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Content of flavan-3-ol monomers and gallic acid in grape seeds by variety and year

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Summary

The content of flavan-3-ol monomers and gallic acid in grape seeds in the samples from the gene collection of Viticulture Research Station Karlštejn (Czech Republic) was investigated. Presence of catechin, epicatechin, epigallocatechin, epicatechin gallate and gallic acid was confirmed in these samples, other flavan-3-ol monomers like gallocatechin or epigallocatechin gallate were below limit of detection. As major flavan-3-ol monomers catechin and epicatechin with 85 % were detected. Average content of catechin in grape seed was 4454 ± 148 µg·g^{-1}, 3085 ± 98 µg·g^{-1} epicatechin, 600 ± 41 µg·g^{-1} epigallocatechin, 457 ± 19 µg·g^{-1} gallic acid, and 352 ± 16 µg·g^{-1} epicatechin gallate. Variety had main impact on phenolic content followed by vintage. Average sum of flavan-3-ol monomers in grape seeds in white varietes was 7601 ± 273 µg·g^{-1} and 10869 ± 430 µg·g^{-1} in red varieties, with 10050 ± 425 µg·g^{-1} in 2012 and 7846 ± 219 µg·g^{-1} in 2013 were found on average in all varieties, respectively. The highest phenolic content was characteristic for 'Pinot Noir', 'Muskat Donskoi', 'Aromriesling' and 'Hibernal' and may contribute to their health properties.

K e y w o r d s : grape seeds; flavan-3-ols; gallic acid; variety and year.

Introduction

Wine production is one of the most important agricultural activities throughout the world (Devesa-Rey *et al.* 2011). This consequently led to the search for possible waste recovery solutions. In the wine industry, large amounts of grape marc are produced, with every 100 kg of processed grapes approximately 20-25 kg of grape waste (Sessa *et al.* 2012). More specifically, grape marc residues have been found to be of particular interest. In fact, the worldwide generation of this residue was estimated to be approximately 7 Mt per year (Benetto *et al.* 2014). The main organic wastes produced in modern wine industries include grape pomace (62 %), lees (14 %), stalk (12 %) and dewatered sludge (12 %). Currently, only a minimal amount of these residues are recycled (Cotoras *et al.* 2014). Grape marc, a complex lignocellulosic material, is one of the most abundant and worthless winery wastes, generated after the pressing process (Devesa-Rey *et al.* 2011). The extraction of bioactive components from the waste of food industries is valuable from the environmental and economical points of view and can be considered as a low-cost source of natural antioxidants (Shojaee-Aliabadi *et al.* 2013). The medicinal actions of phenolics are mostly ascribed to their antioxidant capacity, free radical scavenging and chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways and also have anti-inflammatory and cardioprotective effect (Soobrattee *et al.* 2005, Gessner *et al.* 2013).

Material and Methods

P l a n t m a t e r i a l : Grape seed samples were provided by Viticulture Research Station Karlštejn (Crop Research Institute, Prague, Czech Republic), in Bohemian region. Samples were harvested in the 2012 and 2013 years. Grapes were harvested in full ripeness. Flavan-3-ol monomers and gallic acid were determined in seeds from 70 varieties of grapevine. Thirty-four varieties were the same for comparison in both years and 17 varietes were different in 2012 from 19 varietes in 2013.

C h e m i c a l s : Methanol (p.a.) and acetonitrile (HPLC grade) from LachNer s.r.o. (Neratovice, Czech Republic), pyrogallol, gallocatechin, acetic acid (glacial) and gallic acid from Sigma-Aldrich Chemie (Steinheim, Germany), catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and epigallocatechin from Extrasynthese (Lyon, France), ultrafiltrated water.

P r e p a r a t i o n o f s a m p l e s : Powdered grape seeds (0.2 g) were extracted in five stages with 50 mL (5x10 mL) of 70 % methanol with (0.2 %) pyrogallol as antioxidant. Extraction was carried out in an ultrasonic bath for better and faster solid-liquid extraction of phenolic compounds. After 10 min. in the ultrasound bath the sample in the cuvette was placed into a 5810R centrifuge (Eppendorf AG, Hamburg, Germany), and then centrifuged at 8228 g for 5 min and after supernatant was collected. Each extract was analysed separately and previously filtered through PVDF filters with a pore size of 0.45 µm. Assays were performed in three parallel repetitions.

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H i g h p e r f o r m a n c e l i q u i d c h r o m a t o g - r a p h y a n a l y s i s: HPLC analysis was performed on an Dionex 3000 Ultimate system with a PDA detector (Thermo Fisher Scientific, Dionex, Sunnyvale, CA, USA). Chromatographic analysis was performed on an ChromSep OmniSpher C18 column (4.6 × 250 mm, 5µm; Varian, USA) with ChromSep RP guard column (10 × 3 mm; Varian, USA). The mobile phase consisted from acetonitrile and water acidified by acetic acid (0.1 %); gradient elution was applied starting from 10 % acetonitrile and 90 % water, to final 90 % acetonitrile and 10 % water. HPLC super gradient acetonitrile (Lachner, Ltd. Neratovice, Czech Republic) was used. Time of analysis was 36 min with flow 0.8 $mL·min^{-1}$, column was temperated on 25 °C and injection volume was 4 µL. Gallic acid and flavan-3-ols were quantified at wavelenght 280 nm. Limits of detection (LOD) of assessed flavan-3-ols were 20 $µg·g^{-1}$, 100 $µg·g^{-1}$, 30 $µg·g^{-1}$, 40 $µg·g^{-1}$ and 30 $µg·g^{-1}$ for gallic acid, epigallocatechin, catechin, epicatechin and epicatechin gallate, and their retention times were 5.3 min, 11.3 min, 12.2 min, 13.4 min and 17.6 min, respectively.

Results and Discussion

Gallic acid, catechin, epicatechin, epigallocatechin, and epicatechin gallate were found in the samples of grape seed (Table, complete data will be demonstrated in 'Supplementary material"). Gallocatechin and epigallocatechin gallate were below the limit of detection. Catechin with average 51 % share and epicatechin with 34 % share were found as major flavan-3-ols. Remaining 15 % were distributed between gallic acid (5 %), epigallocatechin (6 %), and epicatechin gallate (4 %). The highest phenolic content showed 'Pinot Noir' with catechin levels of 20959 ± 175 $µg·g^{-1}$ in 2012 and 15569 ± 317 $µg·g^{-1}$ in 2013, and epicatechin of 9364 ± 119 $µg·g^{-1}$ in 2012 and 9812 ± 288$µg·g^{-1}$ in 2013, respectively. High levels of catechin were also found in 'Aromriesling' (16535 ± 768 $µg·g^{-1}$ in 2012, 17782 ± 712 $µg·g^{-1}$ in 2013), 'Pinot Gris' (12572 ± 372 $µg·g^{-1}$ in 2012), 'Hibernal' (10742 ± 318 $µg·g^{-1}$ in 2012), and 'Pinot Precoce Noir' (8603 ± 572 $µg·g^{-1}$ in 2012), respectively. The highest level of epicatechin was found in 'Muskat Donskoi', namely 13397 ± 507 $µg·g^{-1}$.

The sum of flavan-3-ol monomers for each variety showed high differences, but the percentage representation of individual flavan-3-ols and gallic acid was similar, with a negative correlation between catechin and epicatechin. The biggest differences between varieties were observed in catechin and epicatechin contents. Also other authors reported levels of dominant polyphenols in this range. In grape seeds from 'Tuscan' (Italy) catechin content was between 67.4 ± 5.1 mg·100 g^{-1} in 'Foglia Tonda' and 205.7 ± 19.0 mg·100 g^{-1} in 'Montepulciano', epicatechin between 47.2 ± 3.5 mg·100 g^{-1} in 'Foglia Tonda' and 205.7 ± 3.1 mg·100 g^{-1} in 'Canaiolo' (Iacopini *et al.* 2008). Contents of polyphenols in the vine growing area Tuscany were lower than contents in the vine growing area Karlštejn (Czech Republic) probably for growing area climatic differences. But differences between individual varieties were conclusive. In grape seeds from Santa Catarina (Brazil) catechin content was between 24.12 ± 0.37 mg·100 g^{-1} in 'Isabel' and 117.00 ± 1.25 mg·100 g^{-1} in 'Negro Amaro', epicatechin between 17.78 ± 0.14 mg·100 g^{-1} in 'Isabel' and 47.50 ± 0.65 mg·100 g^{-1} in 'Pinot Noir' (Rockenbach *et al.* 2011). Also in the vine growing area Santa Catarina contents of polyphenols were lower than in Karlštejn, since grapes and seeds ripening in a warmer climate have generally lower polyphenol content. But the differences between the varieties were even higher than in Tuscany. In grape seeds from Castilla-La Mancha (Spain) catechin content varied between 82 ± 62.6 mg·1000 g^{-1} in 'Cencibel' and 500 ± 6 mg·1000 g^{-1} in 'Gewürztraminer', that of epicatechin between 60 ± 33.2 mg·1000 g^{-1} in 'Cencibel' and 310 ± 122 mg·1000 g^{-1} in 'Chardonnay', and epicatechin gallate between 13 ± 7.1 mg·1000 g^{-1} in 'Cencibel' and 70 ± 41.1 mg·1000 g^{-1} in 'Merlot', respectively (Rodríguez Montealegre *et al.* 2006). In grape seeds from Castilla-La Mancha the lowest polyphenol content was found in compared studies. But the differences between varieties were highly significant.

In studies reported by other authors, epicatechin and catechin were found dominant in the seeds of the vine in agreement with this study. Epicatechin gallate was also detected in small levels in these studies and other polyphenols were not reported. In agreement with our finding the impact of the variety was very important, and in addition, growing locations also proved to be a highly significant factor. Polyphenol content in the grape seeds from the Karlštejn location was higher than levels reported in samples in the aforementioned studies. This could be due to the fact that it is an area of higher altitude and latitude than usual for vine growing.

Conclusion

Catechin was found as a major flavan-3-ol monomer in grape seed with 51 % followed by epicatechin with 34 %. Minor flavan-3-ols were epigallocatechin with 6 % and epicatechin gallate with 4 %. The remaining 5 % were gallic acid. Ranges of catechin content were from 254 ± 28 $µg·g^{-1}$ to 20959 ± 175 $µg·g^{-1}$, epicatechin from 543 ± 15 $µg·g^{-1}$ to 16421 ± 121 $µg·g^{-1}$, epigallocatechin from 219 ± 44 $µg·g^{-1}$ to 1813 ± 124 $µg·g^{-1}$, epicatechin gallate from 41 ± 2 $µg·g^{-1}$ to 2606 ± 4 $µg·g^{-1}$ and gallic acid from 133 ± 5 $µg·g^{-1}$ to 1313 ± 49 $µg·g^{-1}$, respectively. The most important factor was effect of the variety, as the variety with the highest flavan-3-ol level ('Pinot Noir') contained 30 times more flavan-3-ols than the variety with the lowest content ('Kerner'). Effect of the year was shown to be least important, because total difference between years 2012 and 2013 was only 27 %. Between the content of catechin and epicatechin a negative correlation was demonstrated. In samples with higher percentage of catechin lower percentage of epicatechin was determined, and conversely. 'Pinot Noir', 'Muskat Donskoi', 'Aromriesling' and 'Hibernal' showed the highest phenolic content and therefore their products may contribute to human health.

Table

Representation of flavan-3-ols monomers in grape seeds of vine varieties in 2012 and 2013
(complete data are shown in 'Supplementary material")

Variety (VIVC code)	CAT [μg·g^{-1}]		EC [μg·g^{-1}]		Sugar level [%]	
	2012	2013	2012	2013	2012	2013
Albalonga (226)	4184 ± 110	655 ± 31	3255 ± 94	914 ± 8	18.2	21.0
Andre (456)	5750 ± 371	5728 ± 87	4131 ± 276	3961 ± 36	18.4	18.0
Auxerrois (792)	2101 ± 51	5150 ± 188	3291 ± 87	6084 ± 173	17.9	18.5
Bacchus W. (851)	1566 ± 19	1445 ± 169	1719 ± 67	1707 ± 49	17.2	16.0
Devin (3537)	4793 ± 71	6925 ± 124	2757 ± 63	2895 ± 34	18.6	18.8
Fratava (22807)	2036 ± 73	4203 ± 160	2572 ± 57	4635 ± 151	18.6	19.6
Hibernal (4711)	10742 ± 318	9588 ± 531	3685 ± 129	3159 ± 63	19.6	20.0
Chard. B. (2455)	3803 ± 116	1755 ± 54	5655 ± 168	3351 ± 120	18.6	18.4
Kerner (6123)	438 ± 24	254 ± 28	787 ± 44	543 ± 15	19.3	18.6
Muskat D. (8271)	5353 ± 112	2356 ± 65	3549 ± 64	2057 ± 40	17.8	17.5
Muskat Do. (8272)	5290 ± 299	7775 ± 74	10373±507	16421 ± 121	18.1	16.0
MOPR (13641)	2142 ± 25	785 ± 76	1977 ± 30	816 ± 93	21.0	19.6
Neronet (13980)	1232 ± 36	927 ± 35	1863 ± 102	1505 ± 32	17.6	17.5
Palava (8875)	4770 ± 138	3060 ± 37	3749 ± 222	3390 ± 28	20.0	16.0
Pinot B. (9272)	3349 ± 79	2643 ±53	4313 ± 74	3943 ± 99	20.1	19.0
Pinot G. (9275)	12572 ± 372	6688 ± 289	5354 ± 112	4333 ± 187	21.0	18.4
Pinot N. (9279)	20959 ± 175	15569 ± 317	9364 ± 119	9812 ± 288	18.6	21.0
Pinot P.N. (9280)	8603 ± 572	2718 ± 46	6609 ± 463	2044 ± 35	17.7	18.1
Port. Gra. (9623)	4174 ± 298	6684 ± 106	2157 ± 189	3361 ± 18	17.7	15.5
Port. Gru. (9624)	1777 ± 58	4960 ± 110	1124 ± 41	2175 ± 46	16.5	15.6
Aromriesling (637)	16535 ± 768	17782 ± 712	2940 ± 70	4057 ± 67	20.4	17.9
Riesling R (10076)	1087 ± 20	358 ± 31	1370 ± 21	606 ± 52	18.2	18.6
Riesling W. (10077)	1074 ± 11	1439 ± 28	1303 ± 37	1946 ± 52	17.8	16.2
Saint Laur. (10470)	2721 ± 21	6132 ± 137	1707 ± 29	2218 ± 40	18.4	19.5
Sauvig. B. (10790)	3163 ± 87	1743 ± 75	2617 ± 106	1962 ± 45	20.1	21.6
Savagnin B. (17636)	12382 ± 135	6398 ± 295	3224 ± 45	2292 ± 95	20.0	19.3
Silvaner F. (-)	1874 ± 43	1490 ± 26	1958 ± 71	2030 ± 16	16.2	18.3
Silvaner G. (11805)	919 ± 14	2123 ± 70	1353 ± 62	3033 ± 37	19.1	20.0
Savagnin R. (10797)	3182 ± 167	4661 ± 191	1671 ± 88	1635 ± 67	19.6	20.6
Veltliner G. (12930)	5539 ± 196	9148 ± 280	1993 ± 132	2033 ± 37	20.2	18.6
Veritas (12985)	6522 ± 235	2573 ± 41	4450 ± 142	2349 ± 20	20.0	23.4
Welschries. (13217)	6025 ± 118	1732 ± 48	5665 ± 123	1724 ± 24	15.3	16.0
Zahoranka (22402)	1637 ± 80	3483 ± 118	1103 ± 121	1714 ± 12	15.6	16.3
Zenit (13424)	7612 ± 119	3220 ± 24	3869 ± 51	2289 ± 19	20.5	18.0

GA = gallic acid; EGC = epigallocatechin; CAT = catechin; EC = epicatechin; ECG = epicatechin gallate; < LOD – below limit of detection.
List of varieties:
'Albalonga' (226); 'Andre' (456); 'Auxerrois' (792); 'Bacchus Weiss' (851); 'Devin' (3537); 'Fratava' (22807); 'Hibernal' (4711); 'Chardonnay Blanc' (2455); 'Kerner' (6123); 'Muskat Desertnyi' (8271); 'Muskat Donskoi' (8272); 'MOPR' (13641); 'Neronet' (13980); 'Palava' (8875); 'Pinot Blanc' (9272); 'Pinot Gris' (9275); 'Pinot Noir' (9279); 'Pinot Precoce Noir' (9280); 'Portugieser Grau' (9623); 'Portugieser Gruen' (9624); 'Aromriesling' (637); 'Riesling Rot' (10076); 'Riesling Weiss' (10077); 'Saint Laurent' (10470); 'Sauvignon Blanc' (10790); 'Savagnin Blanc' (17636); 'Silvaner Froelich' (-); 'Silvaner Gruen' (11805); 'Savagnin Rose' (10797); 'Veltliner Gruen' (12930); 'Veritas' (12985); 'Welschriesling' (13217); 'Zahoranka' (22402); 'Zenit' (13424).

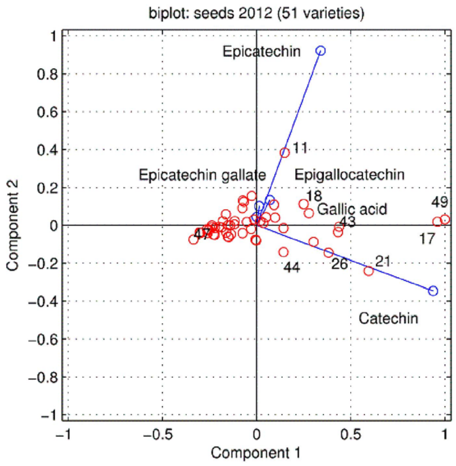

Fig. 1: List of varieties: 11-'Muskat Donskoi' (8272); 17-'Pinot Noir' (9279); 18-'Pinot Precoce Noir' (9280); 21-'Aromriesling' (637); 26-'Savagnin Blanc' (17636); 43-'Pinot Meunier' (9278); 44-'Hans (5286); 47-'Parthenocissus Quinquefolia Linne' (20506); 49-'Pinot Noir Swiss' (-); (-) Not registered in the Vitis International Variety Catalogue (VIVC). Detailed information is given in the Table.

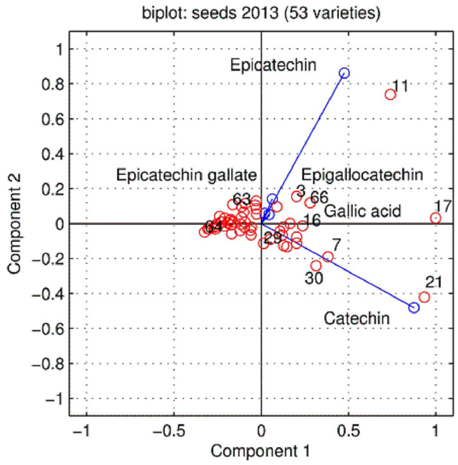

Fig. 2: List of varieties: 3-'Auxerrois' (792); 7-'Hibernal' (4711); 11-'Muskat Donskoi' (8272); 16-'Pinot Gris' (9275); 17-'Pinot Noir' (9279); 21-'Aromriesling' (637); 29-'Savagnin Rose' (10797); 30-'Veltliner Gruen' (12930); 63-'Muscat Hamburg (8226); 64-'Muscat Rannii' (16522); 66-'Neuburger' (8501). Detailed information is given in the Table.

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