Polyphenolic composition of grape stems

Bozena PRUSOVA*, Josef LICEK, Michal KUMSTA, Mojmir BARON, Jiri SOCHOR

Mendel University in Brno, Department of Viticulture and Enology, Valticka 337, CZ-691 44, Lednice, Czech Republic; prusova.bozena@email.cz (*corresponding author); licek.josef@seznam.cz; michal.kumsta@mendelu.cz; MojmirBaron@seznam.cz; jiri.sochor@mendelu.cz

Abstract

This study is focused on the study of polyphenolic compounds in grape stems as by-product of winemaking industry. Two white varieties of Grüner Veltliner and Sauvignon and two red varieties of Blauer Portugieser and Cabernet Moravia were selected for the study. Antioxidant activity, concentration of total polyphenols and concentration of individual phenolic compounds were determined. The results show a higher concentration of polyphenols and higher values of antioxidant activity in red varieties. The Blauer Portugieser variety contained the highest concentrations of syringic acid 1.346 mg.L\(^{-1}\), caffeic acid 20 mg.L\(^{-1}\), ferulic acid 1.192 mg.L\(^{-1}\), coumaric acid 3.231 mg.L\(^{-1}\), trans-resveratrol 14.195 mg.L\(^{-1}\), catechin 79.314 mg.L\(^{-1}\) and epicatechin 33.205 mg.L\(^{-1}\). Cabernet Moravia contained the highest concentration of protocatechuic acid 1.201 mg.L\(^{-1}\), the Sauvignon variety reached the highest concentration of gallic acid 4.015 mg.L\(^{-1}\) and hydroxybenzoic acid 0.076 mg.L\(^{-1}\). The highest values of alpha-amino acids were determined in the Blauer Portugieser variety 165.3 mg L\(^{-1}\) and the lowest in the Grüner Veltliner variety 33.3 mg L\(^{-1}\). The highest concentration of ammonia nitrogen was 214 mg L\(^{-1}\) for the Blauer Portugieser variety and the lowest concentration of ammonia nitrogen was measured in Cabernet Moravia 35.7 mg L\(^{-1}\).

Keywords: antioxidant activity; grape stems; polyphenols; winemaking by-products

Introduction

Grape polyphenolics vary in chemical structure and activity and may be fundamentally categorized into two major classes: flavonoids and nonflavonoids. Flavonoids, the most abundant polyphenolics in grape, are distributed throughout the peel, seed, and stem, and include anthocyanins, proanthocyanidins (procyanidins and prodelphinidins), and flavan-3-ols (Garrido-Banuelos et al., 2019). In contrast, hydroxycinnamic acids, the most abundant non-flavonoids in wine, include caftaric acid and coutaric acid (Lu and Foo, 1999). Most of these polyphenolic compounds occur as glycosylated derivatives in plants and foods and undergo enzymatic transformations in the gut before intestinal absorption (Bang et al., 2015). In vinification, bioactive polyphenolic compounds are partially extracted while the majority remain as glycosides embedded in the grape peel, pulp, or seed (Chafet et al., 2005). Additionally, the amount of polyphenols released into the final wine product greatly depends on the fermentation process, suggesting that an insufficient extraction technique
prevents the liberation of phytochemicals that are essentially confined in grape cell walls and pulp cell vacuoles (Yacco et al., 2016).

For this reason, the waste from wine production is also an important source of phenolic compounds with non-negligible antioxidant capacity (Dineiro García et al., 2009). Grape stems consist of polyphenols: flavonoids, stilbenoids and proanthocyanidins (Makris et al., 2008; Karvela et al., 2009), and thus are considered to be a significant source of antioxidants (Anastasiadi et al., 2009).

Investigation into the application of antioxidants, such as polyphenols, has been increasing due to their health benefits (Di Donato et al., 2017). In recent years, special attention has been given to the isolation of natural compounds from waste materials of the food and wine industry and their reuse or conversion into new products (Maier et al., 2009). Bioactive compounds, such as polyphenols, can be obtained from grape stems by using different extraction methods. Optimal conditions for the extraction, including the type of solvent, process duration and temperature, determine the performance and quality of the obtained compounds (Pintać et al., 2018).

In recent years, new technologies for more efficient and environmentally friendly extraction, such as the use of ultrasound, microwaves and pulsed-electric fields, have been studied. Most of them are expensive, difficult to scale up for larger amounts of extracted material or energy intensive (Okolie et al., 2019). Eco-friendly technologies, such as hydrothermal treatment, have some advantages due to the absence of organic solvents and related corrosion problems, as well as being easy to operate and cost effective (Sepúlveda et al., 2018). Moreover, some studies reported higher bioactive content after hot-water extractions compared to solvent extraction (Kabir et al., 2015).

On a laboratory scale and for a smaller volume of studied material, classical extraction methods using organic solvents are still used. Many studies have used response surface methodologies (RSM) to optimise polyphenolic extraction conditions. However, the extraction conditions for RSM are generally limiting for scale-up and industrial applications, and the polyphenolic content is usually evaluated by generic methods such as total phenolic, flavonoid, tannin, total flavone content and antioxidant/antiradical assays (Di Donato et al., 2017).

The major goal of the present study was to characterise individual polyphenolic compounds in grape stalks in white and blue grape varieties.

**Materials and Methods**

**Material**

The aim of the study was the evaluation of polyphenols as antioxidants in grape stems of the varieties *Vitis vinifera* L., namely the varieties Grüner Veltliner, Sauvignon blanc, Blauer Portugieser and Cabernet Moravia M-43. The material was obtained from the Rozlinky (Škvice) vineyard in the Czech Republic. The age of all the vines from which the stems come is in the range of 10-12 years. The basic analytical parameters are in Table 1.

**Table 1. Basic analytical parameters of grape must**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Date of harvest</th>
<th>Sugar content [°NM]</th>
<th>pH</th>
<th>Total acidity [g.L(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gruner Veltliner</td>
<td>1.10.2019</td>
<td>21,50</td>
<td>3.28</td>
<td>7.80</td>
</tr>
<tr>
<td>Sauvignon</td>
<td>27.9.2019</td>
<td>22.80</td>
<td>3.15</td>
<td>8.10</td>
</tr>
<tr>
<td>Blauer Portugieser</td>
<td>5.10.2019</td>
<td>23.20</td>
<td>3.55</td>
<td>5.80</td>
</tr>
<tr>
<td>Cabernet Moravia</td>
<td>4.11.2019</td>
<td>23.80</td>
<td>3.68</td>
<td>4.50</td>
</tr>
</tbody>
</table>


**Extraction method**

The stems were crushed and homogenised for 20 seconds. Subsequently, 10 g of this homogenate was weighed and transferred quantitatively to a volumetric flask. Ninety ml of 75% methanol was used for the extraction. The extraction was carried out in the dark and cold on an IKA KS 260 Basic shaker for two hours. 50 µL of 75% sulphur dioxide was added to the sample to prevent oxidation.

Individual spectrophotometric determinations were performed on a MIURA ONE automatic biochemical analyser (I.S.E. S.r.l.; Guidonia, RM, Italy). The samples were centrifuged and diluted 1:10 with water. To ensure objectivity of the results, all samples were measured three times, and results were reported as an average of these measurements. The individual methods were adapted to the analyser used, with incubation at 37 °C and incubation times adapted to the instrument’s operating cycles.

For purposes of HPLC analysis, extracts were centrifuged and diluted 1:10 with 100 mM HClO₄ and directly analysed. To ensure the objectivity of the results, all samples were measured three times, and results were reported as the average of these measurements.

**Antioxidant activity**

In each variant, the values of the antioxidant activity were observed. The procedure has been described previously (Sochor et al., 2010a). During this procedure, a 150 µL volume of the reagent (0.095 mmol 2,2-diphenyl-1-picrylhydrazyl, DPPH) was incubated with 15 µL of the wine sample. The absorbance was measured at 505 nm for 10 minutes and the output ratio was calculated as a difference between the absorbance values measured at the 10th minute and the 2nd minute of the assay procedure. This determination was performed in triplicate in 2 mL samples. Antiradical activity was determined based on a calibration curve, using allic acid (GA; 10-300 mg.L⁻¹) as a standard. The results are expressed in equivalents of gallic acid (GA).

**Determination of reducing power (FRAP)**

The ferric reducing/antioxidant power (FRAP) method was modified to determine the reduction ability of the ferric ions. The pH of 198 µL of base buffer containing 200 mmol sodium acetate was adjusted to 3.6 with acetic acid. Twelve µL of sample, 20 µL of 20 mM FeCl₃ solution and 20 µL of 10 mmol TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl were added. After 600 seconds, the absorbance at 620 nm was measured. The reducing power was calculated from the calibration curve using gallic acid (GA; 10-300 mg.L⁻¹) as a standard (Pulido et al., 2000).

**Determination of total flavanols**

Total flavanols concentration was determined using a method based on a reaction with p-dimethylaminocinnamaldehyde (DMACA). In contrast to the widely used reaction with vanillin, this method does not interfere with anthocyanins. In addition, it provides greater sensitivity and selectivity.

To 240 µL reagent (0.1% DMACA and 300 mmol HCl in MeOH), 10µL sample was added, with a reaction time of 600 seconds. The absorbance at 620 nm was then measured. The concentration of total flavanols was determined based on a calibration curve using epicatechin as a standard (10-200 mg.L⁻¹). The results are expressed as mg.L⁻¹ equivalents of catechin.

**Determination of alpha-amino nitrogen**

The primary amino groups are derivatised by ophthalaldehyde and N-acetyl-L-cysteine (OPA/NAC) to form isoindoles in the basic medium. These derivates were detected spectrophotometrically at 340 nm. The absorbance was proportional to the amount of primary amino nitrogen in the sample. Yeast non-assimilable amino nitrogen (e.g. acylated or blocked amines, proline and hydroxyproline) and ammonia nitrogen were not detected in this reaction. Therefore, yeast assimilable nitrogen compound (YANC) determination required independent assays of primary amino nitrogen and ammonia nitrogen. The analysis was performed using a Miura one® device (I.S.E. S.r.l. Via Luigi Einaudi, Italy), which is a spectrophotometer equipped with an
Determination of ammonia nitrogen
The enzyme glutamate dehydrogenase (GLDH) catalyses the condensation of ammonia and α-chetoglutarate to L-glutamate with the concomitant oxidation of nicotinamide adenine dinucleotide (NADH)
Equation 1: \( \text{NH}_3 + \alpha\text{-chetoglutarate} + \text{NADH} + H^+ \rightarrow \text{L-Glutamate} + \text{NAD}^+ \)
The oxidation of NADH causes a decrease in absorbance at 340 nm, which is proportional to the amount of ammonia in the sample. The analysis was performed using the Miura one® device equipped with an autosampler as described above. Determination was performed in triplicate in 2 mL samples.

Determination of individual antioxidant components
Acetonitrile (ACN) was HPLC super gradient purified. Catechin, epicatechin, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, gallic acid, syringic acid, p-coumaric acid, trans-resveratrol, trans-piceid, coffee acid, ferulic acid, piceatannol, rutin, myricetin, quercetin, caemferol, isorhamnetin and perchloric acid were obtained from Sigma Chemical Co. (St. Louis, MO). Malvidin 3,5-diglucoside was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ). Other chemicals used were p.a. quality from a local supplier (Lachema, Penta). Cis-resveratrol and cis-piceid were prepared by photoisomerisation from their trans isomers.

Instrumentation:
Bim High Pressure System Shimadzu LC-10A, Controller system: SCL-10 Avp, 2 pumps: LC-10ADvp, Column thermostat with manual injection valve Rheodyne: CTO-10ACvp, DAD detector: SPD-M10Avp, Software: LCsolution

Separation conditions:
Column: Alltech Alltima HP C18 3 µm; 3 x 150 mm, separation temperature: 50 °C, sample injection volume: 20 µl, mobile phase flow rate: 0.9 ml/min, mobile phase A: 15 mM HClO4, mobile phase B: 15 mM HClO4, 80% ACN

Gradient programme:
0.00 min 3% B, 3.00 min 6% B, 15.00 min 24% B, 18.00 min 30% B, 19.50 min 36% B, 21.00 min 48% B, 21.50 min 60% B, 22.00 min 60% B, 22.01 min 0% B, 23.99 min 0% B, 24.00 min 3% B
The total time between two samples was 27 minutes. Data in the range of 200–520 nm were recorded for 24 minutes.

Determination of individual components based on standard calibration curves: catechin; epicatechin (200 nm), vanilla acid; protocatechuic acid; 4-hydroxybenzoic acid (260 nm), gallic acid; syringic acid; cis-piceid; cis-resveratrol (280 nm), p-coumaric acid; trans-piceid; trans-resveratrol (310 nm), coffee acid; ferulic acid and its derivatives; piceatannol (325 nm), anthocyanins (520 nm).
The hydroxycinnamic acid derivatives were calibrated with the basic acids from which they were derived. Anthocyanins were calibrated to malvidin-3,5-diglucoside.

Statistical analysis
Statistical analyses and figures were generated using Excel 2016 software packages (manufactured by Microsoft Office, USA) and Statistica 10 statistical software (Copyright © StatSoft). Differences between means and contribution to the homogenous groups were determined using Fishers least significant differences test (LSD tests), level of significance was \( \alpha = 0.05 \).
**Results and Discussion**

The first step in modern practices of winemaking is de-stemming resulting in a high fraction of waste material constituted by stems. Afterwards, grapes are pressed to obtain must and grape pomace (mainly skins and seeds) with another high fraction of waste. Grape stems constitute from 3 to 6% of the raw matter processed in a winery (Cabanis, 2000). All results are obtained after extraction with methanol and are expressed in mg.L\(^{-1}\) of methanol extract, so values may differ from those from other studies.

**Determination of antioxidant activity**

It has been shown that grapevine extract is a promising alternative to sulphur dioxide, which is used in wineries for the so-called sulphurisation of wine to preserve it. The antioxidant and antimicrobial effects of sulphur dioxide are similar in nature to the effects of phenolic substances extracted from the wine cane. Use of wine cane extracts is not only economically but also ecologically advantageous. An important benefit of this finding could be the partial elimination of the use of sulphur dioxide, which is often associated with the emergence of certain diseases; therefore, quantitative limits for the use of sulphur dioxide have already been introduced in the past (Ruiz-Moreno et al., 2015).

Figure 1 shows the values of antiradical activity by the DPPH method in the following varieties: Grüner Veltliner, Blue Portugieser, Sauvignon and Cabernet Moravia. The figure shows that the lowest values, on average 458 mg.L\(^{-1}\), were measured for the Grüner Veltliner variety. Other values were quite comparable (in the range of 705 mg.L\(^{-1}\) to 888 mg.L\(^{-1}\)).

Results of the determination of reducing power using gallic acid as a standard for the FRAP method are shown on the Figure 2 for the following varieties: Blauer Portugieser variety (775.6 mg.L\(^{-1}\)) and Cabernet Moravia (765.9 mg.L\(^{-1}\)), with lower values obtained for Grüner Veltliner (529 mg.L\(^{-1}\)) and Sauvignon (486.8 mg.L\(^{-1}\)).

**Figure 1.** Values of antioxidant activity (DPPH)

Results of this study show significant contribution of grape stems to total antioxidant activity of grapes. The higher values of antioxidant activity were recorded in the blue varieties: Blue Portugieser and Cabernet Moravia. The lower values were recorded in white varieties. The DPPH and FRAP methods show different results. Due to different extraction methods, extraction solvents or protocols and different grape varieties, the data are not comparable (Sochor et al., 2010b).
The study by Leal et al. (2020) determined antioxidant activity of grape stems of five white grapevine varieties by two different methods, DPPH and ABTS. For both methods, there were significant differences between the samples. The lower antioxidant activity for both assays was $0.63 \pm 0.03$ and $0.37 \pm 0.03$ mmol Trolox g$^{-1}$ dw (dry weight), for ABTS and DPPH, respectively, with higher antioxidant activity for the ABTS method ($1.17 \pm 0.09$ mmol Trolox g$^{-1}$ dw) and for the DPPH method ($0.56 \pm 0.02$ mmol Trolox g$^{-1}$ dw).

A study by Domínguez-Perles (2014) presented lower values of antiradical activity in white varieties (Viosinho) using the ABTS method ($0.049$ mmol Trolox g$^{-1}$ dw). The study was conducted to analyse the effect of extraction conditions on the extraction of total phenolics, flavonoids, ortho-diphenols and anthocyanins as well as to assess the ABTS$\_+$ scavenging capacity, which were considered as response variables, since the antioxidant activity is positively correlated with the polyphenolic content (Anastasiadi et al., 2012).

Results of the study by Domínguez-Perles (2014) suggested that optimal extraction of phenolic molecules may not reflect the highest antioxidant capacity. This may be explained by the potential for aqueous based solvents to contribute to solubilising a larger range of compounds, some of which may have little or no antioxidant activity (Anwar et al., 2013).

The study by Silva et al. (2018) evaluated the antioxidant activity of skins, seeds and stems in the blue varieties, Preto Martinho and Touriga Nacional, using the ABTS and DPPH methods, expressed in ìmol Trolox equivalent. Higher antioxidant capacity was found for the seed extracts, followed by stems and skins. In both varieties, a higher total polyphenols concentration was determined for the seed extracts when compared to the skin and stem extracts, which likely explains the higher antioxidant activity observed for the former.

This is in good agreement with previous studies that evaluated the antioxidant capacity and total polyphenols concentration of several grape varieties, and reported a high correlation between these parameters, pointing to the fact that the antioxidant activity of wines is mainly due to its phenolic compounds (Paixão et al., 2007).

The study by Queiroza et al. (2017) evaluated the radical scavenging capacity of the isolated compounds from the grape stems, using the DPPH and ABTS methods. Higher antioxidant activity was determined in the coloured flavonoids (anthocyanins), malvidin-3-O-glucoside, malvidin-3-O-(6-O-caffeoyl)-glucoside and quercetin-3-O-glucoside.
Determination of total polyphenols

Recently, grapevines have been studied from the point of view of possible sources of polyphenolic substances because they possess antioxidant, antimicrobial and anticancerogenic activity. The total content of phenolic substances (reported in milligrams of gallic acid (GA) per gram of dry sample) in the extracts of the stems is different mainly depending on the variety of Vitis vinifera as well as the type of extract preparation. The influence of the extraction temperature was studied by Wenzel et al. (2015), and the ideal temperature (163 ± 0.9 to 260 ± 1.5 °C) was determined.

Figure 3 shows the determination of total flavanol concentration on the basis of a calibration curve using epicatechin as a standard for the following varieties: Grüner Veltliner, Blauer Portugieser, Sauvignon and Cabernet Moravia. The results show that the highest concentrations of total flavanols were measured in the Blauer Portugieser (498.5 mg.L⁻¹) and Cabernet Moravia (449.4 mg.L⁻¹) varieties. The values for the white varieties of Grüner Veltliner and Sauvignon were lower, as in all previous determinations. The values of catechin equivalent were measured in the Grüner Veltliner variety (183.4 mg.L⁻¹) and in the Sauvignon variety (214.6 mg.L⁻¹).

![Figure 3. Concentration of total flavanols in grape stems expressed as mg.L⁻¹ epicatechine](image)

Llobera and Cañellas (2007) report the total content of phenolic substances extracted from the stems as 116 ± 2 mg GA g⁻¹ dry matter.

Anastasiadi et al. (2012) determined the total phenolic content of 367-587 mg GA g⁻¹ dry matter, using a methanol:water:HCl (90:5:5 v/v) extraction mixture. The same methodology, using shags from other varieties of Vitis vinifera, was chosen by Sahpazidou et al. (2014), which found the total phenolic content of the extracts to be 318-415 mg GA dry matter.

Although the content of polyphenols is associated with more colourful varieties, it turns out that even white varieties are an important source of polyphenols. The phenolic content of the grapes depends mainly on the variety and not on the colour of the grape (Yang et al., 2009).

Leal et al. (2020) studied the phenolic content of white variety grape stem extracts. The total phenol content varied between 94.71 ± 4.65 (Rabigato) and 123.09 ± 5.02 (Malvasia Fina) mg GA g⁻¹ dw. Also, Anastasiadi et al. (2012) reported a lower content of total phenols for the white varieties, Asyrtiko, Aidani and Athiri, with 11.146, 7.220 and 4.808 mg GA g⁻¹ dw, respectively. Furthermore, high values were shown by Sahpazidou et al. (2014), with 372 mg GA g⁻¹ dw for the white variety Assyrtiko. Concerning the ortho-diphenols, this content varied between 80.62 ± 3.69 (Viosinho) and 116.18 ± 2.67 (Malvasia Fina) mg GA g⁻¹.

dw. The study carried out by Domínguez-Perles et al. (2014) showed values of 36.54 mg GA g\(^{-1}\) dw for Viosinho.

These differences in phenolic composition can be explained by the specific characteristics of each variety, by the climate and biotic factors as well as by the viticultural practices (Portu et al., 2018). The growing conditions have a great effect on the phenolic composition of plants. In lower altitude sites, samples of grape stems revealed a higher content of total phenols, ortho-diphenols and flavonoids. The low altitude region is characterised by some stress factors, such as thermal and water stress as well as the Atlantic influence on climate, resulting in abundant rain. This situation can explain the induction of secondary metabolites (Gouvinhas et al., 2020). Despite these differences, grape stems contain a high concentration of phenolics compounds, in some cases, higher than the fruits (grapes) or the other by-products, revealing the importance of this waste as a rich source of phenolic compounds (Teixeira et al., 2014).

The content of polyphenols in wine also depends on the method of vinification. Their concentration can be increased by longer maceration, or in whole bunch winemaking processes (Baron et al., 2017; Sanmartin et al., 2019).

**Determination of yeast assimilable nitrogen**

In addition to phenolic substances, stems are also an important source of nitrogen substances, which serve as nutrition for yeast during fermentation. The amino acid and ammoniacal nitrogen content are shown in Figures 4 and 5. While the blue variety Blauer Portugieser had the highest content, another blue variety, Cabernet Moravia, had the lowest content. The results can be useful in whole-bunch winemaking.

![Figure 4. Concentration of alpha-amino acids](image-url)
Figure 5. Concentration of ammonia nitrogen

Figure 4 shows the determination of alpha-amino acids on the basis of a calibration curve, using glycine as a standard for the Grüner Veltliner, Blue Portugal, Sauvignon and Cabernet Moravia varieties. The highest values were determined for the Blauer Portugieser variety (165.3 mg.L\(^{-1}\)). The second highest results were measured in the Sauvignon variety (55.8 mg.L\(^{-1}\)). The lowest results were measured in the Grüner Veltliner variety (33.3 mg.L\(^{-1}\) on average).

Figure 5 shows the ammonia nitrogen content of the Grüner Veltliner, Blauer Portugieser, Sauvignon and Cabernet Moravia varieties. The highest measured content was 214 mg.L\(^{-1}\) for the Blauer Portugieser variety, with 144.5 mg.L\(^{-1}\) ammoniaal nitrogen for the Grüner Veltliner variety, and 114.3 mg.L\(^{-1}\) for the Sauvignon variety. The lowest content of ammonia nitrogen was measured in Cabernet Moravia (35.7 mg.L\(^{-1}\)).

Sanmartin et al. (2019) studied the impact of co-fermentation on intact grape clusters and stalks. Both chemical and sensory profiles of wines were discussed soon after racking off as well as after 10 months of ageing with particular attention to phenolic and aromatic compounds. Our results show that co-fermentation of intact grape clusters and stalks can be profitably applied in order to improve the nutraceutical features of Syrah wines as well as to emphasise their aromatic expression, thus significantly speeding up their ageing phase.

Determination of individual antioxidant components

The wine industry is one of the most important agro-economic activities in the countries of southern Europe. This results in the production of a large number of by-products, rich in proanthocyanidins, flavonols, hydroxyxymannic acid derivatives and anthocyanins. These data suggest that grape stems are a rich source of healthy phytochemicals that could be used as food for animals (Barros et al., 2014).

The highest content (4.015 mg.L\(^{-1}\)) of gallic acid was found in the Sauvignon variety (Figure 6). The lowest content of gallic acid was in the Blauer Portugieser variety (0.822 mg.L\(^{-1}\)). Figure 7 shows concentration of syringic acid, which was evaluated to be highest in the Blauer Portugieser variety (1.346 mg.L\(^{-1}\)). The lowest concentration of this acid was found in the Sauvignon variety (0.349 mg.L\(^{-1}\)).
Gallic acid in stems of red varieties in different studies ranged in concentration from 0.07-33.00 mg·g\(^{-1}\)·dw, determined by HPLC-DAD (Anastasiadi et al., 2012; Apostolou et al., 2013; Di Lecce et al., 2014). In white varieties, the concentration range was 0.01-0.03 mg·g\(^{-1}\)·dw, determined by HPLC-DAD (Cetin et al., 2011), and 1.05-22.60 μg·g\(^{-1}\)·dw, determined by HPLC-DAD (Anastasiadi et al., 2012; Apostolou et al., 2013). Syringic acid in red varieties was 32.20 mg·g\(^{-1}\)·dw, determined by HPLC-DAD and in white varieties ≤0.10 μg·g\(^{-1}\)·dw, HPLC-DAD (Apostolou et al., 2013). Results depended on extraction techniques and methods of determination.

As with most other acids, the concentration of caffeic acid (Figure 8) was again highest in the Blauer Portugieser variety (20 mg·L\(^{-1}\)). The coumaric acid content of the Grüner Veltliner variety was not higher than 1 mg·L\(^{-1}\) in all three samples tested (Figure 9).
In different studies, in red varieties, caffeic acid was determined to be ≤0.60 mg·g⁻¹·dw (Apostolou et al., 2013); 0.60-1.90 μg·g⁻¹·dw (Cetin et al., 2011). White varieties were determined to be ≤0.05 μg·g⁻¹·dw (Apostolou et al., 2013); 1.00-1.50 mg·g⁻¹·dw (Cetin et al., 2011). The concentration of coumaric acid in red varieties was determined to be 0.04-0.90 mg·g⁻¹·dw (Apostolou et al., 2013); 0.90-2.20 μg·g⁻¹·dw (Cetin et al., 2011), and in white varieties in concentrations of 0.01-0.08 μg·g⁻¹·dw (Apostolou et al., 2013), all determined by HPLC-DAD.

Figure 8. Concentration of caffeic acid

Figure 9. Concentration of coumaric acid

Figure 10 shows the highest concentration of protokatechuic acid was in the Cabernet Moravia variety (1.201 mg.L⁻¹). In the Grüner Veltliner variety, the content of protokatechuic acid was 0.426 mg·dm⁻³. The lowest measured values were again in the Blue Portugal variety (0.253 mg.L⁻¹). The content of 4-hydroxybenzoic acid was evaluated as very low in the stems of the Grüner Veltliner, Blauer Portugieser, Sauvignon and Cabernet Moravia varieties (Figure 11). The highest content of this acid was measured in the Sauvignon variety, on average 0.076 mg.L⁻¹.
Figure 10. Concentration of protocatechuic acid

The higher concentration of trans-resveratrol was in Blauer Portugieser, the lowest concentrations were measured in both green varieties and also in the blue Cabernet Moravia variety (Figure 12). The lowest content of ferulic acid was measured in the Grüner Veltliner variety (0.005 mg.L⁻¹) (Figure 13). Ferulic acid in stems of the red variety was ≤2.50 (mg·g⁻¹·dw HPLC-DAD) (Apostolou et al., 2013).

Trans-resveratrol in stems of red varieties in different studies was determined to be in the range of ≤0.09-124.10 mg·g⁻¹·dw, and in white varieties was ≤0.02 mg·g⁻¹·dw (Cetin et al., 2011; Anastasiadi et al., 2012; Apostolou et al., 2013).

Figure 11. Concentration of hydroxybenzoic acid
The values of catechin were evaluated as the highest by the chromatographic method in comparison with the other substances. Its highest concentration was measured in the Blauer Portugieser variety (Figure 14). The high levels of epicatechin were measured in the Blauer Portugieser variety, and were more than ten times higher than those of the other varieties, Grüner Veltliner, Sauvignon and Cabernet Moravia (Figure 15). The highest content of epicatechine was also in the variety Blue Portugal.
Catechin concentrations in stems of red varieties in different studies using HPLC-DAD or HPLC-UV were determined to be 0.71-85.80 mg·g\(^{-1}\)·dw; 1.24-2.58 μg·g\(^{-1}\)·dw; and 0.12-1.27 mg·g\(^{-1}\)·dw. White variety stem concentrations ranged from 46.50-98.30 μg·g\(^{-1}\)·dw; 0.13-2.89 mg·g\(^{-1}\)·dw; 3.85-18.58 μg·g\(^{-1}\)·dw and 9.30-133.90 mg·g\(^{-1}\)·dw (Cetin et al., 2011; Anastasiadi et al., 2012; Gonzáles-Centeno et al., 2012; Apostolou et al., 2013; Spatafora et al., 2013; Sá et al., 2014).

Epicatechin concentrations in stems of red varieties were determined to be ≤1.00-13.30 mg·g\(^{-1}\)·dw, and ≤0.11 mg·g\(^{-1}\)·dw. White variety stem concentrations ranged from ≤4.00-0.58 μg·g\(^{-1}\)·dw; 0.04-1.13 mg·g\(^{-1}\)·dw and 0.50-5.80 mg·g\(^{-1}\)·dw (Cetin et al., 2011; Anastasiadi et al., 2012; Gonzáles-Centeno et al., 2012; Apostolou et al., 2013; Spatafora et al., 2013; Sá et al., 2014).

**Conclusions**

The result of this study is a comparison of the concentrations of all-important phenolic substances within different grape varieties. Large differences can be observed not only between white and red varieties, but also within red varieties. These results can serve as a basis for further research and experiments with coniferous fermentation. This is exactly what the study according to (Pascual et al., 2016) dealt with, which evaluated the influence of grape seeds and cones on the composition, color and astringency of wine. Grape stems are also a
source of yeast assimilable nitrogen. The concentration of nitrogen in amino acids ranged from 33.3 mg.L⁻¹ in white varieties to 165 mg.L⁻¹ in blue varieties. The concentration of ammonia nitrogen ranged from 35.7 mg.L⁻¹ in the blue variety Cabernet Moravia to 214 mg.L⁻¹ in Blauer Portugieser variety. These concentrations are not negligible and can partially contribute to the total amount of YAN in the case of whole-bunch fermentation.

The results of HPLC analysis showed the highest measured values of the tested substances in the Blauer Portugieser variety. Only the content of 4-hydroxybenzoic acid and gallic acid was highest in the Sauvignon variety, and the highest content of protocatechuic acid was detected in the Cabernet Moravia variety.

**Authors’ Contributions**

BP - determination of antioxidant activity and total flavanols, writing of article; JL - sample preparation, statistical analysis; MK - determination of antioxidants by HPLC; MB - determination of basic analytical parameters, writing of article; JS - head of experiment, supervision of the article.

All authors read and approved the final manuscript.

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**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

**References**


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