HPLC-MS and Spectrophotometric Assay of the Phenolic Content of Macedonian Red and White Grape Varieties

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INTRODUCTION

Phenolic compounds are very important grape constituants, which belong at two main groups: flavonoids (anthocyanins, flavan-3-ols, flavonols, dihydroflavonols) and non-flavonoids (hydroxycinnamic and hydroxybenozic acids, stilbenes). These compounds are mainly present in grape skins and seeds. In this work, the phenolic content of four typical Macedonian grape varieties, Vranec and Merlot (red) and Smederevka and Chardonnay (white) was studied, applying spectrophotometry and HPLC-MS analyses.

EXPERIMENTAL

-Aceton/water: 80/20 (*v/v*)
-Ultrasonic bath: 15 min
- Extraction on magnetic agitator: 30 min
-Centrifugation: 15 min
(twice extracted)

Spectrophotometric determination of: total phenols (TP), total anthocyanins (TA), total flavonoids (TF) and total flavan-3-ols ($TF_{3\text{-ols}}$)

HPLC-ESI-IT-MS

RESULTS

Fig. 1. Red and white grape varieties

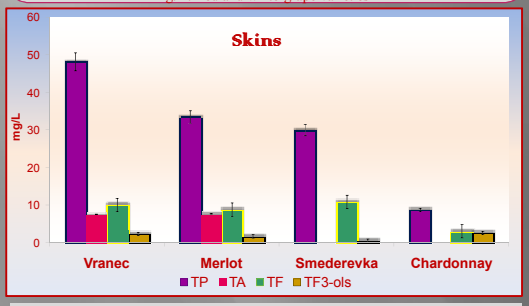

Fig. 2. Concentration of TP, TA, TF and TF3-ols in **skins** of Merlot, Vranec, Smederevka and Charodnnay grapes

Table 1. MS identification of phenolic compounds present in grape

Grape	Anthocyanins			Flavonols	Flavan-3-ols	Phenolic acids and derivatives
Pulp						Caftaric acid (*m/z* 311)[r]
Seeds					(+)-Catechin (*m/z* 289)[r,w] (-)-Epicatechin (*m/z* 289)[r,w]	Gallic acid (*m/z* 169)[r]
Skins	Monoglucosides	Acetylglucosides	*p*-Coumaroylglucosides			
	Dp-3-glc (*m/z* 465)[r]	Dp-3-acetylglc (*m/z* 507)[r]	Dp-3-*p*-coumglc (*m/z* 611)[r]	My-3-glc (*m/z* 481)[r]	(+)-Catechin (*m/z* 289)[r,w]	Caftaric acid (*m/z* 311)[r,w]
	Cy-3-glc (*m/z* 449)[r]	Cy-3-acetylglc (*m/z* 491)[r]	Cy-3-*p*-coumglc (*m/z* 595)[r]	My-3-glcA (*m/z* 495)[r]	(-)-Epicatechin (*m/z* 289)[r,w]	Coutaric acid (*m/z* 295)[r,w]
	Pt-3-glc (*m/z* 471)[r]	Pt-3-acetylglc (*m/z* 521)[r]	Pt-3-*p*-coumglc (*m/z* 625)[r]	Q-3-glc (*m/z* 465)[r,w]	Procyanidin B1 (*m/z* 577)[r,w]	Fertaric acid (*m/z* 325)[r,w]
	Pn-3-glc (*m/z* 463)[r]	Pn-3-acetylglc (*m/z* 505)[r]	Pn-3-*p*-coumglc (*m/z* 609)[r]	Q-3-glcA (*m/z* 479)[r,w]		Gallic acid (*m/z* 169)[r]
	Mv-3-glc (*m/z* 493)[r]	Mv-3-acetylglc (*m/z* 535)[r]	Mv-3-*p*-coumglc (*m/z* 639)[r]	Quercetin (*m/z* 303)[r]		
				La-3-glc (*m/z* 495)[r]		
				Sy-3-glc (*m/z* 509)[r]		

Labels- Dp: delphinidin, Cy: cyanidini Pt: petunidin, Pn: peonidin, Mv: malvidin, My:myricetin, Q: quercetin, La: laricitrin, St: syringetin, glc: glucose, Aclglc: acetylglucoside, *p*-coumglc: *p*-coumaroylglucoside, glcrA glucuronide, r: red wines (Vranec and Merlot), w: white wines (Smederevka and Chardonnay)

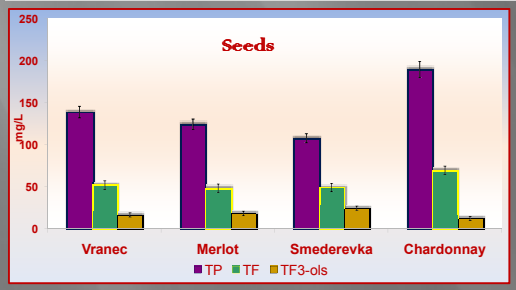

Fig. 3. Concentration of TP, TA, TF and TF3-ols in **seeds** of Merlot, Vranec, Smederevka and Charodnnay grapes

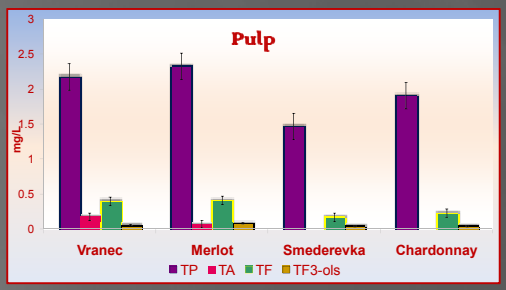

Fig. 4. Concentration of TP, TA, TF and TF3-ols in **pulp** of Merlot, Vranec, Smederevka and Charodnnay grapes

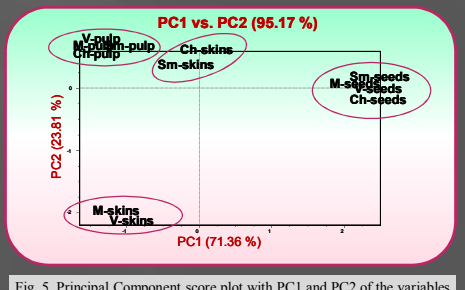

Fig. 5. Principal Component score plot with PC1 and PC2 of the variables based on the spectrophotometric data for TP, TA, TF and $TF_{3\text{-ols}}$ in skins, seeds and pulp of Vranec, Merlot, Smederevka and Chardonnay grapes

Total phenolics, anthocyanins, flavonoids and flavan-3-ols contents were higher in Vranec grapes compared to Merlot variety. Pulp and seeds of Chardonnay contained higher polyphenolic contents than Smederevka. HPLC-ESI-IT-MS confirmed the presence of 15 anthocyanins as monoglucosides, acetylglucosides and *p*-coumaroylglucosides, as well as the presence of the flavan-3-ol monomers, flavonols and the dominant esters of hydroxycynnamic acids (caftaric, coutaric and fertaric acids).

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