

## EFFECTS OF AGING IN OAK BARRELS ON THE TRANS-RESVERATROL AND ANTHOCYANIN CONCENTRATION OF RED WINES FROM HUNGARY

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Polyphenol compounds in grapes and wines are of paramount importance: they have a key role in determining wine quality, and also the beneficial health effects of moderate red wine consumption are well-known. The polyphenol concentration of wines is determined mostly by: a) their concentration in the grapes and b) the production technology, particularly the time and type of aging. Our goal was to determine the trans-resveratrol and anthocyanin contents of Hungarian red wines under different manufacturing conditions, by monitoring the 24-month aging process – using barrique and oak barrels – with semi-annual sampling, without considering vintage. We have chosen to determine polyphenol components that could originate from either the grapes, or be produced during the wooden barrel aging. Both the aging time and the wine variety had non-negligible effects on the changes in the concentrations of the studied components, whereas the wooden barrel type had not.

**Keywords:** polyphenol, wooden barrel aging, wine technology, Blaufränkisch

### Abbreviations

TP: Total polyphenol concentration

ANT: Anthocyanin

LEU: Leucoanthocyanin

CAT: Catechin

INT: Colour intensity

TON: Colour tone

P% - Polymerization

CPI: *cis*-Piceid

TPI: *trans*-Piceid

CRES: *cis*-Resveratrol

TRES: *trans*-Resveratrol

Polyphenols are one of the most important compound classes in wines, originating mostly from the grape, and to some extent from the barrels used for aging. For wine quality, the most important group is the flavonoid phenols, particularly catechin, leucoanthocyanin, and anthocyanin monomers. Grape is particularly rich in flavonoids, which are capable of

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polymerization and condensation (WATERHOUSE, 2002). Flavonoids are particularly strong antioxidants, and are responsible for the sensory changes and browning of wines as well (SINGLETON & ESAU, 1969). The bitter and astringent taste is determined by catechins and leucoanthocyanines (KENNEDY et al., 2006). Resveratrol is a stilbene-type non-flavonoid phenol. It is mostly present in the grape skin; consequently, the resveratrol content of wines is determined primarily by the applied grape processing technology – pectin-degrading enzyme treatment, CO<sub>2</sub> maceration, application of hyperoxidation, and the size of the press. *trans*-Resveratrol occurs naturally in the grape skin as its glycoside, called piceid. During alcoholic fermentation, the  $\beta$ -glycosidase activity of yeast releases *trans*-resveratrol (BAVARESCO et al., 2016). *trans-cis* rearrangement can occur as a result of the isomerase activity of yeast, while piceid-resveratrol conversion was observed for malolactic fermentation as well (KEITA et al., 2004). On one hand, resveratrol is a floral immune substance, participating in the defence mechanism of the grape against fungal infections (YANG et al., 2009). On the other hand, in humans it has a protective role against cardiovascular diseases, as well as anti-inflammatory, antiviral, antibacterial, and antioxidant properties (KING et al., 2006; KISBENEDEK et al., 2014). As demonstrated by SEIGNEUR and co-workers (1990) and GAUTAM and co-workers (2000), *trans*-resveratrol has an important inhibitory role against leukemic cells, and was found to be effective against certain cancerous conditions (GUERRERO et al., 2009). Resveratrol was observed to inhibit  $\beta$ -amyloid aggregation in Alzheimer's disease (RIVIERE et al., 2010), and to protect neurons from oxidative stress (MIZUTANI et al., 2000).

Our goal was to chemically monitor the aging phases of wine, to observe and quantify the changes in polyphenol concentrations. We have investigated the total polyphenol contents of three different wine varieties from the same region. The wines were produced under manufacturing conditions with controlled fermentation, and aged in different oak barrels. During the 24-month wooden barrel aging, the wines were sampled semi-annually to determine their polyphenol content, without accounting for vintage. The components to be determined were selected to be representative of polyphenols that are introduced either naturally, from the grapes, or during the applied technology, or from the wooden barrel aging. Present study deals with the fine composition of the Kadarka, Kékfrankos (Blaufrankisch), and Cabernet Franc varieties, focusing on the concentrations of *trans*-piceid and *trans*-resveratrol during the two-year wooden barrel aging. Hungarian wines were also analysed from two wine regions, from Villány and Eger, with the highest *trans*-resveratrol contents in Merlot and in Cabernet Sauvignon, and *trans*-piceid content in Blaufrankisch from Villány wine region (MONTSKO et al., 2010).

The aim of the study was to find answers to the following questions: how the total polyphenolic content – and, in particular, the concentration of *trans*-piceid, *trans*-resveratrol, and anthocyanins – changes over the two-year wooden barrel aging; and does the applied wooden barrel type have any effect on the amount of polyphenols over the two-year aging process.

## 1. Materials and methods

Three characteristic wine varieties of the Szekszárd wine region in Southwest Hungary (Kadarka, Kékfrankos, and Cabernet Franc) were studied. The wines have not received any treatment other than racking and basic sulphuring. The wines were sulphured up to 20–25 mg l<sup>-1</sup> of free sulphur concentration. Harvest dates were different for the three varieties: early October for Cabernet Franc, mid-October for Kadarka, and late October for Kékfrankos. For

each variety, healthy base material was processed. Alcoholic fermentation was carried out under controlled conditions for 8–14 days, at 18–24 °C temperature, using UVAFERM BDX variety-specific yeast. Following fermentation, the different varieties (Kadarka, Kékfrankos, and Cabernet Franc) were stored in separate oak and barrique barrels for 24-month aging, while the control samples were stored in steel barrels. Sampling was carried out twice a year – in April 2014, September 2014, April 2015, and September 2015 –, both for the wooden barrel and for the control samples.

Spectrophotometric measurements were carried out on a MOM Spektromom 195 device. The total polyphenol content was determined with the Folin-Ciocalteu phenol reagent and published as gallic acid equivalents (KÁLLAY & TÖRÖK, 1997). Anthocyanin content was measured at 550 nm according to the modified method of FLANZY and co-workers (1969), following dilution with 96 % ethanol, containing 2 v/v% HCl. Leucoanthocyanines were determined spectrophotometrically after heating with a 40/60 mixture of hydrochloric acid and butanol according to the modified method of FLANZY and co-workers (1969). The concentration of both components were expressed in malvidin-3,5-diglycoside equivalents. Catechin content was measured spectrophotometrically at 500 nm, after reaction of the alcohol-diluted wine with vanillin (REBELEIN, 1965). Colour index and colour tone (wine hue) were measured according to the Hungarian standard (MSZ 14848:1979) based on the work of SUDRAUD (1958). Absorbances for colour index and colour tone were measured at 420 nm and 520 nm, respectively. Colour index is calculated as the sum of the absorbances for the two wavelengths, while colour tone is expressed by the ratio of the absorbances. The qualitative and quantitative analyses of resveratrols and anthocyanin monomers were carried out with high-performance liquid chromatography (HP Series 1050) in the research laboratory of the Department of Oenology at Szent István University.

Determination of resveratrols was carried out according to the method described by KÁLLAY and TÖRÖK (1997). After filtration, the wines were injected directly into the HPLC device working in isocratic mode. For filtration, a Sartorius membrane filter with 0.45 µm pore diameter was used. Determination of anthocyanin monomers was carried out according to the method described by KÁLLAY and TUSNÁDY (2001). Similarly to resveratrols, no sample preparation was needed other than micro-filtration. Prior to injection, the wine samples were filtered with a Sartorius membrane filter with 0.45 µm pore diameter. The used solvents (HPLC grade) and chemicals (analytical grade) for the spectroscopic and HPLC measurements were purchased from VWR International (Radnor, PA, USA). The applied standards were obtained from Sigma-Aldrich group (Saint Louis, MO, USA).

Quantitative results were evaluated with several standard statistical methods, such as correlation statistics and factorial analysis of variance (ANOVA). The latter is based on the pairwise comparison of the average values of the different groups of samples. STATISTICA 13 software (Dell Inc., Tulsa, OK, USA) was used for the analysis.

For all three varieties, 500-litre oak barrels and 225 litre barrique barrels, produced from sessile oak (*Quercus petraea*), were applied for the 24-month aging process. We have used first-load oak barrel for Kadarka, and five-year, fourth-load barrel for Kékfrankos and Cabernet Franc. The barrique barrels were first-load for Kadarka and third-load for Kékfrankos and Cabernet Franc. The oak and barrique barrels used for the aging of Kadarka were produced from two varieties of Hungarian sessile oak (Zemplén and Mecsek) in 50–50% ratio, their stave seasoning has lasted for 36 months and they received toasting afterwards. Those used for Kékfrankos and Cabernet Franc were produced purely from the Zemplén variety, with minimum 24 months of stave seasoning.

## 2. Results and discussion

From the studied varieties, the starting *trans*-resveratrol concentration of Kékfrankos is the highest (this is in agreement with earlier findings). Table 1 summarizes the changes in the concentrations of *trans*-piceid and *trans*-resveratrol over time for the three wine varieties. *trans*-Piceid and *trans*-resveratrol concentrations display an increasing tendency, because of the small extent of sulphuring during the two-year observation. Kékfrankos clearly exhibits higher concentrations in the case of *trans*-resveratrol. Phenolic compounds are introduced into the wine primarily from the grapes and must (while keeping their bioactivities); this can be further promoted by grape processing and alcoholic fermentation. Wooden barrel aging can also increase the starting concentration of resveratrol, as demonstrated by the data for Kadarka and Kékfrankos. In addition, resveratrol can be released from piceid during fermentation and aging.

Table 1. Changes in *trans*-piceid and *trans*-resveratrol concentrations at the four sampling dates for the three varieties

Sample	<i>trans</i> -Piceid concentration changes at the 4 sampling dates (mg l <sup>-1</sup> )				<i>trans</i> -Resveratrol concentration changes at the 4 sampling dates (mg l <sup>-1</sup> )			
	2014. 04.	2014. 09.	2015. 04.	2015. 09.	2014. 04.	2014. 09.	2015. 04.	2015. 09.
Kadarka control	0.4	0.4	0.9	1.41	0.4	n.d.	4.2	3.36
Kadarka oak		0.4	0.8	0.81		0.7	4.5	3.50
Kadarka barrique		0.3	0.7	0.97		0.5	3.0	4.58
Kékfrankos control	0.3	0.8	1.4	1.41	3.5	3.8	5.7	4.69
Kékfrankos oak		0.7	1.5	0.58		1.3	6.3	4.17
Kékfrankos barrique		0.7	1.2	0.60		1.8	4.4	4.60
Cabernet Franc control	0.3	0.2	0.7	1.46	1.3	1.4	0.5	1.14
Cabernet Franc oak		0.3	0.8	1.74		0.3	0.4	4.42
Cabernet Franc barrique		0.3	0.4	0.44		0.3	0.5	0.64

We have calculated the basic statistical properties of polyphenol content and the non-parametric Spearman rank-correlation coefficients between the variables. (The abbreviated variable names are explained in the Abbreviations section.) The basic properties and the correlation coefficients are summarized in Tables 2 and 3, respectively. The higher standard deviations for some variables can be explained with differences between the wine varieties. To proceed, it was necessary to rescale the variables, for which standardization was employed.

There is a significant positive correlation between catechin (CAT) and leucoanthocyanin (LEU) concentrations; these have decreased due to aging, owing to polymerization (Table 3). The decrease of CAT concentration is presumably due to the polymerization of catechin monomers to tannins of various degrees of polymerization (and molecular mass). The decrease of LEU concentration is due to polymerization or oxidative reactions.

Colour tone (TON) and polymerization (P) exhibit a negative correlation: with longer storage times polymerization increases, while colour tone decreases or remains unchanged. This can be explained by the polymerization reactions of anthocyanins. The loss of anthocyanins during aging cannot be considered a loss of colour, as most of the anthocyanin monomers polymerize (for red wines receiving months of aging, 30–40% of the colorants are polymers). This quantity can be considered stable in the absence of physical, chemical, or microbiological perturbations. During a change in this equilibrium, the polymerization degree might increase.

Table 2. Basic statistical properties of the variables

Features	Valid <i>N</i>	Mean	Std. Dev.
TP (mg l <sup>-1</sup> )	30	2301	554.5
ANT (mg l <sup>-1</sup> )	30	232.7	92.40
LEU (mg l <sup>-1</sup> )	30	1840	415.5
CAT (mg l <sup>-1</sup> )	30	1466	450.2
INT (Au)	30	36.52	21.73
TON	30	4.349	2.655
P%	30	17.10	25.60
CPI (mg l <sup>-1</sup> )	30	0.262	0.182
TPI (mg l <sup>-1</sup> )	30	0.764	0.439
CRES (mg l <sup>-1</sup> )	30	1.061	0.861
TRES (mg l <sup>-1</sup> )	30	2.515	1.939

Std. Dev.: standard deviation, *N*: number of samples. The full names of the variables can be found in the Abbreviations.

Table 3. Spearman rank correlation coefficients

Var.	TP	ANT	LEU	CAT	INT	TON	P%	CPI	TPI	CRES	TRES
TP	1.00	0.15	0.57	0.39	-0.42	0.01	-0.22	0.51	0.15	0.31	0.29
ANT	0.15	1.00	0.24	0.45	0.27	0.54	-0.66	-0.27	-0.34	-0.06	0.13
LEU	0.57	0.24	<b>1.00</b>	<b>0.73</b>	-0.39	-0.09	-0.29	0.20	-0.05	0.16	0.26
CAT	0.39	0.45	<b>0.73</b>	<b>1.00</b>	-0.31	-0.07	-0.18	0.07	-0.05	0.29	0.34
INT	-0.42	0.27	-0.39	-0.31	1.00	0.51	-0.40	-0.28	-0.24	-0.26	-0.34
TON	0.01	0.54	-0.09	-0.07	0.51	<b>1.00</b>	<b>-0.74</b>	-0.07	-0.27	-0.40	-0.35
P%	-0.22	-0.66	-0.29	-0.18	-0.40	<b>-0.74</b>	<b>1.00</b>	0.17	0.41	0.41	0.28
CPI	0.51	-0.27	0.20	0.07	-0.28	-0.07	0.17	1.00	0.59	0.59	0.44
TPI	0.15	-0.34	-0.05	-0.05	-0.24	-0.27	0.41	0.59	<b>1.00</b>	<b>0.72</b>	<b>0.64</b>
CRES	0.31	-0.06	0.16	0.29	-0.26	-0.40	0.41	0.59	<b>0.72</b>	<b>1.00</b>	<b>0.86</b>
TRES	0.29	0.13	0.26	0.34	-0.34	-0.35	0.28	0.44	<b>0.64</b>	<b>0.86</b>	<b>1.00</b>

Notes: The range of the coefficients is between -1 and +1. The most correlated variables are marked with a bold font.

Based on the correlation coefficients, there is a strong correlation between *trans*-piceid (TPI) and *cis*-resveratrol (CRES) and between *cis*-resveratrol and *trans*-resveratrol (TRES). Concentrations of the less stable *cis*-resveratrol were much lower than *trans*-resveratrol, but for both an increase in concentration could be observed at the beginning, followed by a gradual decrease at the end of the aging process.

We have applied factorial analysis of variance (ANOVA) to the standardized variables. The dataset contained a total of 11 dependent variables (measured values) and two factors (wine variety, barrel type). Effects of the two factors were analysed together and separately. Based on the results in Suppl. Material Table S1, barrel type (oak, barrique, and control) did not result in statistically significant difference ( $\alpha=0.05$  in each case), unlike wine variety. In addition, the two factors together did not convey a significant difference, either. Supplementary material is available online at <https://akademai.com/doi/suppl/10.1556/066.2019.0004>.

Figure 1 presents an illustrative example of the similarity of the concentrations (in this case that of *trans*-resveratrol), when plotted against barrel types. Barrel type 1 was the initial sample. The *trans*-resveratrol concentration of Kadarka has barely changed compared to the starting sample, and the two other varieties did not display significant differences between barrel types either (including the steel barrel control samples). The markers on Figure 1 denote the average values in each case.

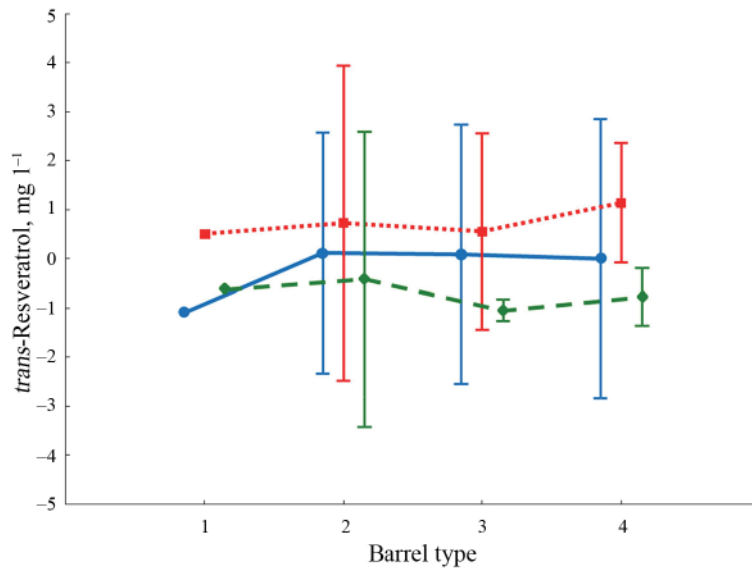


Fig. 1. Factorial ANOVA of *trans*-resveratrol with the factors of barrel type and wine varieties. Barrel types: 1: basic sample; 2: oak barrel; 3: barrique barrel, 4: control. Kadarka is marked with (blue) continuous line, Kékfrankos is marked with a (red) dotted line and Cabernet Franc is marked with a (green) dashed line. Vertical bars denote 0.95 confidence intervals

—●—: Kadarka; —■—: Kékfrankos; —◆—: Cabernet Franc

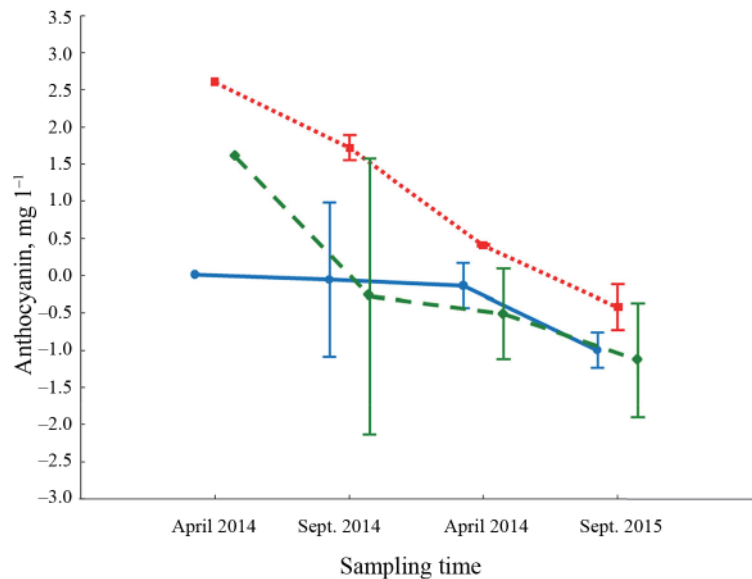


Fig. 2. Factorial ANOVA with the factors of sampling time and wine variety for anthocyanin concentrations. The semiannual measurements are denoted starting with April 2014 and ending with Sept. 2015. Kadarka is marked with (blue) continuous line, Kékfrankos is marked with (red) dotted line and Cabernet Franc is marked with (green) dashed line. Vertical bars denote 0.95 confidence intervals

—●—: Kadarka; —■—: Kékfrankos; —◆—: Cabernet Franc

On the other hand, there is a significant difference at the  $\alpha=0.05$  level based on measurement time (sampling time) and wine variety, as summarized by the factorial ANOVA results in Suppl. Material Table S2.

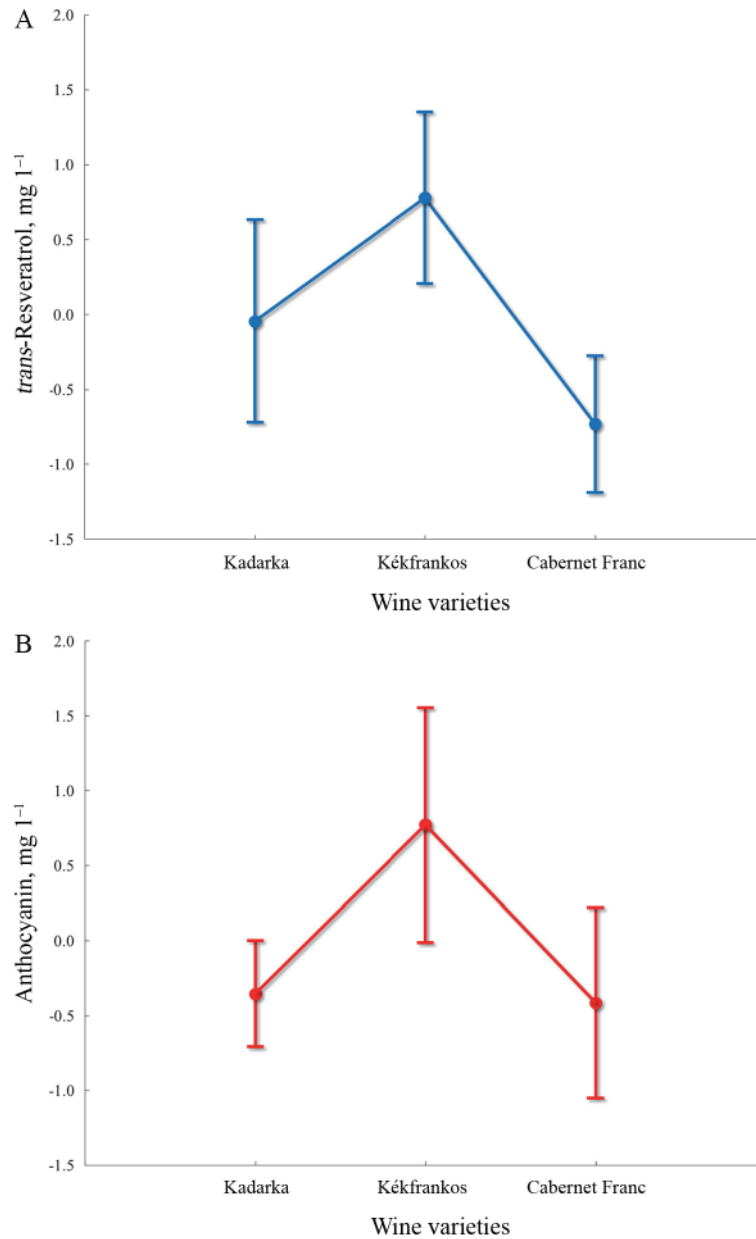


Fig. 3. Factorial ANOVA for the different wine varieties based on A: *trans*-resveratrol and B: anthocyanin concentrations. Vertical bars denote 0.95 confidence intervals



Figure 2 illustrates the decrease of anthocyanin concentration over time for the different varieties. This decrease can be considered specific and characteristic in comparison to the other variables. On the other hand, anthocyanin concentrations have decreased due to the long wooden barrel aging process and the extensive polymerization of polyphenols.

This result prompted us to investigate the *trans*-resveratrol and anthocyanin concentrations of the Kékfrankos variety separately, compared to the other two varieties, as shown in Figure 3AB. It is clear that Kékfrankos displays statistically significantly higher *trans*-resveratrol and anthocyanin concentrations compared to the other two varieties. Despite the fact that we have not accounted for vintage in this study, our results are in agreement with those of NIKFARDJAM and co-workers, 2006, in particular that the variety is a prime determinant of the concentrations of *trans*-resveratrol and anthocyanin. On the other hand, the *trans*-resveratrol content can be influenced by the harvest date as well, based on the work of GEANA and co-workers (2015).

### 3. Conclusions

The effects of manufacturing conditions, particularly the type and length of wooden barrel aging were examined for three wine varieties (Kadarka, Kékfrankos, and Cabernet Franc). The varieties have displayed different starting polyphenol concentrations. After aging, anthocyanin concentrations have decreased due to the long wooden barrel aging process, and the extensive polymerization of polyphenols. From the three varieties, Kékfrankos displayed the highest initial concentration of anthocyanin. *Trans*-resveratrol was also, on average, a more prominent constituent in this variety. Our findings are in good agreement with earlier results. Based on statistical analysis, the type and size of oak and barrique barrels used for the aging process did not have a significant effect on the changes in polyphenol concentrations.

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