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VARIETAL DISTRIBUTIONS OF STILBENES IN GRAPE CANE OF *VITIS VINIFERA* L.

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Grape cane is a waste product from viticulture, which can be used as a source of stilbenes, such as resveratrol and viniferins with high antioxidant values. These stilbenes have also important healthy effects for humans. Resveratrol and viniferins are known as phytoalexins since 1977. Biomass of grape canes in annual pruning is a very valuable source of stilbenes, e.g. *trans*-resveratrol, and *trans*-ε-viniferin in dry grape canes. The main goal of this article was to compare the distribution of resveratrol and viniferins in the grape cane varieties of Laurot, Hibernal, Malverina and Chardonnay. The highest content of *trans*-resveratrol was found in Hibernal (6,111 mg kg^{-1}); for *trans*-ε-viniferin and r2-vinifein, the highest levels were found in Malverina (2,211 and 654 mg kg^{-1}). These compounds can be obtained from this waste product (grape cane), by easy extraction process in winemaking or food-processing industry.

Keywords: *Vitis vinifera* L., varieties, grape canes, stilbenes, distribution

In wine production and viticulture technology there are a few waste plant materials: grape canes, grape marc, young lateral shoots and leaves (Balík et al., 2008). It is known from references that waste plant materials form a good source of stilbene-based substances in many more applications, such as pharmaceutical, cosmetic and food industries (Rayne et al., 2008; Xue et al., 2014).

Resveratrol, as a major stilbene derivative, has been studied in detail over the most recent decades (Langcake et al., 1976, 1977; Sotheeswaran et al., 1993). According to references, the substance is responsible for the so-called "French paradox" connected with wine consumption (Renaud et al., 1992; Catalgol et al., 2012). Next to resveratrol, there are other biologically active compounds, such as viniferins (Aaviksaar et al., 2003; Richard et al., 2011), piceid (Pawlus et al., 2013), pinosylvin (Park et al., 2012) and pterostilbene (McCormack et al., 2013).

According to references, alcohols are some of the best solvents for stilbene extraction out of the protic group of solvents (Rayne et al., 2008). We studied distribution of three types of stilbenes in different varieties of *Vitis vinifera* L. Extraction at 50 °C for more than two hours has a very high yield as observed in the previous study (Soural et al., 2015). Using higher temperature to increase the efficiency of extraction can destroy stilbenes. This work is focused on studying and comparing Laurot, Malverina, Hibernal and Chardonnay varieties and their extracting stilbene distribution from grape canes by ethanol.

Material and methods

Varieties and locations

Samples of grape canes of four *Vitis vinifera* L. cultivars, i.e. Laurot, Hibernal, Malverina and Chardonnay were studied. Description of varieties and growing sites: 1) Laurot is a blue inter-specific grape variety, a cross of ('Merlot' × 'Seibel 13 666') × ('Blaufränkisch' × 'St. Laurent'); grown in the Wine Region Moravia, the Znojemská Wine Sub-region, Znojmo District, Znojmo-Oblekovice wine commune; 2) Hibernal is a white inter-specific grape variety, a cross of "Seibel 7053' × 'Riesling', grown in the Wine Region Moravia, the Velkopavlovická Wine Sub-region, Břeclav District, Rakvice wine commune; 3) Malverina is a white inter-specific grape variety, a cross of 'Merlan' × 'Rakish', grown in the same location as Hibernal; 4) Chardonnay is a white original variety, grown in the Wine Region Moravia, the Slovacká Wine Sub-region, Břeclav District, Kostice wine commune. All grape canes as one year old wood were pruned in same month – March 2013. Type of middle vine training Rhine-Hesse was used in all varieties. Localities with geological and weather conditions (sum of effective temperatures above 10 °C) in year of pruning are in table 1.

Extraction

After pruning, grape canes of all varieties were cut to the approximate length of 10 cm, then frozen and dried within a lyophilisation process and powdered using a knife grinder to achieve a size of around 1 mm. 250 mg of powdered grape canes and 3 ml of methanol were immediately used

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Table 1 Locality and their soil-geological settings and weather conditions

Wine commune	GPS	Soil and geological settings	Sum of effective temperatures (°C)	Rainfall totals (mm)
Znojmo – Oblekovice	48° 49' 26.4"N 16° 05' 30.8"E	gravel-sand, sandy-loamy chernozems	1347	523
Rakvice	48° 51' 46.5"N 16° 48' 00.3"E	loess and clay, loamy chernozems	1340	431
Kostice	48° 44' 28.8"N 16° 58' 28.5"E	sand and clay, loamy-sandy chernozems	1459	696

Table 2 Duncan's test for stilbenes in mg kg^{-1} d.w. of grape cane (One-way ANOVA)

Varieties	Stilbenes	Laurot	Malverina	Hibernal	Chardonnay
Laurot	*trans*-resveratrol *trans*-ε-viniferin r2-viniferin		0.0013 0.0120 0.0001	0.0001 0.2817 0.0279	0.4160 0.3405 0.0326
Malverina	*trans*-resveratrol *trans*-ε-viniferin r2-viniferin	** * **		0.0003 0.0553 0.0002	0.0006 0.0479 0.0001
Hibernal	*trans*-resveratrol *trans*-ε-viniferin r2-viniferin	** n.s. *	** n.s. **		0.0001 0.8544 0.0010
Chardonnay	*trans*-resveratrol *trans*-ε-viniferin r2-viniferin	n.s. n.s. *	** * **	** n.s. **	
Average	*trans*-resveratrol *trans*-ε-viniferin r2-viniferin	1,604.5 1,738.3 159.9	3,507.9 2,210.7 654.4	6,111.0 1,903.2 231.9	1,275.6 1,877.2 90.7

** p <0.01, * p <0.05, n.s. = not significant. Values are means calculated from three measurements

for extraction at 50 °C during 2 hours and 45 min. After extraction, the mixtures were centrifuged for 10 min at 3,500 rpm and at laboratory temperature. The supernatants were transferred into calibrated tubes; 1 ml of pure methanol was added to the solid residues, mixed and this new mixture was centrifuged once again. Subsequently, this rinse procedure was repeated a single time. All the supernatants were combined and the final volume of each supernatant mixture was adjusted to make 5 ml.

A similar process of extraction was done in the previous study with the Cabernet Moravia variety, when extraction yields were comparable with additional extraction steps (Soural et al., 2015).

Quantification of stilbenes

All the extract samples were analysed using the HP 1050 (Ti-series) HPLC instrument (Hewlett Packard, Palo Alto, CA, USA) on a column Luna C18(2) (Phenomenex, Torrance, CA, USA) with parameters: 3 μm, 150 × 2 mm, the temperature of 25 °C, the flow rate of 0.250 ml min^{-1}. Mobile phase A was 5% acetonitrile +0.1% o-phosphoric acid (in vol.%) in water; mobile phase B was 80% acetonitrile +0.1% o-phosphoric acid (in vol. %) in water. The gradient was increased from 20% mobile phase B to 80% of B during 20 min and from 80% of B to 100% of B during 5 min. Injection volume was 5 μl. Two detectors were used for analysing: a diode array detector G1315B (DAD, Agilent, Prague, the Czech Republic) with the detection of wavelengths at 220 and 315 nm and a scanning range of 190–600 nm, and a fluorescence detector G1321A (FLD, Agilent, Prague, the Czech Republic) with excitation wavelength of 315 nm, emission wavelength of 395 nm, and scanning of emission in the 300–600 nm range. Finally, the method of quantification was validated in terms of linearity, limits of detection, and repeatability (Tříska et al., 2012, 2013). Identifications of stilbenes (*trans*-resveratrol, *trans*-ε-viniferin and r2-viniferin) were done previously by HRMS (High Resolution Mass Spectrometr) and H-NMR (Nuclear Magnetic Resonance) as presented by Soural et al. (2015).

Statistical analysis

All extracts of grape canes with stilbenes were measured three times. Mean values, standard deviations and significant vs. non-significant differences (p <0.01 or p <0.05) were analysed by ANOVA, applying the Tukey multiple range test for making comparisons with Statistica Cz 12 and MS Excel 2010 software.

Results and discussion

Contents of stilbenes in extracts of grape cane for all varieties *Vitis vinifera* L. were calculated to achieve amounts of mg kg^{-1} of dried grape cane.

The contents of *trans*-resveratrol in different varieties showed statistical differences by Duncan's test of one-way ANOVA in all four measured varieties, except Chardonnay vs. Laurot. A minimum of *trans*-resveratrol (1,276 mg kg^{-1} d.w.) was present in the Chardonnay and the Laurot variety with a similar content (1,604 mg kg^{-1}). A maximum of *trans*-resveratrol (6,111 mg kg^{-1}) was found in Hibernal. Malverina's value (3,508 mg kg^{-1}) was near to the average of all four varieties (3,125 mg kg^{-1}). Between Hibernal and Chardonnay,

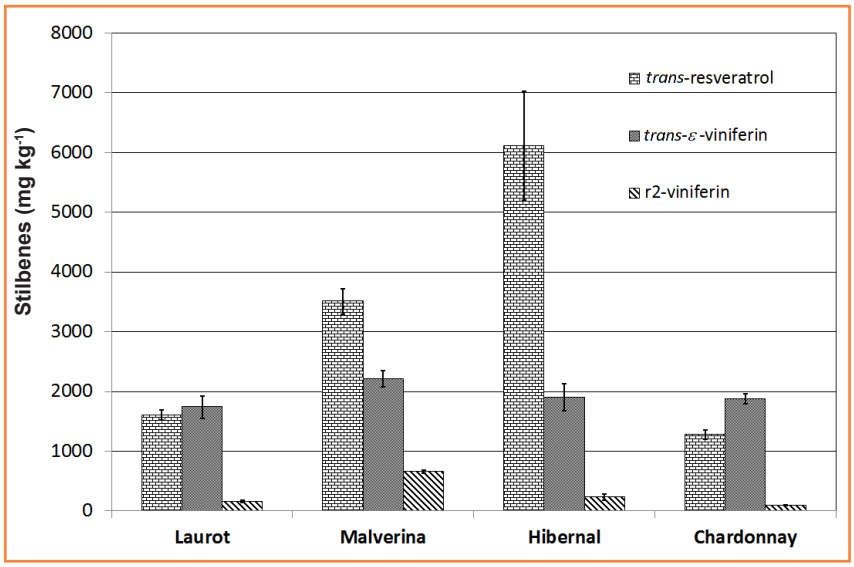

Figure 1 Stilbenes distribution in the grape canes of four varieties *Vitis vinifera* L.

as maximum vs. minimum values of *trans*-resveratrol, there was almost five times disparity (exactly more than 379%).

In case of contents of *trans*-ε-viniferin in these varieties there were no statistical differences except between Malverina and two varieties of Laurot and Chardonnay. Contents of *trans*-ε-viniferin were almost same: Laurot (1,738 mg kg^{-1} d.w.), Hibernal (1,903 mg kg^{-1}) and Chardonnay (1,877 mg kg^{-1}) with values around 1,800 mg kg^{-1} d.w. Only varieties Malverina had significant higher content (2,211 mg kg^{-1}), but equalled only around 20%, approximately.

Among the last-measured stilbenes there was r2-viniferin, with very different contents in all varieties. The maximum was found in the grape cane of Malverina (654 mg kg^{-1} d.w.), while minimum in Chardonnay (91 mg kg^{-1}) – this is more than seven times disparity. The Laurot (160 mg kg^{-1}) and Hibernal (232 mg kg^{-1}) varieties had approximately four times or three times lesser content of r2-viniferin than Malverina. Between Malverina and Chardonnay (as maximum vs. minimum) of r2-viniferin, there was approximately seven times disparity (exactly more than 621%).

Standard deviation values along with contents are shown in Figrue 1.

Malverina showed higher contents of oligomers of resveratrol (*trans*-ε-viniferin as dimer and r2-viniferin as tetramer); these results are of statistical significance. The lowest amounts of sums of stilbenes 3,244 ±170 mg kg^{-1} d.w. were found in Chardonnay in contrast with the highest amounts of stilbenes 8,246 ±1,190 mg kg^{-1} in cv. Hibernal. Comparing Hibernal with Cabernet Moravia with the value of 8,500 mg kg^{-1} (Soural et. al., 2015) showed similar contents of stilbenes in the two different varieties. Pinot noir with the content of 6,900 mg kg^{-1} (Vergara et al., 2012) showed similar results as Malverina with the value of 6,373 ±375 mg kg^{-1}.

Contents of stilbenes are not depending only on the variety but mainly on the storing condition or the process of preparation before extracting, drying, etc. For example, content of ε-viniferin in Chardonnay was found to be 2,089 ±334 mg kg^{-1} d.w. as published by Lambert et al. (2013) which is similar to our result of 1,887 ±89 mg kg^{-1} as reported herein, but contents of *trans*-resveratrol were totally different: 190 ±87 mg kg^{-1} (Lambert et al., 2013) and our value was only 1,276 ±75 mg kg^{-1}. This big difference can be caused by various drying processes.

In the study published by Lambert et al. (2013) drying occurred at the temperature of 40 °C during 15 days period but in our case there was immediately freezing and lyophilisation; in our previous study (Soural et. al., 2015) drying took place at the room temperature (22 °C approximately) for 2.5 months in darkness. In the study of Houillé et al. (2015) we can generally see increasing contents of stilbene over time and higher temperature of storing.

Next to storing conditions, Wine Sub-regions can play role, although all samples are from one Wine Region Moravia. Some differences in: weathers, soils, geological settings and types of arotechnics, can change contents of stilbenes. These effects are in more detail studied in our further publication (in a review process) but with grape cane pruned in years 2014–2016. Not only varieties differences must be studied in this phenomenon.

Conclusion

The present study compared four varieties of *Vitis vinifera* L.: Laurot, Hibernal, Malverina and Chardonnay in terms of sourcing viniferins and *trans*-resveratrol from grape canes as waste plant materials from wine production and viticulture technology. This obtaining of stilbenes can improve economic side of winemakers or can be applied in food-processing industry. Distribution of these stilbenes is highly variable; there was more than 7 times disparity between Malverina and Chardonnay in case of r2-viniferin. Similar disparity (5 times) occurred between Hibernal and Chardonnay in the case of contents of *trans*-resveratrol; very similar results were obtained, however, in the case of *trans*-ε-viniferin, with the disparity being only 1.27 times between Malverina and Laurot. A maximum of these tree stilbenes was found in the Hibernal variety, the value being 8,246 ±1,190 mg kg^{-1} d.w. of grape cane.

Comparing the results of this study against bibliography showed differences in distribution of stilbenes. Different contents of stilbenes in the same variety can be produced by differences in storage-drying process, weather and soil-geological settings. These phenomena should be explored in more detail.

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