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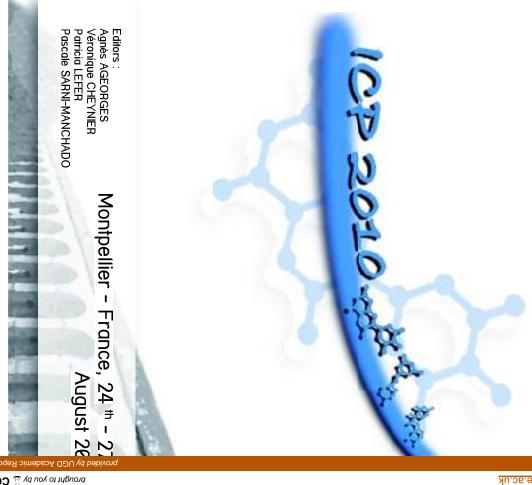
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## Influence of maceration time, $SO_2$ and yeast strain on the content of phenolic compounds in wines from Vranec and Merlot varieties

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Abstract. Red wines from V. vinifera Vranec and Merlot grapes produced using a series of identical fermentation conditions for all wines (maceration times of 3, 6 and 10 days, two doses of sulphur dioxide, 30 and 70 mg/L SO2, and two yeasts for fermentation, Macedonian yeast-Vinalco and French yeast-Levuline) were subject of investigation. The concentration of phenolic acids, anthocyanins and flavan-3ol monomers was determined by reversed-phase HPLC, while analysis of proanthocyanidins was performed by HPLC after acid-catalysed cleavage in the presence of phloroglucinol. Vranec wines were richer in anthocyanins and hydroxycinnamic acids and showed higher content of condensed tannins in comparison to the Merlot wines obtained under the same technological conditions. Vranec wines reached highest concentrations of anthocyanins after 6 days of maceration, while gradual increase of proanthocyanidins was observed both for Vranec and Merlot wines with increased pomace contact. The highest contents in acylated anthocyanins and proanthocyanidins were found in Vranec wines fermented with French yeast, macerated for 10 days. Differences in the extraction kinetics between the two yeast strains can probably be attributed to differences in their fermentation kinetics. Higher doses of SO2 resulted in increased extraction of all phenolic compounds. Statistical treatments of the data, using cluster analysis and principal component analysis, showed a clear separation of the samples, mainly according to the variety, followed by sub-grouping related to the maceration time and SO<sub>2</sub> content.

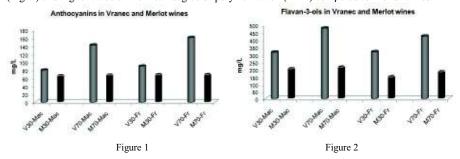
**Introduction.** Oenological practices affect the extraction of phenolic compounds from grapes and their subsequent reactions in wine. In red wine production, studies are primarily focused on the influence of maceration on the extraction of grape pigments and tannins. Anthocyanins are the first components to be extracted from the grape skins together with the skin tannins, while seed tannin extraction, which is more dependent on increasing ethanol content because of their lower solubility in water, starts towards the midpoint of alcoholic fermentation and continues until pressing during the postfermentation phase [1,2]. Addition of SO<sub>2</sub> at crushing increases the transfer of polyphenols into the must [3] and acts as a protector against enzymatic oxidative reactions. The purpose of this research was to study the phenolic composition of Vranec wines (widely cultivated variety in R. Macedonia) obtained under different wine-making conditions and to compare it to phenolic composition of Merlot wines (as a widespread variety) made in the same way.

**Materials and Methods.** Grapes from *Vitis vinifera* L., Vranec and Merlot varieties (2007 vintage) harvested at optimal maturity (22 and 20 °Brix, respectively) were processed including addition of two doses of aqueous solution of potassium metabisulfite, 30 and 70 mg/L, and two yeasts for fermentation, Vinalco (Bitola, R. Macedonia) and Levuline CHP (Bordeaux, France). Maceration time of 3, 6 and 10 days was applied, yielding 12 lots in total for each variety. Wines were mechanically "pumped over" twice a day during fermentation and after maceration, separated from the pomace, stabilized at -4°C for two weeks and bottled.

A Waters 2690 HPLC system was used for analysis of phenolic compounds by direct injection, and elution was performed by previously proposed method [4]. For tannin analysis, the reaction with phloroglucinol was performed as previously described [5]. Tannins after phloroglucinolysis were analyzed on a same C18 column, applying linear gradient of water/formic acid (98:2) and acetonitrile/water/formic acid (80:18:2), at a flow rate of 1 mL/min at 30 °C [4].

Statistical treatment including Cluster Analysis and Principal Component Analyses was performed with statistical software TANAGRA 1.4.28 (Lyon, France).

**Results and Discussion.** Higher levels of hydroxycinnamic acids and of anthocyanins, especially non acylated ones, were found in Vranec then in Merlot wines, irrespective of the wine-making techniques (Fig. 1). Vranec and Merlot wines had high levels of (+)-catechin and (-)-epicatechin, compared to other Merlot wines, as well as to other red cultivars [6]. Vranec wines showed higher contents of flavan-3-ols (Fig. 2) and higher value of the mean degree of polymerization (mDP) compared to Merlot wines.



**Figure. 1.** Anthocyanin contents determined by HPLC analysis of Vranec and Merlot wines obtained under different vinifications, macerated for 6 days

**Figure 2.** Flavan-3-ol contents determined by HPLC in Vranec and Merlot wines obtained under different vinifications, macerated for 6 days

Labels-V: Vranec; M: Merlot; 30: 30 mg/L SO<sub>2</sub>; 70: 70 mg/L SO<sub>2</sub>; Mac: Macedonian yeast, Vinalco; Fr: French yeast, Levuline

The highest anthocyanin concentrations were reached in Vranec wines macerated for 6 days, and in Merlot wines after maceration of 10 days in presence of higher dose of  $SO_2$ . Concentration of flavan-3-ol monomers and proanthocyanidins were highest in the wines macerated for 10 days. The content of epigallocatechin and epicatechin-3-O-gallate units (expressed in mg/L) increased with increased maceration time for all wines. The mDP slightly from the third to the sixth day of maceration and after remain almost constant in the wines macerated for 10 days. The content of hydroxycinnamic acids was not influenced by the maceration time but was increased with increased  $SO_2$  levels. Higher concentrations of anthocyanins and proanthocyanidins were found with higher content of  $SO_2$ , confirming that  $SO_2$  increases the extraction of pigments and tannins and limits oxidation.

Higher levels of anthocyanins and tannins were found in Vranec wines fermented with French yeast and in Merlot wines fermented with Macedonian yeast, indicating that different yeast strains could have different fermentation rates on different grapes, with subsequent influence on phenolic extraction.

Cluster analysis and principal component analysis showed separation of the samples mainly according to the variety, and then, sub-grouping by the maceration time and  $SO_2$  dose.

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