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Qualitative and Quantitative Analysis of Phenolic Acids using High Performance Liquid Chromatography (HPLC) from Organic and Conventional Grapes

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Abstract. Organic farming is currently practiced world wide and involve plants which are cultivated without using synthetic pesticides, herbicides or fertilizers and promotes biodiversity, biological cycles and improve the product quality. Qualitative and quantitative analysis of phenolic acids using high performance liquid chromatography (HPLC) from four table grape varieties ('Timpuriu de Cluj', 'Chasselas doré', 'Napoca' and 'Muscat Hamburg') and five wine grape varieties ('Aromat de Iaşi', 'Traminer roz', 'Riesling Italian', 'Fetească regală' and 'Muscat Ottonel') cultivated in organic and conventional systems were studied. The phenolic acids identified from grape skin samples were: gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, resveratrol and quercetin. For all varieties analized the concentrations of ferulic acid, resveratrol and quercetin were higher in conventional grapes than in organic ones (except 'Timpuriu de Cluj'). Among the varieties analyzed we can distinguish the variety 'Napoca', which achieved the highest concentrations of ferulic acid (12.1±1.1mg/kg-organic; 14.9±1.3mg/kg-conventional), resveratrol (4.9±0.5 mg/kg-organic; 5.3±0.8 mg/kg-conventional) and quercetin (16.0±0.8mg/kg-organic; 16.0±1.3mg/kg-conventional) followed at significant differences by 'Muscat Hamburg'and 'Timpuriu de Cluj'.

Keywords: HPLC, organic farming, phenolic acids, wine and table grapes

INTRODUCTION

Grapes, members of the family Vitaceae, are consumed as table fruit, wine, juice and raisins. The benefits of consuming grapes and its derivated products have gained further recognition with discovery of phenolics. The phenolics in grapes are classified in two groups: the flavonoids and non-flavonoids. In flavonoids group are present flavan-3-ols (catechin), flavonols (quercetin) and anthocyanins. The nonflavonoids group contain gallic acid, hydroxycinnamates and stilbenes (resveratrol) (Yang *et al.*, 2009). Phenolic compounds were indicated to reduce coronary heart deseases (Goldberg *et al.,* 1995)**,** some cancer types and various dermal disorders (Yilmaz and Toledo, 2004). The antioxidant activity of grapes is direct correlated with total phenolic content as it is demonstrated by some papers (Orak, 2007; Gómez-Plaza *et al.*, 2006). Phenolic compounds are synthetized from the early stage of berry maturation and decline towards ripening. Berry skin is the part where most phenolic accumulation occurs (Poudel *et al.,* 2008). Organic agriculture does not use synthetic pesticides and fertilizers (Briar *et al.,* 2007) just ecological products during the cultivation. It was shown that organic cultivation influenced the phenolic content and antioxidant activity (Dani *et al.,* 2007; Mulero *et al.*, 2010).

The nutritional quality of grapes are influenced by environmental, post-harvesting conditions but also genotype is very important to the variation (Connor *et al.*, 2002).

The aim of this study was to compare phenolic acids content of some table and wine grape (skin samples) cultivated in organic and conventional systems.

MATERIALS AND METHODS

1. Biological material and cultivation system

Four table grape varieties (‘Timpuriu de Cluj’, ‘Chasselas doré’, ‘Napoca’ and ‘Muscat Hamburg’) and five wine grape varieties (‘Aromat de Iaşi’, ‘Traminer roz’, ‘Riesling Italian’, ‘Fetească regală’ and ‘Muscat Ottonel’) were tested in 2010, Cluj county, Romania, under two types of cultural practices: organic and conventional. Types of culture were differentiated by treatment for diseases, especially downy mildew of grapevine, caused by *Plasmopara viticola* (Berk. & Curt.) which is one of the most serious diseases of grapevine worldwide (Agrios, 2005) and by different fertilizing for organic practices and for the conventional. In the conventional system were used chemical fungicides: Ridomil Gold MZ 68 WP (metalaxyl-M 4% + mancozeb 64%), Melody Duo 66.8 WP (iprovalicarb 5.5% + propineb 61.3%) , Curzate manox SC (cymoxanil 5% + copper 25% + mancozeb 18%), Quadris max SC (azoxystrobin 22.9%), Folpet 50 WP (folpet 50%) and Dithane M 45 (mancozeb 80%). For the organic treatments were applied ecological products: bordeaux mixture 0.5% + spraying with purine of greater nettle (*Urtica dioica* L.) fermented 1/20 dilution, copper sulphate 1%, Kocide 101 WP (copper hydroxide + metallic copper 50%), bordeaux mixture 1%, soluble sulphur 0.4% and biocontrol agent *Trichoderma harzianum*.

2. Grape samples

All the grape samples were collected during ripening of grapes (at technological maturity) from the vines grown at the experimental vineyards in Cluj county, Romania. Vines were grafted on the same rootstock Selection Openheim nr.4 (SO-4). The grape berries (aproximatively 150) were randomly taken from each sample from different parts of various clusters and proccesed in the same day into laboratory. Choosing neighbouring plots give us the posibility to compare organic and conventional vineyards in the same soil and climate conditions.

3. Polyphenols extraction

For polyphenols extraction 10 g of skin grapes, in three replicated each, was extracted by grinding the sample 1 min at 20,000 rpm in a blender (Ultra-Turrax Miccra D-9 KT Digitronic, Germany) with 10 ml of acidified methanol (85:15 v/v, MeOH:HCl). The homogenate was centrifuged at 3500 rpm for 10 min. The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless. The filtrates were combined in a total extract and dried using rotary evaporator at 40 ˚C.

4. Separation of phenolic acids

Total polyphenol extract obtained was injected into HPLC and mobile phase gradient was used with flow 1ml/min. (Tab. 1). Separation was done on a Supelcosil LC 18 type collar size 250 mm x 4.6 mm x 5 microns, a wavelength of 330 nm. For each injection and 20 ml extract was used at a working temperature of 25°C.

Tab. 1

Gradient solvents program used for HPLC separation

Solvent type	Time (min.)				
	0	10	30	45	55
Solvent A %	100	85	50	15	100
Solvent B %	0	15	50	85	0

Solvent A : methanol/ glacial acetic acid / water in a ratio of 10/2/88
Solvent B : methanol/ glacial acetic acid / water in a ratio of 90/3/7

RESULTS AND DISCUSSIONS

1. Qualitative analysis of phenolic acids in grape varieties tested, using high performance liquid chromatography (HPLC)

Phenolic acids present in grapes were identified using high performance liquid chromatography (HPLC). For their identification was used, co-chromatography with standards of phenolic acids and were compared absorption spectra obtained for each separate type of phenolic acid. Chromatogram obtained for the separation of phenolic acid standards is shown in the figure below (Fig. 1). In Fig. 2 are presented chromatograms of polyphenols from 'Fetească regală' variety.

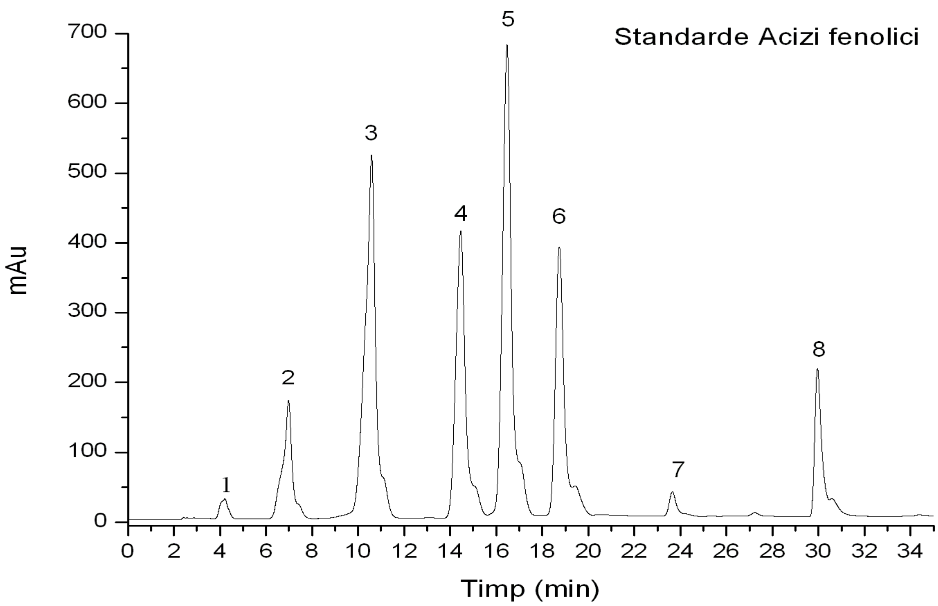
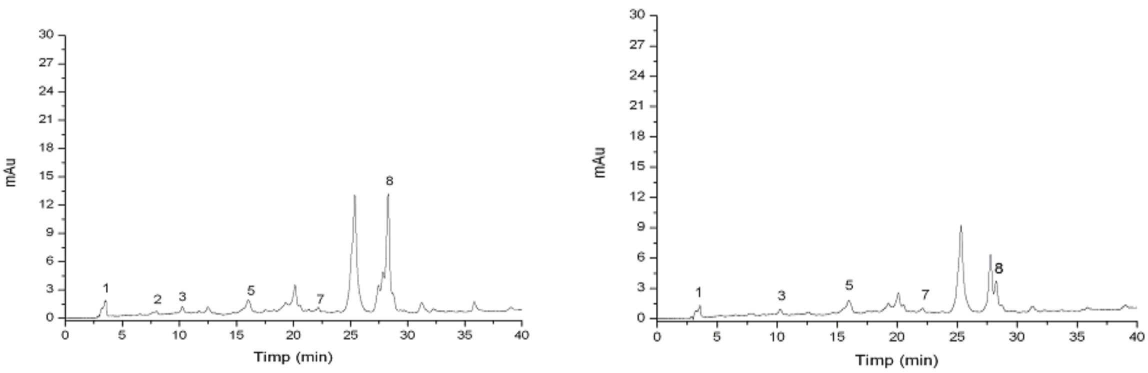


Fig. 1. HPLC chromatogram for phenolic acid standards



a b

Fig. 2. The HPLC chromatogram of polyphenols from Fetească regală conventional (a) and organic (b) type

Regarding chromatograms obtained from HPLC can be observed the presence of different phenolic acids: gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, resveratrol, quercetin (Tab. 2).

Tab. 2

Retention time for phenolic acids standards t_r (min.)

Peak number	Standard name	Retention time t_r (min.)
1.	Ascorbic acid	4,21
2.	Gentisic acid	6,98
3.	Caffeic acid	10,58
4.	*p*-Coumaric acid	14,45
5.	Ferulic acid	16,46
6.	*o*-Coumaric acid	18,74
7.	Resveratrol	23,65
8.	Quercetin	29,95

2. Quantitative analysis of phenolic acids in grape varieties tested, using high performance liquid chromatography (HPLC)

Grape phenolics can be found in large quantities especially in grapes skin (Singleton, 1982). They are classified in two groups: flavonoids (catechins, quercetin and anthocyanins) and non-flavonoids (gallic acid, ferrulic acid, resveratrol).

In grapes, quercetin is mainly localised in skins, while the resveratrol is present in grape skins and seeds (Yang *et al.*, 2009). It is known that both genetic factors as well as environmental conditions play an important role in qualitative and quantitative composition of phenolic acids (Yang *et al.*, 2009; Anastasiadi *et al.*, 2010). Generally the amount of phenolic acids is higher in red grape skins compared with white grapes, because the last ones lose their ability to produce anthocyanins.

Among phenolic acids determined in grapes skin, three of them: ferrulic acid, quercetin and resveratrol are found in larger amounts compared to other phenolic acids quantified (gentisic acid, caffeic acid, *p*-cummaric, etc).

Tab. 3

The phenolic acids concentration (mg/kg*)* in grape skin extract

Variety	Color	Organic			Conventional		
		Ferulic acid (mg/kg)	Resveratrol (mg/kg)	Quercetin (mg/kg)	Ferulic acid (mg/kg)	Resveratrol (mg/kg)	Quercetin (mg/kg)
'Aromat de Iaşi'	white	1.9±0.3 ^{f}	0.51±0.1 ^{ef}	1.8 ±0.2 ^{e}	2.0±1.2 ^{f}	0.7±0.03 ^{d}	2.3 ±1.0 ^{f}
'Traminer roz'	white	4.3±1.1 ^{d}	1.2±0.9 ^{de}	3.8±1.0 ^{d}	5.1±0.9 ^{d}	1.1±0.8 ^{d}	4.3±2.0 ^{e}
'Riesling italian'	white	6.1±0.1 ^{c}	2.1±0.1 ^{cd}	3.1±0.6 ^{d}	6.9±2.1 ^{c}	2.8±0.5 ^{c}	3.2±0.8 ^{ef}
'Feteasca regală'	white	2.3±1.1 ^{ef}	0.9±0.3 ^{e}	5.9±0.9 ^{c}	2.1±0.3 ^{f}	1.2±0.2 ^{d}	15.1±1.7 ^{b}
'Muscat Ottonel'	white	3.3±0.8 ^{de}	0.3 ±0.1 ^{f}	3.2 ±1.1 ^{d}	4.8 ±1.1 ^{d}	1.2±0.3 ^{d}	3.6 ±0.9 ^{ef}
'Timpuriu de Cluj'	white	10.6±1.3 ^{b}	7.2±0.7 ^{a}	8.9±0.8 ^{b}	14.1±0.9 ^{a}	7.3±1.2 ^{a}	6.1±2.3 ^{d}
'Napoca'	red	12.1±1.1 ^{a}	4.9±0.5 ^{b}	16.0±0.8 ^{a}	14.9±1.3 ^{a}	5.3±0.8 ^{b}	16.0±1.3 ^{a}
'Chasselas doré'	white	2.2±0.4 ^{ef}	0.38±0.02 ^{f}	3.5±1.0 ^{d}	3.1±0.8 ^{e}	0.49±0.1 ^{e}	3.8±0.7 ^{ef}
'Muscat Hamburg'	red	9.9±1.6 ^{b}	2.9±1.2 ^{c}	8.9±2.3 ^{b}	12.0 ±3.1^{b}	3.1±0.6 ^{c}	9.7±3.2 ^{c}

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

The ferulic acid, resveratrol and quercetin contents of nine grape extracts were quantified (Tab. 3).

For all the analyzed varieties the concentrations of ferulic acid, resveratrol and quercetin were higher in conventional grapes than in organic ones (except 'Timpuriu de Cluj'). Our results are in accordance with those obtained by Juana Mulero *et al.* (2010).

It is interesting to note that 'Muscat de Hamburg', although it is a red grape, registered for ferrulic acid, resveratrol and quercetin (in both organic and conventional system) significantly lower values compared with some white varieties: 'Timpuriu de Cluj' and 'Feteascā regală'. This result confirms the one obtained by Yang *et al.* (2009), where in Baco Noir the content of total phenolic acids and flavonoids was lower than white varieties Riesling and Vidal Blanc. This highlights the fact that the content of phenolic acids is mainly influenced by variety, rather than the skin color.

Among the varieties analyzed we can distinguish the variety 'Napoca', which achieved the highest concentrations of ferulic acid (12.1±1.1mg/kg-organic; 14.9±1.3mg/kg-conventional), resveratrol (4.9±0.5 mg/kg-organic; 5.3±0.8 mg/kg-conventional) and quercetin (16.0±0.8mg/kg-organic; 16.0±1.3mg/kg-conventional) followed at significant differences by 'Muscat de Hamburg' and 'Timpuriu de Cluj'. The lowest values were obtained for 'Aromat de Iaşi' variety, in both organic and conventional systems.

CONCLUSIONS

For almost of studied grape varieties the phenolic acids concentration (grape skin samples) showed higher and significant differences in conventional system then in organic ones. 'Napoca' variety (red) exhibit highest phenolic acids concentration in both types of grapes, organic and conventional while 'Aromat de Iaşi' has the lowest values.

There were obtained significant differences regarding the phenolic acids concentration among grape variety and the results showed that the phenolic acids content of different grape depends mainly on the varietal differences, not on grape skin colour.

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