaction. The best antioxidant effect and the highest total phenolic content belonged to the Vranac wine, vintage 2020. *B. cereus* appeared to be the most sensitive bacteria. The Blatina wines of both harvest years demonstrated the lowest antimicrobial and the antioxidant activities. Probiotic cultures proved to be resistant to the effects of wine. Pearson's test revealed a reliable correlation between the antioxidant properties and the antimicrobial effect on *B. cereus* and, in one case, on *S. aureus* and *P. aeruginosa*.
All grapevine varieties in this research proved to be suitable for the production of quality wines in the Mostar area.

Keywords: Red grape varieties, wine, must, physicochemical properties, antioxidant activity, antimicrobial activity

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INTRODUCTION

The quality of grapes and wine depends on the variety, the agro-ecological conditions of the vineyard, and the production technologies. The structural and physicochemical characteristics of grape clusters and berries define the ampelographic and technological properties of grape varieties [1, 2].

Wine is one of the oldest and most widespread alcoholic beverages. As a rule, it contains alcohol, sugars, acids, tannins, minerals, proteins, organic acids, volatile compounds, and phenolic compounds [3]. Antioxidant activity is one of the most important properties of red wines. It is associated with polyphenols, e.g., flavonoids, phenolic acids, stilbenes, coumarins, etc. [4]. The polyphenol content of wine depends on the grape variety, vineyard location, cultivation system, climate, soil type, grapevine production practices, harvesting time, production process, and ageing. The polyphenol molecules behave as antioxidants against free radicals. They increase the antioxidant capacity in the human body.

In addition, they affect the sensory profile of wines [5]. Anthocyanins contribute to color while flavan-3-ols are responsible for bitterness and astringency [5, 6]. Vintage has a different effect on antioxidant properties of red wines, even if they share the same production conditions, vineyard location, cultivation system, climate, soil type, harvesting time, and ageing [5].

The antimicrobial activity of red wines against pathogenic and opportunistic microorganisms is well documented and mostly associated with the content of various polyphenolic compounds in red wine, e.g., anthocyanins, flavonoids, stilbenes, catechins, and phenolic acids [7, 8]. The inhibitory effect also depends on the type of bacteria. The content of phenolic acids, resveratrol, and some flavonoids was found to correlate with the inhibitory activity of wine against *Clostridium perfringens* and *Micrococcus flavus* [9]. Antimicrobial activity of wine against *Listeria inocua* and *Proteus vulgaris* was reported to depend on the catechin content. However, none of the abovementioned components correlated with the activity against *Klebsiella pneumonia* strains. Apart from polyphenols, the antimicrobial effect of wines is known to depend on other components and variables, e.g., organic acids, low pH, alcohol, and acetates [10]. In relation to pathogenic microorganisms, wine components do not inhibit the digestive tract microbiota: plant phenols actually have a stimulating effect on their growth [11].

The physicochemical characteristics of grapes from Bosnia and Herzegovina, especially those from the region of Mostar, remain as understudied as the antioxidant and antimicrobial properties of the local wines. This research aimed at analyzing the effect of grapevine variety and production season on these properties.

STUDY OBJECTS AND METHODS

The study involved grapes and wine varieties of Blatina, Vranac, Cabernet Sauvignon, and Merlot, harvested in the area of Mostar (43°20'N; 17°48'E) in Bosnia and Herzegovina in 2020–2021.

Climate indicators. Mostar is located in the southwestern part of Bosnia and Herzegovina. The area owes its warm Mediterranean climate to the Adriatic Sea [12]. Figures 1a and b give the basic meteorological data, average monthly temperatures, extreme daily temperatures, and total monthly precipitation during the growing seasons of April – October 2020–2021 [13]. Both research years were similar in terms of air temperature, with occasional extreme daily temperatures as high as ≥ 30°C in May – September. The amount of precipitation was quite low, especially in June – July 2021.

Physical characteristics of grape clusters and berries. The analysis included examination of the basic physical characteristics of grape clusters and berries. The average weights of 10 grape clusters and 100 grape berries, g, were measured using a digital scale (KERN 440, Germany).

Physicochemical characteristics of must and wine. The quality analysis of the basic physicochemical parameters of the must took place during the first stage of microvinification. It covered the following parameters. The percentage of total soluble solids – sugar (TSS, °Brix) was measured with a digital refractometer (Atago-Pal-3, Japan). The total titratable acidity was determined by the neutralization method. The pH value of the must was measured with a pH-meter (Hanna HI2211, USA).

Microvinification was the same for all grape varieties and followed the classic protocol for red wines. After crushing the grapes, we protected the resulting must from oxidation by adding Vulcasulph, a commercial preparation produced by Vulcascot, Austria, in the amount of 10 g/100 kg. After that, we added Vitamon Combi yeast food (Erbslöh, Germany). The inoculation involved the Oenoferm Color selection yeast culture (Erbslöh, Germany) in the quantities recommended by the manufacturer. Fermentation took place at 20–23°C, with must submersion performed twice a day until the

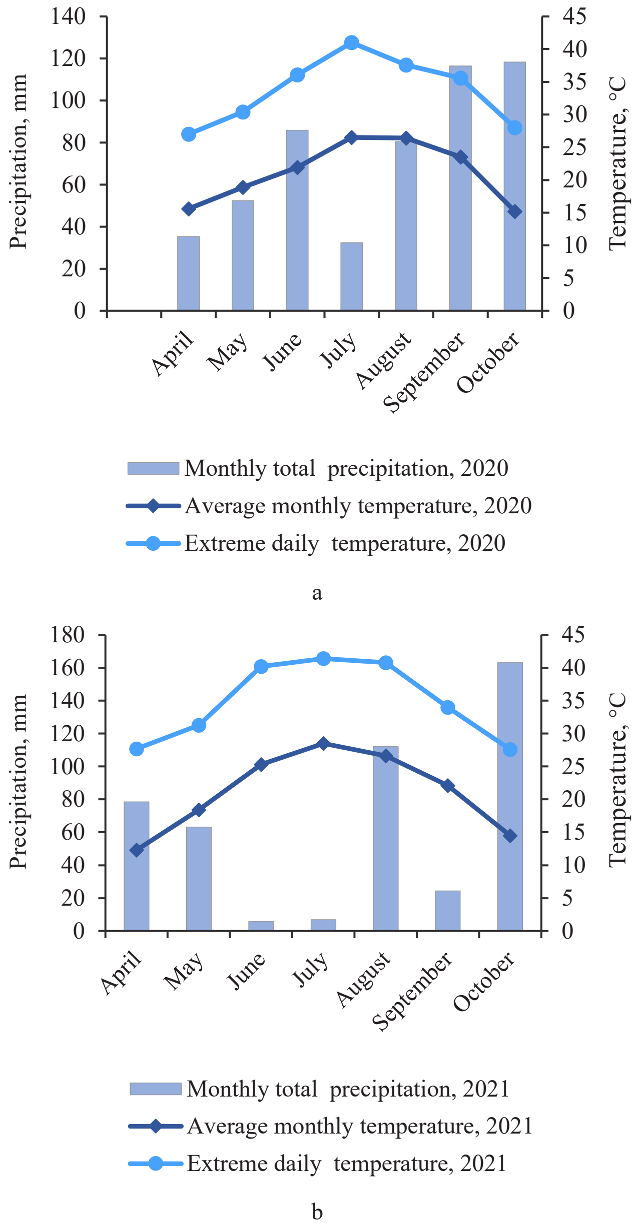

Figure 1 Meteorological data in 2020 (a) and in 2021 (b)

level of remaining sugar in the wine was between 1.0 and 2.0 g/L. The young wine was analyzed after five months of storage in stainless steel tanks. The analysis followed standard procedures and covered the following physicochemical parameters: ethanol, total extract, sugar-free extract, reducing sugars, total acidity, volatile acidity, and pH value [14].

Antioxidant activity of wines. The total phenolic content was determined by the Folin-Ciocalteu colorimetric method [15]. The non-flavonoid content was determined by the formaldehyde method whereas the flavonoid content was calculated as the difference between total phenols and non-flavonoids [16]. The antioxidant activity analysis involved DPPH, ABTS, and FRAP assays [17, 18]. The total anthocyanin content was determined by the spectrophotometric method as described by Mitrevska *et al.* [19].

Antimicrobial activity of wines. The antimicrobial activity test included the following nutrient media: Mueller Hinton agar, Mueller Hinton broth, Nutrient agar, Sabouraud agar, De Man Rogosa and Sharpe agar, and agar (1.5%).

Microbial cultures: the experiment involved five bacterial cultures, namely *Escherichia coli* WDCM 00013, *Pseudomonas aeruginosa* WDCM 00024, *Staphylococcus aureus* WDCM 00034, *Bacillus cereus* WDCM 00151, *Lactobacillus plantarum* 299v, as well as two yeast cultures (*Candida albicans* WDCM 00054 and *Saccharomyces boulardii* DBVPG 6763).

Microbial culture media preparation: the bacterial cultures of *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*, as well as the *Candida albicans* yeast culture, were prepared from the logarithmic phase by the direct colony suspension method (M07. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; M11. Methods for antimicrobial susceptibility testing of anaerobic bacteria) [20].

Preparing standardized inoculum from the logarithmic phase (*E. coli*, *P. aeruginosa*, and *B. cereus*). The cultures inoculated on Nutrient agar slant and Mueller Hinton broth agar plates were incubated for 24 h at 37°C. After incubation, we transferred 3–5 isolated colonies from the agar plate to a test tube with 5 mL of Mueller Hinton Broth. The tubes with the inoculated bacteria were left to incubate for 2–6 h. The culture incubation time was the same for each experiment.

Preparing standardized inoculum for *S. aureus* and *C. albicans*. *S. aureus* and *C. albicans* were inoculated from agar slant (nutrient agar for *S. aureus* and Sabouraud agar for *C. albicans*) on the corresponding agar plates (Mueller Hinton agar for *S. aureus* and Sabouraud agar for *C. albicans*) with an inoculation loop. The agar plates were left to incubate at 37°C (*S. aureus*) and 30°C (*C. albicans*) for 24 h. After incubation, we collected two or three colonies directly from the Mueller Hinton agar and Sabouraud agar to be transferred to the Mueller Hinton broth (*S. aureus*) and the physiological solution (*C. albicans*). The density of the microbial cultures was determined spectrophotometrically (625 nm for bacteria and 530 nm for yeast), and 0.5 McFarland standard (1.5×10^8 CFU/mL) was used for comparison. By diluting the cultures, we adjusted their density to 1.5×10^6 CFU/mL.

Preparing *L. plantarum* inoculum. A capsule of Flobian (Abela Pharm, Belgrade) was added to 99.9 mL of saline solution and vortexed at 300 rpm for 30 min. The density of the culture for inoculation was adjusted to 1.5×10^6 CFU/mL by dilution [21].

Preparing *S. boulardii* inoculum. A capsule of Bulardi Probiotic (Abela Pharm, Belgrade) was suspended in 40 mL of saline solution, shaken, and adjusted to a density of 1.5×10^6 CFU/mL by dilution [22].

Antimicrobial activity testing. To determine the antimicrobial activity of wine, we used the agar dilution method to obtain minimum inhibitory, minimum bactericidal, and minimum fungicidal concentrations (M07 and M11) [20–22]. We performed a series of dilutions on agar by adding an appropriate amount of wine to the previously dissolved and cooled (45°C) Mueller Hinton agar, so that the final concentration of wine in the media would be 10, 20, and 30% (v/v). After shaking the media with wine, we poured the samples into sterile Petri dishes. Upon media setting, the microbial cultures were inoculated onto the surface of the agar plates in drops of 10 μL. The Petri dishes with *L. plantarum* were covered with a layer of 1.5% agar, dissolved, and cooled to 45°C. The inoculated Petri dishes were then incubated at 37°C for 24 h. After incubation, we measured the growth of the cultures on the media with wine. The lowest concentration of wine in the media with no visible culture growth was determined as minimum inhibitory. All Petri dishes with no visible culture growth underwent re-inoculation. After scraping the inoculated spots with a sterile inoculation loop, we re-inoculated them on Nutrient agar, Sabouraud agar, De Man Rogosa, and Sharpe agar. The petri dishes were incubated at 37°C for 24 h. The lowest wine concentrations with no growth of inoculated colonies were determined as minimum bactericidal or fungicidal concentrations. A medium without wine was used as positive control, and ethanol served as negative control in concentrations that corresponded to the concentration of ethanol in the wine.

Statistical analysis. The results were expressed as means ± standard deviation. The statistical analysis involved a one-way analysis of variance (ANOVA). Significant differences between the results were determined by the Duncan's multiple range test. The differences were considered significant at $p < 0.05$. Relationships between antioxidant and antimicrobial activity were established using the Pearson correlation test.

RESULTS AND DISCUSSION

Basic physical characteristics of grapes. The quality and typicality of the wine depend on, among other things, the variety and the quality of berries. This research featured two domestic (Blatina and Vranac) and two international (Cabernet Sauvignon and Merlot) grape varieties. The study of intervarietal differences included the basic physical indicators of grape clusters and berries (average weight of 10 representative grape clusters and 100 grape berries), as well as how these qualities depended on the production year, variety, and the interaction of these two factors.

Table 1 shows that the average grape cluster weight was statistically higher in 2021. An inter-varietal comparison showed that the Blatina variety had the highest grape cluster weight (463.97 g), followed by the Vranac variety (414.17 g): in 2021, their weights were statistically significantly higher than those of the other varieties. The Cabernet Sauvignon variety had the lowest grape cluster weight in both years. The mentioned parameter strongly depended on both year and variety, as well as on their interaction. The highest weight of 100 grape berries also belonged to the Blatina variety (330.00 g), followed by the Vranac variety (290.04 g), while the lowest weight was recorded in Cabernet Sauvignon in both research years (120.63 and 126.30 g, respectively). In contrast to the grape cluster weight, the berry weight depended only on the variety. The research period showed no statistically significant difference between the varieties regarding the average weight of 100 grape berries (232.75 and 226.35 g).

The varietal differences were best illustrated by the lowest values of grape cluster and grape berry weights in the Cabernet Sauvignon variety, which did not contradict the description of the technological potential for this variety published in the official catalog of grapevine varieties [23]. Similar results were found by Ivanišević *et al.* while Russian research teams reported somewhat lower grape mass [24–26]. However, the large variation in the grape cluster weight for the Blatina variety during the research period could be linked to varietal specificity. Functionally female flower causes poor fertilization, which results in a lower percentage of fruit setting and, therefore, leads to a lower grape cluster weight [27, 28]. In the case of the Vranac variety, the locality also had a positive effect on the above-mentioned variables. Our results for this variety were in accordance with a recent multi-year qualitative study of Vranac grapes in Herzegovina [29]. In fact, our results were very close to those published for another study that took place in Montenegro, i.e., the primary production region for Vranac [30, 31]. Similarly, the results we obtained for the Merlot variety indicated a positive effect of the environment. Our values were higher compared to those reported for other regions [32, 33].

Basic physicochemical properties of must. The composition and sensory profile of wine depend on the composition and the ratio of primary and secondary metabolites in grapes. Table 2 sums up the basic physicochemical parameters, total soluble solids, total titratable acidity, and pH values of the musts for each variety and harvest year, as well as their interaction.

The statistical analysis of total soluble solids in the must revealed a statistically significant ($p < 0.001$) difference between the grape varieties. For all varieties, the total amount of soluble solids was significantly ($p < 0.001$)

Table 1 Basic physical parameters of grape clusters and grape berries

Variety	Grape cluster weight, g	Weight of 100 grape berries, g
	2020	
Blatina	229.01 ± 22.27^{bcd}	320.00 ± 1.07^{ab}
Vranac	379.22 ± 28.75^{a}	280.41 ± 0.97^{b}
Cabernet Sauvignon	192.93 ± 10.73^{cd}	120.63 ± 0.32^{d}
Merlot	325.91 ± 37.79^{ab}	200.06 ± 0.71^{c}
	2021	
Blatina	463.97 ± 27.51^{a}	330.00 ± 1.33^{a}
Vranac	414.17 ± 31.72^{a}	290.04 ± 1.02^{b}
Cabernet Sauvignon	149.53 ± 11.18^{d}	126.30 ± 0.31^{d}
Merlot	283.52 ± 11.26^{bc}	150.87 ± 0.39^{d}
Year (Y)	9.59**	1.08^{ns}
Variety (V)	58.97***	223.12***
Y×V	17.55***	3.76*
	Mean values (± standard error)	
	Year	
2020	281.77 ± 18.98^{b}	232.75 ± 1.27^{ns}
2021	327.69 ± 21.34^{a}	226.35 ± 1.44^{ns}
	Variety	
Blatina	346.49 ± 28.65^{b}	326.00 ± 0.84^{a}
Vranac	396.70 ± 21.21^{a}	285.23 ± 0.69^{b}
Cabernet Sauvignon	171.23 ± 7.55^{c}	126.30 ± 0.22^{d}
Merlot	304.72 ± 20.55^{b}	179.38 ± 0.62^{c}

$^{a–d}$ – different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test

***, **, * – significant at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively

^{ns} – not significant

Table 2 Basic physicochemical parameters of grape musts

Year	Variety	Total soluble solids, %			Total titratable acidity, g/L			pH		
		$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE
2020	Blatina	18.71	±	0.14	6.95	±	0.04	3.01	±	0.01
	Vranac	26.02	±	0.05	4.21	±	0.09	3.29	±	0.03
	Cabernet Sauvignon	24.42	±	0.09	4.96	±	0.04	3.71	±	0.02
	Merlot	25.48	±	0.22	4.60	±	0.04	3.44	±	0.02
2021	Blatina	17.24	±	0.17	8.05	±	0.05	3.28	±	0.01
	Vranac	22.59	±	0.09	5.59	±	0.07	3.54	±	0.01
	Cabernet Sauvignon	23.82	±	0.09	4.83	±	0.06	3.56	±	0.04
	Merlot	24.11	±	0.12	5.80	±	0.05	3.35	±	0.01
F_{year}, p_{year}		290.86**, $p < 0.001$			403.22**, $p < 0.001$			17.77**, $p < 0.001$		
$F_{variety}$, $p_{variety}$		135.71**, $p < 0.001$			877.66**, $p < 0.001$			134.08**, $p < 0.001$		
$F_{year*variety}$, $p_{year*variety}$		36.16**, $p < 0.001$			50.54**, $p < 0.001$			44.41**, $p < 0.001$		
$LSD_{year*variety}$		0.37			0.16			0.06		

higher in 2020 than in 2021. The highest value of total soluble solids was observed in the Vranac must in 2020. Regarding the total titratable acidity in the must, the varieties also showed a statistically significant ($p < 0.001$) difference. In general, the total titratable acidity values for all musts were lower ($p < 0.001$) in 2020 than in 2021, with the exception of the Cabernet Sauvignon must, which had a higher total titratable acidity in 2020. The statistical analysis of pH values also revealed a statistically significant ($p < 0.001$) difference between the varieties under analysis. The pH values for all varieties were significantly ($p < 0.001$) higher in 2021 than in 2020. The lowest pH belonged to the musts obtained from the Blatina and Vranac varieties in 2020.

The harvest year had a statistically significant effect ($p < 0.001$) on all variables. The interaction between the variety and the harvest year also proved highly significant ($p < 0.001$). To sum up, the variety factor affected all physicochemical quality indicators. Other studies, which included a greater number of varieties, also confirmed that the content and composition of sugar and acids in grapes largely depended on the variety [34, 35].

Although the effect of climatic factors was not the subject of this research, we reviewed the specifics of the weather conditions during the research period. According to the meteorological data in Figs. 1a and b, the average monthly air temperatures during the research period were relatively high, with occasional extreme daily temperatures as early as in May and low precipitation during the ripening period.

Warm environment was reported to raise sugar concentration and reduce the content of malic acid salts in grapes [36]. However, according to the same author, temperatures ≥ 33°C could lead to a decrease in sugar concentration although low acidity could also be manifested under lower temperatures. The higher temperatures recorded during our research were probably one of the factors that triggered an increase in total soluble solids (23.82–26.02°Brix) and a decrease in total titratable acidity (4.21–5.59 g/L) in most varieties except Blatina. The low content of total soluble solids was registered for all must samples obtained in 2021, which was particularly evident in the case of the Vranac variety. This phenomenon could also be linked to the stressful weather conditions in June and August 2021 when the monthly precipitation was as low as 5.7 and 7.0 mm, respectively, and the extreme daily temperatures were as high as 40.8 and 41.4°C, respectively [13].

In addition to the climatic conditions, the quality of all grape varieties in this research was affected by pedological and agrotechnical methods, as well as by the ampelotechnical measures in the production years. The total soluble solids we obtained for the Blatina must were relatively consistent with those reported in [37, 38] whereas our total titratable acidity data were higher. Maraš *et al.* also confirmed the low total titratable acidity demonstrated by the Vranac variety, which also grows in warmer climates, e.g., in Montenegro [39]. Banjanin, who studied grapes in Trebinje, Herzegovina, in 2016–2018, reported lower total soluble solids in Cabernet Sauvignon grape juice (22.8%) and a significantly higher total titratable acidity (6.83–9.15 g/L) compared to our results [29]. This difference illustrates, to some extent, the effect of the harvest year on the parameters under study. Other authors, who compared different Merlot clones or the yield and quality of Merlot grapes grafted onto different rootstocks, reported a higher total titratable acidity (5.77–10.00 g/L) but lower total soluble solids (15.61–22.20°Brix) [40, 41].

Basic physicochemical characteristics of wine. Table 3 shows the basic physicochemical parameters of wine quality in the harvest years. The ethanol content in all wines was higher in 2020, which was quite predictable from the total soluble solids in the must. The same situation was observed with the total extract. Alcohol determines the stability and sensory properties of wine, but the content of the extract is also significant [42]. This parameter makes it possible to divide wines into light

Table 3 Basic chemical composition of Blatina, Vranac, Cabernet Sauvignon, and Merlot wines

Parameters	Blatina, 2020	Blatina, 2021	Vranac, 2020	Vranac, 2021	Cabernet Sauvignon, 2020	Cabernet Sauvignon, 2021	Merlot, 2020	Merlot, 2021
Ethanol, % (v/v)	9.91 ± 0.04	9.54 ± 0.04	15.43 ± 0.04	13.36 ± 0.04	15.16 ± 0.04	14.28 ± 0.00	15.20 ± 0.00	14.43 ± 0.04
Total extract, g/L	25.80 ± 0.00	22.55 ± 0.15	31.80 ± 0.00	28.00 ± 0.10	33.90 ± 0.00	29.40 ± 0.00	30.60 ± 0.10	24.50 ± 0.00
Sugar-free extract, g/L	25.70 ± 0.00	22.55 ± 0.15	31.00 ± 0.00	27.50 ± 0.16	31.86 ± 0.00	29.15 ± 0.00	29.96 ± 0.10	24.48 ± 0.01
Reducing sugar, g/L	1.10 ± 0.00	0.80 ± 0.00	1.80 ± 0.00	1.50 ± 0.06	3.04 ± 0.00	1.25 ± 0.00	1.64 ± 0.00	1.02 ± 0.01
Total acidity, g tartaric acid/L	6.34 ± 0.00	6.94 ± 0.02	5.23 ± 0.00	6.96 ± 0.00	4.50 ± 0.02	7.30 ± 0.00	5.27 ± 0.00	6.37 ± 0.02
Volatile acidity, g acetic acid/L	0.20 ± 0.01	0.18 ± 0.01	0.22 ± 0.00	0.31 ± 0.01	0.30 ± 0.01	0.26 ± 0.00	0.25 ± 0.01	0.24 ± 0.01
pH	3.57 ± 0.00	3.36 ± 0.00	3.50 ± 0.00	3.58 ± 0.00	3.83 ± 0.01	3.52 ± 0.00	3.91 ± 0.01	3.40 ± 0.00

(≤ 20 g/L) and full-bodied (≥ 30 g/L) [43]. Given that the total extract content in our research ranged from 31.1 to 33.9 g/L, all the wine samples, except Blatina, could be characterized as full-bodied.

The largest variations were recorded for the total acidity for most wine samples. The total acidity of wine correlated with the initial (observed) values of the total titratable acidity of must. This phenomenon was especially pronounced in Cabernet Sauvignon (2021), closely followed by Vranac and Merlot. The acidity of the wine usually depends on the most abundant organic acids, i.e., L-tartaric, L-malic, and citric acids. Their level is known to change during fermentation [44, 45]. In addition to these acids, other acids also appear during fermentation, but in lower quantities. They are the products of different strains of wine yeasts and/or bacteria activity (succinic, lactic, acetic, etc.).

In addition to all these factors, the wines in our research came from grapes produced in warm Mediterranean climate. As a result, the volatile acidity in all wines was low (0.18–0.31 g/L) and stayed within permissible limits [46]. The increase in total acidity in certain wines and years might be linked to the higher content of some other acids that developed during fermentation. Contrary to the above, the Blatina wine samples showed a decrease in the total acidity in both years, compared to the initial values of the total titratable acidity in the must. According to certain literary references, such phenomena are associated with malolactic fermentation, which can go unnoticed – simultaneously or subsequently with alcoholic fermentation [47, 48]. Additionally, the Blatina wine samples demonstrated a lower total acidity caused by longer maceration [49]. However, the Blatina samples had a higher pH. In our research, the pH values in all wine samples were more or less higher than those in the must samples, except for the Cabernet Sauvignon wine sample (2021). The values of reducing sugars ranged from 0.80 to 3.04 g/L, which made it possible to define the wine samples as dry wines [46].

The lowest ethanol content belonged to the Blatina wine harvested in both research years (9.91 and 9.54%, respectively). However, other Blatina studies performed in Herzegovina managed to achieve a higher alcohol content (12.1–13.2%) because the level of total soluble solids in the must was quite high [50, 51].

The wines of the Vranac variety were particularly high in alcohol in 2020. These data exceeded the alcohol content in wines of this variety reported by several authors from Montenegro and Serbia, which ranged from 13.00 to 15.38% (v/v) [52–54]. However, the alcohol content in certain Vranac wines from the Tikveš region, North Macedonia, was as high as 15.83–16.44% [55]. Some other authors reported a lower alcohol content in wines of this variety, which was confirmed by our results for 2021 [39, 56]. Although the total extract content and the total acidity of the Vranac wine samples depended on the research year, our values stayed within the framework reported by the previously mentioned authors, excluding the higher wine total acidity in 2021. Regarding the pH of the Vranac wine, the differences between the research years were defined as insignificant (3.50 and 3.58, respectively).

The analysis of Cabernet Sauvignon indicated the high oenological potential of this international variety grown in the conditions of the Herzegovinian vineyards. Cabernet Sauvignon showed the highest total extract content and sugar-free extract, as well as a very high average alcohol content. On the other hand, we observed large annual variations in total acidity (4.50 and 7.30 g/L). The initial concentrations of total titratable acidity in the must (4.96 and 4. 81 g/L) demonstrated a significantly larger deviation in the second research year. The pH of the wine did not change significantly compared to the pH of the must. The relatively high content of alcohol and total extract in Cabernet Sauvignon wines was also reported by other authors [57, 58]: the total acidity and pH ranged from 5.40 to 6.70 g/L and from 3.22 to 3.73 g/L, respectively. Their results only partially corresponded with ours, excluding the lower total acidity of Cabernet Sauvignon in 2020. Considering the global presence of Cabernet Sauvignon, a Russian research team managed to increase the content of alcohol and extract in wine made from grapes grown in the Krasnodar Region [59].

In this research, the average alcohol content in the Merlot wine sample (14.82%) was higher than the average alcohol content in all other wines. The year of 2020 showed a relatively high content of the total extract in the wine. The differences in the total titratable acidity of must between the harvest years (4.60 and 5.80 g/L, respectively) also manifested themselves in the total acidity of the wine (5.27 and 6.37 g/L, respectively). In addition, a slight increase in the pH value of the wine correlated with the pH value of the must in 2020. The year of 2021, on the contrary, demonstrated much more uniform values. Our research yielded higher values of alcohol, extract, and total acidity for Merlot grapes and wine than a recent study on other Merlot grapes from Herzegovina and a multi-year study of Merlot clones [60, 61].

Antioxidant activity. Table 4 illustrates the content of total phenolics, non-flavonoids, flavonoids, and total anthocyanins. The total phenolic content was 2.69–5.77 g GAE/L in 2020 and 2.37–4.17 g GAE/L in 2021. The average total phenolics showed the same order for both years: Vranac > Cabernet Sauvignon > Merlot > Blatina. The content of polyphenolic compounds in grapes and wine depends on several factors, e.g., climate, location, and agricultural conditions of grape production and origin, as well as on the winemaking phase [3, 5, 62–64]. The total phenolics for Vranac and Cabernet Sauvignon varied between 1623 and 2485 mg/L in the 2015 vintage, and between 1551 and 2227 mg/L in the 2016 vintage [64]. For Merlot wines, these values varied between 1.51 and 7.55 mmol GAE/L in the 2017 vintage, and from 4.07 to 5.28 mmol GAE/L in the 2018 vintage [65]. For Blatina, the range was from 1786.71 to 2235.59 mg GAE/L [48].

In our study, the content of non-flavonoids and flavonoids ranged from 0.83 to 3.20 g GAE/L in 2020 and from 0.29 to 3.90 g GAE/L in 2021. The difference was probably caused by the grape composition. Flavonoids and non-flavonoids shape the sensory profile of wine by giving it either the typical long-aged taste or the astringency and bitterness of young wines [19].

Anthocyanins are responsible for the bright red color [66]. The total anthocyanins ranged from 194.65 to 376.17 mg/L in 2020 and from 223.95 to 532.66 mg/L in 2021. The lowest content belonged to Blatina while the highest belonged to Vranac and Cabernet Sauvignon.

As for the effect of maceration time, the maximal value of total anthocyanins was 200.23 mg/L: it was registered in the Blatina wine samples after 12 days of skin maceration [48]. Pajović Šćepanović R *et al.* reported values from 439 to 586 mg/L for red wines of the 2008–2010 vintages [63]. These differences in the total anthocyanin content might be explained by the

Table 4 Total phenolics, non-flavonoids, flavonoids, and total anthocyanins

Year	Variety	Total phenolics, g GAE/L			Non-flavonoids, g GAE/L			Flavonoids, g GAE/L			Total anthocyanins, mg/L		
		$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE
2020	Blatina	2.69^{aA}	±	0.05	2.40^{aA}	±	0.08	0.29^{aA}	±	0.13	194.65^{aA}	±	0.10
	Vranac	5.77^{bB}	±	0.09	1.87^{bB}	±	0.07	3.90^{bB}	±	0.07	366.15^{bB}	±	0.77
	Cabernet Sauvignon	4.80^{cC}	±	0.03	3.20^{cC}	±	0.04	1.59^{cC}	±	0.07	376.17^{cC}	±	0.50
	Merlot	3.96^{dD}	±	0.04	1.48^{dD}	±	0.04	2.48^{dD}	±	0.08	279.40^{dD}	±	0.25
2021	Blatina	2.37^{aE}	±	0.07	0.83^{aE}	±	0.01	1.54^{aC}	±	0.08	223.95^{aC}	±	0.62
	Vranac	4.17^{bF}	±	0.06	0.88^{aE}	±	0.05	3.29^{bE}	±	0.12	532.66^{bE}	±	0.38
	Cabernet Sauvignon	3.95^{cD}	±	0.09	1.17^{bF}	±	0.06	2.77^{cF}	±	0.03	513.02^{cF}	±	0.86
	Merlot	3.07^{dG}	±	0.02	0.84^{aE}	±	0.09	2.23^{dG}	±	0.10	233.42^{dG}	±	0.41

$^{a–d}$ – Different letters within the same column indicate statistically significant difference at p < 0.05 by Duncan's test for the same year;
$^{A–E}$ – Different capital letters within the same column indicate statistically significant difference at p < 0.05 by Duncan's test

Table 5 Antioxidant activity of wines

Year	Variety	FRAP, mmol Fe^{2+}/L			DPPH (IC_{50}, %), μL			ABTS (IC_{50}, %), μL		
		$\bar{x}$	±	SE	$\bar{x}$	±	SE	$\bar{x}$	±	SE
2020	Blatina	10.57^{aA}	±	0.24	2.40^{aA}	±	0.07	0.78^{aA}	±	0.06
	Vranac	53.25^{bB}	±	0.62	1.21^{bB}	±	0.08	0.37^{bB}	±	0.09
	Cabernet Sauvignon	19.88^{cC}	±	0.61	1.46^{cC}	±	0.06	0.41^{bB}	±	0.05
	Merlot	29.91^{dD}	±	0.64	1.64^{dD}	±	0.06	0.59^{cC}	±	0.07
2021	Blatina	17.22^{aE}	±	0.43	3.14^{aE}	±	0.09	0.86^{aA}	±	0.05
	Vranac	31.40^{bF}	±	0.81	1.39^{bC}	±	0.06	0.38^{bB}	±	0.02
	Cabernet Sauvignon	34.26^{cG}	±	0.65	1.69^{cD}	±	0.05	0.37^{bB}	±	0.02
	Merlot	21.58^{dH}	±	0.41	2.21^{dF}	±	0.07	1.02^{cD}	±	0.04

$^{a–d}$ – Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test for the same year;
$^{A–H}$ – Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test

variations under weather conditions between the growing seasons, especially in rainfall.

The antioxidant activities of wines were analyzed by FRAP, DPPH, and ABTS assays (Table 5).

Total antioxidant activity determined by FRAP assay ranged from 10.57 to 53.25 mmol Fe^{2+}/L for the year of 2020, and from 17.22 to 31.40 mmol Fe^{2+}/L for the year of 2021. In fact, red wines tend to demonstrate a wide range of FRAP values, which means it depends on the total phenolic content [5]. The effect of phenolic compounds on the antioxidant activity of red wines had a high correlation, and the FRAP assay results reported in [5] as 10.54–62.77 mmol Fe^{+2}/L were in accordance with ours (Table 5).

The DPPH (IC_{50}, %) activity ranged from 1.21 to 3.14 µL. Đorđević *et al.* reported 37–62.1% anti-DPPH radical activity for Vranac wines [66]. Radonjić *et al.* also reported stronger DPPH scavenging and reducing ability in Vranac wines [6]. Mitić *et al.* determined slightly higher values of 71.30–83.53% for Cabernet Sauvignon wines from the Balkan region [67]. Many authors consider that DPPH radical activity correlates with the total phenolic content [5, 66, 67].

According to the ABTS assay, the values ranged from 0.37 to 1.02 µL. Cabernet Sauvignon showed stronger antioxidant activity than Vranac and Kratošija red wines as reported by Pajović Šćepanović *et al.*, who considered that the total phenolic content correlated with the ABTS scavenging activity [66].

Antimicrobial activity: minimum inhibitory, bactericidal, and fungicidal concentrations. Table 6 illustrates the agar dilution method. The antimicrobial activity was tested on four types of pathogenic and opportunistic bacteria. The most pronounced antimicrobial activity, i.e., the lowest minimum inhibitory concentration of ≤ 10%, belonged to Vranac, Cabernet Sauvignon, and Merlot in relation to *Bacillus cereus* while the minimum bactericidal concentration was ≥ 30%. The Blatina wine samples showed the weakest antimicrobial activity on all microorganisms in this test. Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* showed similar sensitivity in relation to the analyzed wines with the minimum inhibitory concentration of 20%. Gram-negative *Escherichia coli* was the least sensitive to all analyzed wine varieties, compared to other pathogenic and opportunistic bacteria, with the minimum inhibitory concentration of 20 and 30% and the minimum bactericidal concentration of ≥ 30%. Probiotic cultures of *Lactobacillus plantarum* and *Saccharomyces boulardii* were not sensitive to the wine samples.

The antimicrobial effect of red wines on Gram-positive and Gram-negative pathogens is associated with phenolics [9]. According to many authors, phenolic components are more effective against Gram-positive bacteria than against Gram-negative ones [7]. In our research, Gram-positive *B. cereus* had the highest sensitivity to the tested wine concentrations; the lowest minimum inhibitory concentrations belonged to Vranac, Cabernet Sauvignon, and Merlot. These wines had a higher total phenolic content than Blatina, which had a weaker effect on *B. cereus*. Minimum bactericidal concentrations exceeded 30% in all wines, except for Vranac 2021 with its 30%. Our results were in agreement with those published by other authors [68]. However, these authors linked the inhibitory effect of wine on *B. cereus* to organic acids in the wine rather than to phenolic components. The content of gallic acid, caffeic acid, resveratrol, quercetin, quercetin-3-glucoside, and malvidin-3-glucoside also correlated with the antimicrobial activity of Vranac wine against Gram-negative bacteria, including *E. coli* and *P. aeruginosa* [9]. In our experiment, *P. aeruginosa* and *S. aureus* were both sensitive to the wines. The antimicrobial activity was similar in all samples, regardless of the contents of phenolics, alcohol, and organic acids.

The exact mechanism of antimicrobial activity of wine has not been fully explained [10]. Wine contains alcohols, organic acids, and various phenolic components; in addition, its pH is low. The combination of organic

Table 6 Antimicrobial activity of the wines under study

Microorganisms	Concentrations, % (v/v)	Blatina, 2020	Blatina, 2021	Vranac, 2020	Vranac, 2021	Cabernet Sauvignon, 2020	Cabernet Sauvignon, 2021	Merlot, 2020	Merlot, 2021
Staphylococcus aureus	MIC	20	20	20	20	20	20	20	20
	MBC	> 30	30	30	30	20	> 30	20	> 30
Bacillus cereus	MIC	20	20	< 10	< 10	< 10	< 10	10	< 10
	MBC	> 30	> 30	> 30	30	> 30	> 30	> 30	30
Escherichia coli	MIC	20	30	30	20	30	20	30	20
	MBC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
Pseudomonas aeruginosa	MIC	20	20	20	20	20	20	20	20
	MBC	30	30	30	30	30	20	30	30
Lactobacillus plantarum	MIC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
	MBC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
Candida albicans	MIC	> 30	> 30	> 30	> 30	> 30	> 30	30	> 30
	MBC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
Saccharomyces boulardii	MIC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
	MBC	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30

MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration

Table 7 Pearson's correlation coefficient of antioxidant and antimicrobial activity of wine

MIC		*Staphylococcus aureus*		*Bacillus cereus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Lactobacillus plantarum*		*Candida albicans*		*Saccharomyces boulardii*	
		MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
Total phenolic content	Pearson's correlation	0.029	–0.458*	–0.722**	0.124	0.356	–0.010	0.029	–0.033	–0.010	–0.010	–0.043	–0.010	0.214	0.214
	Sig. (2-tailed)	0.891	0.024	0.000	0.565	0.088	0.964	0.891	0.877	0.964	0.964	0.843	0.964	0.314	0.314
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Total nitrogen content	Pearson's correlation	0.017	–0.281	0.004	0.517**	0.323	–0.025	0.017	0.187	–0.025	–0.025	0.048	–0.025	–0.183	–0.183
	Sig. (2-tailed)	0.937	0.184	0.985	0.010	0.123	0.909	0.937	0.381	0.909	0.909	0.825	0.909	0.391	0.391
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Total flavonoid content	Pearson's correlation	0.016	–0.242	–0.724**	–0.272	0.107	0.009	0.016	–0.177	0.009	0.009	–0.079	0.009	0.353	0.353
	Sig. (2-tailed)	0.939	0.255	0.000	0.198	0.620	0.966	0.939	0.408	0.966	0.966	0.714	0.966	0.091	0.091
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
FRAP	Pearson's correlation	0.007	–0.215	–0.615**	0.036	0.226	–0.008	0.007	–0.210	–0.008	–0.008	–0.081	–0.008	0.279	0.279
	Sig. (2-tailed)	0.976	0.313	0.001	0.867	0.289	0.970	0.976	0.325	0.970	0.970	0.708	0.970	0.186	0.186
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
DPPH	Pearson's correlation	0.034	0.317	0.803**	0.085	–0.046	–0.049	0.034	0.117	–0.049	–0.049	0.157	–0.049	–0.171	–0.171
	Sig. (2-tailed)	0.876	0.131	0.000	0.694	0.830	0.821	0.876	0.585	0.821	0.821	0.464	0.821	0.424	0.424
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
ABTS	Pearson's correlation	–0.090	0.388	0.524**	–0.239	–0.154	0.066	–0.090	0.353	0.066	0.066	0.020	0.066	–0.213	–0.213
	Sig. (2-tailed)	0.675	0.061	0.009	0.261	0.473	0.760	0.675	0.090	0.760	0.760	0.926	0.760	0.317	0.317
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Total acidity	Pearson's correlation	–0.001	–0.111	–0.677**	–0.204	–0.231	0.002	–0.001	–0.523**	0.002	0.002	0.187	0.002	0.249	0.249
	Sig. (2-tailed)	0.995	0.605	0.000	0.340	0.277	0.994	0.995	0.009	0.994	0.994	0.381	0.994	0.241	0.241
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24

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

* – Correlation is significant at the 0.05 level (2-tailed);

MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration acids and alcohol with a low pH has a significantly better antibacterial effect than each of these factors separately [10]. Different phenolic components are known to exhibit a synergistic effect that contributes to a better antimicrobial activity of wine than individual phenolic compounds. Phenolic compounds were also reported to exhibit a synergistic effect with a low pH, alcohol, and organic acids [10]. All these factors mean that the antimicrobial activity of wine depends on the variety, growing conditions, concentration, and type of microorganism.

Candida albicans, *L. plantarum*, and *S. boulardii* showed no sensitivity to the wines in our research. Although *C. albicans* is known to be sensitive to some phenolic components, it was reported resistant to most wines and wine extracts [68]. Plant polyphenols and phenolic components have a stimulating effect on microorganisms that are part of the intestinal microbiome, e.g., *L. plantarum* and *S. boulardii* [11]. Dueñas *et al.* studied phenolic compounds in wine and red wine extracts, e.g., (+) catechin, anthocyanins, etc. [69]. They found out that these substances could stimulate the growth of bacteria of the *Lactobacillus – Enterococcus* spp. group. Vilela *et al.* revealed that *S. boulardii* has a high tolerance to alcohol and organic acids [70].

Table 7 illustrates the degree of correlation between the measured antioxidant and antimicrobial activity of wines using Pearson's test. The correlation was proven in the case of *B. cereus* bacteria (the largest number of cases), as well as *S. aureus* and *P. aureginosa* bacteria (one case each). This finding once again confirms that the antimicrobial activity of wine does not come only from the content of phenolic compounds, but is a combination of several different factors.

CONCLUSION

The statistical analyses confirmed a strong effect of the harvest year and variety, as well as their interaction, on the physicochemical properties of grape must. The highest total phenolic content, as well as the best antioxidant properties, belonged to Vranac wines of both vintages (2020 and 2021). All wines showed satisfactory antimicrobial properties, and the strongest activity was recorded against *Bacillus cereus*. The probiotic strains used in this research showed resistance to all wines. The Pearson test revealed a correlation between antioxidant and antimicrobial effects against *B. cereus*, as well as against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (one case each), while other cases demonstrated no correlation. All grapevine varieties in this study (Blatina, Vranac, Cabernet Sauvignon, Merlot) proved to be suitable for the production of quality wines from grapes grown in the area of Mostar.

CONTRIBUTION

Conceptualization: T. Jovanović-Cvetković and A. Savić. Methodology: T. Jovanović-Cvetković, A. Savić, L. Topalić-Trivunović, A. Velemir, and R. Grbić. Investigation: A. Savić, L. Topalić-Trivunović, A. Velemir, and R. Grbić. Data curation: T. Jovanović-Cvetković, L. Topalić-Trivunović, A. Velemir, and R. Grbić. Original draft: T. Jovanović-Cvetković, A. Savić, L. Topalić-Trivunović, A. Velemir, and R. Grbić. All authors read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

REFERENCES

1. Jovanović-Cvetković T, Sredojević M, Natić M, Grbić R, Akšić MF, Ercisli S, *et al.* Exploration and comparison of the behavior of some indigenous and international varieties (*Vitis vinifera* L.) grown in climatic conditions of Herzegovina: The influence of variety and vintage on physico-chemical characteristics of grapes. Plants. 2023;12(4):695. https://doi.org/10.3390/plants12040695
2. Gnilomedova NV, Anikina NS, Kolesnov AYu. A review of methodological approaches to authenticating the geographical origin of wines. Food Processing: Techniques and Technology. 2023;53(2):231–246. (In Russ.). https://doi.org/10.21603/2074-9414-2023-2-2429: https://elibrary.ru/AUQCPD
3. Gutiérrez-Escobar R, Aliaño-González MJ, Cantos-Villar E. Wine polyphenol content and its influence on wine quality and properties: A review. Molecules. 2021;26(3):718. https://doi.org/10.3390/molecules26030718
4. Kubyshkin A, Ogai Y, Fomochkina I, Chernousova I, Zaitsev G, Shramko Yu. Polyphenols of red grape wines and alcohol-free food concentrates in rehabilitation technologies. In: Wong J, editor. Polyphenols. IntechOpen; 2018. pp. 99–120. https://doi.org/10.5772/intechopen.76655
5. Kesić A, Smajlovic B, Ibrišimović Mehmedinović N, Hodzic Z, Šestan A, Dedić J, *et al.* The content of total polyphenols of the selected red wines from the territory of Tuzla canton in correlation with antioxidant activity. International Journal of Research Methodology. 2019;2(1):141–151.
6. Radonjić S, Maraš V, Košmerl T. The importance of total polyphenols content in red wine. Third International Mediterranean Congress on Natural Sciences, Health Sciences and Engineering; 2019; Podgorica. Podgorica; 2019. p. 231–240.
7. Kauffmann AC, Castro VS. Phenolic compounds in bacterial inactivation: A perspective from Brazil. Antibiotics. 2023;12(4):645. https://doi.org/10.3390/antibiotics12040645

8. Santoro HC, Skroza D, Dugandžić A, Boban M, Šimat V. Antimicrobial activity of selected red and white wines against *Escherichia coli*: In vitro inhibition using fish as food matrix. Foods. 2020;9(7):936. https://doi.org/10.3390/foods9070936

9. Radovanović AN, Jovančičević BS, Radovanović BC, Mihajilov-Krstev T. Antimicrobial effectiveness of selected Vranac wines against six gram-positive and six gram-negative bacterial strains. Tropical Journal of Pharmaceutical Research. 2014;13(5):819–824. https://doi.org/10.4314/tjpr.v13i5.24

10. Boban N, Tonkić M, Budimir D, Modun D, Sutlović D, Punda-Polic V, *et al.* Antimicrobial effects of wine: Separating the role of polyphenols, pH, ethanol, and other wine components. Journal of Food Science. 2010;75(5):M322–M326. https://doi.org/10.1111/j.1750-3841.2010.01622.x

11. Milutinović M, Dimitrijevic-Branković S, Rajilić-Stojanović M. Plant extracts rich in polyphenols as potent modulators in the growth of probiotic and pathogenic intestinal microorganisms. Frontiers in Nutrition. 2021;8:688843. https://doi.org/10.3389/fnut.2021.688843

12. The climate of Bosnia and Herzegovina [Internet]. [cited 2023 Oct 10]. Available from: https://www.fhmzbih.gov.ba/latinica/KLIMA/klimaBIH.php

13. Federal hydrometeorological institute of Bosnia and Herzegovina [Internet]. [cited 2023 Oct 10]. Available from: http://www.fhmzbih.gov.ba

14. Compendium of international methods of wine and must analysis. Paris; 2021. 673 p.

15. Kupina S, Fields C, Roman MC, Brunelle SL. Determination of total phenolic content using the Folin-C assay: Single-laboratory validation, first action 2017.13. Journal of AOAC International. 2018;101(5):1466–1472. https://doi.org/10.5740/jaoacint.18-0031

16. Correa Uriburu FM, Cattaneo F, Maldonado LM, Zampini IC, Alberto MR, Isla MI. *Prosopis alba* seed as a functional food waste for food formulation enrichment. Foods. 2022;11(18):2857. https://doi.org/10.3390/foods11182857

17. Rodríguez-Vaquero MJ, Vallejo CV, Aredes-Fernández PA. Antibacterial, antioxidant and antihypertensive properties of polyphenols from Argentinean red wines varieties. Open Journal of Pharmacology and Pharmacotherapeutics. 2020;5(1):001–006. https://doi.org/10.17352/ojpp.000010

18. Rumpf J, Burger R, Schulze M. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. International Journal of Biological Macromolecules. 2023;233:123470. https://doi.org/10.1016/j.ijbiomac.2023.123470

19. Mitrevska K, Grigorakis S, Loupassaki S, Calokerinos AC. Antioxidant activity and polyphenolic content of North Macedonian wines. Applied Sciences. 2020;10(6):2010. https://doi.org/10.3390/app10062010

20. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens. 2021;10(2):165. https://doi.org/10.3390/pathogens10020165

21. Geng D-H, Liu L, Zhou S, Sun X, Wang L, Zhou X, *et al.* Effects of *Lactobacillus plantarum* inoculum on the fermentation rate and rice noodle quality. Journal of Oleo Science. 2020;69(9):1031–1041. https://doi.org/10.5650/jos.ess20003

22. Chelliah R, Kim E-J, Daliri EB-M, Antony U, Oh D-H. In vitro probiotic evaluation of *Saccharomyces boulardii* with antimicrobial spectrum in a *Caenorhabditis elegans* model. Foods. 2021;10(6):1428. https://doi.org/10.3390/foods10061428

23. Catalogue of grapevines cultivated in France [Internet]. [cited 2023 Oct 15]. Available from: https://www.plantgrape.fr/en

24. Ivanišević D, Kalajdžić M, Drenjančević M, Puškaš V, Korać N. The impact of cluster thinning and leaf removal timing on the grape quality and concentration of monomeric anthocyanins in Cabernet-Sauvignon and Probus (*Vitis vinifera* L.) wines. OENO One. 2020;54(1):63–74. https://doi.org/10.20870/oeno-one.2020.54.1.2505

25. Aleinikova N, Didenko P, Shaporenko V, Didenko L, Belash S. The effect of mineral nutrition systems as an element of cultivation technology of wine grape varieties on their productivity in the conditions of Crimea. IOP Conference Series: Earth and Environmental Science. 2023;1206:012026. https://doi.org/10.1088/1755-1315/1206/1/012026

26. Arestova N, Ryabchun I. Influence of biostimulants on productivity and quality of grapes. E3S Web of Conferences. 2020;210:05001. https://doi.org/10.1051/e3sconf/202021005001

27. Jovanovic-Cvetkovic T, Micic N, Djuric, G, Cvetkovic M. Pollen morphology and germination of indigenous grapevine cultivars Žilavka and Blatina (*Vitis vinifera* L.). AgroLife Scientific Journal. 2016;5(1):105–109.

28. Jovanović-Cvetković T, Šutalo V, Kupe M, Ercisli S, Životić A, Pašalić B. Influence of interaction effects of the different pollenizers on the Blatina variety (*Vitis vinifera* L.) grape cluster and seed characteristics. Plants. 2022;11(3):420. https://doi.org/10.3390/plants11030420

29. Banjanin T. Characterization of quantitative and qualitative characteristics of Blatina vine variety in agroecological conditions of Trebinje. Ph.D. Thesis. Belgrade; 2022.

30. Maras V, Tomic M, Kodzulovic V, Knezevic B, Raicevic D, Cizmovic M. Yield and quality of grapes and wine of the cultivars "Vranac", "Primitivo" and "NegroAmaro". Acta Horticulturae. 2012;931. https://doi.org/10.17660/ActaHortic.2012.931.43

31. Popović T, Raičević D. Yield and quality of grapes of autochthonous variety Vranac in agroecological conditions of Podgorica subregion. In: Jacimovic M, editor. The First International Conference on Vranac and Other Montenegrin Indigenous Sorts of Grapevine. Podgorica: Montenegrin Academy of Sciences and Art; 2021. pp. 197–206.

32. Gombau J, Pons-Mercadé P, Conde M, Asbiro L, Pascual L, Gómez-Alonso S, *et al.* Influence of grape seeds on wine composition and astringency of Tempranillo, Garnacha, Merlot and Cabernet Sauvignon wines. Food Science and Nutrition. 2020;8:3442–3455. https://doi.org/10.1002/fsn3.1627

33. Bubola M, Rossi S, Váczy KZ, Hegyi ÁI, Persic M, Zdunić G, *et al.* Modification of cv. Merlot berry composition and wine sensory characteristics by different leaf area to fruit ratios. Applied Sciences. 2023;13(9):5456. https://doi.org/10.3390/app13095465

34. Liu H-F, Wu B-H, Fan P-G, Li S-H, Li L-S. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. Journal of the Science of Food and Agriculture. 2006;86:1526–1536. https://doi.org/10.1002/jsfa.2541

35. Zhong H, Yadav V, Wen Z, Zhou X, Wang M, Han S, *et al.* Comprehensive metabolomics based analysis of sugar composition and content in berries of 18 grape varieties. Frontiers in Plant Science. 2023;14:1200071. https://doi.org/10.3389/fpls.2023.1200071

36. Coombe BG. Influence of temperature on composition and quality of grapes. Acta Horticulturae. 1987;206. https://doi.org/10.17660/ActaHortic.1987.206.1

37. Banjanin T, Lisov N, Petrović A, Ranković-Vasić Z, Blesić M. The quality of grape and wine of Merlot and Blatina varieties in the agroecological conditions of the Trebinje vineyard. VIII International Symposium on Agricultural Sciences. Banja Luka: Faculty of Agriculture University of Banja Luka; 2019. p. 69–75.

38. Jovanović-Cvetković T, Grbić R, Grobelnik Mlakar S, Bosančić B, Cvetković M. Physicochemical evaluation of the grape and wine of the Blatina, Trnjak and Vranac in different vintage. AgroLife Scientifc Journal. 2023;12(1):105–115. https://doi.org/10.17930/AGL2023113

39. Maraš V, Kodžulović V, Mugoša M, Raičević J, Gazivoda A, Šućur S, *et al.* Clonal selection of autochthonous grape variety Vranac in Montenegro. In: Badnjevic A, editor. Proceedings of the International Conference on Medical and Biological Engineering 2017. Singapore: Springer; 2017. pp. 787–790. https://doi.org/10.1007/978-981-10-4166-2_118

40. Sivcev B, Rankovic-Vasic Z, Petrovic A, Jancis R, Milisic K. Fruit characteristics of the Merlot clones in Belgrade wine Growing Region, Serbia. Journal of Advancements in Plant Science. 2018;1(2).

41. Tecchio MA, da Silva MJR, Sanchez CAPC, Callili D, Vedoato BTF, Hernandes JL, *et al.* Yield performance and quality of wine grapes (*Vitis vinifera*) grafted onto different rootstocks under subtropical conditions. Crop Production and Management. 2022;81:e1622. https://doi.org/10.1590/1678-4499.20210214

42. Yang J, Lee J. Current research related towine sensory perception since 2010. Beverages. 2020;6(3):47. https://doi.org/10.3390/beverages6030047

43. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Alcohol and extract wine. In: Zoecklein B, Fugelsang KC, Gump BH, Nury FS, editors. Wine analysis and production. Springer; 1999. pp. 97–113.

44. Lima MMM, Choy YY , Tran J, Lydon M, Runnebaum RC. Organic acids characterization: Wines of Pinot noir and juices of "Bordeaux grape varieties". Journal of Food Composition and Analysis. 2022;114:104745. https://doi.org/10.1016/j.jfca.2022.104745

45. Vicente J, Baran Y, Navascués E, Santos A, Calderón F, Marquina D, *et al.* Biological management of acidity in wine industry: A review. International Journal of Food Microbiology. 2022;375:109726. https://doi.org/10.1016/j.ijfoodmicro.2022.109726

46. International Code of Oenological Practices. Paris; 2022. 393 p. (In French).

47. Ribereáu-Gayon P, Dubordieu D, Donèche B, Lonvaud A. Treatise on oenology – Microbiology of wine, Vinifications. Paris: Dunod; 2004. 498 p. (In French).

48. Paramithiotis S, Stasinou V, Tzamourani A, Kotseridis Y, Dimopoulou M. Malolactic fermentation – Theoretical advances and practical considerations. Fermentation. 2022;8(10):521. https://doi.org/10.3390/fermentation8100521

49. Herjavec S, Jeromel A, Maslov L, Jagatić Korenika AM, Mihaljević M, Prusina T. Influence of different maceration times on the anthocyanin composition and sensory properties of blatina wines. Agriculturae Conspectus Scientificus. 2012;77(1):41–44.

50. Lavrić M, Prusina T. The influence of the vintage year on Blatina wine quality. Proceedings of 55th Croatian & 15th International Symposium on Agriculture; 2020; Vodice. Zagreb: University of Zagreb; 2020. p. 501–504.

51. Jagatić Korenika A-M, Tomaz I, Preinar D, Lavrić M, Šimić B, Jeremol A. Influence of *L. thermotolerans* and *S. cerevisiae* commercial yeast sequential inoculation on aroma composition of red wines (Cv Trnjak, Babic, Blatina and Frankovka). Fermentation. 2021;7(4):4. https://doi.org/10.3390/fermentation7010004

52. Gašović B, Đaković J, Radonjić S, Maraš V, Kodžulović V. Characteristics and quality of grapes and wines of the Vranac variety. In: Jacimovic M, editor. The First International Conference on Vranac and Other Montenegrin Indigenous Sorts of Grapevine. Podgorica: Montenegrin Academy of Sciences and Art; 2021. pp. 267–277.

53. Jakšić D, la Notte P, Giannini PB, Perović V, Cagnazzo A. Montenegrin Vranac vine variety in the most eastern point of the Vranac cultivation area – Knjaževac wine-growing region (Serbia). In: Jacimovic M, editor. The First International Conference on Vranac and Other Montenegrin Indigenous Sorts of Grapevine. Podgorica: Montenegrin Academy of Sciences and Art; 2021. pp. 207–225.

54. Sošić S, Pajović-Šćepanović R, Raičević D, Popović T. Quality of wines Vranac and Kratošija in the vintage 2021. Agriculture and Forestry. 2023;69(1):127–137. https://doi.org/10.17707/AgricultForest.69.1.11

55. Ivanova-Petropulos V, Ricci A, Nedelkovski D, Dimovska V, Parpinello GP, Versari A. Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines. Food Chemistry. 2015;171:412–420. https://doi.org/10.1016/j.foodchem.2014.09.014

56. Raičević D, Mijović S, Popović T, Pajović-Šćepanović R. The influence of variety and vintage on the chemical composition and sensory properties of red wines in Podgorica subregion (Montenegro). Journal of Agricultural, Food and Environmental Sciences. 2017;71(1):157–164.

57. Eder R, Pajović Šćepanović R, Raičević D, Popović T, Korntheuer K, Wendelin S, *et al.* Study of the effects of climatic conditions on the phenolic content and antioxidant activity of Austrian and Montenegrin red wines. OENO One. 2023;57(3):69–85. https://doi.org/10.20870/oeno-one.2023.57.3.7450

58. Xu S, Zhu J, Zhao Q, Gao J, Zhang H, Hu B. Quality evaluation of Cabernet Sauvignon wines in different vintages by ^{1}H nuclear magnetic resonance-based metabolomics. Open Chemistry. 2021;19(1):385–399. https://doi.org/10.1515/chem-2020-0126

59. Antonenko MV, Guguchkina TI, Prakh AV, Kolesnov AYu, Zenina MA. Research of physical and chemical characteristics of grapes from different regions of Krasnodar territory for their use as standards of authenticity of wine production. Fruit Growing and Viticulture of South Russia. 2019;55(1):95–106. https://doi.org/10.30679/2219-5335-2019-1-55-95-106

60. Banjanin T, Ranković-Vasić Z, Nikolić D, Anđelić B. Influence of climatic factors on the quality of Merlot grapevine variety in Trebinje region vineyards (Bosnia and Herzegovina). Agrofor. 2019;4(2):95–101. https://doi.org/10.7251/AGRENG1902094B

61. Miele A. Wine composition of Merlot and Cabernet Sauvignon vine clones under the environmental conditions of Serra Gaúcha, Brazil. Food Science and Technology. 2021;41(Suppl.1):116–122. https://doi.org/10.1590/fst.10520

62. Muñoz-Bernal ÓA, Vazquez-Flores AA, de la Rosa LA, Rodrigo-García J, Martínez-Ruiz NR, Alvarez-Parrilla E. Enriched red wine: Phenolic profile, sensory evaluation and in vitro bioaccessibility of phenolic compounds. Foods. 2023;12(6):1194. https://doi.org/10.3390/foods12061194

63. Pajović Šćepanović R, Madžgalj V, Vukoslavljević V. Assay of polyphenolic in Montenegrin Vranac wines. Mitteilungen Klosterneuburg. 2019;69:65–75.

64. Pajović Šćepanović R, Wendelin S, Raičević D, Eder R. Characterization of the phenolic profile of commercial Montenegrin red and white wines. European Food Research and Technology. 2019;245:2233–2245. https://doi.org/10.1007/s00217-019-03330-z

65. Tzanova M, Atanassova S, Atanasov V, Grozeva N. Content of polyphenolic compounds and antioxidant potential of some Bulgarian red grape varieties and red wines, determined by HPLC, UV, and NIR spectroscopy. Agriculture. 2020;10(6):193. https://doi.org/10.3390/agriculture10060193

66. Đorđević N, Novaković M, Pejin B, Živković M, Savić A, Mutić J, *et al.* An insight into chemical composition and biological activity of Montenegrin *Vranac* red wine. Scientia Horticulturae. 2018;230:142–148. https://doi.org/10.1016/j.scienta.2017.11.033

67. Mitić MN, Kostic DA, Pavlović AN, Micić RJ, Stojanović BT, Paunović DĐ, *et al.* Antioxidant activity and polyphenol profile of Vranac red wines from Balkan region. Chemical Industry. 2016;70(3):265–275. https://doi.org/10.2298/HEMIND150130032M
68. Čomić LR, Radojević ID, Vasić SM, Mladenović KG, Grujović MŽ. Traditionally made red wines produced from an autochthonous grapevine variety as a source of biologically active compounds and their antioxidant potential. Journal of Food and Nutrition Research. 2020;59(4).
69. Dueñas M, Cueva C, Muñoz-González I, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C, *et al.* Studies on modulation of gut microbiota by wine polyphenols: From isolated cultures to omic approaches. Antioxidants. 2015;4(1):1–21. https://doi.org/10.3390/antiox4010001
70. Vilela A, Fernanda Cosme F, Inês A. Wine and non-dairy fermented beverages: A novel source of pro- and prebiotics. Fermentation. 2020;6(4)113. https://doi.org/10.3390/fermentation6040113

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