HPLC Analysis of Hydroxycinnamic Acid Derivatives in Smederevka and Chardonnay Wines

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INTRODUCTION

Phenolic compounds are considered as main factors responsible for the quality of grapes, and thus for their corresponding wines, and also, they are specific for different cultivars. Wine phenolics belong to two main groups: nonflavonoids and flavonoids. The major nonflavonoid phenolic compounds of white wines are hydroxycinnamic acid derivatives, such as caffeoyltartaric (caftaric) acid, *p*-coumaroyltartaric (*p*-coutaric) acid and feruloyltartaric (fertaric) acid (Fig. 1). For white wine production, maceration is kept to a minimum and seldom lasts more than few hours. White wines are usually made at low temperatures (14–18 °C). The juice runs freely from the crushed grapes, which are protected with SO_2 to prevent the enzymatic oxidation.

Hydroxycinnamic acid derivatives	R_1	M_r
Coutaric acid	H	296
Caftaric acid	OH	312
Fertaric acid	OCH_3	326

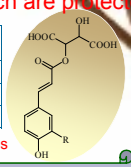

Fig. 1. Structures of hydroxycinnamic acids derivatives

The presented study represents the first attempt to analyze Macedonian white wines for their polyphenolic acid content with HPLC: Smederevka, as typical for the Balkan region and the most wide spread variety at Macedonian vineyards, and Chardonnay, as well known grape variety, in order to compare them and correlate the contents with the winemaking protocols.

MATERIALS AND METHODS

Macedonian white wines, Smederevka and Chardonnay (*Vitis vinifera* L.) were subject of investigation. Winemaking procedures for both varieties included addition of two doses of SO_2 (50 and 100 mg/L) and two yeasts for fermentation (Vinalco and Levuline). A reversed phase liquid chromatographic method was used for identification and quantification of hydroxycinnamic acid derivatives in the wines. Separation of the components, by direct injection of the wines into HPLC (Waters 2690 system), was performed using reversed-phase Atlantis dC18 column, monitored at 320 nm. The mobile phase consisted of water/formic acid (99:1; solvent A), and acetonitrile/water/formic acid (80:19:1; solvent B). In addition, HPLC-MS (Waters 2690 system equipped with ThermoFinnigan LCQ Advantage ion trap mass spectrometer) analysis was carried out to confirm the identity of the separated compounds, recording the spectra in negative ion mode.

RESULTS AND DISCUSSION

MS and UV-Vis identification

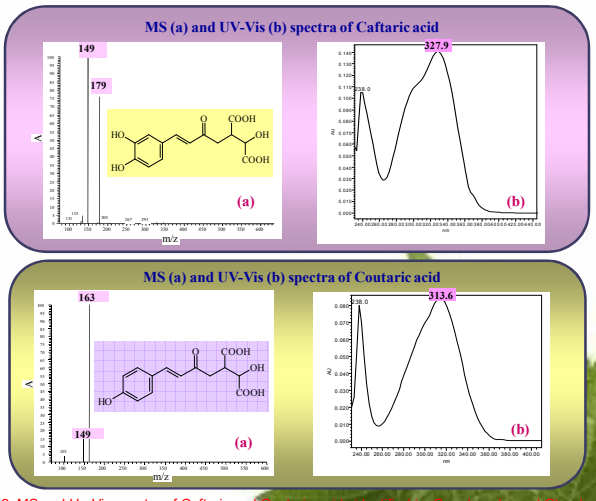

Fig. 2. MS and Uv-Vis spectra of Caftaric and Coutaric acids identified in Smederevka and Chardonnay wines

Hydoroxycinnamic acid derivatives, caffeoyltartaric (caftaric) acid at *m/z* 311 (fragment ions: *m/z* 179, 149) and maximum absorbance at 327.9 nm) and *p*-coumaroyltartaric (coutaric) acid at *m/z* 295 (fragment ion at *m/z* 163, 149 and maximum absorbance at 313.6 nm) have been detected in the wines. These compounds produce same fragment ion [M-H-132]$^-$ which corresponds to loss of tartaric acid residue.

Thus, molecular ion [M–H]$^-$ at *m/z* 311 after fragmentation produces two fragments, [M–H]$^-$ at *m/z* 179, corresponding to caffeic acid and [M–H]$^-$ at *m/z* 149, obtained after elimination of tartaric acid. This compound was identified as *trans*-caffeyltartaric acid or *trans*-caftaric acid.

Molecular ion [M–H]$^-$ at *m/z* 295 giving two fragment ions, [M–H]$^-$ at *m/z* 162.9 corresponding to the *p*-coumaric residue and [M–H]$^-$ at *m/z* 149, corresponding to the tartaric acid residue, was identified as *cis*-*p*-coumaroyltartaric acid or *cis*-coutaric acid.

Quantification

Table 1. Content of hydroxycinnamic acid derivatives in Charodnnay wines

Compounds	Ch-Mac-50	Ch-Mac-100	Ch-Fr-50	Ch-Fr-100
trans-Caftaric acid	57.14	132.18	81.00	142.18
cis-Coutaric acid	25.71	31.37	33.10	32.35
trans-Coutaric acid	30.90	47.03	28.08	51.35
Total	113.75	210.58	142.18	225.88

Table 2. Content of hydroxycinnamic acid derivatives in Smederevka wines

Compounds	Sm-Mac-50	Sm-Mac-100	Sm-Fr-50	Sm-Fr-100
trans-Caftaric acid	3.62	7.17	3.33	11.78
Coutaric acid (*trans*+*cis*)	11.43	26.87	13.77	27.81
Total	15.05	34.04	17.1	39.59

Labels: Ch–Charodnnay, Sm–Smederevka, Mac–Macedonina yeast, Vinalco, Fr–French yeast, Levuline, 50– 50 mg/L SO_2, 70 – 70 mg/L SO_2

Chardonnay wines were richer with phenolic acid derivatives compared to Smederevka wines. The dominant component in Chardonnay wines was *trans*-caftaric acid, while, *trans*-coutaric dominated in Smederevka wines. Regarding the influence of SO_2, wines with higher dose of SO_2 contained higher levels of acids, since SO_2 suppress the activity of oxidases, preventing oxidation of these readily oxidizable phenols and wine browning. The influence of the yeast on the content of hydroxycinnamic acid derivatives was not significantly different. Principal component analysis (Fig. 3) was performed in order to check if the studied wines can be distinguished, observing separation of the samples according to the variety and SO_2 doses.

Statistical analysis

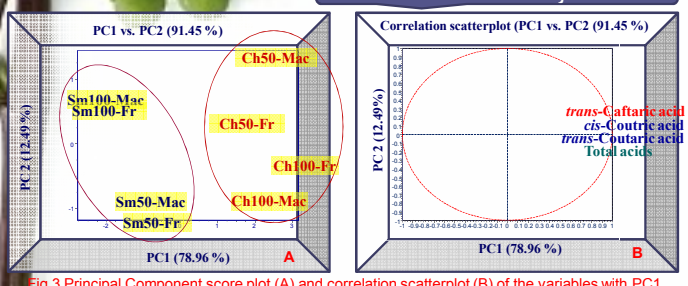

Fig.3 Principal Component score plot (A) and correlation scatterplot (B) of the variables with PC1 and PC2 based on hydroxycinnamic acids for the analyzed Smederevka and Chardonnay wines

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