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Influence of Pectolytic Enzymes and Selected Yeast Strains on the Chemical Composition of Blackberry Wines

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The aim of this study was to determine concentrations of individual organic acids, polyphenolic and aromatic compounds in blackberry wine, and to define the influence of different yeast strains (Uvaferm BDX and Lalvin 71B) and pectolytic enzymes (Lallzyme OE and Lallzyme EX-V) on the chemical composition and quality of the wine. Blackberry wines were produced in five variants, depending on yeasts and enzymes used: BDX OE, BDX EX-V, 71B OE, 71B EX-V, and Control without the addition of selected yeasts and enzymes. All blackberry wine variants were defined by a relatively high sum of organic acids. The citric acid was the predominant one, which concentrations ranged from 5.42 to 7.31 g/L. The concentration of gallic acid ranged from 19 to 37 mg/L and was in dependence of the yeast strain used. The concentration of procyanidin B_2 which was the predominant flavan-3-ol compound, ranged from 103 to 117 mg/L, and there were no significant differences between individual wine variants in the experiment. Rutin is the predominant compound in the flavonol group, followed by quercetin-3-*O*-glucoside. The predominant one among the anthocyanins was cyanidin-3-*O*-glucoside whose concentrations ranged from 134 to 229 mg/L. According to the obtained results, the yeast strain and pectolytic enzymes had a significant impact on the concentration of individual anthocyanins in the analyzed wines. The predominant group of aromatic compounds was monoterpenes, among which linalool was the most prominent in all of blackberry wine variants, except in Control.

INTRODUCTION

Among the fruit wines in Croatia, the most widespread are blackberry wines produced by the fermentation of sugar present in the blackberry juice or mash [Amidžić Klarić *et al*., 2011b]. Most of them are marketed as dessert wines, which, due to the high content of alcohol and unprocessed sugars, are very often inharmonious in taste. In addition, the technology for the production of blackberry wines is not standardized, which often results in the emergence of products on the market that differ significantly in flavor and aroma and therefore in overall quality. One of the prerequisites for quality wine production is the implementation of controlled alcohol fermentation with the use of selected yeast strains. During the yeast strain selection, it is necessary to take into account their technological and qualitative properties such as the fermentation speed and kinetics and their influence on the chemical composition and sensory properties of the wine [Jackson, 2014]. In order to determine the influence of yeasts on the aromatic profile of wine, it is necessary to fully understand their role in the fermentation process as it is confirmed by the researches dealing with the mentioned problem [Jackson, 2014]. The main volatile products of yeast metabolism ethanol, glycerol and carbon dioxide make a relatively small, but still fundamental contribution to the taste of wine. The major groups of compounds that form "the bouquet of fermentation" are the organic acids, higher alcohols and esters, and to a lesser extent, aldehydes [Rapp & Versini, 1991]. Pectolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze pectin compounds, present mainly in plants [Jayani *et al*., 2005]. Enzymes play an important role in the process of wine production by increasing extraction of grape compounds, increasing yields, facilitating filtration and intensifying the taste and color of wine [Sieiro *et al*., 2012]. A large number of commercially available pectolytic enzymes offer us the possibility to study them primarily through various intensity of extraction of colorants, but also some flavor precursors [Jackson, 2014].

Blackberry fruit wines are recognized as a valuable source of nutrients in human nutrition. They are of particular interest due to the high concentrations of anthocyanins and other polyphenolic compounds and their antioxidant properties as confirmed by a large number of scientific papers [Amidžić Klarić *et al*., 2011a; Gao *et al*., 2012; Ortiz *et al*., 2013]. Polyphenolic compounds are secondary plant metabolites that are made of aromatic rings on which one or more hydroxyl groups are bound and appear in the seeds and fruits of many angiosperms [Vinson *et al*., 2005]. They are very important

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wine quality factors, as they affect the color of wine, sensory characteristics such as bitterness and astringency, oxidation reactions, reactions with proteins and changes of wine during the maturation [Kennedy, 2008; Moreno-Arribas & Polo, 2009].

Blackberry aroma is one of the main properties that affect product quality whether the fruits are used in fresh or processed form. The aroma of blackberry wine is a result of the interaction of various volatile compounds that could be classified into seven different groups, according to Gao *et al*. [2012], namely: alcohols, esters, organic acids, aldehydes, ketones, terpenes and others.

Organic acids are products of an incomplete sugar oxidation that takes place mainly in leaves and still unripe fruits. Their composition and concentration directly influence the taste and the stability of wine. There are different literary data on the composition of organic acids in blackberry fruits, but the data on the composition of individual organic acids in blackberry wine are very rare. Worobo & Splittstoesser [2005] describe malic acid as the most common acid in blackberry, whose degradation in the process of malolactic fermentation has a direct impact on the reduction of total acidity of blackberry wine [Petravić-Tominac *et al*., 2013]. According to the study of organic acids in the fruits of blackberry in the genotypes represented in Turkey, the predominant organic acid was citric, followed by malic [Gazioglu Sensoy *et al*., 2013]. Certain yeast strains are able to use malic acid as an energy source and thus modify its content in the end product [Jackson, 2014].

Previous scientific studies on the blackberry wine in Croatia are rather sparese. The studies published so far have mainly involved defining the mineral composition of the wine, the presence of heavy metals, methanol and polyphenolic compounds having antioxidant activity and vasodilation effect [Amidžić Klarić *et al*., 2011a,b, 2017; Mudnić *et al*., 2012]. The aim of this study was to define the influence of different yeasts (Uvaferm BDX *Saccharomyces cerevisiae* and Lalvin 71B *Saccharomyces cerevisiae*) and pectolytic enzymes (Lallzyme OE and Lallzyme EX-V), on the chemical composition and quality of blackberry wines made out of Thornfree cultivar, throughout the two years of research.

MATERIALS AND METHODS

Materials

The fruits of blackberry were harvested over a period of two years, 2011 and 2012, from the plantation of blackberry (*Rubus fruticosus*, *Rosaceae*) cultivar Thornfree situated on an agricultural farm in northwestern Croatia.

Wine preparation

The fruits of the blackberry were crushed and the fruit mash was poured into 15 small tanks. The initial concentration of sugar in blackberry juice was about 40°Oe. 10 kg of sugar per 100 L was added to the fruit mash, which increased the concentration to about 80–90°Oe. Vinification was carried out according to the classic method of production of red wines. The maceration lasted for 6 days during which the "cap" needed to be sunk on a daily basis. Alcoholic fermentation was controlled by adding the selected yeasts Uvaferm BDX *Saccharomyces cerevisiae* and Lalvin 71B *Saccharomyces cerevisiae*, produced by Lallemand, Montréal, QC, Canada (www.lallemandwine.com).

As a controlled variant, the classic technology for the production of red wines was used, but without the addition of selected yeast and enzymes, *i.e.* epiphytic microflora was used. At the beginning of the maceration, we added the pectolytic enzymes, Lallzyme OE and Lallzyme EX-V produced by Lallemand, Montréal, QC, Canada (www.lallemandwine.com). In one variant we added the enzyme Lallzyme OE and in the second the enzyme Lallzyme EX-V in an amount of 1 g to 100 L of blackberry mash. Lallzyme OE is a pectinase enzyme with a very strong secondary activity of hemicellulase and cellulase. It was developed for the purpose of increasing the extraction of colorants, tannin and flavor precursor. Lallzyme EX-V is a pectolytic enzyme with very active and concentrated secondary activity that acts on the cellular structure of the fruit cell membrane. It was developed for the purpose of improving extraction of colorants and tannins for wines that will age longer.

After finishing the main fermentation, the fruit mash was pressed and the obtained wine was left for a low and quiet fermentation. When the sugar level dropped to about 8 g/L, the wines were sulfured with a 5% sulfuric acid solution at 100 mL/hL concentration and the first racking was conducted. At the end of the first racking, a wine chemical analysis was carried out.

Determination of individual organic acids

Individual organic acids (g/L) were determined according to the method described by Frayne [1986] on an Agilent Series 1100 instrument. Analyses were performed isocratically at a 0.6 mL/min flow and 65°C column temperature with a 300 × 7.8 mm i.d. Aminex HPX-87H cation exchange column (Bio-Rad Laboratories, Hercules, CA), using 0.065% H_3PO_4 as the mobile phase and a Diode Array Detector set to 210 nm. Quantification of organic acids was done by the external standard method.

Determination of individual polyphenols

HPLC separation, identification and quantification of wine phenolic compounds were performed according to method described by Tomaz & Maslov [2016] on an Agilent 1100 Series system (Agilent, Germany), equipped with DAD, FLD and coupled to an Agilent ChemStation (version B.01.03) data-processing station. The grape skin extracts were injected (20 μL) on a reversed-phase column Luna Phenyl-Hexyl (4.6 × 250mm; 5 μm particle (Phenomenex, Torrance, USA), heated at 50°C. The solvents were water/phosphoric acid (99.5:0.5, v/v, solvent A) and acetonitrile/water/phosphoric acid; 50:49.5:0.5, v/v/v, solvent B), and the flow rate was 0.9 mL/min. The linear gradient for solvent B was: 0 min, 0%; 7 min, 20%; 35 min, 40%; 40 min, 40%; 45 min, 80%; 50 min, 100%; 60 min, 0%. Hydroxybenzoic acids were detected at 280 nm, hydroxycinnamic acids at 320 nm, flavonols at 360 nm and anthocyanins at 518 nm. Flavan-3-ols were detected at λ_{ex}=225 nm and λ_{em}=320 nm. Phenolic compounds were identified by matching the retention time of each

chromatographic peak with external standards and DAD spectrum. Quantification of individual phenolic peaks was done by the external standard method.

Determination of aroma compounds

Analysis of wine volatile compounds was performed according to the method described by Maslov *et al*. [2017]. In brief, isolation of volatile compounds was done by applying solid phase extraction procedure on LiChrolut EN cartridges (200 mg/3 mL, Merck, Darmstadt, Germany). The GC analysis was performed on an Agilent 6890 system coupled with 5973N mass spectrometer with the column ZB-WAX (60 m × 0.32 mm i.d., with 0.5 μm film thickness, Phenomenal, Torrance, USA). The flow rate of helium was 1 mL/min. The mass spectrometer was operated in an electron ionization mode at 70 eV with selected ion monitoring (SIM) with selected ions. Quantification of all examined compounds was done by the external standard method.

Statistical analysis

Statistical analysis of analytical data was conducted in all samples using SAS v 9.3 Statistical Software (2012, SAS Institute Inc., Cary, NC, USA). The significance of differences between the variants for the content of individual organic acids and individual phenolic and aromatic compounds was determined by the analysis of variance (ANOVA). The mean values were compared using the Duncan's multiple range test [Duncan, 1955]. Differences among treatments mean that the values with $p<0.05$ were considered statistically significant. All measurements were performed three times and the obtained results represent mean ± standard deviation of parallel measurements (mean ± SD).

The canonical discriminant analysis shows the structure of differences between the variants with regard to the content of certain phenolics and differences between the variants with regard to the content of certain aromatic compounds. Due to the position of the centroid, *i.e.* the mean values of the canonical variables variates, scatter plots were created using the first two canonical variables.

RESULTS AND DISCUSSION

In this section we present the results of two-year research on blackberry wines. Wines were produced in five variants, differing in yeasts and enzymes used: Variant 1 (BDX OE): Uvaferm BDX *S.c*. and Lallzyme OE; Variant 2 (BDX EX-V): Uvaferm BDX *S.c*. and Lallzyme EX-V; Variant 3 (71B OE): Lalvin 71B *S.c*. and Lallzyme OE; Variant 4 (71B EX-V): Lalvin 71B *S.c*. and Lallzyme EX-V; and Variant 5: Control – produced without the addition of selected yeasts and enzymes. Yeast Uvaferm BDX was selected due to its ability to form stronger, more pronounced types of red wines, while yeast Lalvin 71B was selected for the production of fresh, fruity red wines that are more quickly ready for market entry and consumption.

Organic acids

Concentrations of individual organic acids in wines from both production years are shown in Table 1. Regardless of production year, all blackberry wine variants had a relatively high sum of organic acids, though in both years the variant 71B OE stood out, as the values were the lowest. The reason for this is the slightly lower concentrations of citric, malic and succinic acids whose concentrations, though not significantly, were the lowest in 2011.

The predominant organic acid was citric acid which concentrations ranged from 5.42 g/L (71B OE, 2011) to 7.31 g/L (BDX OE, 2012). These results are in line with the data found in literature [Gazioglu Sensoy *et al*., 2013], which state the citric acid as the predominant one. The con-

TABLE 1. Concentrations of organic acids in blackberry wines (g/L).

Compounds	Year	Wine variants				
		BDX OE	BDX EX-V	71B OE	71B EX-V	CONTROL
Citric acid	2011	5.75±0.22^{ab}	5.74±0.15^{ab}	5.42±0.17^{b}	5.75±0.41^{ab}	6.00±0.10^{a}
	2012	7.31±0.14^{a}	6.97±0.08^{b}	5.44±0.13^{d}	5.79±0.03^{c}	7.03±0.18^{b}
Malic acid	2011	1.34±0.59^{a}	1.15±0.64^{a}	0.97±0.52^{a}	1.17±0.62^{a}	0.74±0.08^{a}
	2012	1.53±0.11^{a}	1.52±0.09^{a}	1.31±0.03^{b}	1.37±0.07^{b}	1.42±0.01^{ab}
Tartaric acid	2011	0.79±0.08^{ab}	0.65±0.07^{c}	0.85±0.06^{a}	0.69±0.06^{bc}	0.57±0.04^{c}
	2012	1.02±0.01^{a}	1.32±0.78^{a}	1.01±0.01^{a}	0.74±0.04^{a}	0.75±0.02^{a}
Succinic acid	2011	0.28±0.10^{a}	0.27±0.04^{a}	0.17±0.05^{a}	0.30±0.09^{a}	0.22±0.01^{a}
	2012	0.30±0.03^{a}	0.26±0.05^{a}	0.29±0.05^{a}	0.26±0.02^{a}	0.27±0.02^{a}
Lactic acid	2011	0.21±0.07^{bc}	0.33±0.08^{b}	0.13±0.03^{c}	0.30±0.11^{b}	0.48±0.04^{a}
	2012	0.41±0.06^{a}	0.45±0.13^{a}	0.45±0.15^{a}	0.51±0.02^{a}	0.42±0.01^{a}
Σ Organic acids	2011	8.38±0.84^{a}	8.15±0.90^{a}	7.55±0.78^{a}	8.21±1.21^{a}	8.01±0.25^{a}
	2012	10.58±0.10^{a}	10.51±1.01^{a}	8.50±0.09^{b}	8.68±0.01^{b}	9.89±0.23^{a}

Variant 1 (BDX OE): Uvaferm BDX *S.c*. and Lallzyme OE, Variant 2 (BDX EX-V): Uvaferm BDX *S.c*. and Lallzyme EX-V, Variant 3 (71B OE): Lalvin 71B *S.c*. and Lallzyme OE, Variant 4 (71B EX-V): Lalvin 71B *S.c*. and Lallzyme EX-V and Variant 5: CONTROL – without the addition of selected yeasts and enzymes.

Values are presented as means of three repetitions ± standard deviation. The mean values marked with different letters between the variants differ at the $p<0.05$ level, using Duncan's multiple-range test.

centrations of malic acid ranged from 0.74 g/L (CONTROL, 2011) to 1.53 g/L (BDX OE, 2012). There was no statistically significant difference in malic acid concentration between the individual variants in the year of 2011. Considering concentrations of tartaric and lactic acid, the difference across variants were observed in 2011 variants, while there were no statistically significant differences across the variants in 2012 wines. The concentration of succinic acid ranged from 0.17 g/L (71B OE, 2011) to 0.30 g/L (71B EX-V, 2011 and BDX OE, 2012) and there were no statistically significant differences across the variants in both production years. In 2011, none of the variants in the experiment stood out in the total sum of concentrations of organic acids, although Petravić-Tominac *et al*. [2013] determined the influence of yeasts on the level of acidity in wine, with significant differences in malic and lactic acid concentrations observed in wines produced by Fermol Rouge® and Fermol Mediterranee® yeasts. The results of 2012 are consistent with the data above, where the wines obtained with the BDX yeast had a significantly higher concentration of malic and citric acids irrespective of the pectolytic enzyme used.

Phenolic compounds

Concentrations of phenolic compounds in the studied wines are shown in Table 2. The statistically significant differences across the individual variants in the experiment were recorded in the values of gallic acid. The gallic acid was the predominant phenolic acid in the obtained blackberry wines, which was in accordance with earlier published studies [Amidžić Klarić *et al*., 2011a]. Its concentrations ranged from 19 mg/L (BDX OE, 2011) to 37 mg/L (CONTROL, 2012), and was lower than the values obtained by Amidžić Klarić *et al*. [2011a], *i.e.* from 28.14 to 122.41 mg/L. In obtained results significant influence of the BDX yeast was noticed. Wines produced with BDX yeast in both years of our study had the lowest level of gallic acid. This may be due to more pronounced adsorptive properties of its cell wall, whereby this acid may be binding during the accumulation process. Chlorogenic acid with its concentration ranging from 5.06 g/L (BDX EX-V, 2011) to 12 g/L (71B OE, 2012) in the examined wines was the second predominant phenolic acid. In both years of research, wine variants 71B OE had slightly higher concentration of this acid, although the differences were not statistically significant. In the case of *p*-coumaric acid concentrations, there were no statistically significant differences across the individual variants. The reported caffeic acid values ranged from 3.86 to 6.12 mg/L, which is in accordance with the research of Amidžić Klarić *et al*. [2011a], where the impact of the variant was not pronounced.

The concentrations of *trans*-resveratrol in the obtained blackberry wines were relatively high (0.69 to 1.08 mg/L) if compared to the average values of *trans*-resveratrol in red wines ranging from 0.35 to 1.99 mg/L depending on the cultivar, and in white wines where its concentration was significantly lower and ranged from 0.005 to 0.57 mg/L [Gerogiannaki-Christopoulou *et al*., 2006]. The analyzed variants of wines differed from each other in terms of *trans*--resveratrol concentration. Its lowest concentration was determined in control wines, which were produced without the addition of the selected yeasts and pectolytic enzymes, while the variant 71B OE stood out in both examined years with its significantly higher concentrations. Yeasts produce both endogenous and exogenous β-glucosidase and their activity differs between strains [Delcroix *et al*., 1994]. Studies conducted by Vrhovsek *et al*. [1997] have shown that yeasts with higher β-glucosidase activity increase the level of *cis*- and *trans*-resveratrol and decrease the concentration of *trans*-resveratrol glucoside in Pinot noir musts. Yeasts are also known adsorbents of polyphenols and are different by capacity to adsorb polyphenols, which may be another factor influencing concentrations of stilbenes in wine. Besides absorption by the cell walls, Vacca *et al*. [1997] proposed resveratrol might be absorbed and then metabolized by yeast cells.

Procyanidin B_2 is the predominant compound in the group of flavan-3-ols, *i.e.* compounds which are responsible for the sense of bitterness and astringency in grapes and wine [Kennedy *et al*., 2006]. Considering the concentration of procyanidin B_2, there were no statistically significant differences among some variants, although we could notice that the control samples stood out with high values in both production years (111 and 115 mg/L). In both production years, the highest concentrations of catechin were measured in the control samples (65 and 67 mg/L), and the lowest ones in BDX OE variants (56 and 57 mg/L). Although there were no statistically significant differences in both cases, we noticed that in wines produced under the influence of the EX-V enzyme, some higher values were measured in comparison to the wines produced under the influence of the OE enzyme. Enzyme EX-V was selected for its enhanced extraction of polyphenolic compounds for a stronger, more expressive type of wine. According to the literature data, catechin concentrations in blackberry wines produced in Croatia range from 0 to 51.46 mg/L [Ljevar, 2016], which is in accordance with our data. In his research, Gao *et al*. [2012] stated the catechin concentration of 12.87 mg/L in blackberry wine produced in China by the traditional wine making technology, and its significantly higher concentration (25.85 mg/L) in wine produced by the carbonic maceration technology.

The predominant compound in the group of flavonols was rutin, which concentration ranged from 15 mg/L (71B OE, 2011) to 25 mg/L (CONTROL, 2012). In the study of blackberry wine, Chinese authors determined a significantly lower rutin concentration of 5.52 mg/L [Gao *et al*., 2012]. The highest concentrations of quercetin-3-*O*-glucoside were determined in wines of 71B EX-V variant in both production years (1.92 and 2.38 mg/L). In literature data, authors describe berry fruits as a rich source of flavonoids, where quercetin is the most important dietary representative of flavonols [Nijveldt *et al*., 2001]. The concentrations of quercetin aglycone assayed in Croatian blackberry wines ranged from 0.81 to 21.67 mg/L [Amidžić Klarić *et al*., 2017]. In her study on blackberry wines, Ljevar [2016] also stated the values of quercetin aglycone in a fairly wide range from 0.38 to 17.94 mg/L.

The predominant one among the studied anthocyanins was cyanidin-3-*O*-glucoside (134 mg/L to 229 mg/L) followed

TABLE 2. Concentrations of polyphenolic compounds in blackberry wines (mg/L)

Compounds	Year	Wine variants				
		BDX OE	BDX EX-V	71B OE	71 B EX-V	CONTROL
Non-flavonoid polyphenolics						
Gallic acid	2011	19±0.7^{b}	27±8.4^{ab}	32±5.4^{a}	30±0.4^{a}	36±0.1^{a}
	2012	20±0.8^{b}	28±8.7^{ab}	33±5.6^{a}	32±0.6^{a}	37±0.1^{a}
Chlorogenic acid	2011	6.08±0.61^{a}	5.06±1.07^{a}	10±5.7^{a}	6.81±0.92^{a}	6.86±0.12^{a}
	2012	6.93±0.69^{a}	5.77±1.22^{a}	12±6.5^{a}	8.82±0.82^{a}	7.65±0.20^{a}
Caffeic acid	2011	4.88±0.15^{a}	3.86±0.58^{b}	4.42±0.46^{ab}	4.63±0.58^{ab}	4.93±0.05^{a}
	2012	5.67±0.18^{ab}	4.48±0.67^{c}	5.13±0.54^{bc}	6.12±0.69^{a}	5.71±0.07^{ab}
p-Coumaric acid	2011	0.66±0.09^{a}	0.22±0.08^{a}	0.21±0.06^{a}	0.24±0.06^{a}	0.39±0.10^{a}
	2012	1.28±0.17^{a}	0.42±0.04^{a}	0.41±0.11^{a}	1.25±0.24^{a}	0.78±0.16^{a}
trans-Resveratrol	2011	0.91±0.04^{b}	0.84±0.01^{c}	1.00±0.03^{a}	0.94±0.03^{b}	0.69±0.00^{d}
	2012	0.98±0.05^{b}	0.91±0.01^{c}	1.08±0.03^{a}	1.04±0.07^{ab}	0.74±0.00^{d}
Σ Non-flavonoid polyphenols	2011	32±1.0^{c}	37±8.6^{bc}	48±6.3^{a}	43±1.8^{ab}	49±0.4^{a}
	2012	35±1.1^{c}	40±9.0^{bc}	51±7.0^{a}	49±3.2^{ab}	52±0.5^{a}
Flavan-3-ols						
Catechin	2011	56±4.0^{b}	59±6.5^{ab}	59±6.7^{ab}	61±1.6^{ab}	65±1.0^{a}
	2012	57±4.1^{b}	61±6.7^{ab}	60±6.8^{ab}	63±2^{ab}	67±1.0^{a}
Epicatechin-3-*O*-gallate	2011	18±6.3^{a}	14±5.3^{a}	14±5.9^{a}	11±0.3^{a}	13±0.2^{a}
	2012	21±7.2^{a}	16±6.0^{a}	16±6.7^{a}	13±1.2^{a}	15±0.2^{a}
Procyanidin B_2	2011	103±11^{a}	105±12^{a}	108±12^{a}	110±3^{a}	111±5^{a}
	2012	108±11^{a}	110±13^{a}	113±12^{a}	117±5^{a}	115±4^{a}
Σ Flavan-3-ols	2011	177±21^{a}	179±24^{a}	181±24^{a}	182±4^{a}	190±6^{a}
	2012	186±23^{a}	187±25^{a}	189±26^{a}	193±8^{a}	197±5^{a}
Flavonols						
Rutin	2011	20±2.2^{a}	19±1.0^{ab}	15±2.8^{b}	19±3.0^{ab}	22±1.0^{a}
	2012	23±2.4^{a}	21±1.2^{ab}	17±3.2^{b}	22±3.3^{ab}	25±1.1^{a}
Quercetin-3-*O*-glucoside	2011	1.68±0.09^{ab}	1.52±0.16^{b}	1.60±0.31^{ab}	1.92±0.21^{a}	1.52±0.03^{b}
	2012	2.02±0.11^{ab}	1.82±0.19^{b}	1.91±0.37^{ab}	2.38±0.37^{a}	1.98±0.01^{ab}
Kaempferol	2011	0.23±0.10^{a}	0.16±0.01^{ab}	0.05±0.01^{ab}	0.18±0.06^{ab}	0.0
	2012	0.23±0.11^{a}	0.17±0.01^{ab}	0.06±0.00^{b}	0.16±0.04^{ab}	0.02±0.01^{b}
Izorhamnetin	2011	0.69±0.11^{a}	0.58±0.03^{a}	0.50±0.15^{a}	0.64±0.15^{a}	0.49±0.07^{a}
	2012	0.81±0.12^{a}	0.68±0.03^{a}	0.59±0.17^{a}	0.78±0.21^{a}	0.58±0.05^{a}
Σ Flavonols	2011	23±2.4^{a}	21±1.1^{ab}	18±3.3^{b}	22±3.5^{ab}	24±1.1^{a}
	2012	26±2.7^{a}	24±1.2^{ab}	20±3.8^{b}	25±4.0^{ab}	27±1.2^{a}
Anthocyanins						
Cyanidin-3-*O*-glucoside	2011	141±2^{b}	134±2^{b}	207±14^{a}	219±13^{a}	145±2^{b}
	2012	147±2^{b}	139±2^{b}	215±14^{a}	229±15^{a}	150±2^{b}
Cyanidin-3-*O*-xyloside	2011	11±0.3^{d}	11±0.2^{d}	16±0.4^{b}	18±0.1^{a}	12±0.2^{c}
	2012	13±0.4^{c}	14±0.2^{c}	20±0.5^{b}	24±3.1^{a}	15±0.1^{c}
Cyanidin-3-*O*-malonylglucoside	2011	28±0.5^{e}	31±0.4^{c}	32±0.1^{b}	33±0.1^{a}	30±0.2^{d}
	2012	30±0.6^{d}	33±0.4^{d}	34±0.1^{b}	36±1.5^{a}	32±0.3^{c}
Cyanidin-3-*O*-dioxaloylglucoside	2011	6.33±0.16^{c}	6.86±0.08^{b}	7.04±0.20^{ab}	7.27±0.07^{a}	6.81±0.20^{b}
	2012	7.22±0.19^{c}	7.82±0.09^{b}	8.03±0.23^{b}	8.67±0.64^{a}	7.93±0.31^{b}
Σ Anthocyanins	2011	187±3^{b}	183±2^{b}	262±14^{a}	277±13^{a}	194±3^{b}
	2012	198±3^{c}	194±2^{c}	277±15^{b}	298±19^{a}	205±3^{c}

Variant 1 (BDX OE): Uvaferm BDX *S.c*. and Lallzyme OE, Variant 2 (BDX EX-V): Uvaferm BDX *S.c*. and Lallzyme EX-V, Variant 3 (71B OE): Lalvin 71B *S.c*. and Lallzyme OE, Variant 4 (71B EX-V): Lalvin 71B *S.c*. and Lallzyme EX-V and Variant 5: CONTROL – without the addition of selected yeasts and enzymes.

Values are presented as means of three repetitions ± standard deviation. The mean values marked with different letters between the variants differ at the $p<0.05$ level, using Duncan's multiple-range test.

by cyanidin-3-*O*-malonylglucoside at concentrations from 28 mg/L to 36 mg/L. The fact that cyanidin-3-*O*-glucoside is predominant among anthocyanins in blackberry wines was confirmed in the previously published literature data [Ljevar, 2016]. In the study on blackberry wines in Serbia, the authors stated cyanidin-3-*O*-glucoside as the predominant among the anthocyanins in most of the examined wines (209.3 mg/L to 403.8 mg/L). In one of the four examined wines, cyanidin--3-*O*-xyloside was listed as being the predominant among anthocyanins (99.7 mg/L) followed by cyanidin-3-*O*-rutinoside (39.3 mg/L) and cyanidin-3-*O*-glucoside (35.4 mg/L) [Mitic *et al*., 2013]. In our study on cyanidin-3-*O*-xyloside, its concentrations ranged from 11 mg/L in BDX OE variant (2011) to 24 mg/L in 71B EX-V variant (2012), which is somewhat lower in relation to the values of cyanidin-3-*O*--xyloside measured in blackberry wines from Serbia ranging from 42.8 mg/L to 99.7 mg/L [Mitic *et al*., 2013]. The total concentration of the individual anthocyanins ranged from 183 mg/L (BDX EX-V, 2011) to 298 mg/L (71B EX-V, 2012). Generally, the 71B EX-V variants of wines had the highest values of all individual anthocyanins in both production years, followed by 71B OE variant of wines. According to the obtained results, the yeast strain had a larger impact on the concentrations of individual anthocyanins in relation to the pectolytic enzymes used. However, the established differences can be explained by the fact that the anthocyanins are highly reactive and unstable compounds [Ćujić *et al*., 2013] and their stability is influenced by pH, storage temperature, chemical structure, concentration, light, presence of oxygen, solvents, presence of enzymes, flavonoids, proteins and metal ions [Ćujić *et al*., 2013].

Discriminant analysis

The canonical discriminant analysis found that the first three canonical variables explained 99.91% of the variability between variants on the observed characteristics. The first two canonical variables were used to construct graph shown in Figure 1, where the distance among the variants is shown on the basis of the mentioned canonical variables. The most positive correlations between the first canonical variable, which explains 78.32% of the total variability among variants, were observed with *trans*-resveratrol (0.80), and the most negative correlations with catechin (-0.96), procyanidin B_2 (-0.74) and gallic acid (-0.71). It follows that the variants that are in the positive correlation with the 1st canonical variable (BDX OE and 71B OE) have a higher content of *trans*-resveratrol, while those variants that are negatively correlated with the 1st canonical variable (71B EX-V and CONTROL) have higher contents of catechin, procyanidin B_2 and gallic acid.

The second canonical variable, which explained 21.30% of the total variability between the variants, had the most posi-

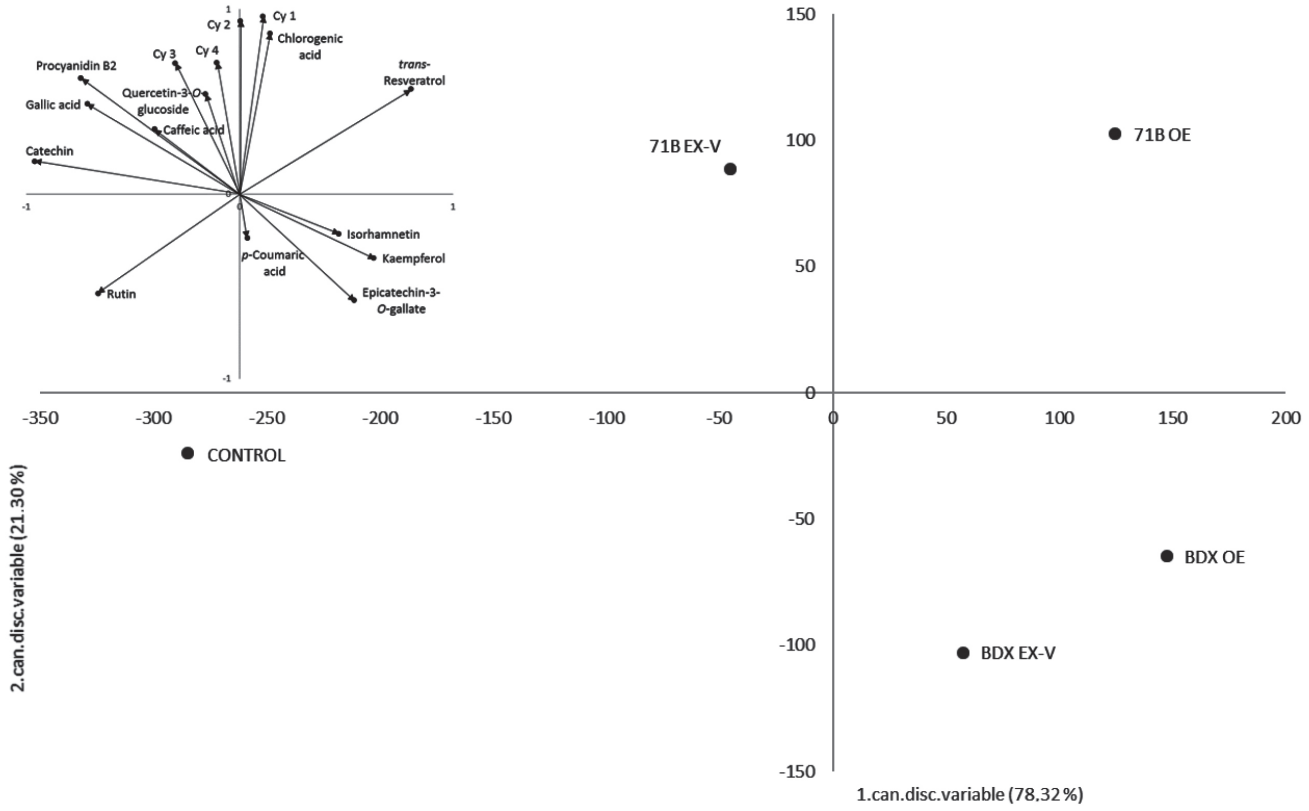

FIGURE 1. Distribution of the 5 tested variants Variant 1: Uvaferm BDX *S.c.* and Lallzyme OE (BDX OE), Variant 2: Uvaferm BDX *S.c.* and Lallzyme EX-V (BDX EX-V), Variant 3: Lalvin 71B *S.c.* and Lallzyme OE (71B OE), Variant 4: Lalvin 71B *S.c.* and Lallzyme EX-V (71B EX-V) and Variant 5: CONTROL - without the addition of selected yeasts and enzymes, on the surface defined by the first two canonical discriminant variables calculated on the basis of the composition of 16 individual phenolic compounds with the direction of action of 16 variables within the first two canonical variables shown as vectors.

Cy 1: cyanidin-3-*O*-glucoside; Cy 2: cyanidin-3-*O*-xyloside; Cy 3: cyanidin-3-*O*-malonylglucoside; Cy 4: cyanidin-3-*O*-dioxaloylglucoside.

tive correlations with anthocyanins, cyanidin-3-*O*-glucoside (0.96) and cyanidin-3-*O*-xyloside (0.94), and with chlorogenic acid (0.87). The most negative correlation with the 2nd canonical variable had epicatechin-3-*O*-gallate (-0.57) and rutin (-0.53).

Aromatic compounds

Table 3 presents concentrations of 14 tested aromatic compounds in the studied wines. In both years, the predominant group of aromatic compounds was monoterpenes. Linalool was the predominant monoterpene alcohol in all the variants except in Control. Its content in all analyzed variants of wines (except in CONTROL wines) was above the sensory threshold of 25.2 μg/L [Ferreira *et al*., 2000], which certainly influences the release of desirable floral aromas in wine. Following the linalool, citronellol concentrations ranged from 0.78 μg/L in CONTROL up to 19 μg/L in the BDX EX-V variant. Citronellol gives specific citrus notes to the wine aroma, while α-terpineol can give a scent of lilies [Clarke & Bakker, 2004]. Patrignani *et al*. [2016] indicate the important influence of yeast strains that carry out the vinification process, on the release of linalool, α-terpineol and citronellol. The mentioned compounds are released into wine as a result of yeast β-glucosidase activity [Fia *et al*., 2005]. If we look at the total sum of the analyzed aromatic compounds, we find that the CONTROL wines had far lower concentrations in comparison to all the other variants. Higher values of total monoterpenes were in wines that were treated with pectolytic enzymes in relation to wines which were produced without the addition of enzymes. That is confirmed by results of a previous scientific study [Rusjan *et al*., 2009]. Among the variants used, there was a tendency for a greater influence of 71B yeast on the release of *trans*-rose oxide while the pectolytic EX-V enzyme had a greater influence on α-terpineol concentration. This may also be due to a change in the content of terpene during alcoholic fermentation resulting from the joint action of several factors involving mutual conversions, *i.e.* formation of terpene oxides, enzymatic and chemical hydrolysis of glycosidically bound terpenes and adsorption of terpene on the cell walls of yeast [Darriet, 1992].

Of the analyzed C13 norisoprenoids, in Table 3 we have only shown α-ionone concentrations, while β-ionone and β-damascenone that contribute to the fruit aromas in wine and may disguise some undesirable "unripe" aromas of methoxypyrazine [Pineau *et al*., 2007], have not been identified in any the wine samples.

The predominant compound in a group of higher alcohols was 1-hexanol which concentrations ranged from 0.44 μg/L (CONTROL, 2012) to 1.87 μg/L (BDX OE, 2012). 2-Hexenol was not identified in any sample. Out of the given values of higher alcohols and aldehydes, we can see that the BDX OE variant stands out with the highest concentration of 1-hexanol and furfuryl alcohol. The lowest concentrations of 1-hexanol in both production years and the lowest one of furfuryl alcohol in the second year of production were measured in the Control wines. The synthesis of higher alcohols in the process of alcohol fermentation is greatly influenced by the type and yeast strain that carry out the fermentation [Singh & Kunkee, 1976], so we can conclude that yeast BDX and 71B stimulated the synthesis of higher alcohols. Concentrations of 2-hexenal were the highest in CONTROL wines and in the 71B EX-V variant. Furfural is an aldehyde whose origin in wine is mainly related to fermentation and aging of wine in the barrel, but may also arise as a product of carbohydrates degradation [Moreno-Arribas & Polo, 2009]. In our study, the highest concentrations of this aldehyde were measured in CONTROL from 2011 (1.10 μg/L), while the values in all wines in 2012 were slightly lower (0.82 to 0.83 μg/L). The measured values of furfural were far below its detection threshold of 14,100 μg/L [Ferreira *et al*., 2000], so the contribution of this aldehyde to the aromas of caramel in wine is almost insignificant.

In the concentrations of the only analyzed lactone, γ-nonalactone, there was no difference among the variants. γ-nonalactone is a compound whose aroma reminds of coconut and cooked peach [Buettner, 2017]. It is one of the components of peach, pineapple and coconut aroma. According to the present literature data, this compound is present in wine in very low concentrations, but given the low sensitivity threshold even its very small concentrations can contribute to the aroma of wine [Nakamura *et al*., 1988]. Although there were no statistically significant differences between the examined variants, we can notice that it was least represented in the control wines. The highest concentrations were in BDX OE and BDX EX-V variants from 2011 (0.39 μg/L), where it did not exceed the detection threshold of 30 μg/L [Nakamura *et al*., 1988]. However, the studies show that, even below the detection threshold, lactones contribute to wine aromas through the synergistic effect [Cooke *et al*., 2009].

Discriminant analysis

The canonical discriminant analysis found that the first three canonical variables explained 99.36% of the variability between variants on the observed characteristics. The first two canonical variables were used to construct graph shown in Figure 2, where the distance among the variants is shown on the basis of the mentioned canonical variables. The most positive correlations between the first canonical variable, which explained 78.05% of the total variability among variants, existed with citronellol (0.79) and the most negative correlations with γ-nonalactone (-0.66) and furfural (-0.51). It follows that the variants that are in positive correlation with the 1st canonical variable (BDX EX-V and 71B EX-V) had a higher content of citronellol, while those variants that were negatively correlated with the st 1st canonical variable (BDX OE and 71B OE) had higher contents of γ-nonalactone and furfural.

The second canonical variable, which explained 15.36% of the total variability between the variants, had the most positive correlations with *trans*-rose oxide (0.90) and linalool (0.86), while the most negative correlation had the following aromatic compounds: 1-hexanol (-0.92), furfural (-0.82), furfuryl alcohol (-0.96), nerol (-0.66) and α-ionone (-0.89).

CONCLUSIONS

According to the results of the study on the chemical composition of the blackberry wines of Thornfree cultivar, we can conclude that the predominating organic acid is citric, followed by malic acid. Statistically significant differences in the concentrations of these acids were recorded in wines produced

TABLE 3. Concentrations of aromatic compounds in blackberry wines (μg/L).

Compounds	Year	Wine variants				
		BDX OE	BDX EX-V	71B OE	71B EX-V	CONTROL
		Monoterpenes				
cis-Rose oxide	2011	1.65 ±0.28^{bc}	1.93±0.24^{b}	1.28±0.13^{c}	1.77±0.39^{b}	2.61±0.10^{a}
	2012	1.75±0.16^{ab}	1.73±0.21^{ab}	1.49±0.0^{b}	1.66±0.26^{ab}	1.97±0.12^{a}
trans-Rose oxide	2011	5.86±2.09 ^{b}	7.65±4.08^{ab}	9.14±0.95^{ab}	11±1.1^{a}	1.76±0.09^{c}
	2012	6.34±2.62^{b}	6.75±3.43^{b}	11±0.0^{a}	11±0.1^{a}	1.33±0.08^{c}
Linalool	2011	48±13.2^{ab}	58±3.3^{a}	42±3.3^{b}	52±8.0^{ab}	1.09±0.03^{c}
	2012	51±9.0^{a}	52±1.9^{a}	49±2.6^{a}	49±3.3^{a}	0.83±0.01^{b}
α-Terpineol	2011	1.09±0.02^{c}	1.73±0.34^{abc}	1.28±0.34^{bc}	2.10±0.86^{ab}	2.23±0.1^{a}
	2012	1.16±0.10^{a}	1.56±0.37^{a}	1.52±0.52^{a}	1.94±0.66^{a}	1.69±0.08^{a}
Citronellol	2011	9.65±7.49^{ab}	19±9.1^{a}	7.41±5.82^{ab}	8.41±1.28^{ab}	1.03±0.05^{b}
	2012	11±4.5^{ab}	16±6.9^{a}	8.58±5.01^{ab}	8.05±1.95^{ab}	0.78±0.07^{b}
Nerol	2011	1.71±0.28^{a}	1.46±0.21^{ab}	1.47±0.18^{ab}	1.60±0.51^{a}	1.04±0.09^{b}
	2012	1.84±0.42^{a}	1.30±0.07^{a}	1.71±0.27^{a}	1.50±0.37^{a}	0.79±0.06^{b}
		C-13 norisoprenoids				
β-Damascenone	2011	n.d.	n.d.	n.d.	n.d.	n.d.
	2012	n.d.	n.d.	n.d.	n.d.	n.d.
α-Ionone	2011	1.03±0.22^{a}	0.95±0.20^{a}	0.77±0.10^{a}	0.87±0.15^{a}	1.06±0.10^{a}
	2012	1.08±0.14^{a}	0.84±0.10^{b}	0.89±0.02^{b}	0.82±0.07^{b}	0.80±0.01^{b}
		Higher alcohols				
1-Hexanol	2011	1.75±0.29^{a}	1.35±0.20^{b}	1.37±0.06^{b}	1.23±0.30^{b}	0.58±0.01^{c}
	2012	1.87±0.27^{a}	1.21±0.16^{b}	1.59±0.09^{a}	1.15±0.18^{b}	0.44±0.09^{c}
2-Heksenol	2011	n.d	n.d	n.d	n.d	n.d
	2012	n.d.	n.d.	n.d.	n.d.	n.d.
Furfuryl alcohol	2011	1.15±0.07^{a}	1.08±0.18^{ab}	0.83±0.22^{b}	1.02±0.14^{ab}	1.04±0.02^{ab}
	2012	1.22±0.05^{a}	0.96±0.08^{b}	0.95±0.15^{b}	0.96±0.05^{b}	0.79±0.06^{c}
		Aldehydes				
2-Hexenal	2011	0.99±0.13^{b}	1.09±0.14^{b}	1.19±0.49^{b}	1.82±0.17^{a}	1.80±0.10^{a}
	2012	1.05±0.04^{bc}	0.98±0.04^{c}	1.36±0.42^{ab}	1.72±0.05^{a}	1.36±0.03^{ab}
Furfural	2011	0.78±0.07^{cd}	0.93±0.09^{b}	0.72±0.07^{d}	0.87±0.08^{bc}	1.10±0.0^{a}
	2012	0.83±0.0^{ab}	0.83±0.0^{ab}	0.833±0.01^{a}	0.82±0.01^{b}	0.83±0.0^{ab}
		Lactones				
γ-Nonalactone	2011	0.39±0.21^{a}	0.39±0.06^{a}	0.15±0.02^{a}	0.28±0.18^{a}	0.09±0.02^{a}
	2012	0.27±0.14^{a}	0.26±0.11^{a}	0.16±0.06^{a}	0.25±0.15^{a}	0.07±0.0^{a}
		Total aromatic compounds				
Total	2011	74±6.2^{b}	95±15.6^{a}	68±9.0^{b}	84±10.9^{ab}	15±2.3^{c}
	2012	79±4.2^{a}	85±7.0^{a}	79±8.6^{a}	79±4.0^{a}	12±1.8^{b}

Variant 1 (BDX OE): Uvaferm BDX *S.c*. and Lallzyme OE, Variant 2 (BDX EX-V): Uvaferm BDX *S.c*. and Lallzyme EX-V, Variant 3 (71B OE): Lalvin 71B *S.c*. and Lallzyme OE, Variant 4 (71B EX-V): Lalvin 71B *S.c*. and Lallzyme EX-V and Variant 5: CONTROL – without the addition of selected yeasts and enzymes.

Values are presented as means of three repetitions ± standard deviation. The mean values marked with different letters between the variants differ at the $p<0.05$ level, using Duncan's multiple-range test.

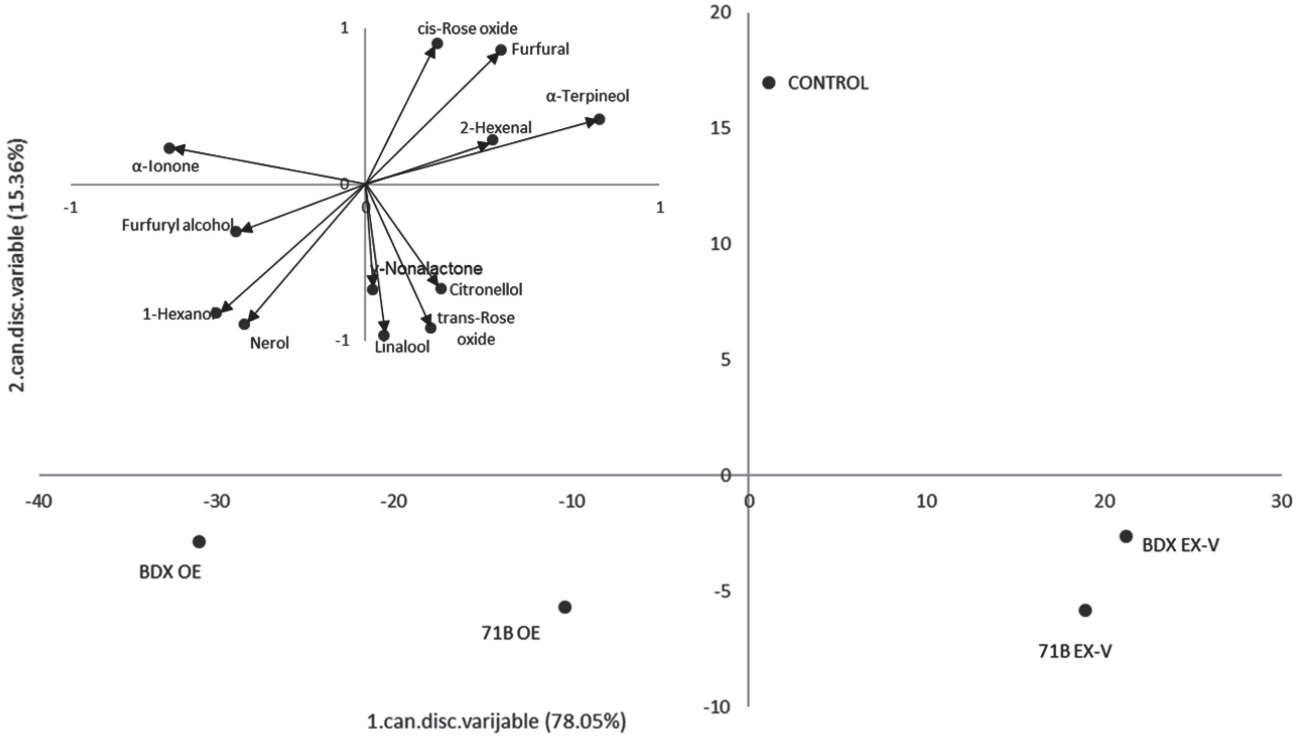

FIGURE 2. Distribution of the 5 tested variants Variant 1: Uvaferm BDX *S.c.* and Lallzyme OE (BDX OE), Variant 2: Uvaferm BDX *S.c.* and Lallzyme EX-V (BDX EX-V), Variant 3: Lalvin 71B *S.c.* and Lallzyme OE (71B OE), Variant 4: Lalvin 71B *S.c.* and Lallzyme EX-V (71B EX-V) and Variant 5: CONTROL - without the addition of selected yeasts and enzymes, on the surface defined by the first two canonical discriminant variables calculated on the basis of the composition of 12 individual aromatic compounds with the direction of action of 12 variables within the first two canonical variables shown as vectors.

under the influence of different yeast strains in 2012. Among the phenolic acids, the gallic acid stood out with the highest concentrations. Concentrations of this phenolic acid were affected to the greatest extent by yeast strains used in the study compared to the pectolytic enzymes. If we compare the concentrations of *trans*-resveratrol in the obtained blackberry wines with the average values in red wines, we can conclude that blackberry wines represent a good source of this non-flavonoid. The predominant compound among the analyzed flavan-3-ols was procyanidin B_2, and in the flavonol group, rutin. The predominant one among the anthocyanins was cyanidin-3-*O*-glucoside whose concentrations were the highest in wines produced with 71B yeasts. The predominant group of aromatic compounds were monoterpenes, where the significant influence of pectolytic enzymes as well as yeast strains was determined. The predominant monoterpene was linalool, followed by citronellol, α-terpineol and *trans*-rose oxide.

We can conclude that use of different pectolytic enzymes and selected yeast strains can significantly affect the chemical composition and quality of blackberry wines. Considering the obtained results it would be desirable for future studies to extend the research to wines made by other blackberry cultivars as well as wild blackberry.

RESEARCH FUNDING

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

REFERENCES

1. Amidžić Klarić D., Klarić I., Mornar A., Polyphenol content and antioxidant activity of commercial blackberry wines from Croatia: Application of multivariate analysis for geographic origin differentiation. J. Food Nutr. Res., 2011a , 50, 199–209.
2. Amidžić Klarić D, Klarić I., Velić D., Dragojević I.V., Evaluation of mineral and heavy metal contents in Croatian blackberry wines. Czech J. Food Sci., 2011b, 29, 260–267.
3. Amidžić Klarić D., Klarić I., Velić D., Velić N., Marček T., Evaluation of quercetin content, colour and selected physico-chemical quality parameters of Croatian blackberry wines. Pol. J. Food Nutr. Sci., 2017, 67, 75–84.
4. Buettner A., Springer Handbook of Odor. 2017, Springer International Publishing AG. Switzerland, pp. 151–152.
5. Clarke, R.J., Bakker J., Volatile components. 2004, *in*: Wine Flavour Chemistry, 2nd Edition, Blackwell Publishing, Oxford, UK, pp. 166–171.
6. Cooke R.C., Van Leeuwen K.A., Capone D.L., Gawel R., Elsey G.M., Sefton M.A., Odor detection thresholds and enantiomeric distributions of several 4-alkyl substituted γ-lactones in Australian red wine. J. Agr. Food Chem., 2009, 57, 2462–2467.
7. Ćujić N., Kundaković T., Šavikin T., Anthocyanins – The chemical analysis and biological properties. Lek. Sirov, 2013, 33, 19–37 (in Croatian; English abstract).

8. Darriet P., Recent advances in wine microbiology in relation to the organoleptic properties and the wine alterations. 1992, *in*: Les acquisitions récentes en microbiologie du vin. Incidences sur les propriétés organoleptiques et les altérations du vin (ed. P. Darriet). Tec-Doc Lavoisier, Paris, pp. 111–121 (in French).
9. Delcroix A., Gunata Z., Sapis J.C., Salmon J.M., Bayonove C., Glycosidase activities of three enological yeast strains during winemaking: effect on the terpenol content of muscat wine. Am. J. Eno. Vitic., 1994, 45, 291–296.
10. Duncan D.B., Multiple range and multiple F tests. Biometrics, 1955, 11, 1–42.
11. Ferreira V., Lopez R., Cacho J.F., Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agr., 2000, 80, 1659–1667.
12. Fia G., Giovani G., Rosi I., Study of β-glucosidase production by wine-related yeasts during alcoholic fermentation. A new rapid fluorimetric method to determine enzymatic activity. J. Appl. Microb., 2005, 99, 509–517.
13. Frayne R.F., Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. Am. J. Eno. Vitic., 1986, 37, 281–287.
14. Gao J., Xi Z., Zhang J, Guo Z, Chen T., Fang Y., Influence of fermentation method on phenolics, antioxidant capacity and volatiles in blackberry wines. Anal. Lett., 2012, 45, 2603–2622.
15. Gazioglu Sensoy R.I., Gundogdu M., Sensoy S., Celik F., Dogan A., HPLC analysis of blackberry fruits for organic acid and sugar contents. III Intern. Symp. Pomeg. Minor Medit. Fruits, Acta Horticulare, 2013, 1089, 77–81.
16. Gerogiannaki-Christopoulou M., Athanasopoulos Kyriakidis N., Gerogiannaki I.A., Spanos M., *trans*-Resveratrol in wines from the major Greek red and white grape varieties. Food Contr., 2006, 17, 700–706.
17. Jackson R., Wine Science – Principles and Applications. 2014, 4th Edition. Academic Press, London, Oxford, Boston, New York, San Diego, pp. 6, 359–379; 7, 465–479.
18. Jayani R.S., Saxena S., Gupta R., Microbial pectinolytic enzymes: a review. Process Biochem., 2005, 40, 2931–2944.
19. Kennedy J.A., Grape and wine phenolic: Observations and recent findings. Cien. Inv. Agr., 2008, 35, 107–112.
20. Kennedy J.A., Saucier C., Glories Y., Grape and wine phenolics: History and perspective. Am. J. Eno. Vitic., 2006, 57, 239–248.
21. Ljevar A., Phenolic composition and antioxidant capacity of fruit wines. Doctoral thesis. Faculty of Food Technology and Biotechnology, University of Zagreb, 2016, p. 125 (in Croatian; English abstract).
22. Maslov L., Tomaz I., Mihaljević Žulj M., Jeromel A., Aroma characterization of predicate wines from Croatia Euro. Food Res. Tech., 2017, 243, 263–274.
23. Mitic M.N., Obradovic M.V., Mitic S.S., Pavlovic A.N., Pavlovic J.L., Stojanovic B.T., Free radical scavenging activity and phenolic profile of selected Serbian red fruit wines. Revista de Chimie, 2013, 64, 68–73.
24. Moreno-Arribas M.V., Polo M.C., Wine chem. and biochem. 2009, Vol. 378. New York: Springer, pp. 4, 108–110; 8, 284–306; 9, 463–464.
25. Mudnic I., Budimir D., Modun D., Gunjaca G., Generalic I., Skroza D., Boban M., Antioxidant and vasodilatory effects of blackberry and grape wines. J. Med. Food, 2012, 15, 315–321.
26. Nakamura S., Crowell E.A., Ough C.S., Totsuka A., Quantitative analysis of γ-nonalactone in wines and its threshold determination. J. Food Sci., 1988, 53, 1243–1244.
27. Nijveldt R.J., van Nood E., van Hoorn D.E.C., Boelens P.G., van Norren K., van Leeuwen P.A.M., Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 2001, 74, 418–425.
28. Ortiz J., Marín-Arroyo M.R., Noriega-Domínguez M.J., Navarro M., Arozarena I., Color, phenolics, and antioxidant activity of blackberry (*Rubus glaucus* Benth.), blueberry (*Vaccinium floribundum* Kunth.), and apple wines from Ecuador. J. Food Sci., 2013, 78, C985–C993.
29. Patrignani F., Chinnici F., Serrazanetti D.I., Vernocchi P., Ndagijimana M., Riponi C., Lanciotti R., Production of volatile and sulfur compounds by 10 *Saccharomyces cerevisiae* strains inoculated in Trebbiano must. Front. Microb., 2016, 7, art no. 243.
30. Petravić-Tominac V., Mesihović A., Mujadžić S., Lisičar J., Oros D., Velić D., Petrović, Z., Production of blackberry wine by microfermentation using commercial yeasts Fermol Rouge® and Fermol Mediterranée®. Agr. Consp. Sci., 2013, 78, 49– 55.
31. Pineau B., Barbe J.C., Van Leeuwen C., Dubourdieu D., Which impact for β-damascenone on red wines aroma? J. Agr. Food Chem., 2007, 55, 4103–4108.
32. Rapp A., Versini G., Influence of nitrogen compounds in grapes on aroma compounds of wine. 1991, *in*: Proceedings of the International symposium on nitrogen in grapes and wines (ed. J.M. Rantz). Davis, CA: Ameri. Soc. Eno. Vitic., pp. 156–164.
33. Rusjan D., Strlič M. S., Košmerl T., Prosen, H., The response of monoterpenes to different enzyme preparations in Gewürztraminer (*Vitis vinifera* L.) wines. S. Afr. J. Eno. Vitic., 2009, 30, 56–64.
34. Sieiro C., da Silva A.F., García-Fraga B., López-Seijas J., Villa T.G., Microbial pectic enzymes in the food and wine industry. Food Ind. Proc. – Meth. Equip., 2012, 11, 201–217.
35. Singh R., Kunkee R.E., Alcohol dehydrogenase activities of wine yeasts in relation to higher alcohol formation. Appl. Environ. Microb., 1976, 32, 666–670.
36. Tomaz I., Maslov L., Simultaneous determination of phenolic compounds in different matrices using phenyl-hexyl stationary phase. Food Anal. Meth., 2016, 9, 401–410.
37. Vacca V., Leccis L., Fenu P., Pretti L., Farris G.A., Wine yeasts and resveratrol content. Biotech. Lett., 1997, 19, 497–498.
38. Vinson J.A., Zubik L., Bose P., Samman N., Proch J., Dried fruits: excellent *in vitro* and *in vivo* antioxidants. J. Am. Coll. Nutr., 2005, 24, 44–50.
39. Vrhovsek U., Wendelin S., Eder R., Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. Am. J. Eno. Vitic., 1997, 48, 214–219
40. Worobo R.W., Splittstoesser D.F., Microbiology of fruit products. Processing fruits. CRC Press, Boca Raton, FL, 2005, pp. 261–284.

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