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The effect of harvest time and vintage year on the phenolic composition of Nero and Bianca wines

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ABSTRACT

Fungal disease resistant (PIWI) interspecific grape varieties are playing an important role as an alternative for organic wine production. Organic (bio) wines are demanded by numerous conscious consumers around the globe. They choose this kind of wines predominantly because of the absence of synthetic pesticides, fertilisers and sustainable agriculture. Resistant grape growing moreover results in additional environmental and health benefits. Nero and Bianca are among Hungary's most promising interspecific grape cultivars gaining international interest recently, there are, however, limited vitivinicultural knowledge on them. Our aim was to examine the flavonoid and anthocyanin composition for both interspecific varieties during different harvest times in two consecutive vintages. The date of harvest and vintage played a significant effect on grape and wine quality.

KEYWORDS

Nero, Bianca, interspecific cultivars, flavonoid, harvest date

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1. INTRODUCTION

Fungal disease resistant (PIWI) or interspecific hybrid grapes originate from cross-breeding of *V. vinifera* and North American (such as *V. riparia*, *V. rupestris*, etc.) and/or Asian *Vitis* species (such as *V. amurensis* etc.) (Hajdu, 2015). The genetic background of these non-Eurasian species is responsible for the high resistance attributes against fungal diseases in the offsprings, including powdery and downy mildew. Nero and Bianca are one of the most promising results of the Hungarian grape breeding programs (Hajdu, 2015). These cultivars have received increasing international attention in recent years (Bellin et al., 2009; Lisek, 2012). There is also a high consumer demand for organic wines and, therefore, its production is increasing considerably (Pedneault and Provost, 2016). Working with traditional *V. vinifera* varieties are difficult for growers when the goal is making wines without pesticides, fertilisers, and other synthetic residues from sustainable viticulture, while these are highly susceptible to fungal diseases and pests (Fuller et al., 2014). Therefore, fungal disease resistant grape varieties are playing an important role as an alternative for organic winemaking with reduced production costs and economic losses (Fragoulis et al., 2009; Becker, 2013; Fuller et al., 2014). However, beside the positive aspects of these cultivars, there are also challenges with them. Interspecific grapes and their wines are rather unknown amongst consumers, which is the result of insufficient wine marketing (Becker, 2013; Fuller et al., 2014). Besides, some consumers have negative preconceptions (eg. foxy flavours, poor quality, etc.) with the wines made from interspecific cultivars (Fuller et al., 2014). Beyond these circumstances, the scientific and growing experiences with these grape varieties are very limited. Improving winemaking technologies are also needed in order to make more pleasant wines from interspecific hybrids. Furthermore, little information is available on how these varieties function and thrive under different climatic conditions, in different areas and vintages, and how these environmental factors affect the quality and phenolic composition of wine. The aim of this study was to investigate the effect of harvest time and vintage on phenolic composition of Nero and Bianca wines.

2. MATERIALS AND METHODS

2.1. Experimental site and vines

Noir-skinned Nero [Eger 2 (Seyve-Villard 12,375 selection) × Gárdonyi Géza (Medoc noir × Csaba gyöngye)] and white-skinned Bianca [Eger 2 (Seyve-Villard 12,375 selection) × Bouvier] are both interspecific crossings. Nero and Bianca vineyards can be found in Eger wine region, Kőlyuktető, Hungary (lat. 47°51′57.2″N, long. 20°22′51.5″E) and were planted in 2000. The cordon trained vines with single arm were grafted onto Teleki-Kober 5BB rootstock, using vertical shoot positioning at 2.4 × 1 m inter- and intrarow spacing, respectively. During pruning, four spurs, each with two nodes were left per vines.

2.2. Experimental design

A trial site of six-six rows was selected for both cultivars. Each row was divided into three blocks. One block contained 25 to 30 vines. At the same harvest time, one block per row was harvested for both cultivars resulting in three replicates. The experiment was done in two consecutive vintages (2013; 2014) and with three harvest dates in both years (Table 1).

Table 1. Harvest dates

Year	Nero			Bianca		
	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
2013	26.08.	02.09.	09.09.	28.08.	04.09.	11.09.
2014	28.08.	04.09.	18.09.	09.09.	17.09.	24.09.

For the standard analytical measurements, 1 kg samples from each harvest date were collected three times at random from several clusters from all of the blocks before vinification. White and red wine microvinification protocol was the same for all treatments and vintages and was done in three replicates.

Briefly: After destemming, crushing, and pressing Bianca grape juice was immediately pumped into 50 L stainless steel fermenting vats with controllable temperature. 20 mg L^{-1} free sulphur dioxide level was adjusted with liquid sulphur dioxide solution (5 v/v%) after grape processing. Then selected active dry yeasts (Uvaferm CM, Lallemand Inc.) with a dosage of 20 g/100 L and yeast nutrients (30 g/100 L) (Uvavital, Lallemand Inc.) were added. The fermentation took place and ended at constant temperature (16 °C). After that, the wines were racked and transported for laboratory assessment.

Destemmed and crushed Nero grapes were co-inoculated with 20 g/100 L Uvaferm CM (Lallemand Inc.) active dry yeast, and with 10 mg L^{-1} lactic acid bacteria (Uvaferm Alpha, Lallemand Inc.) after 24 h of cold maceration at 8 °C. Fermentation took place in 50 L stainless steel vats. The cap was punched down twice a day throughout the skin contact period at constant temperature of 20 °C. The maceration lasted for 21 days, and the wines were pressed at 1.2 bar. Free-run and press wines were mixed. After completion of the malolactic fermentation, the wines were racked and transported for detailed analysis.

2.3. Climatic data

Climatic data were monitored by an automatic weather station (Boreas Ltd. Érd, Hungary), located approximately 200 m from the trial site.

2.4. Standard analytics

The analytical methods recommended by the OIV were used to determine sugar concentration (OIV-MA-AS311-01A), titratable acidity (OIV-MA-AS313-01), and pH (OIV-MA-AS313-15) of the grapes and alcohol content (OIV-MA-AS312-01B), titratable acidity (OIV-MA-AS313-01), and pH (OIV-MA-AS313-15) of the wines. All measurements were done in triplicate.

2.5. Qualitative and quantitative determination of phenolic components in wines by HPLC

The wine samples were analysed without sample pretreatment on a modular Shimadzu HPLC system (Shimadzu, Germany) equipped with a Kinetex 2.6 μ XB-C18 100A (100 × 4.6 mm) column at a flow rate of 1 mL min^{-1} and a Hitachi LaChrom HPLC system (Merck, Hungary) coupled with an Hypersil ODS (250 × 4.6 mm, 5 μm) column with the mobile phase flow rate of 0.8 mL min^{-1} for the flavonoids and anthocyanins, respectively. For separation of different

flavonoid compounds eluent A and B were water and acetonitrile, both supplemented with 1% acetic acid, and the following gradient program was applied: initially 0% B, at 16.40 min 16.3% B, at 16.90 min 18.4% B, at 20.30 min 18.4% B, at 24.90 min 19.4% B, at 27.50 min 20.4% B, at 27.51 min 100% B, at 30.4 min 100% B, at 30.41 min 0% B, and at 37.0 min 0% B. In the case of anthocyanins, the eluent A and B were water/formic acid/acetonitrile, 87:10:3 (V/V%) and 40:10:50 (V/V%), using the following gradient program: at 0 min 6% B, at 15 min 30% B, at 30 min, 50% B, at 35 min 60% B, at 41 min 6%, and at 45 min 6%. Flavonoid [(+)-catechin, (−)-epicatechin, protocatechuic acid, gallic acid, vanillic acid, caftaric acid, *t*-caffeic acid, quercetin-3-O-galactoside, quercetin-3-glucuronide, kaempferol-3-O-glucoside, *t*-resveratrol, and *t*-piceid] and anthocyanin (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside) contents of the samples were identified and quantified using standard reference compounds detected at 280 nm and at 518 nm, respectively.

2.6. Glories indices

The evaluation of Glories indices in the case of Nero was the same as described earlier in our previous work (Villangó et al., 2015).

2.7. Statistical analysis

Statistical analyses were carried out by one-way ANOVA and mean separation was made by Tukey's test.

3. RESULTS AND DISCUSSION

3.1. Analysis of the vintages

Figure 1 shows the most important characteristics (monthly sum of precipitation and monthly mean temperature) of the experimental years (2013; 2014) at Kőlyuktető vineyard. The differences are remarkable between the two vintages. In the first three months, in May and in June of 2013 fell an abundant amount of precipitation. In the following year, the appearance of extreme rainfall was more typical from July to September along with a low mean temperature in August. These factors resulted in a much slower ripening process (both cultivars ripen early), and in the end lower overall grape and wine quality compared to the previous year. It must be highlighted, that despite the much higher amount the precipitation during the vegetation period in 2014, bunches remained healthy in the case of both cultivars. No signs of fungal disease were found.

Weather anomalies (lower or higher mean temperatures than usual, and/or uneven precipitation) were experienced in both years during the experiment. Climatic change and vintage effect under moderate climate conditions, therefore, will have the most powerful effect on grape and wine quality in the following years (Gutiérrez-Gamboa et al., 2021).

3.2. Standard analysis of the grape juice and wines

Sugar content and, therefore, alcohol levels were the most sensitive parameters on harvest date and on vintage effect in the case of both cultivars (Tables 2 and 3). The warm August of 2013 resulted in wines with high alcohol levels and low titratable acidity. Higher acidity and lower

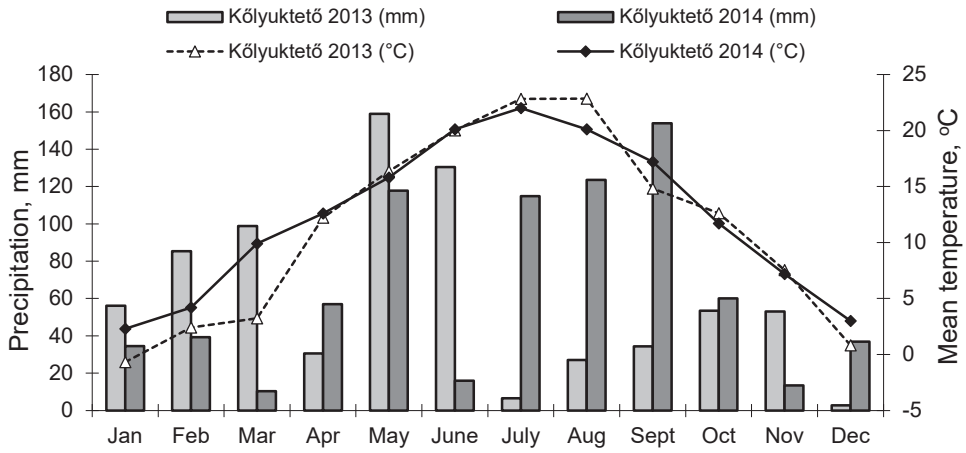

Fig. 1. Vintage characteristics (monthly precipitation and mean temperature)

pH values were representative for the cooler and rainier 2014 in the case of Nero and partly Bianca wines. Interestingly, the amount of titratable acidity was lower in the first two Bianca wines in 2014 compared to the first and second harvest dates of the previous vintage, but the pH values were lower all season long. pH levels around and mostly above 4.0, as seen in Table 3 for Nero wines are not favourable (eg. emerging level of wine faults, spoilage).

Every grape producer has to seriously deal with both parameters (alcoholic content and titratable acidity) in order to keep them between a range, which fits for the chosen wine style, technology and quality. Our results indicate that an earlier harvest date for both cultivars particularly in warmer vintages should be chosen. Overripening must be avoided for both cultivars, as the rapid loss of acidity can result in mild and unbalanced wines along with high alcohol levels, which is generally not preferred by wine consumers (Bucher et al., 2018). The fact that both cultivars have an early ripening period should also be taken into account during the selection of optimal harvest date. Considering the actual vintage characteristics and the desirable wine style is also highly recommended.

3.3. HPLC analysis of the wines

Vintage had the most significant effect on Nero's anthocyanin levels (Table 4). Higher amount of red pigments was measured in the cooler vintage of 2014. This result is in accordance with other findings (Gaiotti et al., 2018; Yan et al., 2020). These works declare that cooler temperatures are favourable for accumulation of grape anthocyanins. Malvidin-3-glucoside was the most abundant anthocyanin component in all measured samples, which is normal, because in most red grapes and red wines this component and its derivatives dominate the anthocyanin profile (Allegro et al., 2021). Total level of anthocyanin was increased from the first harvest to the second one, but a decrease could be detected in both seasons at the third sampling date. This decrease can be explained by the overripening process, where pigments began to degrade (Bigard et al., 2019). Other explanation for the lower anthocyanin concentration at the end of the sampling process would be the high pH level. The higher the pH, the lower the solubility and

Table 2. Standard analysis of the grape juices

Parameters	Vintage	Nero			Bianca		
		1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
Sugar (g L^{-1})	2013	214.4±4.3aα	226.1±3.6bα	264.0±5.6cα	199.3±3.8aα	223.9±3.9bα	238.8±3.4cα
	2014	192.0±2.6aβ	210.7±1.9bβ	231.0±3.4cβ	205.0±3.0aβ	200.2±3.0aβ	242.2±4.2bα
T. acidity (g L^{-1})	2013	5.4±0.1aα	5.1±0.1bα	5.0±0.1cα	7.0±0.1aα	7.4±0.1bα	5.6±0.1cα
	2014	7.1±0.1aβ	7.2±0.1bβ	5.8±0.2cβ	6.7±0.1aβ	6.5±0.1bβ	6.2±0.1cβ
pH	2013	3.87±0.02aα	3.86±0.02aα	3.93±0.02bα	3.44±0.01aα	3.45±0.01aα	3.56±0.01bα
	2014	3.36±0.02aβ	3.67±0.02bβ	3.73±0.02cβ	3.17±0.06aβ	3.43±0.03bα	3.52±0.02cβ

Each value represents the average ± standard error of 3 replicates ($P < 0.05$).
Values marked with different Roman letters mean significant differences between harvest dates within the same year. Different Greek letters mean significant differences between the years within the same harvest dates.

Table 3. Standard analysis of the wines

Parameter	Vintage	Nero			Bianca		
		1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
Alcohol (v/v%)	2013	13.23±0.12aα	13.59±0.15bα	14.25±0.14cα	12.25±0.13aα	13.46±0.09bα	14.51±0.04cα
	2014	11.12±0.10aβ	11.56±0.16bβ	13.56±0.05cβ	12.44±0.06aβ	12.89±0.11bβ	13.50±0.17cβ
T. acidity (g L^{-1})	2013	4.23±0.06aα	3.97±0.06cα	4.03±0.06bα	5.57±0.06aα	5.10±0.10bα	4.70±0.10cα
	2014	3.87±0.06aβ	4.47±0.06cβ	4.07±0.06bα	5.53±0.06aα	4.87±0.06cβ	5.27±0.06bβ
pH	2013	3.85±0.01aα	3.97±0.01bα	4.03±0.01cα	3.48±0.03aα	3.73±0.01bα	3.83±0.05cα
	2014	4.10±0.01aβ	4.13±0.01bβ	4.30±0.02cβ	3.60±0.10aβ	3.80±0.03bβ	3.63±0.06aβ

Each value represents the average ± standard error of 3 replicates ($P < 0.05$).
Values marked with different Roman letters mean significant differences between harvest dates within the same year. Different Greek letters mean significant differences between the years within the same harvest dates.

Table 4. Amounts of anthocyanin components in Nero wines

Parameter (mg L^{-1})	Vintage	Nero		
		1st harvest	2nd harvest	3rd harvest
Delphinidin-3-glucoside	2013	2.05±0.07aα	2.42±0.01aα	1.94±0.22aα
	2014	9.94±0.28aβ	9.37±0.27bβ	8.70±0.57cβ
Cyanidin-3-glucoside	2013	0.79±0.51aα	0.68±0.30aα	0.53±0.26aα
	2014	1.65±0.11aβ	1.70±0.14aβ	1.78±0.17aβ
Petunidin-3-glucoside	2013	10.04±5.31aα	10.64±2.95aα	8.89±2.75aα
	2014	25.80±1.65aβ	24.01±2.40aβ	19.30±2.62bβ
Peonidin-3-glucoside	2013	1.20±0.53aα	1.23±0.28aα	0.88±0.28aα
	2014	2.65±0.13aβ	2.76±0.22aβ	2.64±0.27aβ
Malvidin-3-glucoside	2013	226.25±12.95aα	312.92±4.10bα	298.54±15.59bα
	2014	528.46±51.88aβ	642.49±64.98aβ	492.90±81.79bβ
Σ	2013	240.33±17.88aα	327.89±0.61bα	310.78±16.79bα
	2014	568.50±53.87aβ	680.33±67.76bβ	525.32±85.42aβ

Each value represents the average ± standard error of 3 replicates ($P < 0.05$).
Values marked with different Roman letters mean significant differences between harvest dates within the same year. Different Greek letters mean significant differences between the years within the same harvest dates.

extraction of anthocyanins. This phenomenon was verified with wine-like solutions (Forino et al., 2019), as well as authentic wines (Forino et al., 2020). Beside the previously mentioned environmental, technological, and analytical factors, changes in the berry skin and seed cell wall composition should also be taken into account. Anthocyanins may be bound to other grape skin components such as monosaccharides (galactose, arabinose, etc.) and polysaccharides, especially cellulose. In this sense, the amounts of these compounds have also a great impact on the extractability of anthocyanins as well as the degree of methylation of the pectins (Allegro et al., 2021). This review article among others also demonstrated that the extraction rate of grape pigments depended on numerous factors, especially on the chemical interactions with cell wall material and ripeness grade. Earlier results also indicate that the extraction of phenolic compounds is more related to berry density and berry mechanical properties than harvest date (Rolle et al., 2011). In contrast to anthocyanins, other phenolic substances are synthetised in higher amounts under warmer weather conditions along with more sunshine hours and solar radiation, however, optimal range and thresholds for these secondary metabolites should also be discussed (Torres et al., 2020).

As expected, Nero wines contained much higher levels of flavanols ((+)-catechin, (−)-epicatechin) than Bianca wines (Table 5). Stilbenes (*t*-rezveratrol and *t*-piceid) were only present in Nero wines. Quercetin-3-O-galactoside and kaempferol-3-O-glucoside were also absent in all Bianca wines. The warmer weather in 2013 significantly enhanced the accumulation of (−)-epicatechin, (+)-catechin, gallic acid, caftaric acid, quercetin-3-O-galactoside, quercetin-3-glucuronide, and kaempferol-3-O-glucoside in Nero wines. It can be concluded from the results of standard and HPLC analyses that both cultivars can be used for making lighter wines with limited aging capacity. Moreover, French-American hybrid grapes like Bianca and Nero contain usually more pectin (as uronic acids) in their skin cell walls than other *V. vinifera*

Table 5. Amounts of flavonoid components in wines

Parameter (mg L^{-1})	Vintage	Nero			Bianca		
		1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
(+)-Catechin	2013	270.27±24.72aα	304.55±2.02aα	295.33±18.97aα	10.38±0.72aα	12.03±0.23aα	14.57±1.42bα
	2014	184.14±5.54aβ	197.59±8.43aβ	114.04±12.07bβ	15.41±0.81aβ	3.66±1.75bβ	6.04±0.45bβ
(−)-Epicatechin	2013	94.17±10.43aα	107.93±2.36aα	96.43±8.49aα	1.03±0.05aα	1.54±0.14a	1.75±0.08a
	2014	67.19±4.21aβ	70.10±2.38aβ	32.64±4.97bβ	2.64±0.53β	n.d.	n.d.
Protocatechuic acid	2013	3.14±0.21a	3.31±0.10aα	4.15±0.92bα	1.54±0.47aα	4.72±0.70bα	4.71±0.52bα
	2014	n.d.	1.19±0.22aβ	3.65±0.55bα	2.92±0.45aα	4.25±0.55cα	1.00±0.40bβ
Gallic acid	2013	48.57±1.45aα	44.79±1.28aα	46.62±1.85aα	1.32±0.40aα	2.45±0.10bα	2.41±0.10bα
	2014	28.10±1.30aβ	18.42±3.00bβ	22.94±4.94aβ	2.74±0.17aβ	2.53±0.18aα	1.34±0.05bβ
Vanillic acid	2013	4.37±1.41aα	6.04±1.96aα	6.74±1.81aα	n.d.	n.d.	n.d.
	2014	21.78±1.50aβ	21.10±1.21aβ	22.49±0.45aβ	1.32±0.01a	3.08±0.42b	3.39±0.18b
Caftaric acid	2013	49.61±1.53aα	50.95±10.95aα	42.45±0.41aα	5.64±0.37aα	3.13±1.19bα	3.00±0.14bα
	2014	35.76±0.67aβ	31.57±2.18aβ	25.72±3.49aβ	19.13±0.05aβ	15.11±0.26bβ	0.95±0.15cβ
t-Caffeic acid	2013	5.79±0.43aα	4.50±0.62bα	5.69±0.49aα	n.d.	n.d.	n.d.
	2014	3.19±0.35aβ	4.02±0.27bα	6.57±0.16cα	1.64±0.03a	1.84±0.03b	n.d.
Quercetin-3-O-galactoside	2013	4.78±0.88aα	7.66±3.61aα	16.91±5.75b	n.d.	n.d.	n.d.
	2014	1.92±0.40aβ	1.36±0.05bβ	n.d.	n.d.	n.d.	n.d.
Quercetin-3-glucuronide	2013	6.09±0.58aα	10.74±7.70a	6.02±2.17a	n.d.	n.d.	n.d.
	2014	1.23±0.23β	n.d.	n.d.	1.17±0.01a	1.63±0.39b	n.d.
Kaempferol-3-O-glucoside	2013	8.01±0.76a	9.93±0.64a	6.02±4.97a	n.d.	n.d.	n.d.
	2014	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
t-Resveratrol	2013	2.76±0.59aα	3.60±1.43a	3.74±1.22aα	n.d.	n.d.	n.d.
	2014	1.30±0.40aβ	n.d.	3.15±0.84bα	n.d.	n.d.	n.d.
t-Piceid	2013	n.d.	2.27±0.52	n.d.	n.d.	n.d.	n.d.
	2014	n.d.	n.d.	0.90±0.12	n.d.	n.d.	n.d.

Each value represents the average ± standard error of 3 replicates ($P < 0.05$); n.d. = not detected. Values marked with different Roman letters mean significant differences between harvest dates within the same year. Different Greek letters mean significant differences between the years within the same harvest dates.

Table 6. Nero Glories indices

Vintage/Harvest date	2013			2014		
	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
A1 (mg L^{-1})	888.0±99.7aα	808.5±45.3aα	806.8±40.6aα	607.7±122.4bβ	860.0±61.6aα	439.3±155.8cβ
A3.4 (mg L^{-1})	480.9±19.8aα	404.1±41.1bα	403.7±33.4bα	510.3±46.0aα	546.0±43.5aβ	319.3±56.2bα
CMI%	45.45±4.87aα	49.99±4.84aα	50.02±2.03aα	30.65±18.10aα	36.07±8.64aβ	39.83±9.28aβ
SMI%	74.46±2.91aα	78.08±3.10aα	83.91±0.73bα	82.65±4.23aβ	75.33±5.14aα	83.86±3.59aα

Each value represents the average ± standard error of 3 replicates ($P < 0.05$). Values marked with different Roman letters mean significant differences between harvest dates within the same year. Different Greek letters means significant differences between the years within the same harvest dates.

cultivars, which can have a favourable impact on the release of polyphenols during winemaking because of better cell wall binding (Allegro et al., 2021). This phenomenon has to be taken into consideration during winemaking process (e.g. application of pectolytic enzymes).

3.4. Nero glories indices

Table 6 shows the Glories indices for Nero grapes. Glories indices are used to evaluate the phenolic maturity with the prediction of extractable anthocyanins form grape skins and tannins from seeds (Villangó et al., 2015). Both vintage characteristics and harvest time affected the anthocyanin levels and mobility. In 2013, Nero berries produced higher total amount of red pigments along with lower extractability in the first and third harvest. The third harvest in 2013 predicted higher concentration of extractable anthocyanins compared to the following years last sampling date, but the difference was not significant. The slow extraction rate of polyphenols from seeds was common in both years.

4. CONCLUSIONS

It can be summarised that under the continental climate of Hungary, the most important factor in terms of grape and wine quality is the vintage effect. Alongside with seasonal differences, climate change will also have a powerful effect on grape composition and quality in the near future. Beside these factors, the importance of choosing the optimal harvest date, which can have a big impact both on wine style and quality, should also be mentioned. There is also a need to make further experiments with Nero and Bianca for different vintages, terroirs and climate conditions. Optimising winemaking technologies for interspecific varieties are also a necessity in order to minimalise undesirable sensory characteristics. Further goal is to improve general oenological potential of interspecific wine grapes and, therefore, their overall wine quality.

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