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Characterisation of (poly)phenolic constituents of two interspecific red hybrids of Rondo and Regent (*Vitis vinifera*) by LC–PDA–ESI-MS QTof

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ABSTRACT

The aim of this study was to identify and compare phenolic acids, flavan-3-ols, flavonols, and anthocyanins in two the most popular interspecific hybrids of red grapes, Rondo and Regent, nowadays very popular in red wine production in Poland. The phenolic profiles of these hybrids have not yet been reported. Thirty-three phenolic compounds, including 2 flavan-3-ols, 3 phenolic acids, 5 flavonols, and 23 anthocyanins, were determined in the examined samples using the ultra-performance liquid chromatography photodiode detector-quadrupole/time of flight-mass spectrometry (UPLC-PDA-Q/TOF-MS) method. Major differences were found in the phenolic profiles of investigated cultivars. The Regent hybrid exhibited the highest total phenolics content (27029.75 mg/kg dry matter) but Rondo was characterized by the highest concentration of anthocyanins (19342.36 mg/kg dm). The dominant fraction was anthocyanin compounds, especially acetylated > diglucosylated forms than glucosylated ones. This data represents valuable information that may be useful for oenological practices and to valorise these varieties as sources of bioactive compounds.

1. Introduction

Compared to typical wine countries such as France, Italy, Spain or Germany in Poland much more popular is growing of interspecific hybrids of *Vitis vinifera*. Beside these cultivars in recent time much more attention has been focused on two red hybrids of grapes, Rondo and Regent variety, which account more than 80% of red wine grapes produced in Poland, and are very popular in other countries from this part of Europe. The grape of Rondo was cross of Zarya Severa (a hybrid which has *V. amurensis* in its pedigree) and St. Laurent and registered in 1997 and Regent was cross of Diana, a Silvaner x Müller-Thurgau cross and thus a *V. vinifera* variety, with the interspecific hybrid Chambourcin and registered in 1999.

Both varieties were part of a genetic improvement program carried out by Czech Republic (Rondo) and Germany (Regent), however both finally were completed as experimental planting in Geisenheim Grape Breeding Institute. These interspecific hybrids are well-adapted varieties to cold climatic condition (very popular in Germany, Poland, Sweden, Netherlands, Ireland), more resistant to fungal-pathogens. Additionally, wine obtained from these varieties is characterized by deep red color. Color is an important indicator for evaluating the quality of red wine and is one of the most influential factors when consumers come to choose a wine in all parts of the world. The color of red wine is determined mainly by the composition and concentration of anthocyanins, which are responsible for slight red to dark purple color of wine. Varieties of *V. vinifera* only synthesize monoglucoside anthocyanins, whereas some other species like hybrids usually have diglucoside anthocyanins in significant quantities as well (OIV, 2011). Therefore, the occurrence of anthocyanidin 3,5-diglucosides is a quality indicator used in distinguishing vinifera from non-vinifera grapes and their products. For marketing and import purposes, many European countries test for diglucoside content, with wine testing above a specified limit (e.g., 15 mg/L) being prohibited for sale (OIV, 2011).

In recent years in Poland, there has been a rapid expansion of grape growing, particularly for wine production. Most of it has been concentrated in the west and southwest regions. The climatic conditions vary greatly, longer summer with higher temperature (but not very hot) with increasing altitude and distance from the sea, from east to west, which might impact the accumulation of flavonoid compounds in grapes. Nowadays Poland is a 'new world in

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Europe' for wine producers and consumers. Therefore, in the past few decades, many viticultural researchers have focused on the influence of the grapes growing environment.

Polyphenols from grapes and grape-derived products have been associated with the prevention of numerous diseases including cardiovascular and neurodegenerative diseases as well as several forms of cancer (Pasinetti & Ho, 2010; Aziz, Kumar, & Ahmad, 2003). Epidemiological and experimental evidence supports hypotheses that specific grape polyphenol forms may serve as disease preventative agents (Bertelli & Das, 2009; Renaud & de Lorgeri, 1992). Polyphenols are one of the most important quality parameters of wines and belong to two main groups of compounds, non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoids (anthocyanins, flavanols, flavonols and dihydroflavonols) (Garrido & Borges, 2013).

However, to the best of our knowledge, there has been no report so far on the individual phenolic composition of Rondo and Regent varieties. Considering this, the aim of this work was the identification, quantification and comparison of polyphenolic compounds (anthocyanins, flavonols, phenolic acid and flavanols) by LC-PDA/MS QTof of interspecific red hybrids – Rondo and Regent grapes, nowadays one of the principal red grape varieties cultivated in Poland. Present results provide some information about phenolic compounds, which play an important role in oenology due to their influence on some important sensory properties of wines, such as color, stability, bitterness, and astringency.

2. Materials and methods

2.1. Reagents

Acetonitrile for chromatography analysis was purchased from Merck (Darmstadt, Germany). Formic acid and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Cyanidin-, malvidin-, peonidin-, petunidin- of -3-*O*-glucoside, and -3,5-diglucoside, quercetin-3-*O*-galactoside, -3-*O*-glucoside, -3-*O*-glucuronide, myricetin-3-*O*-glucoside, caftaric acid, (+)-catechin and procyanidin B_2 were purchased from Extrasynthese (Lyon, France).

2.2. Plant material

Grape fruits of two hybrids of *V. vinifera*: 'Rondo' and 'Regent' were used in this study. Grapes were cultivated and collected in 2016 from the vineyard Winnice Wzgórz Trzebnickich localized in Jaksonowice, Poland (51°15′01.2″N 17°14′50.5″E). Grapes were harvested at the optimum ripening stage recommended for wine production. Measurements of 10 randomly chosen bunch of grapes from five plant per cultivar, were carried out. For each cultivar, approximately 1 kg of grapes was collected for analysis. After harvest, the whole grapes were cut and directly frozen in liquid nitrogen and freeze-dried (24 h; Christ Alpha 1–4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). After that homogeneous powders were obtained by crushing the dried tissues using a closed laboratory mill to avoid hydration (IKA 11A; Staufen, Germany). Powders were kept in a refrigerator (−80 °C) until extract preparation.

2.3. Extraction procedure

The powder samples of grapes (0.5 g) were extracted with 5 mL of methanol acidified with 1% acetic acid (v/v) and containing 1% ascorbic acid (g/v) as an antioxidant. The extraction was performed by incubation for 20 min under sonication (300 W, 40 kHz; Sonic 6D, Polsonic, Warsaw, Poland) with occasional shaking. The extraction part was repeated two times.

Next, the slurry was centrifuged at 19,000*g* for 10 min, and the supernatant was filtered through a hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck) and used for analysis.

2.4. Identification of phenolic compounds by the LC-PDA–MS QTof method

Methanolic extracts of two red hybrids grapes were analyzed using an ACQUITY Ultra Performance LC system consisting of an autosampler, binary solvent manager and photodiode array detector (PDA) (Waters Corporation, Milford, MA), with a mass detector G2 Q-TOF mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) as source operating in negative and positive modes. Chromatographic separations was performed on UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm; Waters Corporation, Milford, USA) maintained at 30 °C. Elution was performed at a flow rate of 0.42 mL/min. The gradient started with 99% of 1% aqueous formic acid, isocratic conditions for 1 min, followed by a 11 min linear gradient of 1% to 40% acetonitrile with 1% formic acid was applied. From 12 min the acidified acetonitrile was increased to 100%, followed to 2 min, and then returned to initial conditions at 2 min. Volume injected was 10 μL. Two MS experiments were performer, one in negative mode (phenolic acid, flavan-3-ols, flavonols) and one using positive ionization (anthocyanins) before and after fragmentation. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Analyses were carried out with voltage ramping cycles from 0.3 to 2 V, using full scan mode, and data-dependent MS scanning from *m*/*z* 100 to 1800, with collision induced fragmentation experiments were performed using argon as the collision gas. The capillary and cone voltages were 2500 V and 30 V, respectively. The capillary temperature was set to 300 °C, while the source heater temperature was 100 °C, and desolvation gas (nitrogen) flow rate of 300 L/h. Leucine enkephalin was flow rate of 2 μL/min and was used as the reference compound at a concentration of 500 pg/μL, and *m*/*z* at 554.2615 and 556.2771 were detected for negative and positive ionisation, respectively. The PDA spectra were recorded from 200 to 600 nm in steps of 2 nm, and runs were monitored at the following wavelengths: 280, 320, 360 and 520 nm for flavan-3-ols, phenolic acids, flavonols and anthocyanins, respectively. The PDA spectrum and retention times were compared to those of standards if they avaiable. Calibration curves at concentrations ranging from 0.05 to 0.5 mg/mL ($R^2 \leq 0.9998$) were made from caftaric acid, (-)-epicatechin, (+)-catechin, procyanidin B_2, quercetin-3-*O*-glucoside, myricetin-3-*O*-glucoside, and cyanidin-, malvidin-, peonidin-, petunidin- of -3-*O*-glucoside, and -3,5-diglucoside as standards. Quercetin and myricetin derivatives were expressed as quercetin- and myricetin-3-*O*-glucoside, respectively, and each identified anthocyanins were expressed as cyanidin-, malvidin-, peonidin-, petunidin- of -3-*O*-glucoside, and -3,5-diglucoside, respectively. All phenolic acids were calculated as caftaric acid. The results were expressed as mg per 100 g dry matter (dm). Data processing was performed using MassLynx 4.0 ChromaLynx Application Manager software. Sample was analyzed in duplicate.

3. Results and discussion

3.1. (Poly)phenolic profile by LC–PDA–ESI-MS QTof

The chromatographic fingerprint of polyphenolic compounds from two red hybrids of *V. vinifera*, Rondo and Regent, was analyzed using LC–ESI-MS/MS (with PDA and Q/Tof detectors) with