Relations between Polyphenols Content and Antioxidant Activity in Vine Grapes and Leaves

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Abstract: The occurrence and content of some polyphenols and the antioxidant activity of compounds present in grape berries, stems and leaves of *Vitis vinifera* L. were evaluated. Three white and three blue varieties of grapevine were investigated. The contents were determined of *trans*-resveratrol, *trans*-piceid, caftaric acid, tryptophan, catechin, epicatechin, total polyphenols, and flavanols, both in healthy material and in the samples of the plant material infested with microorganisms (*Botryotinia fuckeliana* Whetzel anamorph *Botrytis cinerea* Pers.; *Uncinula necator* (Schw.) Burr; *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni). The antioxidant activity of the extracts obtained was determined by different methods: FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and TAC-PCL (Total Antioxidant Capacity of Photochemiluminescence). The content of *trans*-resveratrol varied between 0.3–2.3 mg/kg and 0.7–12.1 mg/kg in non-infested and infested grape berries, respectively. The content of *trans*-piceid between 0.6–2.9 mg/kg and 1.5–6.3 mg/kg in non-infested and infested grape berries, respectively. The content of *trans*-resveratrol varied between 2.5–10.3 mg/kg and 3.7–20.9 mg/kg in healthy and in infected leaves, respectively. The content *trans*-piceid varied between 11.3–58.4 mg/kg and 18.5–60.9 mg/kg in the healthy and in the infected leaves, respectively. The highest content of *trans*-resveratrol was found in stems (16.3–276.3 mg/kg). In young lateral shoots, the highest levels of *trans*-piceid (12.6–99.7 mg/kg) and caftaric acid (474–2257 mg/kg) were determined. The infested grape berries showed a higher antioxidant activity, which was most closely correlated with the content of total polyphenols (correlation coefficient = 0.8336–0.9952).

Keywords: vine grapes; vine leaves; stems; downy mildew; powdery mildew; grey mould; piceid; resveratrol; caftaric acid; catechin; epicatechin; photochemiluminescence; DPPH; FRAP

Antioxidants are generally compounds that are capable, even in small quantities, to prevent or reduce the oxidative destruction of biologically important compounds such as lipids, proteins, and nucleic acids (Halliwell 1990; Madhavi *et al*. 1996). In a number of studies, the health aspects of the consumption of plant polyphenols have been examined (Frankel *et al*. 1993; Blond *et al*. 1995; King *et al*. 2006; He *et al*. 2008) as well as their incidence in vine grapes and wine with a particular emphasis on resveratrol.

The basic mechanism of wine polyphenols antioxidant effect is the same as that of other polyphenols and is based on the ability to provide the hydrogen atom from their hydroxyl groups to the free radical with high oxidative activity.

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Polyphenols of vine grapes and wines are part of complex mixtures of compounds that may react with radicals by different mechanisms and often interact synergistically or inhibitorily (Paganga *et al*. 1996; Riceevans *et al*. 1996; Saucier & Waterhouse 1999).

In recent years, individual compounds with antioxidant activity have been determined, however, the approach to the evaluation of antioxidant activity of natural materials as a whole has been used. For the evaluation of the antioxidant activity of vine grapes and wines, several methods are most often used, based on the elimination of radicals or on the measurement of redox properties of the substances: TEAC-ABTS (Trolox Equivalent Antioxidant Capacity; using 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid), ORAC (Oxygen Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power), DPPH (using 2,2-diphenyl-1-picrylhydrazyl radical) (Fogliano *et al*. 1999; Fernández-Pachón *et al*. 2004). A photochemiluminescence method (PCL) for the quantification of antiradical properties and nonenzymic antioxidants of polycomponent systems was developed by Popov and Lewin (1994, 1996). It is based on the antioxidant-sensitive inhibition of photo-induced autoxidation of luminal which is accompanied by chemiluminescence.

The comparison of polyphenols contents and their antioxidant activity provided by different methods in red, white and sherry wines was reported by Fernández-Pachón *et al*. (2006) and Stratil *et al*. (2008), who also examined the antioxidant contributions of selected polyphenols to the total antioxidant activity. The main chemoprotective polyphenolic compounds in the grape berries, rachis, and pedicels of ten cultivars *Vitis vinifera* L., growing in Southern Moravian vineyards were studied by Mikeš *et al*. (2008).

One of the most studied polyphenols of vine grapes and wines is resveratrol and its glucosidic form piceid. The species and varieties of the genus *Vitis* probably differ in their ability to synthetise these compounds. In the vine grapes of *Vitis rotundifolia*, a significantly higher content of resveratrol than in the vine grapes of *V. vinifera* L. and *Vitis labrusca* was found (Lamikanra *et al*. 1996).

Since stems of vine grapes and leaves of the vines have minimal or no impact on the concentration of resveratrol in wine due to the modern wine technology, there is very limited information on the contents of resveratrol and other polyphenols in these plant parts (Monagas *et al*. 2006). Melzoch *et al*. (2001) found significantly higher levels of *trans*-resveratrol (209 and 440 mg/kg) in stems than in leaves (3.6 and 9.9 mg/kg), particularly with the "Teinturier" varieties. Grape berries contained from 0.7 to 5.8 mg/kg of *trans*-resveratrol which was produced preferentially, compared to *cis*-isomer. The authors suppose that the high variability of resveratrol content in wines may be due to varying degrees of enzymatic hydrolysis of *trans*-piceid, particularly during the alcohol fermentation by beta-glucosidase of yeast and subsequent isomerisation.

Moreno *et al*. (2008) examined the distribution of resveratrol inside the grape berries. Most of resveratrol was present in the skin of grape berries and to a lesser extent in the seeds, whereas the content of *cis*-resveratrol was 10 times lower. Adrian *et al*. (1998) observed that the mould *Botrytis cinerea* Pers. produced an oxidative enzyme of laccase type (stilbene-oxidase) which oxidised resveratrol to other stilbenoid compounds. The main metabolite was resveratrol *trans*-dehydrodimer which might be responsible for mould self-intoxication during the infection of vine grapes.

MATERIAL AND METHODS

Samples preparation. The study was performed on three white varieties (Chardonnay, Welschriesling, and Pinot gris) and three blue varieties (Saint Laurent, André, and Blauer Portugieser) of *Vitis vinifera* L. originating from the vine growing region of Mikulov (south Moravia, Czech Republic) (Table 1). Healthy samples and samples of vine leaves, young lateral shoots, grape stems, and berries infested (at level above 40%) by microorganisms (*Botryotinia fuckeliana* (de Bary) Whetzel anamorph *Botrytis cinerea* Pers.; *Uncinula necator* (Schw.) Burr; *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni were analysed. Frozen stems and leaves (–20°C) were extracted after freeze-drying with 90% methanol.

Regarding the relatively high content of sugars in the grape berries, frozen grape berries were directly extracted with 80% methanol (Mikeš *et al*. 2008). The methanolic extracts obtained were stored at –20°C until their analyses. All tests were run in triplicates. The concentrations of phenols and tryptophan, and the results of antioxidant activity determination were expressed as related to the fresh material basis.

Table 1. Content of selected phenolic compounds and tryptophan in healthy and fungal pathogen infected vine grapes, leaves and stems of different *Vitis vinifera* L. varieties

Variety		Catechin	Epicatechin	*Trans*-resveratrol	*Trans*-piceid	Caftaric acid	Tryptophan
		(mg/kg)					
Saint Laurent	berries(A)	118	63.5	2.3	2.9	traces	9.4
	berries(B)	888	456	1.4	1.5	traces	36.7
	stems	867	81.4	276.1	18.6	nd	65.4
	leaves(A)	nd	nd	8.5	32.9	618	nd
	leaves(C)	nd	nd	8.0	28.3	556	nd
	shoots*	nd	nd	8.1	25.6	694	nd
Blauer Portugieser	berries(A)	467	123	0.4	2.5	traces	9.5
	berries(B)	638	294	1.0	3.6	traces	22.0
	stems	1369	156	42.1	24.2	nd	40.4
	leaves(A)	nd	nd	8.5	48.8	746	nd
	leaves(C)	nd	nd	18.3	60.9	296	nd
	shoots*	nd	nd	8.8	45.7	1274	nd
André	berries(A)	533	235	0.4	0.6	traces	11.2
	berries(B)	1509	801	0.7	1.7	traces	34.7
	stems	1582	186	40.4	12.4	nd	39.8
	leaves(A)	nd	nd	2.5	11.3	514	nd
	leaves(C)	nd	nd	3.7	18.5	292	nd
	shoots*	nd	nd	3.7	12.6	713	nd
Chardonnay	berries(A)	123	144	0.3	1.1	traces	21.6
	berries(B)	471	556	9.6	2.7	traces	24.2
	stems	899	84.4	23.8	12.1	nd	188
	leaves(A)	nd	nd	10.3	45.4	914	nd
	leaves(C)	nd	nd	7.0	36.7	584	nd
	shoots*	nd	nd	15.6	72.9	2257	nd
Welschriesling	berries(A)	61.0	84.3	0.4	1.6	traces	20.5
	berries(B)	355	391	12.1	6.3	traces	19.8
	stems	926	158	16.3	18.7	nd	162
	leaves(A)	nd	nd	8.7	58.4	459	nd
	leaves(C)	nd	nd	20.9	58.4	278	nd
	shoots*	nd	nd	6.5	99.7	820	nd
Pinot gris	berries(A)	481	251	0.6	1.1	traces	21.2
	berries(B)	1275	1047	11.8	3.1	traces	36.4
	stems	973	199	73.7	24.1	nd	319
	leaves(A)	nd	nd	4.0	27.1	391	nd
	leaves(C)	nd	nd	4.2	38.7	460	nd
	shoots*	nd	nd	4.8	25.1	474	nd

A – healthy; B – infection with *Botryotinia fuckeliana* anamorph *Botrytis cinerea*; C – infection with *Uncinula necator*. or *Plasmopara viticola*; *young lateral shoots, nd = not detected

Chromatographic methods. The separation of catechin, epicatechin, *trans*-resveratrol, *trans*-piceid, caftaric acid, and tryptophan was carried out using Luna Phenomenex C18(2) column (2 × 150 mm, 3 µm) (Torrance USA) on Hewlet Packard HPLC model 1050 (Wilmington, USA) with diode-array detector HP 1040A. The injected volume was 5 µl. For chromatographic mobile phases were used (A) acetonitrile:*o*-phosphoric acid:water (5:0.1:94.9) and (B) acetonitrile:*o*-phosphoric acid:water (80:0.1:19.9) with a flow rate of 0.25 ml/min in a gradient from 0 to 45% during 55 minutes.

Determination of total polyphenols and flavanols. Methanolic extracts were diluted in the ratio 1:9 with buffer (42mM tartaric acid, 24mM Na_2HPO_4, 12% ethanol, pH 3.4).

Modified Folin-Ciocalteu method. To 980 µl of water in a 1.5 ml Eppendorf vial, 20 µl of the diluted extract and 50 µl of Folin-Ciocalteu reagent were added and the mixture was thoroughly shaken. After 3 min, 150 µl of sodium carbonate decahydrate solution (20%) was added, the reaction mixture was thoroughly shaken and left for 120 min in the dark at room temperature. Then, the absorbance was measured at 750 nm against the blank prepared for each series of determinations in such way that the sample was replaced by the dilution buffer. The concentration of polyphenols was calculated from the calibration curve using gallic acid.

DMACA method (using p-dimethylaminocinnamaldehyde). To 980 µl of the reagent solution (0.1% DMACA and 300mM HCl in methanol) in a 1.5 ml Eppendorf vial, there was added 20 µl of the diluted extract, the mixture was shaken and left to react for 12 min at room temperature. The absorbance was then measured in the same manner at 640 nm against the blank in which the sample was replaced by the dilution buffer. Total concentration of flavanols was calculated from the calibration curve using catechin.

Determination of antioxidant activity. Methanolic extracts were diluted in ratio 1:9 with the same buffer as in previous methods.

FRAP(AA) method (Ferric Reducing Antioxidant Power). In 1.5 ml Eppendorf vial, 50 µl of ferric ions solution (3mM $FeCl_3$ in solution of 6mM citric acid) was mixed with 20 µl of the diluted extract and the mixture was incubated at 37°C in a heating block for 30 minutes. Then 930 µl of TPTZ (2,4,6-tripyridyl-s-triazine) solution in 50mM HCl was added, the mixture was shaken and after 12 min the absorbance was measured at 620 nm against the blank in which the sample was replaced by the dilution buffer. The reducing power was calculated from the calibration curve using ascorbic acid.

DPPH(Trolox) method (using 2,2-diphenyl-1-picrylhydrazyl radical). To 980 µl of DPPH solution in methanol (150µM), 20 µl of the diluted extract was added, the mixture was shaken and after 30 min the absorbance was measured at 515 nm in comparison with the absorbance using demineralised water. For the determination of the antiradical activity, the difference between the absorbance of the blank (dilution buffer) and that of the sample was used. The antiradical activity was calculated from the calibration curve using Trolox.

TAC-PCL(Trolox) method (Total Antioxidant Capacity of Photochemiluminescence method). The antioxidant capacity was determined by photochemiluminescence method using KIT ACL (400.803) and an instrument Photochem from Analytik Jena AG. Free radicals (superoxide anion radicals) were produced by optical excitation (irradiation) of a photosensitiser (dye). These radicals were partially eliminated by the reaction with the antioxidants present in the sample (diluted extract) and measured by means of the luminescence change. The total antioxidant capacity was quantified by comparison with the Trolox standard (Popov & Lewin 1994, 1996).

Reagents and solvents. Standards: *trans*-resveratrol, *trans*-piceid, catechin, epicatechin, gallic acid, caftaric acid, tryptophan, Trolox and L-ascorbic acid were obtained from Sigma-Aldrich (Prague, Czech Republic) and KIT ACL (400.803) from Analytik Jena AG (Germany). Acetonitrile was purchased from Merck (Prague, Czech Republic) and *o*-phosphoric acid, Folin-Ciocalteu reagents, TPTZ (2,4,6-tripyridyl-s-triazine), *p*-dimethylaminocinnamaldehyde (DMACA), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and $FeCl_3$ from Fluka (Sigma-Aldrich, Prague, Czech Republic).

Statistical methods. The data were evaluated using the method of variance analysis with the Tukey test, and correlation analysis of the program Statgraphics.

RESULTS AND DISCUSSION

Table 1 shows the results of the analysis of the vine grapes, stems, and leaves of the vines of the selected varieties of *Vitis vinifera* L. affected by

downy mildew (*Plasmopara viticola*) or powdery mildew (*Uncinula necator*) The Moravian traditional varieties for the production of white and red wines were tested. Neither catechin nor epicatechin was identified in these plant parts. On the other hand, in comparison with the grape berries, high concentrations of caftaric acid were found. The content of caftaric acid in the young leaves and young lateral shoots of the variety Chardonnay achieved 2257 mg/kg. Higher concentrations of *trans*-resveratrol and *trans*-piceid in the berries infested with gray mould (*Botryotinia fuckeliana*

Table 2. Content of total polyphenols, flavanols and antioxidant activity in healthy and fungal pathogen infected vine grapes and leaves of different *Vitis vinifera* L. varieties

Variety		Polyphenols	Flavanols	TAC-PCL (Trolox)	DPPH(Trolox)	FRAP(AA)
		(mg/g)				
Saint Laurent	berries(A)	3.76	0.67	4.21	5.09	1.30
	berries(B)	12.9	4.00	6.35	16.4	3.35
	stems	24.0	6.75	8.07	35.2	8.48
	leaves(A)	15.1	1.87	9.29	21.9	6.35
	leaves(C)	10.8	1.31	8.03	14.4	4.07
	shoots*	16.1	2.38	7.61	23.7	7.18
Blauer Portugieser	berries(A)	4.55	1.28	5.42	5.71	1.51
	berries(B)	8.30	2.54	5.53	11.3	2.85
	stems	21.8	7.12	8.66	30.5	7.83
	leaves(A)	23.8	3.89	10.9	34.2	11.8
	leaves(C)	11.9	1.84	8.12	17.1	4.85
	shoots*	25.9	4.09	12.8	35.3	11.3
André	berries(A)	6.54	2.03	5.08	9.12	2.43
	berries(B)	15.3	5.55	8.69	20.3	4.65
	stems	27.2	9.16	8.21	35.3	9.39
	leaves(A)	22.1	3.76	9.84	31.4	10.3
	leaves(C)	15.6	2.38	6.56	21.0	6.21
	shoots*	18.1	2.76	8.45	25.3	8.43
Chardonnay	berries(A)	3.59	0.96	3.12	4.66	0.96
	berries(B)	10.5	3.54	4.82	13.4	2.51
	stems	14.5	2.23	8.56	19.9	5.96
	leaves(A)	20.5	3.83	10.5	28.8	9.77
	leaves(C)	16.7	2.99	10.3	24.8	8.12
	shoots*	27.6	4.84	15.8	38.1	12.4
Welschriesling	berries(A)	4.02	0.95	3.25	6.10	1.09
	berries(B)	11.1	3.40	4.76	14.9	2.54
	stems	15.2	5.00	7.38	22.0	5.63
	leaves(A)	16.2	2.44	7.21	22.5	7.29
	leaves(C)	15.6	2.84	8.24	22.7	7.27
	shoots*	20.3	2.99	12.0	26.6	7.75
Pinot gris	berries(A)	3.93	1.49	4.24	5.3	1.25
	berries(B)	15.2	5.99	8.10	20.7	3.97
	stems	13.6	4.36	7.21	18.4	4.77
	leaves(A)	15.5	2.35	8.22	21.6	6.26
	leaves(C)	12.2	1.60	8.70	15.9	4.61
	shoots*	17.8	6.48	11.0	24.4	6.25

A – healthy; B – infection with *Botryotinia fuckeliana* anamorph *Botrytis cinerea*; C – infection with *Uncinula necator*. or *Plasmopara viticola*; *young lateral shoots

Table 3. Correlation analysis between the data determined with all experimental samples (n = 36)

	DPPH	Flavanols	FRAP	*Trans*-piceid	Polyphenols	*Trans*-resveratrol	TAC-PCL
DPPH		***0.0000*	*0.0000*	*0.0009*	*0.0000*	*0.0376*	*0.0000*
Flavanols	*0.6810		*0.0027*	*0.9323*	*0.0000*	*0.0095*	*0.0123*
FRAP	0.9594	0.4860		*0.0001*	0.0000	*0.2304*	*0.0000*
Trans-piceid	0.5297	0.0147	0.6144		*0.0011*	*0.9745*	*0.0000*
Polyphenols	0.9952	0.7067	0.9462	0.5229		*0.0542*	*0.0000*
Trans-resveratrol	0.3479	0.4263	0.2050	0.0055	0.3236		*0.7465*
TAC-PCL	0.8344	0.4131	0.8646	0.7126	0.8336	0.0558	

*Pearson's correlation coefficient; **Significance level of Pearson's correlation coefficient (in italic)

Whetzel anamorph *Botrytis cinerea* Pers.) were recorded in the white varieties Welschriesling (12.1 mg/kg and 6.3 mg/kg, respectively) and Pinot gris (11.8 mg/kg and 3.1 mg/kg, respectively). The estimated high value of *trans*-resveratrol content in the stems of Saint Laurent (276 mg/kg) is close to those given by the above cited authors (Melzoch *et al*. 2001). In addition to phenolic compounds, amino acid tryptophan was identified in both the healthy and damaged grape berries (9.4–21.6 mg/kg and 19.8–36.7 mg/kg, respectively) and stems of all varieties observed (39.8–319 mg/kg).

A higher antioxidant activity in the vine grapes was recorded in the infested grape berries and stems, especially with the blue varieties. In the healthy leaves, higher concentrations of polyphenols and flavanols were found as compared with the infected leaves, this finding correlated with a higher antioxidant activity in the healthy leaves. The highest values of antioxidant activity and contents of polyphenols and flavanols were found in young lateral shoots, which was in relation to their high biochemical activity during the period of growth (Table 2).

The relations between determined parameters are statistical evaluation in Table 3. The highest correlation occurred between the antioxidant activity, regardless of the method used, and total polyphenols content (0.8336–0.9952 mg/kg; $P < 0.0001$), but the antioxidant activity only weakly correlated with the content of *trans*-resveratrol. A relatively wide range of the correlation coefficient between the estimated antioxidant activity and the contents of selected polyphenols was also presented by Fernández-Pachón *et al*. (2006). These results demonstrate that the methodological

Table 4. Contents of observed components in healthy and fungal pathogen infected vine grapes (mean ± standard deviation, n = 6)

Component (mg/kg)	Berries(A)	Berries(B)	Stems
Catechin	297 ± 218^{a}	856 ± 458^{b}	1103 ± 299^{b}
Epicatechin	150 ± 78^{a}	591 ± 282^{b}	144 ± 50^{a}
Trans-resveratrol	0.7 ± 0.8^{a}	6.1 ± 5.6^{a}	78.7 ± 98.7^{b}
Trans-piceid	1.8 ± 0.7^{a}	3.0 ± 2.0^{a}	18.3 ± 5.3^{b}
Component (g/kg)			
Tryptophan	16 ± 6^{a}	29 ± 8^{a}	136 ± 110^{b}
Polyphenols	4.4 ± 1.1^{a}	12.2 ± 2.8^{b}	19.4 ± 5.7^{c}
Flavanols	1.2 ± 0.5^{a}	4.2 ± 1.3^{b}	5.8 ± 2.4^{b}
TAC-PCL(Trolox)	4.2 ± 0.9^{a}	6.4 ± 1.7^{b}	8.0 ± 0.6^{c}
DPPH(Trolox)	6.0 ± 1.6^{a}	16.2 ± 3.8^{b}	26.9 ± 7.7^{c}
FRAP(AA)	1.4 ± 0.5^{a}	3.3 ± 0.9^{b}	7.0 ± 1.8^{c}

A – healthy; B – infection with *Botryotinia fuckeliana* anamorph *Botrytis cinerea*; ^{abc}different letters in the same line indicate significant difference by Tukey test (P = 0.05)

Table 5. Contents of observed components in healthy and fungal pathogen infected leaves (mean ± standard deviation, n = 6)

Component (mg/kg)	Leaves(A)	Leaves(C)	Shoots**
Trans-resveratrol	7.1 ± 3.1^{a}	10.4 ± 7.4^{a}	7.9 ± 4.2^{a}
Trans-piceid	37 ± 17^{a}	40 ± 17^{a}	47 ± 33^{a}
Caftaric acid	607 ± 195^{ab}	411 ± 140^{a}	1039 ± 653^{b}
Component (g/kg)			
Polyphenols	19 ± 4^{a}	14 ± 2^{b}	21 ± 5^{a}
Flavanols	3.0 ± 0.9^{ab}	2.2 ± 0.7^{a}	3.9 ± 1.5^{b}
TAC-PCL(Trolox)	9.3 ± 1.4^{ab}	8.3 ± 1.2^{a}	11.3 ± 3.0^{b}
DPPH(Trolox)	27 ± 5^{a}	19 ± 4^{b}	29 ± 6^{a}
FRAP(AA)	8.6 ± 2.3^{a}	5.9 ± 1.6^{b}	8.9 ± 2.4^{a}

A – healthy; C – infection with *Uncinula necator* or *Plasmopara viticola*; ^{abc}different letters in the same line indicate significant difference by Tukey test (P = 0.05); **young lateral shoots

approaches to determining the antioxidant activity have a complex nature, the resulting values being part of a wider range of compounds and active reaction processes in plant tissues.

The degree of analogy used for determining the antioxidant activity can be evaluated using a set of high correlation coefficients between them (0.8344–0.9594; $P < 0.0001$). On the other hand, specific differences found in the estimated values of antioxidant activity in various experimental samples point to the different principles applied in the determination the antioxidant activity in complex mixtures and unequal mechanisms of radical reactions. The lowest values of FRAP(AA) obtained in all cases and were close to TAC-PCL(Trolox). The values determined by the method of DPPH(Trolox) as compared to the results of the TAC-PCL(Trolox) were in average 2, 2.2 and 3.4 times higher for the grape berries, leaves, and stems, respectively (Tables 4 and 5). Stratil *et al*. (2008) also determined multiple differences between the antioxidant activity measuring the antioxidant activity of white and red wines by the methods of ABTS, DPPH, and FRAP. The highest values of antioxidant activity in comparison with the other cited procedures were obtained using the ORAC method for white and sherry wines (Fernández-Pachón *et al*. 2006).

Statistically significant differences (P = 0.05) between the healthy and mould damaged vine grapes were found in the contents of catechin, total polyphenols and flavanols. As to *trans*-resveratrol and *trans*-piceid, an increase occurred in their contents due to the mould attack, however, the increase was not statistically significant due to the high variability of the determined values, which was apparently influenced by different variety characteristics. A similar trend was observed with healthy and microbially damaged leaves (Tables 4 and 5). Romero-Pérez *et al*. (2001) found a more significant difference in due to the strong attack by powdery mildew. On the other hand, the stems, which are a waste material in the wine-making technology, may be a significant source of *trans*-resveratrol and *trans*-piceid in some varieties, as observed also by Melzoch *et al*. (2001).

CONCLUSIONS

The contents of the selected phenolic compounds and the antioxidant activity were investigated in healthy vine grapes and in vine grapes infested with gray mould, and in healthy vine leaves and leaves infested with downy or powdery mildew of six varieties. In the leaves, regardless of the microbial damage, higher amounts were found of *trans*-resveratrol and *trans*-piceid than were those in grape berries. In accordance with other authors, high levels of *trans*-resveratrol in stems were confirmed. The concentration of total polyphenols was most closely correlated with the antioxidant activity determined. Microbially damaged grape berries showed a higher antioxidant activity compared to healthy grape berries. The analysis pointed out that not only stilbenoid compounds, but also other polyphenols accumulate in vine grapes during the attack by moulds.

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