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Trans-polydatin and *trans*-resveratrol in grape berries grown under organic and conventional production systems

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Abstract: Resveratrol and polydatin are stilbenes with notable antioxidant and health benefits, making them important compounds in health promotion, grapevine production, and oenology. This study aimed to monitor the *trans* isomers of these compounds in twelve grape varieties (red and white), grown under organic production (OP) and conventional production (CP) systems. A novel high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) method was developed and validated, demonstrating sensitivity and reliability for the quantification of the mentioned stilbenes. The method was applied to real samples, enabling the evaluation of the influence of production systems on the presence of the analysed compounds. The research included some of the most widely cultivated domestic and international varieties in the examined region. The obtained results revealed a significant impact of the production system and grape colour on resveratrol and polydatin content. OP consistently yielded a higher content of both compounds, compared to CP. Furthermore, red varieties showed higher overall stilbene levels than white ones. 'Shiraz' variety was an exception, with high levels of both compounds under CP. All the obtained findings highlight the role of the production system in enhancing bioactive compound content in grapes. The study provides valuable insights for optimising viticulture, supporting plant resilience, and improving the nutritional qualities of grape-derived products.

Keywords: agriculture; chromatography; plant protection; polyphenols; stilbenes; viticulture

The extensive use of chemical agents poses risks to health and the environment, prompting a focus on alternative cultivation systems, including ecological, biodynamic, and organic approaches, which aim to minimise chemical inputs and promote long-term sustainability (Baker et al. 2020). Contemporary viticulture increasingly relies on mineral fertilisers and synthetic pesticides, making good agricultural practices essential for sustainable vineyard management (Vukosavljević et al. 2016). The need for those

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practices is particularly relevant in regions with a rich winemaking tradition, such as Serbia, where viticulture has been established since Roman times. Despite its modest size, Serbia is recognised as a notable wine-producing region, combining traditional approaches with contemporary advancements (Korać et al. 2016). The country's subcontinental climate, numerous rivers, and diverse microclimates create ideal conditions for high-quality grape production. Organic viticulture has recently gained traction in the Balkan region, generally reflecting a global shift toward balanced and eco-friendly agriculture (Vukosavljević et al. 2016). Serbia's geography and climate, to a certain extent, support organic production, as these conditions help control vine diseases naturally. In addition, effective alternatives need to be found to enable the production of grapes of the same quality as conventionally produced, but in a more eco-friendly way (Merot et al. 2020). Within this vine cultivation system, natural compounds such as resveratrol and polydatin play a crucial role in the plant's defence mechanism.

Resveratrol (3,5,4'-trihydroxystilbene) is one of the most studied and popular stilbenes, first isolated in 1939 by the Japanese scientist Takaoka (Pezzuto 2019). It has been proven that resveratrol has benefits for human health and positive effects in the treatment of various human diseases (Pezzuto 2019), while simultaneously playing a crucial role in the plants that synthesise it. Alongside, polydatin (3,4,5-trihydroxystilbene-3-β-monoglucoside) also occurs in grapevines as a natural glycoside of resveratrol and its stable precursor, which may be transformed into resveratrol by enzymatic hydrolysis or fermentation (Basholli-Salihu et al. 2016).

These two compounds are present in at least 72 plant species, with grapevine ranking as their most important source in the human diet. Both compounds protect the grapevine as phytoalexins, chemicals generated in response to stressful conditions or pathogen infections, which help in the plant's resistance (Song et al. 2021). Their combined presence is essential to the plant's immune system, while their concentration in grapes differs based on variety, genotype, ripening stage, environmental conditions, etc. Given naturally occurring low concentrations, their synthesis can be induced by a plant's exposure to various biotic and abiotic factors. The main biotic factors are causal agents of some of the most economically significant phytopathogenic vine diseases, including *Botrytis cinerea* (Pers.), *Uncinula necator* (Schwein), *Plasmopara viticola* (Berk. and Curt.; Berl. and Toni), *Guignardia bidwellii* (Ellis), etc. In proximity to their infection site, polydatin and resveratrol concentrations increase significantly. Their localised synthesis and accumulation contribute to mitigating the infection to a certain extent, thereby hindering its further spread. Consequently, synthesis and activation are triggered promptly upon detection of the pathogen, often before the manifestation of visible symptoms (Hasan, Bae 2017). Fungicide applications possibly have a dual impact on polydatin and resveratrol synthesis. Specifically, their use may delay the activation of a plant's immune system, resulting in reduced natural synthesis of these compounds (Dugo et al. 2004). Conversely, synthetic fungicides and other agrochemicals can induce chemical stress in plants, potentially stimulating an increase in polydatin and resveratrol synthesis as a part of the plant's defensive response to stress. Given the pivotal role of the mentioned compounds in protective mechanisms, coupled with their generally low concentrations in grapes, for further research, the extraction and accurate quantification of these compounds is crucial.

The aim of this research was to monitor *trans*-resveratrol and *trans*-polydatin in grapes grown under organic production (OP) and conventional production (CP) systems. The content of these compounds is determined for the *trans*-isomers, as they are commercially available and present in greater quantities in grapes compared to *cis*-isomers. The data obtained for each variety from both systems were analysed and compared to evaluate how different agricultural practices affect the synthesis of bioactive compounds, which could be crucial for gaining new knowledge and a better understanding of vine protection and its responses to stressful conditions. The adaptation, development, and validation of a solid-phase extraction (SPE) method for extracting the mentioned compounds from grapes, along with the development of a high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) method for their determination, were necessary.

MATERIAL AND METHODS

Chemicals. For this research, analytical standards of the analysed compounds (*trans*-polydatin, 98%, and *trans*-resveratrol, 98.37%) were purchased from

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Dr Ehrenstorfer (Germany) and Toronto Research Chemicals Inc. (Canada). Other chemicals, such as acetonitrile and deionised water (HPLC purity), were purchased from Fisher Scientific (USA). Methanol, which was also HPLC purity, was from J. T. Baker (Netherlands), and formic acid (HCOOH), which was LC-MS purity, was from Merck KGaA (Germany).

The stock mix solution of analytical standards was prepared by dissolving both of them in 50% (v/v) methanol containing 0.1% (v/v) HCOOH. After that, the concentrations for *trans*-polydatin and *trans*-resveratrol were 519.4 µg/mL and 536.15 µg/mL, respectively. By further diluting the stock solution, a series of working solutions was prepared, with final concentrations ranging from 0.0519 to 103.88 µg/mL for *trans*-polydatin and 0.0536 to 107.23 µg/mL for *trans*-resveratrol. Afterwards, the solutions were stored in the dark at 4 °C to prevent any degradation or isomerisation.

Samples. This research includes international and domestic varieties (Table 1), enabling the analysis of phenolic compounds in different genotypic and geographical contexts. International varieties such as 'Merlot', 'Pinot Noir', 'Cabernet Sauvignon', 'Cabernet Franc', 'Shiraz', 'Muscat Hamburg', and 'Chardonnay' are known for wide cultivation in viticulture and winemaking worldwide. They are often the subject of research for the characteristic phenolic profile and wine quality they provide. Conversely, domestic varieties such as 'Jagoda', 'Bačka', 'Morava', 'Panonia', and 'Tamjanika', which are represented in Serbia and the Balkans, are adapted to specific climatic conditions and exhibit resistance to certain stress factors.

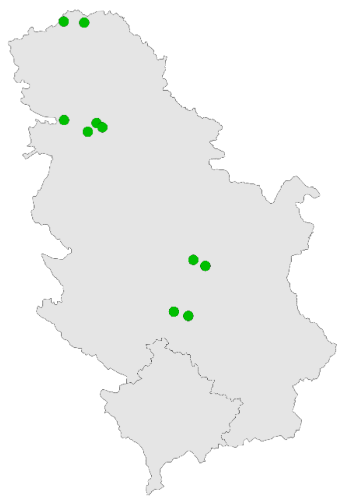

Figure 1. Map of Serbia with pinned vineyard locations where the grapes were sampled

These varieties, especially 'Tamjanika' and 'Morava', have a unique aromatic profile representing an important part of the regional identity in winemaking. In the context of OP and CP, all of the selected varieties allow the examination of the variability of the content of *trans*-resveratrol and *trans*-polydatin.

Grapes were sampled from organic and conventional vineyards during August, September, and October 2023. All varieties were sampled from both cultivation systems, each in four replications, and special attention was paid to ensure that each variety was taken from similar agroecological conditions within OP and CP systems (Figure 1), to reduce the influence of external factors and enable a relevant comparison of examined compounds between the analysed grapes.

Table 1. Grapevine varieties included in this research

No.	Variety	Variety type	Colour	Sampling period
1	Cabernet Franc	international	red	September
2	Cabernet Sauvignon	international	red	October
3	Merlot	international	red	September
4	Pinot Noir	international	red	September
5	Muscat Hamburg	international	red	October
6	Shiraz	international	red	October
7	Morava	domestic	white	August
8	Bačka	domestic	white	August
9	Panonia	domestic	white	August
10	Tamjanika	domestic	white	September
11	Jagoda	domestic	white	September
12	Chardonnay	international	white	September

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Sampling was carried out by winegrowers at the harvesting of each analysed variety. The healthy samples, without visible symptoms of infection, were collected when the grapes reached technological maturity, i.e. when the °Brix values were in the optimal range for each variety, as determined using a refractometer. °Brix values in white varieties ranged from 16 to 22 °Bx, and in red varieties from 19 to 25 °Bx. These ranges correspond to values typically considered to be an appropriate indicator of the moment of harvest, indicating technological maturity (Lytridis et al. 2023). For each variety, samples weighing up to 1 kg were randomly picked, labelled, packed, and stored in the laboratory at –20 °C until further analysis.

Sample preparation and chemical analysis. The 60 g of frozen grape berries were weighed, poured with 180 mL of methanol [0.1 % HCOOH (v/v)], and ground in a laboratory blender (MB 950G mixing mill Kinematica, Kinematica AG, Switzerland). After grinding, the sample was centrifuged (Sigma 2-5, Sigma Laborzentrifugen GmbH, Germany) at room temperature at 2 000 × g for 5 minutes. The Strata^{TM}-X 33 µm Polymeric SPE column (8B-S100-EBJ, Phenomenex, USA) was conditioned with methanol, while equilibration was achieved with 20% (v/v) methanol containing 0.1% HCOOH (v/v). Subsequently, the column was loaded with 3 mL of the supernatant obtained by centrifugation and then washed with 20, 40, and 50% MeOH (v/v) containing 0.1% HCOOH (v/v), respectively, to remove undesirable components. The remaining content on the column was eluted with 4 mL of 80% acetonitrile (0.1% HCOOH) (v/v). The eluent was collected and further analysed using HPLC-DAD. Simultaneous determination of *trans*-polydatin and *trans*-resveratrol in the grape samples was achieved using Agilent 1260 Infinity and Kinetex XB-C18 100Å column (150 mm, 2.1 mm, 2.6 µm) (Phenomenex, USA). Previously, the method has been validated by assessing parameters such as linearity, precision, accuracy, the limit of detection (LOD), and the limit of quantification (LOQ).

Data analysis. The statistical analysis in this study was conducted using Statistica software version 14.0.0.15 (Tibco Software Inc., USA). To evaluate the differences in *trans*-polydatin and *trans*-resveratrol content among various grape varieties and different production systems, a *t*-test, Cohen's *d*, and one-way and two-way ANOVA were conducted, with significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Method validation for *trans*-polydatin and *trans*-resveratrol determination in grapes. To precisely determine polydatin and resveratrol content in plant extracts, chromatography techniques such as HPLC with different detectors are particularly used. HPLC-DAD provides satisfactory sensitivity and selectivity for analysing these compounds in plant extracts (Tzanova, Peeva 2018). The method is favoured due to its simplicity, ease of application, and rapid and accurate quantitative analysis (Gupta et al. 2022). Additionally, this technique offers wide applicability and flexibility in mobile phases, which can be tailored to specific analytical needs (Li et al. 2024). It also reduces the need for high pressures, making it more accessible and practical than other advanced methods. Even if gas chromatography (GC) is a possibility, it is not suitable for polydatin and resveratrol analysis due to their thermolabile nature, which increases the risk of degradation at the high temperatures necessary for volatilisation (Gupta et al. 2022). Combining GC with mass spectrometry (MS) offers significant advantages as it merges GC separation capabilities with MS's powerful identification and confirmation features, but the non-volatile nature of these compounds requires chemical derivatisation to produce their volatile and thermostable derivatives (Viñas et al. 2009; Li et al. 2024).

The HPLC-DAD method for the simultaneous determination of *trans*-polydatin and *trans*-resveratrol in grapes was validated according to the ICH guidelines (2023). The method's advantages are easy implementation and an undemanding sample preparation procedure. The optimal determination of analysed compounds in grapes was achieved under the chromatographic conditions shown in Table 2. Both analysed compounds were identified based on the retention time of the standard solution and their UV spectra at 306 nm wavelength (Figure 2).

The linearity of the detector response relates to the detector's behaviour in response to changes in analyte concentrations. The assessment was done at seven levels with concentrations ranging from 0.0519 to 103.88 µg/mL for *trans*-polydatin and from 0.0536 to 107.23 µg/mL for *trans*-resveratrol. The relationship between the magnitudes of the analytical signals and the concentrations of the standard solutions is presented in Figure 3. The R^2 values were 0.9988 and 0.9989, for *trans*-polydatin and *trans*-

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Table 2. Conditions of chromatographic analysis of *trans*-polydatin and *trans*-resveratrol in grapes

Mobile phase	Acetonitrile (B); 0.1% HCOOH (A)							
Flow rate	0.32 mL/min							
Column temperature	35 °C							
Injected volume	20 µL							
Wavelength	306 nm							
Gradient	*t* (min)	0	4	18	18.2	20	20.2	26
	B (%)	10	10	50	80	80	10	10

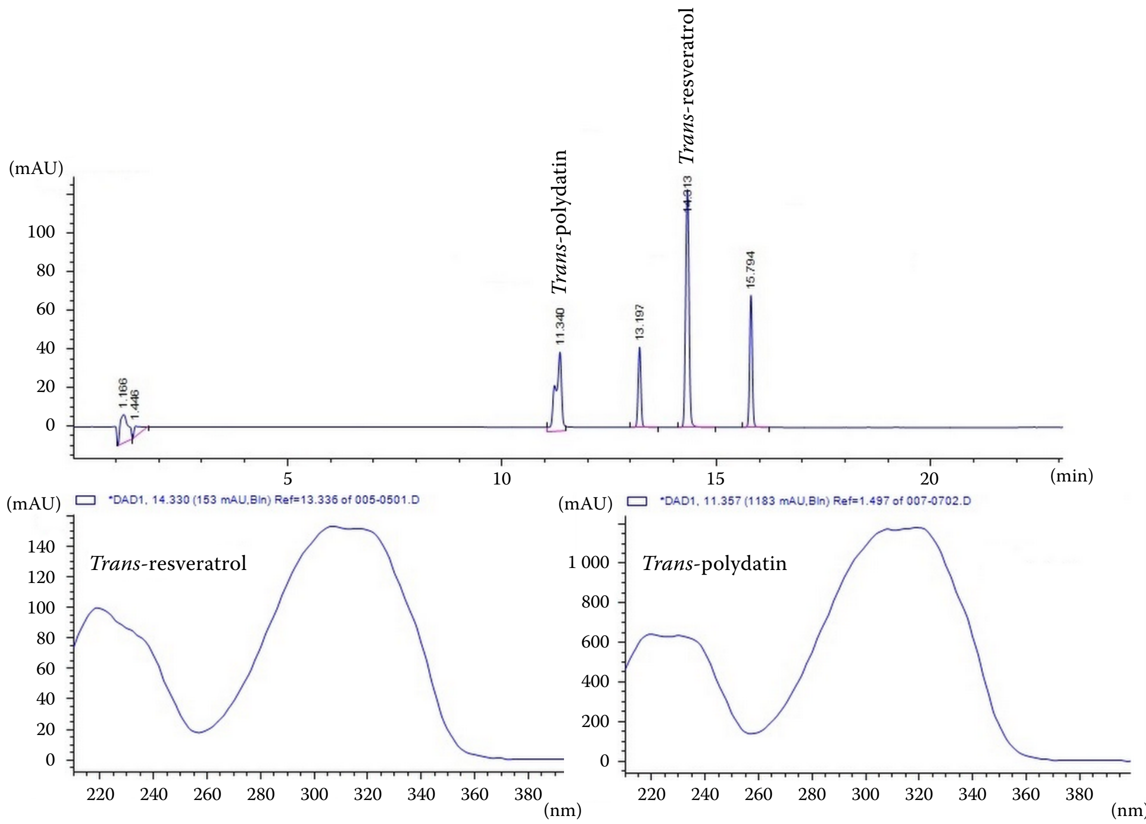

Figure 2. Chromatogram and UV spectra of *trans*-polydatin and *trans*-resveratrol in standard solutions (1.0388 µg/mL and 1.0723 µg/mL, respectively) at 306 nm wavelength

resveratrol, respectively, following the prescribed guidelines and available literature.

The precision of the method was tested through repeatability, which refers to the closeness of agreement between measurements from multiple injections of the same standard solution. It is expressed as the relative standard deviation (RSD%) for a statistically relevant sample set. In this study, repeatability was established through nine injections of the *trans*-polydatin and *trans*-resveratrol mix standard solution (concentrations 1.0388 µg/mL and 1.0723 µg/mL). The obtained results of 0.92% and 0.19% follow the prescribed criteria, which further indicate the satisfactory precision of the method (Table 3).

The limit of detection (LOD) represents the lowest detectable analyte concentration, while the limit of quantification (LOQ) is the lowest concentration that can be accurately quantified. Both parameters were determined using a statistical method based on standard deviation (SD) and a calibration curve. The used formulas were:

$$LOD = 3.3*\sigma/S \quad (1)$$

$$LOQ = 10*\sigma/S \quad (2)$$

where: σ – standard deviation of the response; *S* – the slope of the calibration curve.

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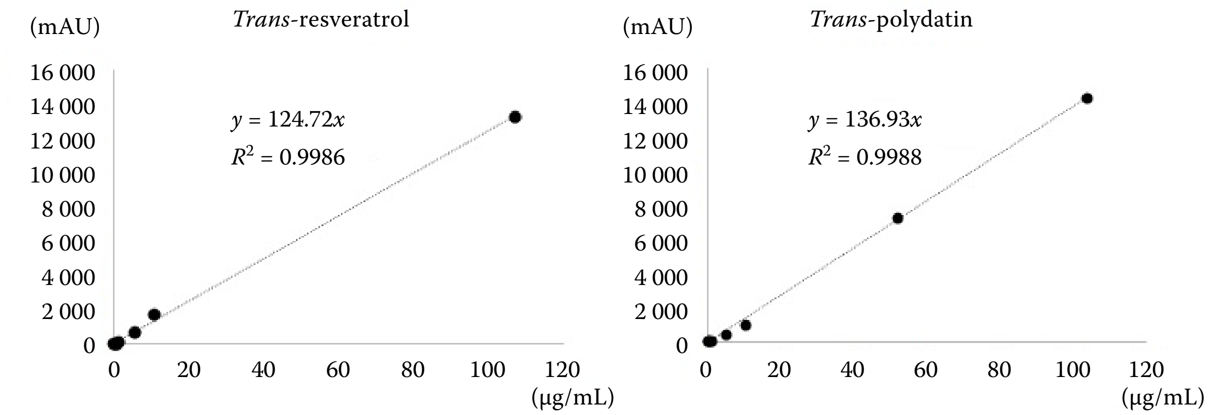

Figure 3. Linearity of detector response for *trans*-polydatin and *trans*-resveratrol at the wavelength of 306 nm

The obtained LOD for *trans*-polydatin and *trans*-resveratrol were 0.0128 µg/mL and 0.0047 µg/mL, while LOQ values were 0.0387 µg/mL and 0.0143 µg/mL, respectively. Low LOD and LOQ values demonstrate the high sensitivity of the proposed method for detecting these stilbenes in grapes.

The method's accuracy refers to the compliance of the achieved results with the actual values of the analyte concentration in the sample, indicating the extraction yield. To evaluate the accuracy of the proposed method, the grape supernatant was spiked before SPE with different volumes (0.01; 0.05; 0.1; 0.2; 0.5 mL) of working solution giving the final concentrations of 1.703; 3.35; 6.493; 14.84; 32.463 µg/mL for *trans*-polydatin, and 1.758; 3.46; 6.702; 15.319; 33.5 µg/mL for *trans*-resveratrol. The achieved recovery of 98.87% for *trans*-polydatin and 100.98% for *trans*-resveratrol reflects the method's efficiency, with minimal matrix effects observed, and complies with data obtained using the HPLC technique shown in the available literature, ranging from 79 to 130.5% (Hashim et al. 2013; Piñeiro et al. 2016; Tzanova, Peeva 2018; Shah, Nayak 2019).

Table 3. Repeatability of detector response (mAU)

Injection	*trans*-polydatin 1.0723 µg/mL	*trans*-resveratrol 1.0388 µg/mL
1	57.71	105.67
2	57.73	105.51
3	58.32	105.80
4	57.29	105.51
5	57.02	105.51
6	57.36	105.68
7	57.81	105.54
8	57.25	105.73
9	56.47	105.11
$\bar{X}$	57.44	105.56
SD (%)	0.53	0.20
RSD (%)	0.92	0.19

$\bar{X}$ – average value; SD – standard deviation; RSD – relative standard deviation

***Trans*-polydatin and *trans*-resveratrol content in organically and conventionally produced grapes.** The validated method was further implemented to determine the *trans*-polydatin and *trans*-resveratrol content in 12 grapevine varieties grown according to the OP and CP concepts (a total of 24 samples). In these, as well as many other grapevine varieties, the content of the mentioned compounds has been analysed primarily from the grape skin and seeds, given that they are present there to the greatest extent (Romero-Pérez et al. 2001; Li et al. 2006). In contrast, this research shows the content of *trans*-polydatin and *trans*-resveratrol in whole grape berries (Tables 4 and 5).

The higher content of *trans*-polydatin was observed in all organically produced varieties, except 'Shiraz', which stood out within CP. When it comes to the content of *trans*-resveratrol, obtained values were higher in five conventionally grown varieties ('Jagoda', 'Tamjanika', 'Shiraz', 'Pinot Noir', and 'Cabernet Sauvignon'), while in all organically grown varieties, the values did not exceed 1 mg/kg (Figure 4).

In Table 4, mean values for each variety show a clear difference between the two production systems, whereby in most varieties the *trans*-polydatin content is higher in OP than CP. That trend is present in varieties 'Cabernet Franc', 'Merlot', and 'Cabernet Sauvignon', where the average content in OP was 10.628, 12.494, and 5.653 mg/kg, respectively, while it was significantly lower in CP (3.689, 1.681, and 3.034 mg/kg). However, a specific

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Table 4. *Trans*-polydatin content (mg/kg) in different grape varieties under organic production (OP) and conventional production (CP)

Trans-polydatin	Mean (OP) ($n = 4$)	Mean (CP) ($n = 4$)	*t*-value	*P*	SD (OP)	SD (CP)	*F*-ratio (variances)	*P* (variances)
Cabernet Franc	10.628	3.689	106.562	< 0.001	0.115	0.060	3.671	0.314
Cabernet Sauvignon	5.653	3.034	50.612	< 0.001	0.077	0.069	1.274	0.847
Merlot	12.494	1.681	130.402	< 0.001	0.157	0.054	8.334	0.115
Pinot Noir	4.487	3.059	22.610	< 0.001	0.104	0.072	2.088	0.561
Muscat Hamburg	2.619	2.271	8.897	< 0.001	0.055	0.055	1.002	0.998
Shiraz	2.419	21.090	–150.910	< 0.001	0.076	0.236	9.675	0.095
Morava	1.979	0.601	104.416	< 0.001	0.019	0.018	1.166	0.902
Bačka	0.187	0.055	26.155	< 0.001	0.008	0.006	1.552	0.727
Panonia	0.262	0.205	4.526	0.004	0.018	0.017	1.104	0.937
Tamjanika	0.869	0.478	8.328	< 0.001	0.080	0.049	2.681	0.439
Jagoda	0.979	0.697	7.421	< 0.001	0.074	0.019	15.292	0.051
Chardonnay	0.390	0.223	9.667	< 0.001	0.016	0.030	3.605	0.320

SD – standard deviation

case is 'Shiraz', where the content of *trans*-polydatin in CP (21.090 mg/kg) is significantly higher than in OP (2.419 mg/kg), as confirmed by a negative *t*-value and a statistically significant result ($P < 0.001$). At the same time, for 'Muscat Hamburg', the variability is completely uniform with standard deviations of 0.055 mg/kg in both production systems, and the content in both these systems was similar (2.619 mg/kg in OP and 2.271 mg/kg in CP). Moreover, results (*F*-ratio) show that in most cases there is no significant difference in variances within OP and CP, as shown by *P*-values for variances all above 0.05. This indicates that the variability between samples is similar in both production systems, except for the variety 'Jagoda', where the *F*-ratio was 15.292, indicating a significant difference in variances between groups ($P = 0.051$).

Due to thick grape skin, 'Shiraz' grapes are genetically predisposed to synthesise and accumulate high levels of polyphenols, including resveratrol and polydatin. This variety exhibits a greater upregulation of the polyphenol pathway compared to other

Table 5. *Trans*-resveratrol content (mg/kg) in different grape varieties under organic production (OP) and conventional production (CP)

Trans-resveratrol	Mean (OP) ($n = 4$)	Mean (CP) ($n = 4$)	*t*-value	*P*	SD (OP)	SD (CP)	*F*-ratio (variances)	*P* (variances)
Cabernet Franc	0.383	0.131	24.872	< 0.001	0.019	0.006	10.204	0.088
Cabernet Sauvignon	0.221	0.551	–37.851	< 0.001	0.007	0.016	5.080	0.215
Merlot	0.474	0.157	23.918	< 0.001	0.025	0.008	8.940	0.105
Pinot Noir	0.895	1.983	–116.231	< 0.001	0.014	0.012	1.464	0.762
Muscat Hamburg	0.627	0.208	69.036	< 0.001	0.011	0.004	8.393	0.114
Shiraz	0.374	4.675	–136.815	< 0.001	0.014	0.061	19.750	0.035
Morava	0.286	0.137	22.734	< 0.001	0.009	0.009	1.012	0.992
Bačka	0.149	0.127	3.956	0.007	0.010	0.005	4.727	0.234
Panonia	0.232	0.135	16.947	< 0.001	0.008	0.008	1.032	0.980
Tamjanika	0.243	0.471	–16.750	< 0.001	0.006	0.027	19.893	0.035
Jagoda	0.194	0.258	–9.104	< 0.001	0.003	0.014	22.392	0.030
Chardonnay	0.299	0.058	33.350	< 0.001	0.012	0.008	2.314	0.509

SD – standard deviation

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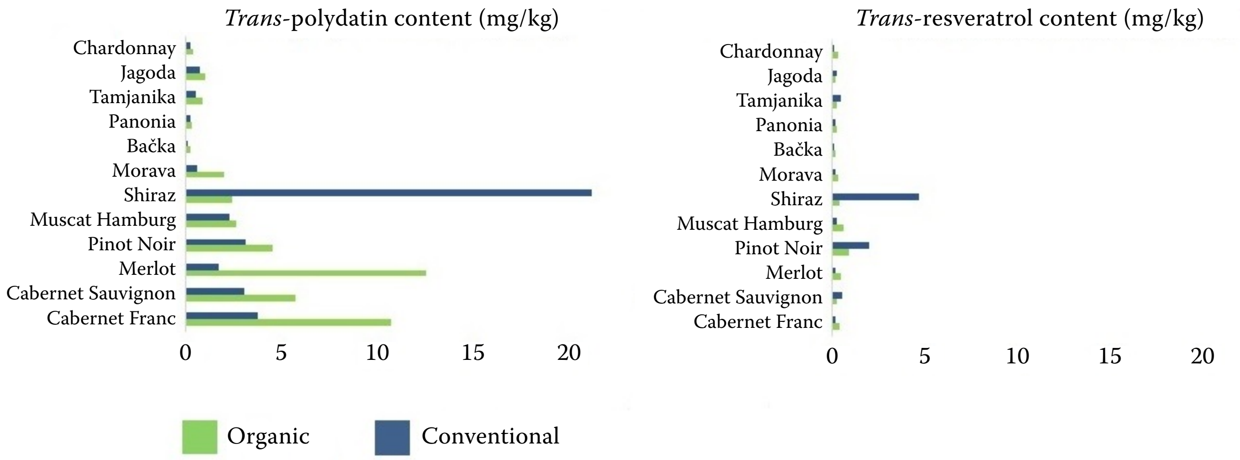

Figure 4. Graphical illustration of *trans*-polydatin and *trans*-resveratrol content in grapes from two production systems

varieties like 'Cabernet Sauvignon'. This includes the higher accumulation of specific compounds, such as piceid and coumaroyl anthocyanins, supported by increased expression of flavonoid biosynthetic genes (Degu et al. 2014). Furthermore, the intensive application of plant protection products at the localities where this variety was grown, due to unstable weather conditions during the ripening period in 2023, may explain the higher content of the analysed compounds in CP.

Results from Table 5 illustrate significant differences in *trans*-resveratrol content between organically and conventionally produced grapes. 'Cabernet Franc' and 'Merlot' showed significantly higher values in OP (0.383 and 0.474 mg/kg) than CP (0.131 and 0.157 mg/kg), with statistical parameters indicating a significant difference between the two production systems. A few white varieties, such as 'Morava' and 'Panonia', consistently showed higher *trans*-resveratrol content in OP, and the variance between the two production systems was relatively similar. In contrast, the 'Pinot Noir' variety shows the opposite, with higher values in CP (1.983 mg/kg), while $P = 0.000$, indicating strong statistical significance. A similar pattern was observed in 'Shiraz' where the content of *trans*-resveratrol in CP (4.675 mg/kg) was significantly higher than in OP (0.374 mg/kg) ($P < 0.001$). The 'Jagoda' variety is particularly interesting because the content of *trans*-resveratrol in CP was higher than in OP, with significant differences in variances suggesting greater variability in CP. 'Chardonnay' exhibited a statistically significant variation between the two production systems, with a *trans*-polydatin content of 0.299 mg/kg in OP compared to 0.058 mg/kg in CP. Very similar results in favor of organic grapevine production, when it comes to the tested compound, were found by Levite et al. (2000). In samples of 'Chardonnay', 'Gamay', and 'Chasselas' the content of *trans*-resveratrol in organic production was 0.3, 32.8, and 5.3 mg/kg, while its content in CP was 0.2, 23.6, and 0 mg/kg, respectively.

To examine the effect of grape colour, a one-way ANOVA was conducted. The results showed highly statistically significant differences ($P < 0.0001$) between the red and white grapes. In each examined production type, red-coloured varieties had a higher content of the analysed compounds, with small deviations (Figure 5).

According to the obtained data, red grape varieties in OP exhibit significantly higher levels of *trans*-polydatin compared to white varieties, with 'Merlot' and 'Cabernet Franc' particularly standing out. Among the white varieties, 'Morava' and 'Jagoda' show relatively higher *trans*-polydatin content, but still below the levels observed in red varieties. Organically grown red varieties, such as 'Pinot Noir' and 'Muscat Hamburg', demonstrate higher concentrations of *trans*-resveratrol compared to white varieties, which aligns with expectations. White varieties have lower concentrations, with 'Chardonnay' and 'Morava' showing the highest levels, while 'Bačka' records the lowest content of this compound. In CP, the data reveal a significantly higher *trans*-polydatin content in red grape varieties compared to white ones, with 'Shiraz' standing out for its exceptionally high levels. White varieties such as 'Jagoda' and 'Morava' contain higher levels of *trans*-polydatin than white varieties but remain below the values observed for red ones. White varieties, such as 'Tamjanika' and 'Jagoda', show higher *trans*-resveratrol levels than red

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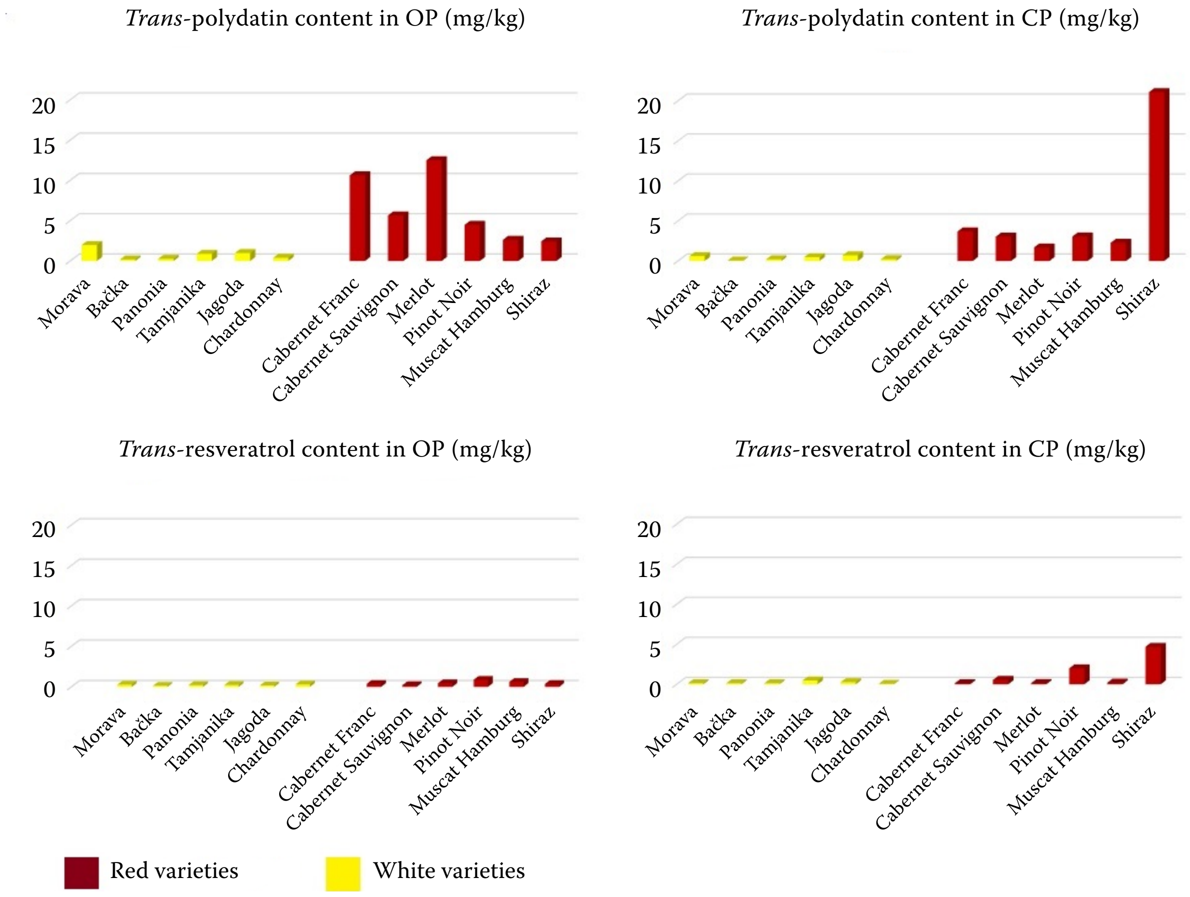

Figure 5. Comparison of the content of *trans*-polydatin and *trans*-resveratrol in white and red varieties from both production systems

OP – organic production; CP – conventional production

varieties like 'Merlot' and 'Cabernet Franc', deviating from the initial hypothesis. These discrepancies may be attributed to the specific characteristics of the grape variety and agroecological conditions, as well as the cultivation method (Căpruciu 2025).

Excluding the production system, it has been observed that the content of *trans*-polydatin is often several times higher than that of *trans*-resveratrol (Gatto et al. 2008; Wang et al. 2013; Kuo et al. 2016). Although the results of our study showed that the analysed grape varieties contain higher amounts of *trans*-polydatin than *trans*-resveratrol, this trend is especially pronounced in red grape varieties, which are generally characterised by a higher content of the tested compounds. Red grape varieties are often richer in phenols (Bavaresco et al. 2016) and characterised by more intense metabolic activities in berry skins, expressing a more pronounced tendency towards the glycoside forms of the mentioned compounds. *Trans*-polydatin is a glycosidic form of *trans*-resveratrol, which plants convert in order to stabilise it, reduce losses due to oxidation and UV radiation, and enable its storage in cells (Regev-Shoshani et al. 2003). During grape ripening, the glycosylation process becomes more pronounced (Geana et al. 2015), as the transition from defence functions to storage promotes the conversion of resveratrol into polydatin via enzymatic reactions. In addition, *trans*-polydatin is synthesised to a greater extent in response to stress, with glycosylation serving as a cell protection response.

A two-way ANOVA was conducted to further examine the influence of grape variety and production system on the content of these compounds. The obtained results are presented in Table 6, which details the variation in *trans*-polydatin and *trans*-resveratrol content depending on the mentioned factors.

The results of this analysis imply that the production system (PS) has a significant effect on the content of analysed compounds ($F = 46.0$; $P < 0.0001$), in-

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Table 6. Analysis of the variance of *trans*-polydatin and *trans*-resveratrol content depending on the grape variety and production system, and the interaction of factors

Source of variation	SS	*df*	MS	*F*	*P*
PS	0.156	1	0.156	46.0	0.0000001
V	779.664	11	70.879	20851.6	0.0000001
Com.	371.642	1	371.642	109332.4	0.0000001
PS × Com.	9.011	1	9.011	2650.8	0.0000001
V × Com.	450.635	11	40.967	12051.9	0.0000001
PS × V × Com.	369.430	11	33.585	9880.1	0.0000001
Error	0.490	144	0.003	–	–

PS – production system; V – variety; Com. – compound; SS – sum of squares; *df* – degrees of freedom; MS – mean square; *F* – *F*-value; *P* – *P*-value

dicating a statistically significant difference between OP and CP. In addition, the variety factor (V) also shows an extremely significant effect ($F = 20\ 851.6$; $P < 0.0001$). This suggests that different grape varieties have a pronounced effect on the levels of the compounds (Com.), underscoring the importance of varietal selection in viticulture. Similar significances were observed in interactions between compounds and other factors (PS × Com., V × Com., and PS × V × Com.), demonstrating the complexity of the relationship between the cultivation system, variety, and *trans*-polydatin and *trans*-resveratrol content. These results additionally confirm the previously highlighted differences in the content of these compounds between white and red grape varieties, as well as the influence of different cultivation systems.

Existing research directly comparing the *trans*-polydatin and *trans*-resveratrol content between organically and conventionally grown grape varieties is scarce. Most studies on phenolic compounds, such as *trans*-resveratrol, either do not include *trans*-polydatin or examine it with less detail, or examine the overall phenolic profile in various production systems, frequently identifying *trans*-resveratrol as a significant antioxidant (Cosme et al. 2018). Our results revealed statistically significant differences in *trans*-polydatin and *trans*-resveratrol content between organically and conventionally grown grape varieties. In particular, higher concentrations of *trans*-polydatin and *trans*-resveratrol were observed in organically produced grapes, which aligns with previous research suggesting enhanced synthesis of secondary metabolites under organic cultivation conditions (Dani et al. 2007). These results are consistent with those of Brandt et al. (2011), who reported higher levels of defence-related compounds, including resveratrol, in organically cultivated fruit. Tassoni et al. (2013) did not observe statistically significant differences in polyphenol content between samples produced using different cultivation methods, suggesting that the response of these compounds may vary depending on specific environmental factors. However, their study highlighted a more pronounced distinction in polyphenol levels between red and white grape varieties, suggesting that varietal differences play a larger role in influencing polyphenol composition than the production system.

To the best of our knowledge, this research is the first of its kind in the Republic of Serbia and the Balkans, focusing on a comparative analysis of *trans*-polydatin and *trans*-resveratrol in international and domestic grape varieties grown in various production systems. Available literature in the mentioned region focuses on the analysis of total phenols, *trans*- and *cis*-resveratrol in commercial Serbian wines (Sošić et al. 2023), as well as trans-resveratrol in the grape skin and seeds (Tzanova, Peeva 2018). In this sense, this research is novel because it focuses on the influence of OP and CP systems on the content of *trans*-polydatin and *trans*-resveratrol in domestic and international grape varieties, which are significantly present in the viticulture of this region.

CONCLUSION

In this research, a fast, simple, precise, and efficient HPLC-DAD method for the simultaneous determination of *trans*-polydatin and *trans*-resveratrol was developed and validated. It was applied to real grape samples to determine their content in international and domestic varieties, within OP and CP systems. This research represents the first Serbian contribution to the understanding of the influence

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of organic and conventional production systems on the content of *trans*-polydatin and *trans*-resveratrol in grapes. The selection of varieties was based on those most commonly represented in the vineyards of the examined region, and each was sampled in both production systems. The obtained results indicate statistically significant differences in the content of the tested compounds between the two cultivation systems, with generally higher values recorded in the organically grown varieties. The exceptions were 'Shiraz' (for both compounds), 'Jagoda', 'Tamjanika', 'Pinot Noir', and 'Cabernet Sauvignon' (for *trans*-resveratrol content), where higher values were recorded in conventionally produced grapes. Also, it was determined that red varieties generally contain more *trans*-polydatin and *trans*-resveratrol, with small deviations in individual varieties.

All the obtained findings contribute to a deeper understanding of the impact of production systems on the content of phenolic compounds, which are holders of the grapevine defence mechanism. Thus, this research provides a foundation for future studies in organic and conventional viticulture, offering valuable insights and guidelines for selecting optimal cultivation strategies that rely on the natural potential of plants, while simultaneously enhancing the nutritional profile and overall quality of the final products.

REFERENCES

Baker B.P., Green A.T., Loker A.I. (2020): Biological control and integrated pest management in organic and conventional systems. Biological Control, 140: 104095.

Basholli-Salihu M., Schuster R., Mulla D., Praznik W., Viernstein H., Mueller M. (2016): Bioconversion of piceid to resveratrol by selected probiotic cell extracts. Bioprocess and Biosystems Engineering, 39: 1879–1885.

Bavaresco L., Lucini L., Busconi M., Flamini R., de Rosso M. (2016): Wine resveratrol: From the ground up. Nutrients, 8: 222.

Brandt K., Leifert C., Sanderson R., Seal C.J. (2011): Agroecosystem management and nutritional quality of plant foods: The case of organic fruits and vegetables. Critical Reviews in Plant Sciences, 30: 177–197.

Căpruciu R. (2025): Resveratrol in grapevine components, products and by-products: A review. Horticulturae, 11: 111.

Cosme F., Pinto T., Vilela A. (2018): Phenolic compounds and antioxidant activity in grape juices: A chemical and sensory view. Beverages, 4: 22.

Dani C., Oliboni L.C., Vanderlinde R., Bonatto D., Salvador M., Henriques J.A.P. (2007): Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food and Chemical Toxicology, 45: 2574–2580.

Degu A., Hochberg U., Sikron N., Venturini L., Buson G., Ghan R., Plaschkes I., Batushansky A., Chalifa-Caspi V., Mattivi F., Delledonne M., Pezzotti M., Rachmilevitch S., Cramer G.R., Fait A. (2014): Metabolite and transcript profiling of berry skin during fruit development elucidates differential regulation between Cabernet Sauvignon and Shiraz cultivars at branching points in the polyphenol pathway. BMC Plant Biology, 26: 188.

Dugo G., Saitta M., Giuffrida D., Vilasi F., la Torre G.L. (2004): Determination of resveratrol and other phenolic compounds in experimental wines from grapes subjected to different pesticide treatments. Italian Journal of Food Science, 16: 305–321.

Gatto P., Vrhovsek U., Muth J., Segala C., Romualdi C., Fontana P., Pruefer D., Stefanini M., Moser C., Mattivi F., Velasco R. (2008): Ripening and genotype control stilvene accumulation in healthy grapes. Journal of Agricultural and Food Chemistry, 56: 11773–11785.

Geana E.I., Dinca O.R., Ionete R.E., Artem V., Niculescu V.C. (2015): Monitoring *trans*-resveratrol in grape berry skins during ripening and in corresponding wines by HPLC. Food Technology and Biotechnology, 53: 73–80.

Gupta M.K., Ghuge A., Parab M., Al-Refaei Y., Khandare A., Dand N., Waghmare N. (2022): A comparative review on High-Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC) & High-Performance Thin Layer Chromatography (HPTLC) with current updates. Current Issues in Pharmacy and Medical Sciences, 35: 224–228.

Hasan M.M., Bae H. (2017): An overview of stress-induced resveratrol synthesis in grapes: Perspectives for resveratrol-enriched grape products. Molecules, 22: 294.

Hashim S.N.N.S., Schwarz L.J., Boysen R.I., Yang Y., Danylec B., Hearn M.T.W. (2013): Rapid solid-phase extraction and analysis of resveratrol and other polyphenols in red wine. Journal of Chromatography A, 1313: 284–290.

ICH guidelines (2023): International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: ICH Harmonized Guideline – Validation of Analytical Procedures Q2(R2), Final Version Adopted on 1 November 2023. [Guideline]. Available at https://www.ich.org/page/quality-guidelines (accessed Nov 11, 2024).

Korać N., Cindrić P., Medić M., Ivanišević D. (2016). Voćarstvo i vinogradarstvo. Novi Sad, Serbia, University of Novi Sad: 1–3. (in Serbian)

https://doi.org/10.17221/231/2024-HORTSCI

Kuo C.-H., Chen B.-Y., Liu Y.-C., Chen J.-H., Shieh C.-J. (2016): Production of resveratrol by piceid deglycosylation using cellulase. Catalysts, 6: 32.

Levite D., Adrian M., Tamm L. (2000): Preliminary Results on Contents of Resveratrol in Wine of Organic and Conventional Vineyards. In: Willer H., Meier U. (eds): Proceedings of the 6th Annual International Congress on Organic Viticulture. Basel, Aug 25–26, 2000: 256–258.

Li W., Yuan H., Liu Y., Wang B., Xu X., Xu X., Hussain D., Ma L., Chen D. (2024): Current analytical strategies for the determination of resveratrol in foods. Food Chemistry, 431: 137182.

Li X., Wu B., Wang L., Li S. (2006): Extractable amounts of *trans*-resveratrol in seed and berry skin in *Vitis* evaluated at the germplasm level. Journal of Agriculture and Food Chemistry, 54: 8804–8811.

Lytridis C., Siavalas G., Pachidis T., Theocharis S., Moschou E., Kaburlasos V.G. (2023): Grape maturity estimation for personalized agrobot harvest by fuzzy lattice reasoning (FLR) on an ontology of constraints. Sustainability, 15: 7331.

Merot A., Fermaud M., Gosme M., Smits N. (2020): Effect of conversion to organic farming on pest and disease control in French vineyards. Agronomy, 10: 1047.

Pezzuto J.M. (2019): Resveratrol: Twenty years of growth, development and controversy. Biomolecules and Therapeutics, 27: 1–14.

Piñeiro Z., Marrufo-Curtido A., Serrano M.J., Palma M. (2016): Ultrasound-assisted extraction of stilbenes from grape canes. Molecules, 21: 784.

Regev-Shoshani G., Shoseyov O., Bilkis I., Kerem Z. (2003): Glycosylation of resveratrol protects it from enzymic oxidation. Biochemical Journal, 15: 157–163.

Romero-Pérez A.I., Lamuela-Raventós R.M., Andrés-Lacueva C., de La Torre-Boronat M.C. (2001): Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. Journal of Agriculture and Food Chemistry, 49: 210–215.

Shah A.A., Nayak Y. (2019): Development, optimization, and validation of RP-HPLC method for the quantification of resveratrol. Indian Journal of Pharmaceutical Education and Research, 53: 356–363.

Song P., Yu X., Yang W., Wang Q. (2021): Natural phytoalexin stilbene compound resveratrol and its derivatives as anti-tobacco mosaic virus and anti-phytopathogenic fungus agents. Science Reports, 11: 16509.

Sošić S., Pajović-Šćepanović R., Raičević D., Popović T. (2023): Quality of wines Vranac and Kratošija in the vintage 2021. Agriculture and Forestry, 69: 127–137.

Tassoni A., Tango N., Ferri M. (2013): Comparison of biogenic amine and polyphenol profiles of grape berries and wines obtained following conventional, organic, and biodynamic agricultural and oenological practices. Food Chemistry, 139: 405–413.

Tzanova M., Peeva P. (2018): Rapid HPLC method for simultaneous quantification of *trans*-resveratrol and quercetin in the skin of red grapes. Food Analytical Methods, 11: 514–521.

Viñas P., Campillo N., Martínez-Castillo N., Hernández-Córdoba M. (2009): Solid-phase microextraction on-fiber derivatization for the analysis of some polyphenols in wine and grapes using gas chromatography-mass spectrometry. Journal of Chromatography A, 1216: 1279–1284.

Vukosavljević V., Žunić D., Todić S., Matijašević S. (2016): Organic viticulture in world, Serbia and region. Acta Agruculturae Serbiae, 21: 155–166.

Wang L., Xu M., Liu C., Wang J., Xi H., Wu B., Loescher W., Duan W., Fan P., Li S. (2013): Resveratrols in grape berry skins and leaves in *Vitis* germplasm. PLoS One 8: e61642.

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