ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 1 2011

Maria LEJA[1], Iwona KAMIŃSKA[1]
and Katarzyna KULCZAK[1]

ANTIOXIDATIVE PROPERTIES IN GRAPES OF SELECTED CULTIVARS GROWN IN POLAND

ANTYOKSYDACYJNE WŁAŚCIWOŚCI WINOGRON WYBRANYCH ODMIAN UPRAWIANYCH W POLSCE

Abstract: Ten grape-vine cultivars (5 white and 5 red skinned) were cultivated in south-east region of Poland. Samples were frozen and for analyzes extracts in 80 % MeOH were prepared. Contains of total phenols, phenylpropanoids, flavonols and anthocyanins were measured, using UV/VIS method. Additionally, radical scavenging activity with $DPPH^{\bullet}$ was determind.

In red skinned fruits content of phenolic compounds was higher than in white skinned ones. Cultivars showed great diversity: the highest level of phenolics was observed in 'Heridan' and the lowest in 'Bianca' cultivars.

Radical scavenging activity (RSA) was also very high in red grapes, especially in skin (over 90 % of free radical was neutralized). Antioxidant activity of white fruit cultivars was ranged between 9 and 50 % ('Bianca' and skin of 'Jutrzenka' cultivars, respectively).

In analyzed tissue correlation ($r = 0.70$) between total phenolics and RSA was found.

Keywords: grapes, antioxidant activity, phenolic compounds

The great number of civilization diseases can be initiated by the free radical action, particularly by the ROS (*Reactive Oxygen Species*) which are generated either during the normal cell function or are the products of environment pollution. Plant tissues are especially rich in the "defense system" against ROS, their antioxidant capacity is closely associated with activity of "free radical scavenging enzymes" (superoxide dismutase, catalase, peroxidases) and with the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherols and ascorbic acid [1].

High biological activity both of fresh grapes and wine produced from them is mostly due to polyphenolic substances. Jeandet et al [2] found correlation between red wine consumption and reduction of coronary heart diseases. Low mortality caused by heart

[1] Department of Plant Physiology, Faculty of Horticulture, University of Agriculture in Krakow, al. 29 Listopada 54, 31–425 Kraków, Poland, phone: +48 12 662 52 07, email: mleja@ogr.ar.krakow.pl; kaminskai@gmail.com

diseases among French people drinking regularly moderate amounts of red wine, despite of their high-fat diet, is called the "French paradox" as described by WHO authorities.

According to Vinson et al [3] the level of phenolics determined in grape fruits, approximately the same as in strawberries, blueberries and plums, strongly depends on grape vine species: red grapes have more phenolic compounds than the white ones. Red wine is considered as a particularly rich source of phenolics [4]. Among phenol compounds found in it the most active antioxidants are phenolic acids (gallic, gentisic, vanillic), trihydroxystilbens (cis and trans resveratrol) and flavonols (catechin, epicatechin, quercetin) [5]. Resveratrol, identified during the last years seems to be especially important in human diet as regulator of lipid metabolism [6] as well as a bacteriostatic and anticancerogenic agent [7]. The same phenolic substances were observed in fresh fruits, particularly in the red ones, however, antioxidant activity measured in fruit extracts was slightly lower in comparison with wines obtained from them. Content of total phenols, flavonols and anthocyanins was estimated in various parts of fruits of popular in Italy cultivar Negro Amaro: the highest level of flavonols was observed in seeds while that of anthocyanins was found in the skin [8].

The aim of the present study was to evaluate antioxidative ability of ten selected vine cultivars, grown in Poland. Total polyphenolics, cinnamic acid derivatives, flavonols and anthocyanins were estimated in different parts of fruits. Additionally *radical scavenging activity* (RSA) was measured.

Material and methods

The experiment was carried out in 2006–2007. The grape fruits were collected in the Golesz vineyard situated in south-east Poland, in Jaslo environment. Ten grape-vine cultivars, five white and five red skinned, mostly cultivated in Polish climate conditions, were taken for the experiment (Table 1).

Fruits were harvested in the second half of September 2006, their technological maturity was estimated by refractometric measurement of sugar level and by determination of titration acidity, expressed as the tartaric acid content.

For laboratory analyses three randomly chosen samples of fruits from each cultivar were taken. Fruits were divided into skin and flesh + skin (after the removing of seeds), in fruits of four selected cultivars flesh + skin together with seeds was analyzed. For the determination of phenolic compounds and radical scavenging activity (RSA) 2.5 g samples were frozen and kept at –20 ^{o}C until analyzed.

Tissue extracts in 80 % methanol were prepared and used for all measurements.

Determination of total phenols, phenylpropanoids (derivatives of cinnamic acid) and anthocyanins was based on UV/VIS spectrum measurements, according to the method of Fukumoto et al [9].

Radical scavenging activity was detected with DPPH$^{\bullet}$ (*1,1-diphenyl-2-picrylhydrazyl*) described by Pekkarinen et al [10]. In these methods the stable free radical is neutralized by antioxidants present in tissue extracts and the decrease of absorbance is measured. RSA is expressed as percent of free radical neutralized for 5 minutes.

The obtained results were statistically verified by two factor analysis of variance, using Duncan's test for the significance level p = 0.05.

Results

According to the presented data (Table 1) content of total phenolics was considerably higher in red fruits in comparison with the white ones. These compounds determined in the skin and in flesh + skin of white grapes did not differ significantly, while in the skin of red fruits their concentration distinctly exceeded level of flesh + skin.

Table 1

Analyzed antioxidant parameters

Grape	Cultivar	Part of fruit	DPPH neutralization [%]	Phenolic compounds [mg · 100 g^{-1} f.m.]			
				Total phenols	Phenyl-propanoids	Flavonols	Antho-cyanins
White skinned fruit	Muskat Odeski	F + S*	26.0 ± 2.3	130.0 ± 6.4	29.3 ± 1.9	36.0 ± 2.1	8.3 ± 0.3
		S	41.0 ± 2.5	183.7 ± 9.8	45.0 ± 3.2	70.0 ± 8.2	8.7 ± 0.9
	Hibernal	F + S	11.6 ± 0.4	63.0 ± 2.0	12.0 ± 0.0	13.5 ± 0.5	3.5 ± 0.5
		S	30.7 ± 0.9	108.7 ± 0.9	24.0 ± 0.6	41.7 ± 2.0	7.0 ± 0.0
	Seyval Blanc	F + S	8.3 ± 2.0	40.5 ± 1.5	7.5 ± 0.5	7.0 ± 0.0	3.0 ± 0.0
		S	34.4 ± 1.9	106.7 ± 15.3	15.3 ± 0.9	20.0 ± 2.5	6.3 ± 0.9
	Jutrzenka	F + S	19.2 ± 1.3	86.3 ± 4.3	19.7 ± 0.3	21.0 ± 1.2	5.7 ± 0.3
		S	28.7 ± 1.1	133.0 ± 7.4	29.0 ± 2.6	46.0 ± 5.2	8.0 ± 0.6
	Bianca	F + S	9.2 ± 0.4	40.7 ± 1.5	6.7 ± 0.3	6.7 ± 0.3	2.3 ± 0.3
		S	15.6 ± 1.7	79.0 ± 4.6	15.7 ± 1.5	22.7 ± 2.3	5.3 ± 0.3
		W**	91.8 ± 0.2	440.0 ± 16.7	18.0 ± 0.6	16.3 ± 0.7	10.3 ± 0.3
Red skinned fruit	Marechal Foch	F + S	41.8 ± 1.3	306.3 ± 27.2	31.3 ± 1.8	29.0 ± 2.1	138.7 ± 8.4
		S	84.1 ± 0.9	668.7 ± 28.3	61.0 ± 3.1	68.7 ± 3.4	295.3 ± 11.3
		W	84.5 ± 1.0	557.0 ± 27.5	35.3 ± 2.3	35.7 ± 1.7	133.0 ± 2.5
	Frontenac	F + S	61.2 ± 0.7	484.0 ± 24.3	35.0 ± 4.0	45.0 ± 2.6	230.3 ± 7.8
		S	85.4 ± 0.4	2065.0 ± 37.2	156.0 ± 3.2	202.0 ± 4.4	976.7 ± 11.7
		W	78.8 ± 0.9	1309.0 ± 60.0	271.7 ± 11.6	94.7 ± 2.3	531.7 ± 16.9
	Heridan	F + S	65.9 ± 0.6	570.3 ± 7.9	51.7 ± 4.1	63.0 ± 4.4	273.7 ± 13.9
		S	79.0 ± 0.3	2034.0 ± 2.6	178.0 ± 2.1	234.7 ± 1.5	986.3 ± 6.9
		W	87.6 ± 0.9	855.3 ± 38.2	62.7 ± 2.9	75.7 ± 7.4	281.0 ± 8.7
	Rondo	F + S	82.5 ± 0.2	686.7 ± 50.7	62.3 ± 3.4	77.0 ± 7.6	291.7 ± 21.7
		S	85.8 ± 0.3	1893.7 ± 125.6	137.7 ± 17.0	161.7 ± 22.4	925.7 ± 94.4
	Swenson Red	F + S	26.1 ± 0.8	117.0 ± 3.2	18.3 ± 0.7	21.3 ± 1.2	37.3 ± 1.8
		S	70.5 ± 1.7	345.7 ± 2.6	36.7 ± 0.9	56.0 ± 3.8	79.0 ± 31.0

* F – flesh; S – skin; ** W – whole fruit.

The total phenol level was strongly differentiated among cultivars (Table 1), the highest and the lowest contents were observed in 'Heridan' (red grapes) and in 'Bianca' (white grapes) cultivars, respectively.

Contents of phenylpropanoids, flavonols and anthocyanins were higher in red grapes than in the white berries, similarly, as in the case of total phenols more of these constituents were determined in the skin as compared with flesh + skin (all red and most of white cultivars) (Table 1). The great variability among cultivars was observed for all groups of phenolic compounds, the highest and the lowest contents of phenylpropanoids, flavonols and, particularly, anthocyanins were found in fruits of 'Heridan' and 'Bianca' cultivars, respectively.

In the case of whole berries (with seeds) of four cultivars (white 'Bianca' and red 'Heridan', 'Marechal Foch', 'Frontenac') the level of total phenols was significantly higher than that of either flesh + skin (all cultivars) or for skin ('Bianca'). This dependence for phenylpropanoids, flavonols and anthocyanins was observed only in berries of 'Frontenac' cv. (Table 1).

Radical scavenging activity determined by DPPH method was very high and in fruits of red cultivars was much higher than in the white ones (Table 1). Similarly as in the case of phenolics, RSA in the skin exceeded that of flesh + skin.

The highest ability of free radical neutralization (over 90 %) obtained by DPPH was noticed in the skin of most of red fruits, the lowest values (about 50 %) were observed in flesh + skin of 'Swenson Red' cv.

In white berries the considerable variability of RSA was found, the highest and the lowest values were noticed in the skin of 'Jutrzenka' (50 %) and in flesh + skin of 'Bianca' (9 %).

In berries of white 'Bianca' cultivar analyzed together with seeds high RSA (91 %) measured by DPPH method exceeded markedly the value of skin and flesh + skin. In three red cultivars free radical neutralization of whole fruits was as high as RSA of skin.

Discussion

According to the presented results the great variability of antioxidative properties depended both on color of berries and on the part of fruit. For the skin of red berries the radical scavenging activity measured by DPPH method was very high and reached 90 % of the free radical neutralization while in the case of white fruits it was distinctly lower (50 %).

The main compounds responsible for high antioxidant activity in grapes are phenolics, especially anthocyanins [2–4, 9]. In the present study dark grapes contained more polyphenols than the white ones which was in agreement with the findings of Vinson et al [3]. The highest level of total phenols (2000 mg · 100 g^{-1} fresh mass) was observed in the skin of red grapes such as Frontenac, Heridan and Rondo and was accompanied by the high concentration of anthocyanins (over 900 mg · 100 g^{-1} fresh mass). In the skin and flesh of white berries the content of total phenolics was much lower (about 100 mg · 100 g^{-1} fresh mass) with a poor level (under 10 mg · 100 g^{-1} fresh mass) of anthocyanins.

According to Meyer et al [11] the relative antioxidant capacity of grape berries and wines is correlated with the level of anthocyanins. In the present investigations the highest correlation (r = 0.983) between total phenolics and RSA was found in white grapes (Fig. 1), for the red ones this value was medium (r = 0.590) (Fig. 2) while the correlation coefficient for all cultivars was r = 0.720, hence, phenol constituents of various structure seem to be associated with neutralization of free radicals.

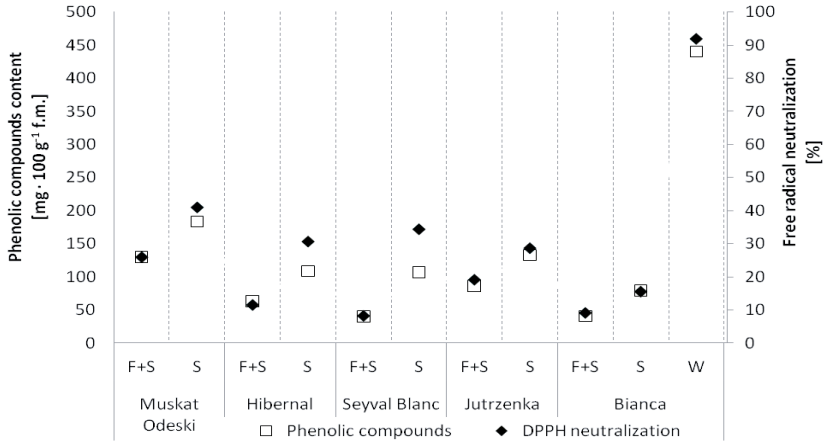

Fig. 1. Correlation between antioxidant activity and phenolics in white skinned grapes

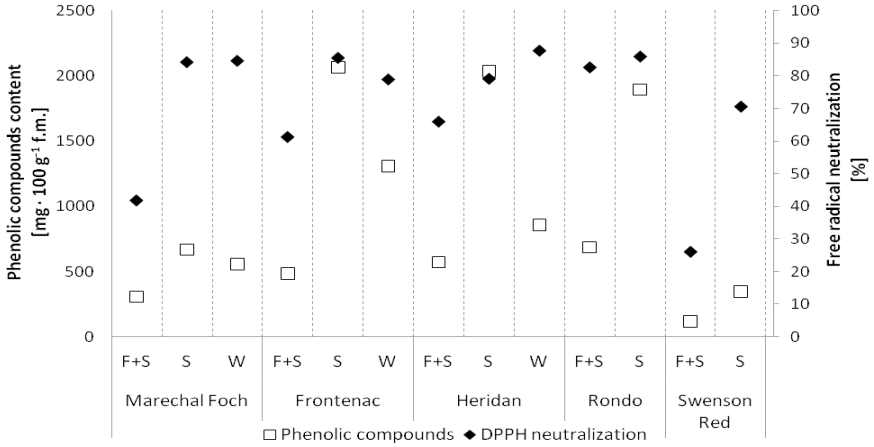

Fig. 2. Correlation between antioxidant activity and phenolics in red skinned grapes

Special attention should be paid to the analysis of whole fruits (including seeds). The antioxidant activity determined either in white (‘Bianca’) or in red (‘Heridan’, ‘Marechal Foch’) was very high (over 90 % of free radical neutralization). According to

the above findings seeds of grape berries seemed to be a rich source of antiradical substances. Similarly, Negro et al [8] who analyzed phenolics in grape seeds found very high content (8.58 g · 100 g^{-1} dry matter) of these compounds.

In general, grape berries of all cultivars grown in Polish climate conditions are rich in health-promoting substances, particularly those of dark color, and may be recommended in the human diet.

Conclusions

1. Grape berries of cultivars grown in Poland are rich in phenolic antioxidants of various structure.
2. In dark berries the amount of phenolic substances is higher in comparison with the white ones.
3. Skin and seeds have more antioxidants than flesh tissue.
4. Radical scavenging activity is strongly correlated with level of phenolics.

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ANTYOKSYDACYJNE WŁAŚCIWOŚCI WINOGRON WYBRANYCH ODMIAN UPRAWIANYCH W POLSCE

Katedra Fizjologii Roślin, Wydział Ogrodniczy
Uniwersytet Rolniczy w Krakowie

Abstrakt: Dziesięć wybranych odmian winogron (5 o białej i 5 o czerwonej skórce) uprawiano w południowo-wschodnim regionie Polski. Z zamrożonych próbek owoców przygotowywano ekstrakty w 80 % metanolu, w których oznaczano sumę związków fenolowych, fenylopropanoidy, flawonole i antocyjany stosując metodę UV/VIS. Ponadto oznaczono zdolność neutralizowania wolnego rodnika za pomocą metody z $DPPH^{\bullet}$.

W owocach o czerwonej skórce zawartość składników fenolowych była większa niż w owocach o skórce białej. Stwierdzono znaczne zróżnicowanie odmianowe: najwyższy poziom fenoli obserwowano w odmianie Heridan, najniższy w odmianie Bianca.

Aktywność antyrodnikowa (RSA) była również bardzo duża w czerwonych winogronach, szczególnie w skórce (ponad 90 % neutralizacji wolnego rodnika). W przypadku odmian o skórce białej aktywność antyoksydacyjna była zawarta w przedziale 9–50 % (Bianca i skórka odmiany Jutrzenka).

W analizowanych tkankach stwierdzono dodatnia korelację (r = 0.70) pomiędzy sumą związków fenolowych a RSA.

Słowa kluczowe: winogrona, aktywność antyoksydacyjna, związki fenolowe