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Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: Effect of variety and enological practices

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ABSTRACT

The content of the stilbenes *trans*-resveratrol and piceid as well as the antioxidant activity of Macedonian red wines from the two main grape varieties Vranec and Merlot have been evaluated. The effects of time of maceration, type of yeast and the level of sulphur dioxide applied on stilbene content and antioxidant activity have been studied. The most important factor in winemaking technology is the maceration time since the highest concentrations of *trans*-resveratrol, piceid and highest antioxidant activity were found following 6 and 10 days of maceration. Concerning the yeast type, higher concentrations of *trans*-resveratrol and piceid have been obtained with French yeast "Levuline CHP" in comparison to Macedonian yeast "Vinalco". In contrast, the higher antioxidant activity of wines from both varieties of grapes was observed by application of Macedonian yeast "Vinalco".

1. Introduction

Stilbenes belong to the non-flavonoid class of wine phenolic compounds and resveratrol is the major stilbene present in grapes and wines (Wilkens, Rentzsch, & Winterhalter, 2008, chap. II). Resveratrol occurs in two isomeric forms, the *trans*- and *cis*-configured isomers. *Trans*-resveratrol or *trans*-3,5,4′-trihidroxystilbene is the most abundant form being mainly located in grape skins. Gluco-conjugated forms of *trans*- and *cis*-resveratrol are known as piceids.

Generally, stilbenes are known as phytoalexins which can be biosynthesised from grapevines as a defence to fungal diseases, such as *Botrytis cinerea*, or abiotic stress and UV irradiation.

The antioxidant and antimicrobial efficiency of resveratrol provides health benefits, such as the prevention of cardiovascular diseases, arteriosclerosis and cancer. Originally, epidemiological studies indicated an inverse relationship between moderate wine consumption and the risk of coronary heart disease, the so-called "French Paradox" (Barnard & Linter, 1992).

Comparisons between levels of resveratrol in different red wines from a single grape variety (mono-varietal red wines) and the level of resveratrol in red wines from different regions was also established. The average level of *trans*-piceid was found to be three times higher than that of *trans*-resveratrol. The content of resveratrol has been determined in white (0.005–0.57 mg/L) and red wines (0.550–2.534 mg/L) from Greece (Gerogiannaki-Christopoulou, Athanasopoulous, Kyriakidis, Gerogiannaki, & Spanos, 2006; Kallithraka, Arvanitoyannis, El-Zajouli, & Kefalas, 2001), in red wines from the Canary Islands (2.06–3.75 mg/L) (Rodríguez-Delgado, González, Pérez-Trujillo, & García-Montelongo, 2002), in wines produced from grapes cultivated in the Snake River Valley (>1.91 mg/L) (Lee & Rennaker, 2007), in red and rosé wines produced in the four designations of origin of Aragon (0.62–3.09 mg/L) (Abril, Negueruela, Pérez, Juan, & Estopañán, 2005), in red wines elaborated from Galician varieties (3.02–36.13 mg/L) (Feijóo, Moreno, & Falqué, 2008), in Brazilian wines (0.04–1.26 mg/L) (Lucena et al., 2010), as well as in a wide range of commercial red and white wines from Japan (0.2–1.5 mg/L), France (3.8–7.4 mg/L)

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(Stervbo, Vang, & Bonnesen, 2007) and Serbia (till 2.5 mg/L) (Atanacković et al., 2012).

The antioxidant potential of wine is largely attributable to its phenolic composition which is determined by its flavonoid content. A common method for determining the antioxidant activity of wines is the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical cation ($ABTS^{\cdot+}$) method (Re et al., 1999). The TEAC (Trolox Equivalent Antioxidant Capacity) value which is measured for wine samples expresses the concentration of a Trolox solution whose antioxidant activity is identical to that of the wine itself. This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to a 1.0 mM solution of the sample under study. Effects of some prefermentative treatments (e.g. addition of SO_2 and ascorbic acid before grape crushing or must hyperoxidation) on the final resveratrol content in red wine have been studied (Castellari, Spinabelli, Riponi, & Amati, 1998). It was demonstrated that lactic acid bacteria can impact resveratrol and piceid levels in wines (Poussier, Guilloux-Benatier, Torres, Heras, & Adrian, 2003). According to the findings of the research group of Vrhovsek, different yeast strains can significantly affect the resveratrol content in wine, except that of the *cis*-resveratrol glucoside (Vrhovsek, Wendelin, & Eder, 1997). Increases in the levels of resveratrol after malolactic fermentation have been shown to be a result of glycoside breakage of piceid primarily concentrated in the grape skins (Pezet & Cuenat, 1996). Further studies have examined the effect of different types of yeast (Vacca, Leccis, Fenu, Pretti, & Farris, 1997) and the improvement of enzymatic hydrolysis with regard to higher levels of resveratrol in wine (La Torre et al., 2004).

The influence of temperature, pre- and post-fermentative factors, the influence of yeast and other factors of the wine-making process on the phenolic compounds of wines has been the focus of many studies (Caridi, Cufari, Lovino, Palumbo, & Tedesco, 2004; Gil-Muñoz, Gómez-Plaza, Martínez, & López-Roca, 1999; Gil-Muñoz, Gómez-Plaza, Martínez, & López-Roca, 1997; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999). The group of Kovac found that the length of maceration time and the addition of high quantities of pomace, seeds and must increased the concentration of catechines and proanthocyanidins in wines (Kovac, Alonso, Bourzeix, & Revilla, 1992). The group of Budić-Leto studied the influence of maceration time on the concentration of polyphenolic compounds in autochthonous cultivar 'Plavac mali' (*Vitis vinifera* L.). The results indicated that prolonged maceration time can increase the concentrations of proanthocyanidins and decrease the content of anthocyanins (Budić-Leto, Gracin, Lovrić, & Vrhovsek 2008).

It is obvious that there are already many studies published on resveratrol and the antioxidative potential of wine, but, until now, there have been no published results about the level of resveratrol and the antioxidant activity of Macedonian wines. Therefore, the main objective of this study was to examine the content of resveratrol and piceid in Vranec and Merlot wines produced under different winemaking conditions, as well as the resulting antioxidant capacity. This included the application of different maceration times (3, 6, and 10 days), SO_2 dosage and a study of the effect of different types of yeast.

2. Materials and methods

2.1. Samples and winemaking process

Twelve red wines from Vranec grape variety (V1–V12) and 12 red wines from Merlot variety (M1–M12) produced at the Experimental Laboratory of the Department for Enology, Institute of Agriculture, Skopje, Macedonia, were the subject of this investigation.

In brief, grapes from both varieties were harvested at optimal maturity (22 °Brix for Vranec and 20 °Brix for Merlot) and, after crushing, the grape mash was divided into 12 lots collected in 25 L plastic fermentation tanks. Aqueous solutions of potassium metabisulphite were added to the mashes of both varieties to give six tanks with 30 mg/L of total SO_2 and six tanks with 70 mg/L of total SO_2. Two yeasts (*Saccharomyces cerevisiae*) were used for fermentation: Vinalco, selected by Yeast Factory, Bitola, R. Macedonia, and Levuline, isolated in the terroirs of Champagne and selected by CIVC 8130 (Interprofessional Committee of Champagne Wines), France. Vinalco (20 g/100 L) was applied to three lots containing 30 mg/L of SO_2 and three other lots containing 70 mg/L of SO_2 of each variety. Levuline (30 g/100 L) was applied to the other lots of both varieties.

Maceration times of 3, 6, and 10 days were applied for wine production of both varieties, each containing two doses of SO_2 and two yeasts for fermentation. After stabilisation for 2 weeks (at −4 °C), wines were bottled and analysed after 2 years of storage.

2.2. Reagents

trans-Resveratrol and *trans*-piceid were obtained from Phytolab, Vestenbergsgreuth, Germany. Distilled water, acetonitrile, methanol, ethanol and glacial acetic acid were purchased from Merck, Germany. All solvents used were HPLC grade. 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) in the crystallised diammonium salt form, horseradish peroxidase type VI-A, hydrogen peroxide 30% (v/v) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a water soluble tocopherol analogue), were also obtained from Merck, Germany.

2.3. TEAC assay

The antioxidant activity of wines was determined using the $ABTS^{\cdot+}$ method for screening of the antioxidant activity as a decolorisation assay applicable to both lipophilic and hydrophilic antioxidants (Re et al., 1999). The assay involves direct production of the blue/green $ABTS^{\cdot+}$ chromophore through reaction between $ABTS^{\cdot+}$ and potassium persulphate. The product has absorption maxima at 414, 645, 734 and 815 nm.

The method employed in this study gives a measure of the antioxidant capacity of red wines produced under different conditions. For this purpose, 10 mL of ABTS solution was prepared. The ABTS solution was made from 38.43 mg of ABTS and 6.90 mg of $K_2S_2O_2$ and made up with Nanopure water to volume. For the calibration curve, 5 mL solution of 12.52 mg of Trolox standard were diluted with ethanol (97%) and four standard solutions were prepared for the calibration curve ($y = 0.2523X + 0.0453$ and $R = 0.9996$) with concentrations of 250, 500, 750, and 1000 mmol/5 mL, respectively. The measured values for antioxidant activity of the wines were recorded after 6 min. A UV/vis spectrophotometer Bruker IFS 66 was used for the analyses. The absorbance was measured at 734 nm at room temperature.

2.4. HPLC analysis

A Chromatograph Agilent Technologies 1200 Series, with Jasco AS-950 sampler, an auto injector (20 μL injection volume) was used for the analyses. Mass Selective Detector type Bruker Daltonics HCT Ultra was used for identification of *trans*-resveratrol (under negative ion mode) and *trans*-piceid. For quantification purposes, a Jasco MD-1510 Multiwavelength Detector was applied. Separation of the components was performed by using a C18 Luna column (5 μm × 4, 6 mm × 25 cm, Phenomenex). The mobile phase flow rate was 0.5 mL/min. The eluents were: solvent A (water:acetic