

INFLUENCE OF 4 YEARS OF AGEING ON SOME PHENOLIC COMPOUNDS IN RED WINES

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Most of red wine's health benefits are attributed to polyphenols, which can express different biological effects. During ageing process, numerous chemical reactions occur, alternating phenolic composition of wine. Therefore, this paper focused on the influence of ageing on the evolution of wine phenolics (phenolic acids, flavonoids, stilbenes, and anthocyanins). Wines from 4 local grape varieties (Frankovka, Portugieser, Probus, and Rumenika) were analysed after 1 month and 4 years of ageing. Monomeric anthocyanin and total phenolic contents and antioxidative potential in aged wines were also determined. Among tested phenolics, main components in aged wines remained gallic acid (2.16–49.55 mg l⁻¹) and catechin (8.39–37.21 mg l⁻¹). The most prominent changes of analysed phenolic compounds during ageing were observed for *p*-coumaric acid (from +173.3% to +763.1%) and malvidin-3-glucoside (from +173.3% to +763.1%). Wine from Rumenika variety maintained the highest content of individual phenols after ageing. Additionally, aged wines had very low content of monomeric anthocyanins. Significant correlation ($r = -0.93$, $P < 0.05$) between total phenolic content and IC₅₀ values in aged wines was also noticed. Obtained results provide useful information about the quality preservation during aging and storage of these products.

Keywords: polyphenols, wine ageing, anthocyanins, gallic acid, catechin

Red wine is a rich source of polyphenols, and it is suggested that these compounds may play a significant role in health benefits of moderate wine consumption. Some of them include decreased cardiovascular mortality, increased bone mineral density, and influence on inflammation mediated chronic disorders (CVEJIĆ HOGERVORST et al., 2018). It is recognized that the effects of phenols intake are more pronounced when they are consumed through diet because of the possible synergistic interactions.

The final phenolic content in wine depends on many factors including grape variety and winemaking technology applied (CVEJIĆ & ATANACKOVIĆ, 2015). Also, the phenolic composition of wine alternates during wine ageing process. Namely, most of the phenolic compounds are highly unstable, and therefore take part in chemical reactions such as condensations, enzymatic and chemical oxidation, polymerization, and copigmentation. These reactions lead to modifications of phenolic profile and antioxidant potential of wine (GINJOM et al., 2010). Also, the evolution of wine colour during ageing is mainly associated with anthocyanin copigmentation and polymerisation, while aged wine astringency is attributed to the participation of flavan-3-ols in the copigmentation reactions (ATANASOVA et

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al., 2002). One of the important grape growing regions in Serbia is located in its northern province, Vojvodina. Besides well-known red grape varieties (Merlot, Cabernet Sauvignon, Cabernet franc, etc.) some local varieties like Probus (cross of Kadarka and Cabernet Sauvignon) and Rumenika (cross of Kadarka and Teran) are also produced here. This region is also recognised for cultivation of central European varieties like Frankovka (Blafränkisch) and Portugizer (Portugieser), and there is no available data about the ageing of the wines from these local varieties.

Considering that wines are usually consumed after reaching appropriate level of maturity, the aim of this study was to examine the influence of aging on wine phenolics by comparing their content in samples aged 4 years with those obtained for the same 1 month old wines. Additionally, total phenolic and monomeric anthocyanin contents, as well as antioxidative capacity in aged wines were determined.

1. Materials and methods

1.1. Samples – grape and wines

The research was carried out with Probus, Frankovka (Blafränkisch), Portugizer (Portugieser), and Rumenika grapes grown in Sremski Karlovci, in the experimental vineyards of Faculty of Agriculture – Institute of Viticulture (University of Novi Sad, Serbia), additional samples of Portugieser and Probus were obtained from Bajlo and Mrdjanin vineyards, respectively.

The grape was hand-harvested at technological maturity in 2011. Processing under laboratory conditions was carried out immediately after harvest. Destemmed and crushed grapes were homogenised, and 7 kg of pomace was transferred into 10 litre casks for each microvinification experiment. Sulphur dioxide (in form of $K_2S_2O_5$) was added at a rate of 50 mg l⁻¹. Maceration and fermentation was conducted using open vessels with “floating cap”. The cap was punched down by hand three times daily. Alcoholic fermentation was conducted by use of commercial yeast *Saccharomyces cerevisiae* (Anchor WE372, South Africa), which was rehydrated and inoculated into the pomace (0.25 g kg⁻¹). Microvinification was carried out at 25 °C for 10 days, after which pomace was pressed and wine was left for completion of fermentation. Wine was racked after 10 days, and then poured into 500 ml glass bottles at room temperature, and sealed with screw cap closures. Concentration of free sulphur dioxide was previously set at about 30 mg l⁻¹ of wine. The storage of wine samples during ageing was performed in the absence of light at 10±2 °C. Following marks were used to denote wines: FI – Frankovka (Blafränkisch) from the Institute, OI - Portugizer from the Institute, OB - Portugizer from Bajlo, PI – Probus from the Institute, PM – Probus from Mrdjanin, and RI – Rumenika from the Institute. Analysis of individual phenolic compounds and anthocyanins was performed on the 1-month old wine samples (six in total, two samples analysed in this study, other data previously published by the same authors) and compared with the same six samples after 4 years of ageing (all analysed in this study).

1.2. Spectroscopic analysis

Spectroscopic measurements were performed on Agilent 8453 UV-Visible spectrophotometer (Germany). All samples were filtered through a membrane filter (0.45 µm) and the analyses were performed in triplicate.

1.2.1. Total phenolic content. Total phenolic content was determined by the Folin–Ciocalteu method (WORARATPHOKA et al., 2007).

1.2.2. The radical scavenging activity. The radical scavenging activities were evaluated after the reaction with DPPH, using the modified method proposed by SOLER-RIVAS and co-workers (2000).

1.2.3. Total monomeric anthocyanins. Total monomeric anthocyanins were determined by pH differential method described by LEE and co-workers (2005).

1.3. HPLC analysis

HPLC analyses were conducted using an Agilent 1100 Series liquid chromatograph (USA) with UV/DAD detector. Samples were filtered after opening (0.45 µm) and directly injected. All measurements were done in triplicate. Chromatographic separation of 16 phenolic compounds was conducted on Poroshell 120 EC-C18 (4.6 × 100 mm, 2.7 µm) column with gradient elution program. Mobile phase was distilled water with 0.1% glacial acetic acid and acetonitrile with 0.1% glacial acetic acid (CVEJĆ et al., 2016). Five anthocyanins were determined according to OIV (2013).

1.4. Statistical analysis

The data were analysed statistically using Excel for Mac 2016 and the Origin 8.1 program (ANOVA). The differences were considered significant at level 0.05.

2. Results and discussion

In this study, effects of aging were examined with focus on phenolics, which are generally present in considerable amounts in wine (gallic, syringic, vanillic, caffeic, and *p*-coumaric acid; catechin, quercetin, resveratrol; malvidine-3-glucoside).

2.1. Effect of aging on the phenolic acid content

Gallic acid, the phenolic acid present in the highest concentration in young and aged wine samples, showed decrease in Portugieser varieties, while obvious increase was recorded for Probus, Rumenika, and Frankovka (Fig. 1). Reduction of gallic acid content during ageing is noticed in several studies (PROESTOS et al., 2005; YILDIRIM & ALTINDIŞLI, 2015; STAVIDROU et al., 2016) and has been attributed to oxidation and polymerisation reactions, which lead to formation of other forms – quinones, procyanidines, and tannins. On the other hand, the slight increase noticed in both samples from Probus variety could be due to hydrolysis of corresponding complexes of this compound and e.g. anthocyanins. Also, gallic tannins can be hydrolysed to gallic acid, particularly during the aging process (STAVIDROU et al., 2016). Concerning other hydroxybenzoic acids, syringic acid content had relatively high values in all analysed samples. Also, this compound obviously increased in almost all varieties (Table 1). On the other hand, vanillic acid behaviour during aging differed among grape varieties, but drastic changes were not noticeable (Fig. 1, Table 1). Vanillic and syringic acid are found in grapes in the form of complexes with anthocyanins. During ageing, with

decomposition of these complexes, an increase can be observed as noticed by STAVIDROU and co-workers (2016).

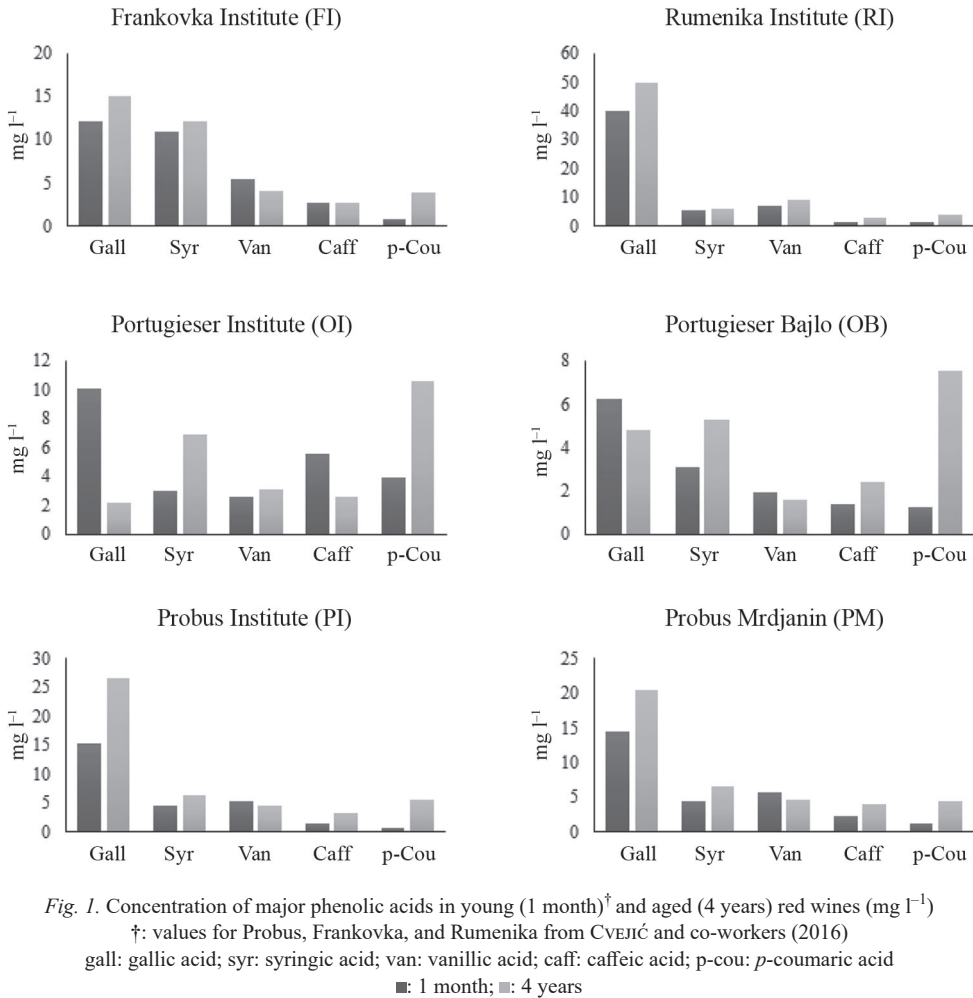


Fig. 1. Concentration of major phenolic acids in young (1 month)[†] and aged (4 years) red wines (mg l⁻¹)
[†]: values for Probus, Frankovka, and Rumenika from CVEJIĆ and co-workers (2016)

gall: gallic acid; syr: syringic acid; van: vanillic acid; caff: caffeic acid; p-cou: *p*-coumaric acid

Among analysed hydroxycinnamic acids, influence of aging was the most pronounced for *p*-coumaric acid, where obvious increase of content was observed for all analysed samples (Table 1). Similar effect is also noticed in bottle aged wines from Greece (STAVIDROU et al., 2016), Turkey (YILDIRIM & ALTINDIŞLI, 2015), Spain (GARCIA FALCON et al., 2007), and Brasil (FERREIRA-LIMA et al., 2016). Also, AGAZZI and co-workers (2018) confirmed that aging (5 years) increases concentration of *p*-coumaric acid. This phenomenon has been attributed mainly to the hydrolysis of coumaric acid during aging process. Similarly, hydrolysis of caftaric acid can lead to increase of caffeic acid. Also, increase of *p*-coumaric acid can occur due to degradation of coumaryl anthocyanins during storage of the wines (GARCIA-FALCON et al., 2007). On the other hand, some researchers observed that *p*-coumaric acid content declined during aging, due to its participation in oxidation and polymerisation reactions

(PROESTOS et al., 2005). Concerning varieties, it is noticeable that variety Rumenika had the highest phenolic acid content in young as well as aged wine with gallic acid dominantly present (Table S1, Fig. 1). Supplement containing Tables S1–S4 are available in the online version.

Table 1. Percentage change of the phenolic compounds content from young (1 month) to aged (4 years) wines

Compound (%) [‡]	FI [†]	OI	OB	PI	PM	RI
Gallic acid	+22.9	−78.7	−23.2	+74.2	+42.1	+23.6
Syringic acid	+10.9	+126.7	+69.1	+40.89	+47.5	+3.9
Vanillic acid	−24.3	+21.7	−16.2	−16.2	−17.5	+31.6
Caffeic acid	−0.75	−53.9	+70.7	+137.4	+67.8	+119.2
<i>p</i> -Coumaric acid	+350.0	+173.3	+508.9	+763.1	+267.2	+135.2
Catechin	−38.3	−38.4	−34.1	+60.0	53.3	−38.4
Quercetin	−19.2	−29.3	−57.4	+46.2	−4.6	+39.4
Resveratrol	−75.2	−42.4	−44.6	−10.7	−64.1	−52.6
Mvd-3-glu	−99.1	−97.9	−97.7	−97.6	−98.6	−99.7

† FI: Frankovka Institute; OI: Portugieser Institute; OB: Portugieser Bajlo; PB: Probus Institute; PM: Probus Mrdjanin, RI: Rumenika Institute

‡: Percentage change was calculated as follows: $\% = (C4 - C1) / C1 \times 100$, where C1 and C4 are concentrations at 1 month and 4 years, respectively

Mvd-3-glu: malvidine-3-glucoside

2.2. Effect of aging on the flavonoid and stilbene content

Among analysed flavonoids, catechin was the most abundant in young as well as in aged wine samples (Fig. 2).

It is shown in Table 1 that catechin content increased in Probus wine, while for other varieties decrease can be observed. Similar observation was made by STAVIDROU and co-workers (2016), where decrease of catechin during ageing occurred for Merlot and Syrah, while for Cabernet Sauvignon an increase was detected. Mentioned decrease can be attributed to oxidation and polymerisation reactions, since catechin and other flavan-3-ols may participate in procyanidin condensation and, combined with anthocyanins, generate polymeric pigments, consequently affecting wine colour. On the other hand, proanthocyanidins may release free flavan-3-ols. *Trans*-resveratrol content in wine declined in all samples (Fig. 2, Table 1). GAO and co-workers (2015) reported similar reduction in resveratrol concentration together with accumulation of polymeric pigments.

Concerning the amount of quercetin, in Portugieser, Frankovka, and Probus Mrdjanin decrease can be observed, while for Probus Institute and Rumenika, the contents of this compound are similar or slightly higher as in aged wines (Fig. 2, Table 1). Increase of quercetin aglycon concentration during bottle storage of wine is mainly attributed to the hydrolysis of the corresponding glucosides. Concerning varieties, Rumenika variety had the highest content of catechin, and in this variety the highest concentrations of total analysed flavonoids and stilbene were determined in both young and aged samples (Fig. 2). Frankovka was the only wine variety in which all tested compounds were quantified (Table S2).

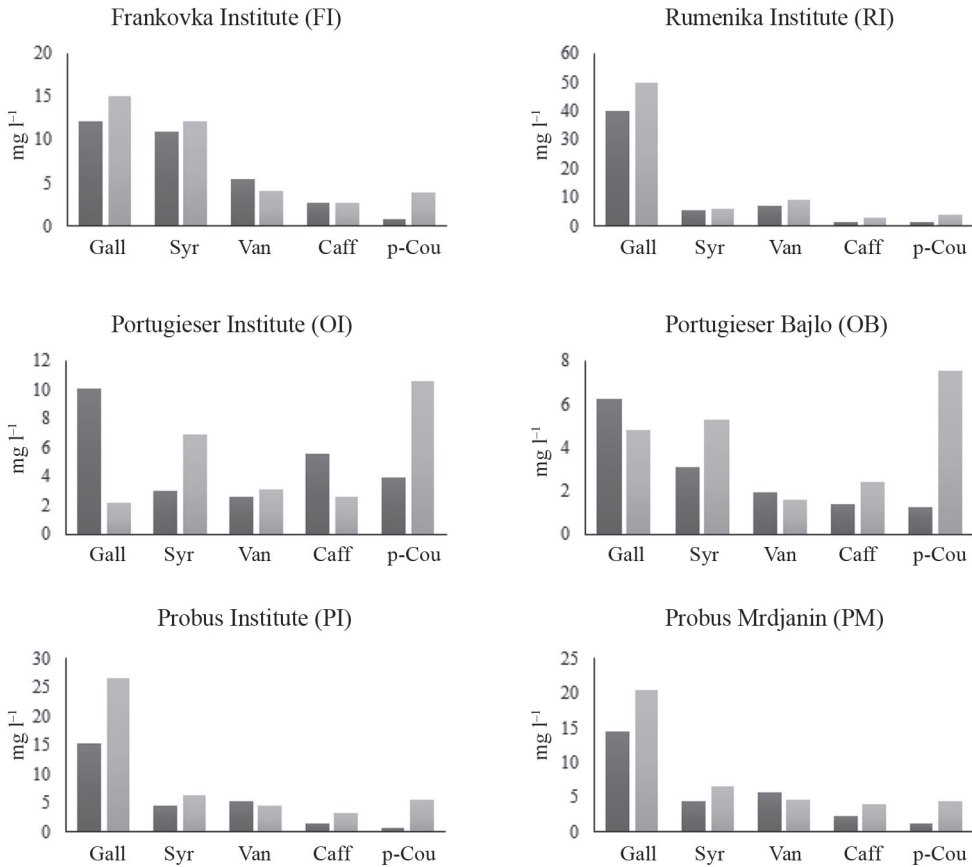


Fig. 2. Concentration of major flavonoids and resveratrol in young (1 month)[†] and aged (4 years) red wines (mg l⁻¹)
[†]: values for Probus, Frankovka, and Rumenika from CVEJIĆ and co-workers (2016)
 ■: 1 month; ■: 4 years

2.3. Effect of aging on the individual anthocyanin content

Obtained results showed that in aged wines only malvidine-3-glucoside (from five analysed individual anthocyanins) was present (Table S3). In young wines, malvidine-3-glucoside was strongly dominant, while other anthocyanins were present in much lower amounts – delphinidin-3-glu 2.3–16.8; cyanidin-3-glu 1.0–1.7, petunidin-3-glu 7.2–25.3, and peonidin-3-glu 2.9–7.3 mg l⁻¹ in PI, PM, FI, and RI samples (CVEJIĆ et al., 2016), and petunidin-3-glu in OI-1.6 mg l⁻¹; OB-1.1 mg l⁻¹ and peonidin-3-glu in OI-1.4 mg l⁻¹ samples. In aged wines, malvidine-3-glucoside is quantified in all samples with the average value of 2.1 mg l⁻¹ (0.70–4.65 mg l⁻¹).

Obvious decrease in malvidin-3-glucoside concentration (over 97%, Table 1) was observed in all aged samples, confirming earlier findings that anthocyanins show constant reduction of concentration during aging period (GAO et al., 2015). Similar observation was made for 5-year-old Mendoza wines, where decrease of anthocyanins was up to 96% (AGAZZI et al., 2018).

2.4. Total phenolic and monomeric anthocyanin content and antioxidant potential

Obtained values for the total phenolic content and antioxidant potential in aged wines are generally in accordance with previously published results for wines from Serbia (ATANACKOVIĆ et al., 2012; MITIĆ et al., 2014) (Table S4). Statistical analysis showed that based on the TPC values, samples are grouped according to grape variety, while for IC_{50} , this kind of grouping is not obvious. Total monomeric anthocyanin content was between 9.79 and 80.71 mg l⁻¹ malvidine-3-glucoside equivalents (Table S4) with the average value of 32.39 mg l⁻¹. Similar low values were published for Bulgarian aged wines Gamza (2–7 years old), where polymeric pigment almost totally replaced monomeric anthocyanins during ageing (TSANOVA-SAVOVA et al., 2002). Spectrophotometrically determined content of monomeric anthocyanins is obviously higher than the content determined by the HPLC method, which could be due to formation of acylated and coumaroylated derivatives of individual anthocyanins, as reported in wines from Spain (PEREZ-TRUJILLO et al., 2011).

Linear correlation test showed significant ($P=0.0043$, $r=-0.930$) negative correlation between total phenolic content and IC_{50} values. Significant correlations between concentration of individual phenolics and IC_{50} values were not noticed. Previous studies reported that correlation of individual phenolic compounds with antioxidant capacity could depend on the grape variety as well as the antioxidant test used. Additionally, total monomeric anthocyanin content exhibited strong correlation with total phenolic content of analysed wine samples ($r=0.873$, $P=0.00045$).

3. Conclusions

The presented study showed that gallic acid and catechin remained main components in young as well as in aged wine. Trends in changes of phenolic compounds content was mainly influenced by grape variety. Generally, consistent influence of ageing was noticed for *p*-coumaric acid and malvidin-3-glycoside, as their content in all samples significantly increased and decreased, respectively. Furthermore, decrease of resveratrol content was observed in all analysed samples. Although some variations in individual phenolic compound contents are noticed, in general, after 4 years of ageing, the total content of wine phenolics (with exception of malvidine-3-glucoside) did not change dramatically. Also, it can be noticed that in aged wines, as well as in young samples, Rumenika wine remained the sample with the highest content of detected phenols.

Supplement containing Tables S1-S4 is available in the online version.

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