MONITORING OF PHENOLIC COMPOUNDS OF WHITE WINES BY HPLC WITH FLUORESCENCE DETECTOR

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The influence of phenolic compounds on white wine aromas is very high. As different grape varieties ripen at different times, their contents vary and therefore the influence of phenolic acids on the final quality of wine aroma is evident. The wide spread of organic compounds is presented in the white wine aromas and some of them have fluorescence character. Four volatile compounds present in Slovakian protected wines (vintage year 2016) were examined by means of fluorescence detector. Direct estimation of these compounds (vanillic acid, gallic acid, caffeic acid and p-coumaric acid) sometimes shows several methodological problems. In this work direct estimation of these phenolic acids was performed by HPLC analysis with fluorescence detector in the four white grape varieties ('Pálava', 'Traminer rosé', 'Pinot gris' and 'Rheinriesling'). The different chromatographic peaks for gallic, vanillic, caffeic and p-coumaric acids were identified and confirmed by comparing their spectral characteristics to standards. The detection limits were calculated of each calibration graph and were 0.9 to 1.6 µg/l.
Keywords: phenolic acids, HPLC, grape varieties

Bestimmung phenolischer Verbindungen in Weißweinen mittels HPLC mit Fluoreszenzdetektor. Der Einfluss phenolischer Verbindungen auf Weißweinaromen ist sehr groß. Da verschiedene Rebsorten zu unterschiedlichen Zeiten reifen, variieren ihre Gehalte, und daher ist der Einfluss von Phenolsäuren auf die Qualität des Weinaromas offensichtlich. Die große Bandbreite organischer Verbindungen zeigt sich in den Weißweinaromen, und einige von ihnen haben Fluoreszenzeigenschaften. Vier flüchtige Verbindungen, die in slowakischen geschützten Weinen (Jahrgang 2016) vorhanden sind, wurden mittels Fluoreszenzdetektor untersucht. Die direkte Bestimmung dieser Verbindungen (Vanillinsäure, Gallussäure, Kaffeesäure und p-Cumarsäure) weist manchmal einige methodologische Probleme auf. In dieser Arbeit wurde die direkte Bestimmung dieser Phenolsäuren durch HPLC-Analyse mit Fluoreszenzdetektor in den vier weißen Rebsorten ('Pálava', 'Roter Traminer', 'Pinot Gris' und 'Rheinriesling') durchgeführt. Die verschiedenen chromatographischen Peaks für Gallussäure, Vanillinsäure, Kaffeesäure und p-Cumarsäure wurden identifiziert und durch Vergleich ihrer spektralen Eigenschaften mit Standards bestätigt. Die Nachweisgrenzen wurden für jede Kalibrierungskurve berechnet und betrugen 0,9 bis 1,6 µg/l.
Schlagwörter: Phenolsäuren, HPLC, Rebsorten

Wine is enjoyed by everyone not just because of its alcohol content, but also because of its aromas and antioxidant components. Wines containing higher amounts of these substances show positive effects on human health (De Nisco et al., 2013). Therefore the determination of phenolic acids is necessary. The techniques used in the winemaking process play a fundamental role in the final quality of the wines. Because of the strong influence of phenolic compounds on white wine quality as well as the decisive role of the released odor-active volatile compounds reflecting the particular variety, climate and soil (Roujou-de-Boubee, 1999; Pasquale Crupi et al., 2013), they were monitored. Four important varieties of *Vitis vinifera* L. were analysed: 'Traminer rosé', 'Pálava', 'Pinot gris' and 'Rheinriesling'. These varieties are used to make white wines with high quality – e. g. wines with predicates like late harvest, grape selection or berry selection, which are wines of protected designation of origin/PDO in Slovakia (Laštincová et al., 2016). The most common way to classify all these highquality wines is by monitoring the content of volatile aroma compounds. The aroma compounds analysis could be used for wine classification, quality control or study of sensory properties (Lakatošová, 2010). During maceration process the interaction between thiols and phenolic compounds in grapes is important (Pavloušek, 2011). During ripening phenolic acids are formed which guarantee aromas and could determine the right date of harvest. The 'Pálava' variety ripens in the second half of August, 'Traminer rosé' ripens from the beginning to the end of September and 'Pinot gris' together with 'Rheinriesling' at the beginning of October. Three different vineyards from the same PDO area (Malokarpatská) in Slovakia were included in this work.

MATERIAL AND METHODS

The white wines (vintage year 2016) were received from famous Slovak wine producers:

- 'Traminer rosé' from Elesko a.s. Modra, SR, date of harvest September 2016, quality varie - tal wine with protected designation of origin, dry;
- 'Pálava' from Elesko a.s. Modra, SR, date of harvest second half of August 2016, quality varietal wine with protected designation of ori gin, dry;
- 'Pinot gris' from winery Topoľčianky s.r.o., To poľčianky, SR, date of harvest second half of October 2016, quality varietal wine with late harvest predicate with protected designation of origin, dry;
- 'Rheinriesling' from Dufrex s.r.o., Hurbanovo, SR, date of harvest second half of October 2016, quality varietal wine with protected designation of origin, dry.

All wine samples were analysed in triplicate in the Central Controlling and Testing Institute in Agriculture in Bratislava. The standard parameters like sugar, alcohol, extract, volatile acids, total acids and total SO_2 were determined according to OIV methods.

The method described by Canas et al. (2003) was followed with some modifications. HPLC analysis was carried out on an Agilent 1260Series HPLC system (Agilent Technologies, Santa Clara, USA) consisting of a quaternary pump equipped with an injection valve (Rheodyne), thermostat, diode array detector and fluorescent detector (Agilent Technologies, Santa Clara, USA). Chromatographic column was LiChrospher 100 RP-18 (5 µm) LiChroCART 250-4 connected with guard column packed with the same sorbent (LiChrospher 100 RP-18 (5 µm) LiCroCART 4-4) (Agilent Technologies, Santa Clara, USA). The mobile phase for gradient elution was a mixture of methanol/water/formic acid (70/28/2 v/v/v) (A) and water/formic acid (98/2 v/v) (B) with gradient elution (0 to 3 min 0 % A, 3 to 25 min 0 to 40 % A, 25 to 43 min 40 to 60 % A, 43 to 55 min

60 % A, 55 to 60 min 60 to 80 % A, 60 to 65 min 80 % A, 65 to 68 min 80 to 0 % A). The flow rate was 1.0 ml/ min. The column temperature was kept constant at 40 °C. Samples were filtered through a 0.45 µm membrane and analyzed by direct injection of 20 µl. The spectrophotometric detector was operated at 280 nm. The fluorescence detector was operated at λex = 278 nm and λem = 360 nm (gallic and vanillic acids) or λex = 278 nm and λem = 454 nm (caffeic and p-coumaric acids). The identities of the different chromatographic peaks were confirmed by comparing their spectral characteristics to standards and retention times. Using UV detection a linear calibration range was 0.5 to 500 mg/l. With fluorescence detection linear calibration range was 5 to 500 µg/l. Detection limits were calculated as three times the standard deviation of the background noise divided by the slope of each calibration graph and were 0.03 to 0.08 mg/l for UV detection or 0.9 to 1.6 µg/l for fluorescence detection. Triplicate analyses were performed and the mean value was determined in all cases. Chromatograms were recorded and the identification of the compounds was achieved.

RESULTS AND DISCUSSION

In all wine samples hydroxybenzoic acids (gallic acid, vanillic acid) and hydroxycinnamic acids (caffeic acid, p-coumaric acid) were analysed by HPLC.

Hydroxycinnamic acids are the main phenolic compounds in white wines and originate from hydroxycinnamic tartaric esters present in grapes. In grapevine, they occur above all in the pulp of berries. Their content is rather independent of the winemaking process and the most important are caffeic, p-coumaric and ferulic acids. Studied were also some important antioxidants that typically occur in wine like gallic and vanillic acids. These hydroxybenzoic acids found in Riesling wines from Slovakia are as significant as in those from Germany or the Czech Republic (Tomaskova et al., 2017). The chromatograms (Fig. 1) with identification of the compounds were achieved by comparing spectra and the retention times of the separated peaks with the retention times of the standards. Quantification was made by the method using calibration of standards as a reference and was based on peak area from HPLC.

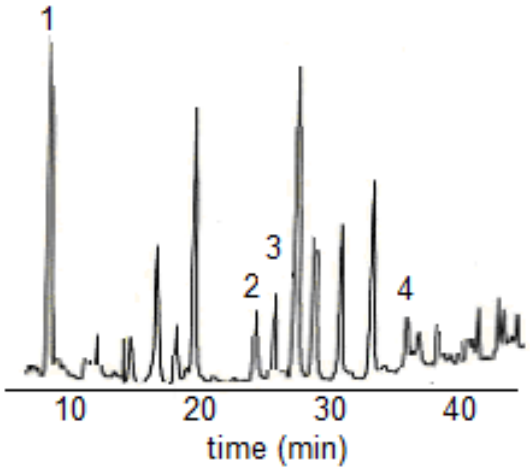

Fig. 1a: 'Pinot gris' ((1) 4.2 mg/l, (2) 1.2 mg/l, (3) 1.3 mg/l, (4) 0.8 mg/l)

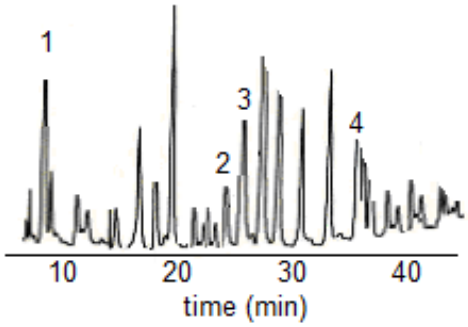

Fig. 1b: 'Traminer rosé' ((1) 1.8 mg/l, (2) 1.6 mg/l, (3) 2.3 mg/l, (4) 1.2 mg/l)

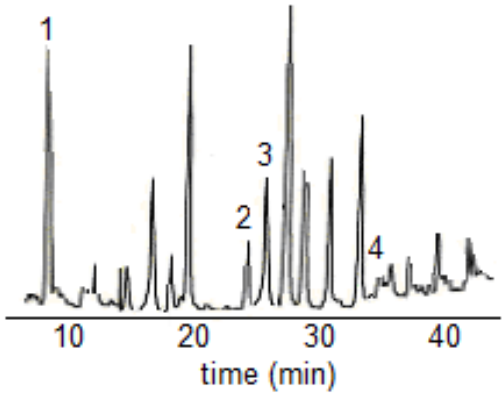

Fig. 1c: 'Pálava' ((1) 2.8 mg/l, (2) 1.2 mg/l, (3) 3.1 mg/l, (4) 0.4mg/l)

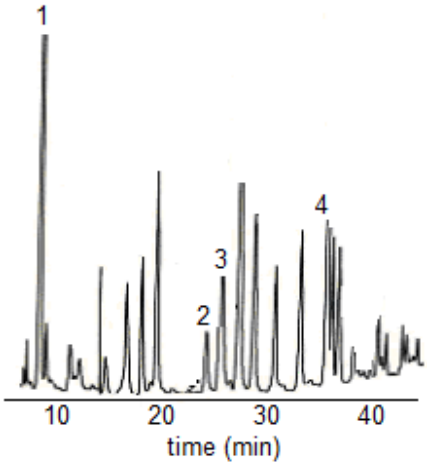

Fig. 1d: 'Rheinriesling' ((1) 5.6 mg/l, (2) 1.8 mg/l, (3) 2.5 mg/l; (4) 1.6 mg/l)

Fig. 1: Phenolic acids detected in the wine samples ((1) = gallic acid, (2) = vanillic acid, (3) = caffeic acid, (4) = p-coumaric acid)

The different amounts of these compounds were detected in different varieties of white wines (Table 1). The varieties of white wines which ripen in October have higher amounts of gallic and p-coumaric acid. They are mainly responsible for the flavor attributes typical for 'Riesling' (Kupsa et al., 2017). Varieties which ripen earlier in August to September like 'Pálava', have higher contents of caffeic acid. For the 'Traminer rosé' terpenes are mainly responsible for flavor, this variety ripens in September, the total acid is about 7.0 g/l (Domin, 2017; Goliáš et al., 2016) and lower concentrations of gallic acid were found. In dry white wines contents of phenolic compounds ranged from 0.4 to 5.6 mg/l and this is in agreement with literature. In 'Pálava' low concentrations of p-coumaric acid were found, but on the other hand there were higher contents of caffeic acid. In 'Rheinriesling' 1.6 mg/l of p-coumaric acid and 5.6 mg/l of gallic acid were found, which is a higher amount than in the other three varieties.

Table 1: Concentrations of phenolic acids and standard parameters in Slovak white wines (vintage 2016)

	Rulandské šedé/Pinot gris	Tramín červený/Traminer rosé	Pálava	Rizling rýnsky/Rheinriesling
Gallic acid mg/l	4.2 ± 0.5	1.8 ± 0.3	2.8 ± 0.2	5.6 ± 0.4
Vanillic acid mg/l	1.2 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.8 ± 0.2
Caffeic acid mg/l	1.3 ± 0.2	2.3 ± 0.2	3.1 ± 0.3	2.5 ± 0.3
p-Coumaric acid mg/l	0.8 ± 0.1	1.2 ± 0.3	0.4 ± 0.1	1.6 ± 0.3
Total SO_2 mg/l	138 ± 4	128 ± 4	125 ± 4	109 ± 4
Sugar g/l	1.6 ± 0.16	6.6 ± 0.6	3.7 ± 0.4	1.9 ± 0.19
Alcohol %vol.	12.37±0.1	12.90±0.1	12.86±0.1	12.38±0.1
Total acidity g/l	6.26±0.06	6.55±0.06	6.21±0.06	5.93±0.06

CONCLUSION AND DISCUSSION

The results here show a difference in the phenolic acids concentration between the examined white wine varieties. The maturity of grapes is usually monitored by means of the sugar concentration. However, the assessment of other parameters such as the phenolic content is also important because the phenolic maturity has an important impact on the organoleptic characteristics of wines (Garcia-Hernandez et al., 2018). Analysis of phenolic compounds in wine using the HPLC method with fluorescence detector could differentiate all the investigated phenolic acids in these dry white wines. The objective of this study was to evalute whether and which phenolic compounds in wine can serve as a source of fluorescence active compounds. They contribute to wine aroma, taste and color and this is a relatively simple method for direct determination of phenolic acids in dry white wines.

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