Scientia Horticulturae 230 (2018) 142–148

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

An insight into chemical composition and biological activity of Montenegrin *Vranac* red wine

N. Đorđević[a], M. Novaković[b], B. Pejin[c,*], M. Živković[b], A. Savić[c], J. Mutić[d], V. Tešević[d]

[a] Laboratory of Molecular Biology and Endocrinology, Institute of Nuclear Sciences "Vinča", University of Belgrade, Mike Petrovića, Alasa 12-14, 11351 Belgrade, Serbia
[b] Centre of Chemistry, Institute of Chemistry, Technology and Metallurg, ICTM, University of Belgrade, Studentski trg 12-16, 11001 Belgrade, Serbia
[c] Department of Life Sciences, Institute for Multidiscipinary Research, IMSI, University of Belgrade, Kneza Višeslava 1, 11030, Belgrade, Serbia
[d] Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11001, Belgrade, Serbia

ARTICLE INFO

Keywords:
Red wine
Vranac
New clones
Phenolics
Anti-2,2-Diphenyl-1-picrylhydrazyl radical activity
Metals

ABSTRACT

This study aimed to describe quality and potential health benefits of three *Vranac* wines obtained from new grape clones (CI, CII and CIII) recently developed and recognised. In this aim, their phenolic profiles, anti-2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical activities along with metal compositions were analyzed. Compared with the commercial one, CII and CIII *Vranac* wines were found to contain both higher contents of total phenolics, flavonoids and monomeric anthocyanins as well as anti-DPPH radical activities. The most abundant phenolics were gallic acid (8.88–16.36 mg/L), catechin (12.42–24.35 mg/L) and epicatechin (4.30–7.79 mg/L). Finally, the metal content of all the analyzed samples was within the toxicological safety limits. Taken all together, Montenegrin *Vranac* wines represent a rich source of both phenolics and minerals exhibiting promising antioxidant potential. CII and CIII wines can be considered for commercialising due to their high amounts of gallic acid, catechin and epicatechin, significant K/Na ratios and favourable contents of essential metals making them products with health added values.

1. Introduction

Balanced nutrition decreases the risk of numerous health problems and thus is of great importance for overall human health welfare. A growing body of data supports the view that use of medicinal food, including red wine, may have beneficial effect on human organism functioning. This type of wine actually represents one of the most important sources of dietary polyphenolic antioxidants, the compounds with proven effects against oxidative stress-related diseases. Among alcoholic beverages, red wine has been reported to have the most protective effect against oxidative stress (Woraratphoka et al., 2007). Its consumption (together with olive oil) was found to be one of the key explanations for the resolving of "French paradox" – low incidence of cardiovascular diseases despite intake of fatty foods (Renaud and de Lorgeril, 1992).

Vranac, autochthonous grape variety and brand of wine characteristic for Montenegro, Serbia and FYR of Macedonia, is exported to the EU. This wine brand frequently used in the Western Balkans is actually protected both as an intellectual property and the product with geographical indication of origin (Republic of Montenegro) since 1977 (WIPO, 2003). Berries, grooving in small bunches, are large and deeply coloured. The fruit is harvested mostly from mid September to mid October.

Polyphenols such as flavonoids, anthocyanins, hydroxybenzoates, hydroxycinnamates and stilbenes, the major wine compounds, are well known for their role in the reduction of risk for diseases related to oxidative stress such as cardiovascular, neurodegenerative and cancer (Jang 1997; Youdim et al., 2002). Moreover, these compounds also exhibit good anti-inflammatory properties (Youdim et al., 2002). Not only phenolic compounds themselves were found to have antioxidant activity, but also some of their metabolites are antioxidants as well (Villaño et al., 2005).

In addition, detailed profiling of wine phenolic compounds is currently in the focus of research due to several more reasons including evaluation of the authenticity of regional products, prediction of their sensory properties and issues related to their oxidative stabilities. Moreover, phenolics are used as markers of the wine processing technology and wine ageing (Gomez-Cordoves and Gonzalez-SanJosé 19953). Together with antioxidant capacity of the wine, the content and composition of polyphenols depend on many intrinsic and extrinsic factors such as grape variety, wine growing region and climatic conditions, viticultural practices as well as winemaking procedures including clarification, stabilisation, storage and ageing conditions and techniques (Gomez-Cordoves and Gonzalez-SanJosé 1995; Zafrilla

* Corresponding author at: Department of Life Sciences, Institute for Multidiscipinary Research, IMSI, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia.
E-mail addresses: brspjn@gmail.com, borispejin@imsi.rs (B. Pejin).

https://doi.org/10.1016/j.scienta.2017.11.033
Received 14 October 2017; Received in revised form 25 November 2017; Accepted 30 November 2017

et al., 2003).

Phenolic compounds found in red wine can be divided into two groups: flavonoids and non-flavonoids. They play a primary role in providing the sensory characteristics of the wine giving it the "oak wood" taste typical for long-aged products, besides being largely responsible for the astringency and bitterness of young wines (Gomez-Cordoves and Gonzalez-SanJosé, 1995; Gonalves and Jordao, 2009). The main phenolic compounds associated with the colour of red wine are anthocyanins (Pejin et al., 2016).

Additionally, major, trace and ultra trace elements are among the components that contribute to wine quality and nutritional value. Their concentrations may be quite variable. It is well known that daily consumption of wine in moderate quantities (1–2 glasses per day) contributes to the requirements for essential elements. However, above optimal level, some elements may have detrimental effects (Nicolini et al., 2004). Certain elements have determinant effects on the final organoleptic properties and, therefore, their concentrations must be monitored during wine manufacturing processes (Catarino et al., 2006). The International Organisation of Vine and Wine (OIV) (OIV, 2016) provides the maximum acceptable values for metal contents in the wine. Furthermore, regulation at national level dealing with acceptable limits of these elements does exist in almost all countries.

Clonal selection has a major importance in upgrading the quality both of the grape and wine (Vujovic et al., 2016a; Vujović et al., 2016). The main objective of this work was to compare quality (phenolic profile, anti-2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical activity and elemental composition) of *Vranac* wines obtained from three new grape clones (CI, CII and CIII) with the commercial wine of the same brand (a mixture of several different clones) bottled for sale due to search for novel dietary products with health added values. At the same time this study brings out first detailed chemical analysis of *Vranac* wines originated from Montenegro.

2. Materials and methods

2.1. Chemicals

Standards of catechin, epicatechin, quercetin, myricetin, siringaldehyde, protocatechuic, 4-hydroxy benzoic, ferulic, ellagic and syringic acids were purchased from Fluka AG (Buch, Switzerland). Kaempherol, vanillin, gallic, caffeic, *p*-coumaric and vanillic acid were supplied from Sigma-Aldrich (Steinheim, Germany). DPPH and acetonitrile (HPLC grade) were delivered from Sigma-Aldrich (Steinheim, Germany). Methanol, Folin-Ciocalteu's reagent and formic acid were purchased from Merck (Darmstadt, Germany). Ultrapure water (TKA Germany MicroPure water purification system, 0.055 μS cm^{-1}) was used in liquid chromatography (LC) analisys. The 0.22 μm Econofilters were purchased from Agilent Technologies (Santa Clara, CA, USA).

Ultrapure water (MilliQ, Millipore) and ultrapure acids (nitric acid, hydrochloric acid, Merck-Suprapure) were used for sample dilution and preparation of standards for Inductively coupled plasma (ICP) analysis. Multi-element standards were prepared in-house by mixing of certified, traceable, ICP grade single element standards (Merck-CertiPUR). Other chemicals and solvents were of analytical grade and supplied by Merck (Darmstadt, Germany).

2.2. Wines

Four *Vranac* wines produced in Montenegro were analyzed. These wines obtained from development sector of vinery "Plantaže 13. juli" A.D. (Podgorica, Montenegro) were produced according to the method described by Radović et al. (2015). The winery sample was labeled as BT wine (commercial wine bottled for sale), while the wines of the newly developed clones (Harvest, 2012) were labeled as CI (clone I), CII (clone II) and CIII (clone III). The samples provided directly from the producer in 750 ml glass bottles were stored at 10 °C in the dark and analyzed immediately after opening.

2.3. Determination of total phenolic content

The total phenolic content in the aforementioned wine samples was determined by the Folin–Ciocalteu procedure (Singleton et al., 1998) with some minor modifications. Wines were diluted in deionised water to give the dilution that will have absorbance between 0.2–0.7 (linear dependence of absorption from concentration) after the reaction with Folin-Ciocalteu (FC) reagent. The volume of 200 μl was mixed with 1000 μl of FC reagent previously diluted with the distilled water in proportion 1:10. After 6 min standing in the dark, 800 μl of 7.5% sodium carbonate solution were added, shaken and left in the dark for next 2 h to react. Asorbance was measured at 740 nm with a spectrophotometer (GBC Cintra 40). All samples were analyzed in three replications. The total phenolic content is expressed as gallic acid equivalents (mg GAE/L).

2.4. Determination of total flavonoid content

The total flavonoid content in the selected wine samples was determined by spectrophotometric procedure using aluminum chloride ($AlCl_3$) as reagent (Chia-Chi et al., 2002). The absorbance was measured at 410 nm with a spectrophotometer (GBC Cintra 40). All samples were analyzed in three replications. The content is expressed as rutin equivalents (mg RTE/L).

2.5. Determination of monomeric anthocyanin content

The anthocyanin content in the wine samples was determined by spectrophotometric pH differential method (Lee et al., 2005). The absorbance was measured on spectrophotometer (GBC Cintra 40) at 520 nm and 700 nm. All samples were analyzed in three replications. The content is expressed as cyanidin-3-glucoside (Cyd-3-glu) equivalents.

2.6. Determination of anti-DPPH radical activity

The antiradical activity of the wine samples was assessed using DPPH assay. A modified original method of Blois (1958) was used (Gorjanović et al., 2010). All wine samples were diluted in deionised water to solution that gives absorbance of DPPH radical remaining after the reaction in range 0.2-0.7. Volumes of 200 μl of the wine solutions were mixed with 1800 μl of methanolic solution of DPPH (0.04 mg/L) and left to stand to react in the dark at room temperature for 30 min. Absorbance was measured at 517 nm on spectrophotometer (GBC Cintra 40). Each wine sample was analyzed in four different dilutions, while each dilution was evaluated in three replications. The results are expressed as $EC_{50}{}^{-1}$ values (the reciprocal dissolution of the wine sample able to scavenge 50% of DPPH·).

2.7. Liquid chromatography–tandem mass spectrometry (LC/MS/MS) analysis

The analysis of phenolic compounds in wine samples was carried out using liquid chromatograph (Waters Acquity UPLC H-Class; WAT-176015007; Milford, MA USA) with ultraviolet detector [Waters 2998 PDA (Photodiode Array)] and interfaced to a mass detector [Waters TQ (Tandem Quadropole), WAT-176001263)]. For acquisition and processing data MassLynx V4.1 software was performed.

Wine samples were filtered and then directly injected onto ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 μm). Elution program used was according to method described previously by Radović et al. (2015). The mobile phase consisted of 0.2% (v/v) formic acid in deionised water (solvent A) and acetonitrile (solvent B), starting from 95% to 84% solvent A (20 min); from 84% to 60% solvent A (28 min); from 60% to