



THE STUDY OF SELECTED COMPONENTS OF GRAPE AND FRUIT WINES

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ABSTRACT

This study aimed to compare chemical properties, in terms of the content of volatile substances, antioxidant compounds, and antioxidant activity (AOA), in samples of fruit and grape wines. For this purpose, the following types of wine were selected, namely fruit wines (apple, strawberry, and elderberry) and grape wines (Müller-Thurgau, St. Lawrence Rosé, and Blue Portugal). Basic analyses of fruit and grape wines were conducted by using the ALPHA method and volatile substances in wines were determined by the GS/MC method. The antioxidant content and AOA were estimated by spectrophotometry, using two types of DPPH method. The results of the experiment showed that the highest values of antioxidant compounds (anthocyanins and flavanols) were found in the samples of Blue Portugal wine and elderberry wine. Significant differences were determined among the wines in antioxidant content, which may have been influenced by the production technology. The results showed that the alcohol content of the wines ranged from 10.99% to 19.49% vol. The highest alcohol content was measured in the elderberry wine samples and the lowest in those of the apple wine. The strawberry wine had the highest titratable acid content, which corresponded to a pH of 3.38. The lowest content was measured in the apple wine samples. Due to the higher acid content of the strawberry wine, a higher residual sugar level of 46.9 g.L⁻¹ was obtained. We noted that the red fruit wines contained a higher proportion of valuable bioactive substances than white grape wines. Wines with superior sensory properties did not contain higher levels of antioxidants or higher AOA. The research results can provide a helpful reference for the widespread use of grape and fruit wines in medical, nutritional, and other fields.

Keywords: antioxidant; analytical parameter; fruit wine; grape wine; volatile substance

INTRODUCTION

Wines produced in the Czech Republic have been among the most highly valued in terms of worldwide parameters, as evidenced by several international awards received by winemakers. The success of winemakers is also valued very positively by consumers, who are starting to prefer wines produced in the Czech Republic over foreign wines (Snopek et al., 2018).

The main target of producing quality and premium wines is expressing the connection, through sensory character, to the place where the grapes were cultivated (Slaghenaufi et al., 2019). Similar to other foods, wine has a taste and aroma that significantly affect the interest of the final consumer. These characteristics of the wine are influenced not only by the cultivar used but also by how the wine has been stored during its maturation. The most valuable compounds in wine are volatile organic and bioactive compounds.

The most studied compounds from this group are terpenes (González-Barreiro et al., 2015). Different methods of wine-making technology have affected substance levels (Baron et al., 2018). Polyphenols,

especially resveratrol, anthocyanin, and catechins, are the most valued wine antioxidants (Snopek et al., 2018).

The current modern trend has been in plant breeding. One scientific area that has been discussed has concentrated on producing large-fruited cultivars and clones with regular fertility and a higher profile of bioactive substances and AOA. After several years of exploring this issue, crop refinement has only been achieved by selecting the most usable and fastest-growing plants. Eventually, the selection of plants studied has influenced the morphological and quantitative properties of crops all around the world (Kaczmarek et al., 2015; Cehula et al., 2019).

Various polyphenolic compounds are valued for plant cell pigmentation, i.e. anthocyanidins, responsible for red, blue, violet, orange, and purple colors (Moreno-Montoro et al., 2015; Vallverdu-Queralt et al., 2015; Barba et al., 2016).

The aim of this study was the comparison of the different bioactive substance content of grape wines and fruit wines. Three kinds of grape wine ('Müller-Thurgau', 'St. Lawrence Rosé' and 'Blue Portugal') and three kinds of fruit wine (strawberry, elderberry, and apple wine) were selected. During the study, attention was given to the

evaluation of AOA by the DPPH method, estimation of the total content of anthocyanins, flavanols, acetic, citric, lactic, malic and tartaric acids, alcohol, fructose, glucose, saccharose, and glycerol, of pH, of total acidity, and sugar content.

Scientific hypothesis

H1: The content of antioxidants depends on the type of wine.

H2: Red wines contain higher levels of antioxidant compounds than white wines.

H3: Wines with significant sensory properties contain more antioxidants than other wines.

H4: By monitoring the occurrence of volatile substances, we can show that grape wines will have a higher content than fruit wines.

MATERIAL AND METHODOLOGY

Characteristics of selected wines:

Müller-Thurgau, 2014: Samples were obtained from the Kubík winery (Velké Bílovice, Czech Republic). It is a high quality, fresh, young wine with a typical yellow-green color, spicy taste, and somewhat pleasant smell.

St. Lawrence Rosé, 2014: The samples were obtained from the same winery. It is a quality deep pink wine, with tones of strawberries in aroma and taste.

Blue Portugal, 2014: We obtained the samples of this wine from the Veverka winery (Čejkovice, Czech Republic). The wine is a soft ruby color, with the aroma of flowers. The tannin content provides it with an attractive expression.

Strawberry wine: This is made from the strawberry *Fragaria annanassa*, cultivar 'Karmen'. The fruits were stripped and crushed and were allowed to macerate for 12 hours before the mash was pressed. The juice was drained by sedimentation. Water and sucrose were added to achieve the desired acid reduction. Nitrogenous substances were also treated with nutrient salt. The juice was then inoculated with noble yeast and placed in an appropriate glass container, fitted with a fermentation plug, and allowed to ferment. After fermentation, the wine was removed from the sludge and filtered. Subsequently, the wine was bottled.

Elderberry wine: The wine was made from elderberries (*Sambucus nigra*, L.). The elderberry fruits were stripped and crushed. The mash was heated to 80 °C for 15 minutes, to destroy the sambunigrin, which causes headaches and nausea. Then the mash was macerated for 48 hours and subsequently pressed. The juice was drained by sedimentation. Water and sucrose were added to achieve the desired acid reduction. Nitrogenous substances were also treated with nutrient salt. The juice was then inoculated with noble yeast and placed in an appropriate glass container, fitted with a fermentation plug, and allowed to ferment. After fermentation, the wine was removed from the sludge and filtered, then subsequently bottled.

Apple wine: The wine was made from the cultivar 'Jonagold'. The apples were stripped of stalks and cores, then crushed. The mash was pressed, and the juice was drained by sedimentation. Water and sucrose were added to achieve the desired acid reduction. Nitrogenous

substances were also treated with nutrient salt. The juice was then inoculated with noble yeast and placed in an appropriate glass container, fitted with a fermentation plug, and allowed to ferment. After fermentation, the wines were removed from the sludge and filtered, then, subsequently, was bottled.

For our research we used the followed methods:

Determination of antioxidant activity by the DPPH method

For the determination procedure, see the description earlier (Sochor et al., 2010). We mixed 150 µL volume of the reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl; DPPH') with 15 µL of wine sample. Each process was repeated twice. All samples were incubated for 10 minutes. After incubation, we measured samples at an absorbance of 505 nm on the spectrometer. The results were expressed as trolox equivalents.

Estimation of anthocyanin content

These measurements were performed using recognized spectrophotometric methods (Zoecklein et al., 1999). The wine sample was placed into a 0.2 cm path-length quartz cuvette, then 200 µL of the sample and 1.8 mL of 1.1 M HCL were added and the resulting solution was thoroughly mixed and kept at room temperature for 180 minutes. A 0.22 M solution of K₂S₂O₅ was used as a blank.

The absorbance was read at 520 nm (A_{520}^{HCl}) for anthocyanins. Total concentrations of anthocyanin (mg.L⁻¹) were calculated as follows:

$$\text{Total content of anthocyanins (mg.L}^{-1}\text{)} = 4 \times \text{dilution} \times [A_{520}^{HCl} - (5/3) \times A_{520}^{SO_2}]$$

Estimation of total flavanol content

Total flavanol content was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method (Li, Tanner and Larkin, 1996; Tomášková et al., 2017). Compared with the widely used vanillin method, a major advantage of this method is that there is no interference from anthocyanins. Furthermore, it provides higher sensitivity and greater specificity. Wine (20 µL) was poured into a 1.5 mL Eppendorf tube and 980 µL of DMACA solution (0.1 % in 1 M HCl in MeOH) was added. The mixture was vortexed and allowed to react at room temperature for 12 minutes. Absorbance at 640 nm was then read against a blank sample prepared similarly but without DMACA. The total flavanol concentration was then estimated from a calibration curve and constructed by plotting known solutions of catechin (1 – 16 mg.L⁻¹) against A_{640} ($r^2 = 0.998$). The results were expressed as mg.L⁻¹ of catechin equivalents.

GC-MS determination of individual volatile compounds and sample preparation

The concentration of individual volatile substances in the wine was determined by the previously unpublished method of extraction with methyl *t*-butyl ether. The following was pipetted into a 25 mL volumetric flask: 20 mL of wine, 50 µL of 2-nonanol solution (500 mg.L⁻¹), and cyclopentanone (25 g.L⁻¹) in ethanol as the internal standard, and 5 mL of saturated (NH₄)₂SO₄ solution.

Table 1 Determination of basic analytical parameters in selected wines.

Type of wine/ parameter	Alcohol (%)	Tit. acids (g.L ⁻¹)	Red. sugar (g.L ⁻¹)	pH	Malic acid (g.L ⁻¹)	Lactic acid (g.L ⁻¹)	Acetic acid (g.L ⁻¹)
Müller-Thurgau	12.31	7.07	7.6	3.49	3.76	0.16	0.26
St. Lawrence rosé	13.22	7.73	7.1	3.4	3.71	0.39	0.34
Blue Portugal	13.41	5.14	0.1	3.54	0.02	2.15	0.51
Apple wine	10.99	3.47	8	3.25	3.22	0	0
Strawberry wine	16.7	8.47	46.9	3.38	3.13	1.12	0.34
Elderberry wine	19.49	4.75	15.2	3.98	3.43	0.5	0.39
SD	±6.14	±2.91	±16.19	±1.35	±1.69	±0.78	±0.19
	Tartaric acid (g.L ⁻¹)	Glycerol (g.L ⁻¹)	Glucose (g.L ⁻¹)	Fructose (g.L ⁻¹)	Sucrose (g.L ⁻¹)	Density	Citric acid (g.L ⁻¹)
Müller-Thurgau	2.83	7.18	3.1	5.51	0.07	0.99	0
St. Lawrence Rosé	2.61	6.4	1.94	5.72	0	0.99	0.03
Blue Portugal	2.47	9.64	0.61	0.24	0.02	0.99	0.05
Apple wine	0.98	7.07	1.65	7.65	0	0.99	0.44
Strawberry wine	0.49	11.93	2.99	44.97	0	1	2.67
Elderberry wine	0	12.22	1.11	13.97	0	0.99	0.08
SD	±1.26	±4.16	±1.16	±15.64	±0.03	±0.38	±0.98

Note: Experiment was replicated 3 times.

The flask contents were then thoroughly mixed, and 0.75 mL of extraction solvent added, MTBE with the addition of 1% neohexane. After thorough mixing of samples, and separation phases, the upper organic layer was transferred to a microtube with a portion of the resulting emulsion, centrifuged and the clear organic phase was dried over anhydrous magnesium sulphate. We continued using the treated extract for GC-MS analysis.

Estimation of acetic, citric, lactic, malic and tartaric acids, alcohol, fructose, glucose, sacharose, and glycerol, of pH, of total acidity, and the sugar content

The aforementioned parameters were estimated using the ALPHA apparatus (Bruker, Germany). The ALPHA spectrometer is a compact FTIR analyser based on the principle of ATR sampling. Physical-chemical parameters of wine samples were determined according to the **EEC Official Method by the European Commission (2003)**, which was used in a similar study by **Condurso et al. (2018)**. This method of sampling considerably simplifies the preparation of samples for analysis. This means that samples of clarified wine were analysed directly, (i.e. without any adjustments), while those of musts and fermenting wine were centrifuged at 13 400 rpm for six minutes. Before the measuring of the first sample, the apparatus was thoroughly rinsed with distilled water and

the background was measured using deionized water as a blank sample. For analysis, 1 mL of clear wine was sampled with a syringe; of this, one half (0.5 mL) was used for the rinsing of the system, and the remaining half (i.e. also 0.5 mL) was used for the subsequent three measurements. Depending on the method of calibration (musts/fermenting wine/fermented wine), the recorded resulting amounts were automatically evaluated by software.

Separation conditions

Column: DB-WAX 30 m x 0.25 mm; 0.25 µM stationary phase (polyethylene glycol). Sample injection volume: 1 µL split ratio 1:5; carrier gas flow rate He:1 ml/min (linear gas velocity 36 cm.S⁻¹); injection chamber temperature: 180 °C. The initial column chamber temperature of 45 °C was maintained for 3.5 minutes, followed by a temperature gradient: up to 90 °C by 12 °C.min⁻¹ held for 0.75 minutes, up to 120 °C by 3 °C.min⁻¹.

Statistic analysis

For this study, we applied ANOVA, simple descriptive statistical methods, and correlation- regression. Statistical analysis was performed using NCSS 2019 software (ver. 19.0.4.; NCSS, LLC Utah, USA).

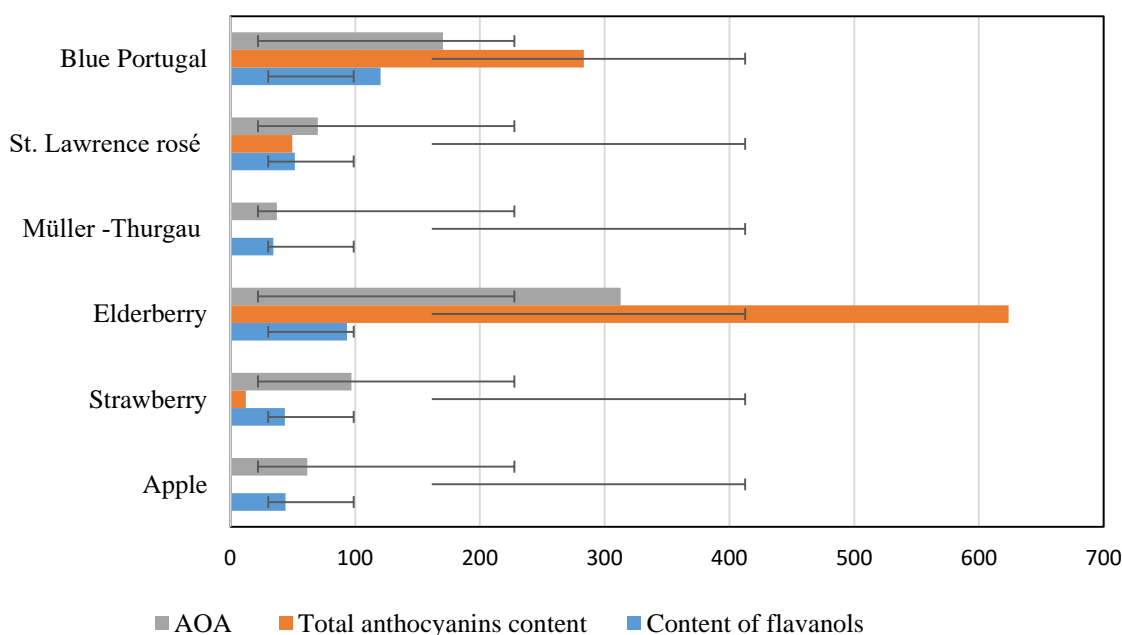


Figure 1 Estimation of Antioxidant activity – AOA (equivalent to TROLOX mg.L⁻¹); total anthocyanins content (mg.L⁻¹) and content of flavanols (mg.L⁻¹). Note: Experiment was replicated 3 times.

RESULTS AND DISCUSSION

Determination of basic analytical parameters in selected wines

The results are shown in Table 1. The highest percentage of alcohol was determined in the elderberry wine samples, while the smallest percentage of alcohol was measured in the apple wine samples. The pH range was from 3.25 (apple wine) to 3.98 (elderberry wine). The lowest content of malic acid was determined in the samples of Blue Portugal and the highest content identified in the samples of Müller-Thurgau.

Lactic acid was maximal level in the samples of Blue Portugal, and minimal level in the apple samples. Zero acetic acid content was determined in the case of the apple wine and tartaric acid content in the elderberry wine samples.

On the contrary, the highest values were measured in the Blue Portugal (for acetic acid) and the Müller-Thurgau (for tartaric acid).

We also measured the value of zero in citric acid content, namely in the Müller-Thurgau, very low values in the St. Lawrence rosé and Blue Portugal, and high values in the strawberry wine. We found that the lowest values were of sucrose, of which we measured values close to zero, but we were able to determine higher fructose levels. The values of glucose were from 0.61 to 3.10 g.L⁻¹.

We observed that in the case of the grape wines, the analytical parameters were generally higher than in the case of the fruit wines. Among the grape wines, due to the analytical parameters, we evaluated Blue Portugal as the highest and, among fruit wines, we found that these values were the highest in the elderberry wine.

In the study by **Robles et al. (2019)**, some organic acids were determined and identified in samples of wine. The study mentions that the following organic acids are found

in Czech white grape wines: tartaric acid (4.4 – 17.4 mM), malic acid (12.4 – 46.4 mM), acetic acid (1.7 – 27.9 mM), and lactic acid (1.4 – 27.4 mM). The values of tartaric acid measured in our study were in the above range. The content of malic acid in the observed samples was below the levels of those in the above study. Only samples of Blue Portugal corresponded in a range of values of lactic acids. There were no similarities in the other grape wine samples to those in comparable research.

Higginson et al. (2015) demonstrate in their study that single berry samples taken from bunches (grape) showed a large variation in the content of tartaric acid and of malic acid across a single vine. This assayed variation is relevant for studies collecting samples of berries for organic acid analysis. We noted that grape berries (but not fruit) predetermined chemical and sensory properties.

Estimation of antioxidant activity, total anthocyanin content and flavanol content

The next aim of this study was to determine AOA, total anthocyanin content, and flavanol content in all the wine samples. After using statistical analysis methods, we came to the following conclusions:

The highest AOA was measured in the elderberry samples and the lowest content in the Müller-Thurgau samples.

Elderberry wine was the highest-scoring sample regarding total anthocyanin content. Otherwise, low levels were measured in the Müller-Thurgau and apple wine samples. The highest values of flavanol content were found in the Blue Portugal and elderberry wine samples, whereas the lowest values were determined in the Müller-Thurgau. All results are shown in Figure 1. Considering the above-measured parameters, we concluded that elderberry wine and Blue Portugal proved to be the wines with the highest content. Conversely, the lowest levels of

measured parameters were demonstrated in the Müller-Thurgau and apple wine samples. By using the ANOVA statistical method, we showed a statistically significant difference between individual types of wine (grape and fruit wines; $p \leq 0.05$). The correlation and regression method showed that all represented pairs of amounts appeared on a single line and that the function had a rotating character. We found that the coefficient was equivalent to +1, which ultimately means a greater degree of interdependence.

By comparing the various constituents of the wines, we assessed that, in terms of AOA, fruit wines contained higher values than grape wines. Furthermore, in terms of the total anthocyanin content, we evaluated fruit wines as higher than grape wines. However, the flavanol content increased with red wines, elderberry wine, and Blue Portugal, as confirmed by other studies (Vilas Boas et al., 2019; Lingua et al., 2015).

Bruno and Sparapano (2007) mentioned that the content of each substance in grapes was influenced by abiotic factors (e.g. by variety, parent soils, geographic situation, locality, and climatic conditions). We agreed with this statement and we added agricultural and horticultural methods and interventions in vineyards during the growing season. The studies by Wirth et al. (2010) and Baron et al. (2017) assert that the bioactive substance content and AOA in wine depends on wine-making technology, e.g. by the pressing, maceration, fermentation, filtration, and/or bottling techniques used. They also declare that these contributions have an effect on the sensory properties of wine, the storage time, and other indicators that promote human health.

The study Čakar et al. (2019) aimed to determine the potential of the strawberry and drupe (apricot, plum, and sweet cherry) fruits for the production of new fruit wines Improved with phenolic compounds. They found general results that showed that the same fruit wine samples presented high redox potentials, marginally lower than strawberry, and plum wine samples.

The antioxidative potential and total phenolic content were tested in elderberry must and wine in the study Schmitzer et al. (2010). The level of total phenolic content in elderberry must and wine was 2004.13 GAEL⁻¹ and antioxidative potential of elderberry wine was equal to red wine. There was a detected correlation between total

phenolic content and antioxidative potential of elderberry wine. Moreover, examinations of elderberry fruits in other studies showed that it contains high biological activity components, primarily polyphenols, mostly anthocyanins, flavonols, phenolic acids, and proanthocyanidins, as well as terpenes and lectins (Sidor and Gramza-Michalowska, 2015).

Mlček et al. (2019) concluded that adequate wine consumption leads to cancer prevention and another health disease. Studies dealing with the content of bioactive substances in beverages made from berries showed that in addition to beneficial effects on human health, these substances have also side effects, e.g. it may cause an allergic reaction for unexplained reasons (Sedláčková et al., 2018; Nunes et al., 2019; Snopek et al., 2019).

Determination of the content of volatile substances in selected samples of fruit and grape wines

Table 2 shows the measured values of individual volatile substances. The fruit wines contained more methanol than the grape wines. The highest level of ethyl acetate was measured in the strawberry wine and other values were similar. Among the terpenes, the most significant that we identified were in the samples of strawberry wine (linalool 100 µg.L⁻¹; α-terpineol 43 µg.L⁻¹ and acetoin 9.7 mg.L⁻¹); compared to the strawberry wine, elderberry wine contained more acetoin, and apple wine was higher in methionol content. Lan et al. (2017) found that changes in taste properties in wine occur at an early stage of fermentation, i.e. 0 – 4 days. The content of aldehydes, ketones, heterocyclic and aromatic compounds were very reduced by fermentation. The same opinion was presented in studies Peng et al. (1997); Lubbers, Verret and Voilley (2001) and Spence, Velasco and Knoefler (2014).

Comparing the values of volatile substances in grape wines, we found that Blue Portugal and Müller-Thurgau were wines with a relatively high content of linalool. Low total terpene content was demonstrated in samples of St. Lawrence rosé. Geraniol, methionol, and acetoin were measured; Müller-Thurgau differed substantially from Blue Portugal with the highest content of ho-trienol and α-terpineol. The only value of nerol was detected in the Blue Portugal sample. We definitively demonstrated fruit wines as wines with much greater values of volatile substances

Table 2 GC-MS (mg.L⁻¹) determined individual volatile compounds.

	Müller-Thurgau	St. Lawrence rosé	Blue Portugal	Apple wine	Strawberry wine	Elderberry wine	SD
Methanol (mg.L ⁻¹)	63.70	48.80	100.20	32.60	83.90	221.40	±71.00
2,3-butanediol (mg.L ⁻¹)	269.00	372.90	1251.30	210.80	2798.10	5087.40	±1883.12
Butyric acid (mg.L ⁻¹)	1.40	2.23	0.64	1.30	3.51	0.74	±1.16
Ethyl acetate (mg.L ⁻¹)	74.70	73.50	62.80	55.50	191.60	72.40	±57.37
Isoamyl acetate (mg.L ⁻¹)	4.43	2.49	0.18	3.85	1.11	1.45	±1.72
Linalool (µg.L ⁻¹)	65.00	10.00	70.00	0.00	100.00	28.00	±39.50
Ho-trienol (µg.L ⁻¹)	24.00	0.00	8.00	0.00	0.00	0.00	±9.07
α-terpineol (µg.L ⁻¹)	17.00	0.00	15.00	0.00	43.00	0.00	±16.12
Nerol (µg.L ⁻¹)	0.00	0.00	3.00	0.00	0.00	0.00	±1.13
Geraniol (µg.L ⁻¹)	10.00	2.00	12.00	0.00	11.00	8.00	±5.30
Methionol (mg.L ⁻¹)	1.64	0.57	5.56	10.72	5.32	1.97	±3.78
Acetoin (mg.L ⁻¹)	0.00	6.40	10.50	1.30	9.70	19.20	±7.05

Note: Experiment was replicated 3 times.

than grape wines. Moreover, we found statistically significant differences between the measured values ($p \leq 0.05$).

Dziadas and Jeleń (2010) measured terpenes in white wines (different cultivars). As an example, we chose Riesling. The results showed that linalool reached a level of 16.6 – 54.4 $\mu\text{g}\cdot\text{L}^{-1}$. Comparing it with Müller-Thurgau, we noted a higher level of linalool. In contrast, the content of α -terpineol was much lower in our examined samples than those of the results from the comparison study. The nerol content, of which almost none of the examined samples allowed an estimate, had been determined with a decisively higher content in the above research. **Tarko et al. (2008)** declared an opinion that red wines had higher antioxidant potential and total polyphenol content than analyzed white grape and fruit wines. The prevailing volatile compounds of wines were higher alcohols, mainly amyl alcohols and isobutanol. Different conditions of double fermentation were examined in a study **Ubeda et al. (2011)**. There was added SO_2 and pectolytic enzymes which increased the level of methanol and acetaldehyde, especially in strawberry purees. In the study **Kong et al. (2019)** results showed that enzyme treatments improved the contents of volatile substances. The levels of terpenes and higher alcohols increased constantly during alcohol fermentation. **Yang et al. (2020)** and **Bhat (2000)** also mentioned the quality of fruit wines is related to the corrected application of enzymes in the process of winemaking.

Ayestarán et al. (2019) studied the effect of the winemaking process on the volatile composition and aromatic profile of Tempranillo Blanco wines.

The results showed that carbonic macerated wines had a higher level of alcohols and carbonyl compounds. We agreed with this statement.

CONCLUSION

We noted that red fruit wines contained a higher proportion of valuable bioactive substances than white grape wines. Wines with superior sensory properties did not contain more important antioxidants or higher values antioxidant activity. However, we stated that white grape wines contained a higher content of volatile substances, but, especially in the red wine samples (grape and fruit), we measured higher values for the research parameters. Relatively few scientific articles are published on fruit wines. Therefore, it was very interesting to evaluate these wines in terms of basic analytical parameters and make a comparison with grape wines.

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