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Research paper

Some chemical characteristics and antioxidant capacity of novel Merlot wine clones developed in Montenegro

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ABSTRACT

The overall aim was to compare the quality of two Montenegrin Merlot wines obtained from new vine clones (VCR1 and VCR 101) along with commercial Merlot wine throughout the consecutive vintages in 2010 and 2011, due to preliminary screening of the potential of novel Merlot vine clones for the wine industry. The content of phenolic compounds was determined by liquid chromatography – tandem mass spectrometry (LC–MS/MS). Elemental composition was analysed using inductively coupled plasma optical emission spectrometery (ICP-OES) and inductively coupled plasma mass spectrometery (ICP-MS)]. Additionaly, antioxidant capacity was assessed by cyclic voltammetry. Compared with the commercial one, both wine clones contained higher percent of major phenolic compounds (namely, gallic acid and catechin) with top values recorded for VCR1 both in 2010 (30.46 ± 0.42 mg/l) and 2011 (28.12 ± 0.29 mg/l). The same wines were enriched with epicatechin, resveratrol, myricetin and quercetin. Furthermore, VCR 1 wine from 2011 stood out for its elemental composition. On the other hand, antioxidant capacity of VCR 101 wines was the highest one for the both vintages. According to the experimental data obtained, both new Montenegrin Merlot wines represent good candidates for the commercialised wines with an indication of geographical origin.

1. Introduction

The purpose of clonal selection of grapevine is to achieve healthy clones capable of obtaining high quality grapes and resistant towards different environmental conditions (Vujović et al., 2016a). As a consequence, more tasteful wines of higher quality have been produced. Indeed, the combination of the selected clones may lead to the wine with specific chemical properties and organoleptic features (Vujović et al., 2016b). Clonal evaluation, the necessary process in clonal selection, includes viticultural analysis (growth and yield components, rot susceptibility), wine analysis (chemical data, colour, tannins) and sensory evaluation. Among numerous vine varieties, Merlot that is produced all over world is among the most important ones (Vujović et al., 2016c). Both Mediterranean and continental climates in Podgorica's viticultural area (Montenegro) enables this variety to preserve its best properties. Moreover, Merlot wine produced in "Plantaže 13. juli" A.D. winery (Podgorica, Montenegro) is exported to countries around the world, so determining the composition of the wine and improving its quality is of wider importance.

Generally, identification of phenolic profiles of red wines is of great importance due to several reasons. Besides the fact that phenolics influence organoleptic features of the wine, such as astringency, bitterness and color stability, they may also play important role in increasing its nutritional value (Pejin et al., 2016; Đorđević et al., 2017). Additionally, phenolic compounds can act as an antioxidant species capturing and/or neutralising free radicals, contributing in such way to the maintaining the homeostasis of the human body (Jang et al., 1997; Youdim et al., 2002). Indeed, numerous studies have indicated that moderate daily wine consumption appears to protect against many chronic disease, like cardiovascular diseases, certain cancers and dementia (Arranz et al., 2012; Garaguso and Nardini 2015). Phenolic compounds, as bioactive food components, provide additional health

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benefits of red wine, classifying it into the functional food.

All wine phenolics can be classified into flavonoid or non-flavonoid compounds. The flavonoids includes anthocyanins, flavanols and flavonols, while the main non-flavonoids are phenolic acid derivatives (hydroxybenzoic and hydroxycinnamic acids along with their derivatives) and stilbenes. Among the analytics used for the wine profiling, liquid chromatography (LC) with tandem quadrupol (TQ) detection is the most commonly applied one (Lambert et al., 2015). It is well known that this analytical method has several advantages including no need for complicated and time consumpting sample preparation coupled with high precission and selectivity.

Nutritional value and organoleptic characteristics of wine greatly depend on its multi-elemental content. Indeed, wine is a source both of essential elements for humans and potentially toxic ones. Elemental composition of wine is influenced by different environmental factors, such as soil composition, pollution of the surrounding environment and climate (Geana et al., 2014). Nonetheless, viticultural practices itself along with processing methods can induce significant changes in its elemental composition and general chemical content (Ivanova-Petropulos et al., 2015a). For these reasons it is important to regularly monitor the content of major and trace elements in wine. For example, ICP-OES and ICP-MS are rapid analytical methods of choice for such analysis due to their high sensitivity and selectivity (Nardi et al., 2009).

Cyclic voltammetry represents one of the novel analytical methods widely used for determination of total antioxidant capacity. It actually represents an electrochemical method performed by linear cycling of the potential of a working electrode in anodic and chatodic direction along with measuring the resulting current. Among other applications, this method is commonly used for the analysis of the plant products and foodstuff (Kilmartin and Zou Waterhouse, 2001; Makhotkina and Kilmartin 2010). Its key advantages are rapidity, simplicity and sensitivity (Lino et al., 2014). Moreover, cyclic voltammetry is low-cost analytical method which does not need prior sample preparation, thus shortening the duration of the analysis. Finally, it only depends on the inherent electrochemical properties of the antioxidants presented in the sample not requesting the presence of oxidisable compounds for measuring antioxidant capacity (Lino et al., 2014).

The aim of this work was to determine phenolic profile, elemental composition and antioxidant capacity of two Montenegrin Merlot wines obtained from new vine clones (VCR1 and VCR 101) along with commercial Merlot wine throughout the consecutive vintages in 2010 and 2011. This was done in order to compare the quality and health added value of aforementioned wines due to preliminary screening of the potential of novel Merlot vine clones for the wine industry.

2. Materials and methods

2.1. Standards and solvents

Acetonitrile (HPLC grade) was delivered from Sigma-Aldrich (Steinheim, Germany). Methanol and formic acid (HPLC grade) were purchased from Merck (Darmstadt, Germany). Standards of protocatechuic, 4-hydroxy benzoic, catechin, epicatechin, quercetin and myricetin were purchased from Fluka AG (Buch, Switzerland). Gallic and caffeic acids, as well as kaempherol and resveratrol, were supplied from Sigma-Aldrich (Steinheim, Germany). Ultrapure water (TKA Germany MicroPure water purification system, 0.055 μS/cm) was used in liquid chromatography (LC) analysis. Econofilters (0.22 μm) were purchased from Agilent Technologies (Santa Clara, CA, USA).

0.1 M KCl (Merck, Darmstadt, Germany) was used as supporting electrolyte in cyclic voltammetry analysis. Multielement standards were prepared in-house by mixing of certified traceable ICP grade single element standards (Merck-CertiPUR). For sample dilution and preparation of standards, ultrapure water (MilliQ, Millipore) and ultrapure acids (nitric acid, hydrochloric acid, Merck-Suprapure) were applied. The other chemicals and solvents used of analytical grade were purchased from Merck (Darmstadt, Germany).

2.2. Wine samples

The samples of commercial wines (2010 and 2011) along with two novel red wine clones of Merlot variety were obtained from developing sector of “Plantaže 13. juli” A.D. winery (Podgorica, Montenegro). The samples were selected according to a vintage (2010 or 2011), and labeled as Merlot bottled, Merlot VCR1 and Merlot VCR 101. All the analysed samples were produced under the same conditions and using standard vinifications (Radović et al., 2015). They were stored at 10 °C in the dark (prior to analysis) and analysed immediately after opening.

2.3. Liquid chromatography − tandem mass spectrometry (LC–MS/MS) profiling of phenolic compounds

After filtration through 0.22 μm Econofilters (Agilent Technologies, Santa Clara, CA, USA), the wine samples analysed were directly injected into liquid chromatograph (Waters Acquity UPLC H-Class; WAT-176015007; Milford, MA USA) with ultraviolet detector [Waters 2998 PDA (Photodiode Array)], interfaced to a mass detector [Waters TQ (Tandem Quadropole), WAT-176001263)]. MassLynx V4.1 software was used for acquisition and data processing.

ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 μm) was used for the separation of the phenolic compounds. The solvents used were 0.2% (v/v) formic acid in deionised water (solvent A) and acetonitrile (solvent B).

Elution program, according to previously described method Radović et al. (2015), was as follow:

- 5% – 16% linear gradient of solvent B (20 min)
- 16% – 40% linear gradient of solvent B (28 min)
- 40% – 70% linear gradient of solvent B (32 min)
- 70% –98% linear gradient of solvent B (36 min)
- Constant at 98% solvent B (45 min)
- 98% – 5% linear gradient of solvent B (46 min)
- Constant at 5% solvent B (55 min) due to reconditioning of the column

Column temperature was maintained at 25 °C, while mobile phase flow rate was 0.7 ml/min. PDA and mass detectors were used for analysing and quantifying of the separated phenolic compounds. The representative chromatographic peaks of the selected phenolic compounds determined in VCR 101 sample of 2011 vintage are presented in Fig. 1.

Operation conditions on mass detector were as follow:

- Temperature of the electrospray ion
- Source: 150 °C
- Capillary voltage: 3.5 kV
- Cone voltage: 20–60 V [depending on the tested component (Table 1)]
- Collision energy: 10 eV–56 eV [depending on the tested component (Table 1)].

IntelliStart feature of MassLynx V4.1 was applied for tuning of the mass spectrometer (MS). Retention times, UV maxima and the multiple reaction monitoring transitions in electrospray ionisation in negative (ESI–) and positive (ESI +) modes were used for identification and quantification of the selected phenolic compounds (mg/l) (Table 1).

2.4. Multielement analysis

ICP-OES (Thermo Scientific, United Kingdom), model 6500 Duo, equipped with a CID86 chip detector was applied for determination of major elements (calcium, sodium, potassium, magnesium) and iron.