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Total antioxidant activity of selected grape cultivars (*Vitis vinifera* L.) studied by electron paramagnetic resonance spectroscopy

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Abstract

The total antioxidant activity of three white grape cultivars (*Vitis vinifera* L.) (Afus Ali, White Chasselas and Chardonnay) and four red grape cultivars (Red Chasselas, Muscat Hamburg, Cardinal and Blue Frankish) conventionally farmed was investigated using electron paramagnetic resonance spectroscopy (EPR), which has the unique ability to directly monitor free radicals and antioxidants in natural environments and systems. Grapes examined in this work originated from the eastern part of continental Croatia (the Slavonia region) and were picked in early September 2010. The spin label used in all of the experiments was a nitroxide-based TEMPO radical (2,2,6,6-tetramethylpiperidine-1-oxyl) with a well-defined EPR spectrum consisting of three equidistant peaks. The obtained results indicated differences in total antioxidant activity among the examined grape cultivars. Muscat Hamburg had the greatest antioxidant activity, very narrowly followed by Afus Ali. The White Chasselas, Cardinal and Red Chasselas cultivars showed moderate antioxidant activity, though still stronger that of the Chardonnay and Blue Frankish cultivars, which were at the end of the list. The experimental results suggested that the table grape cultivars generally had greater antioxidant activity than the wine grape cultivars did. All experimental data obtained from EPR spectra were fitted to a non-linear exponential first-order decay curve using the Levenberg-Marquardt method, in two steps. The high values of R^2 parameters obtained for all of the cultivars confirmed the proposition of exponential first-order decay as a mathematical model describing the dynamic properties of spin labels which diminished in the presence of fresh grape juice.

Key words: Antioxidant activity, electron paramagnetic resonance, free radicals, white grapes, red grapes, data fitting.

Introduction

Fruits and vegetables contain significant amounts of antioxidants, particularly polyphenols and vitamins [8, 14, 15, 17, 18]. Antioxidants are cleaners (scavengers) of free radicals, which are the cause of many diseases, such as cardiovascular diseases, diabetes, cancer, aging-related disorders, Parkinson's and Alzheimer's diseases, etc. While the human body has built-in mechanisms for dealing with oxygen radicals, such as the enzyme superoxide dismutase [3, 13], intake of fruits and vegetables rich in antioxidants ensures extra protection from the impact of radicals on cells as reported by Frankel *et al*. [5].

Due to their importance, antioxidative properties of fruits and vegetables are studied extensively worldwide. Those studies employ different methods, the majority being based on the hydrogen atom transfer and electron transfer reactions as reported by Huang *et al.* [6]. Wang *et al.* [16] investigated total antioxidant activity of twelve fruits and five commercial fruit juices using automated oxygen radical absorbance capacity (ORAC) assay with a peroxyl radical generator. The results they presented indicated that strawberry, plum and orange had the highest ORAC activity. Grape juice had the highest antioxidant activity among the examined fruit juices. Phenolic compounds as antioxidants for low-density lipoproteins (LDL) were studied in prunes and prune juice by Donovan *et al.* [4]. The results, obtained using reversed-phase high-performance liquid chromatography (HPLC), revealed that phenolic compounds from prune and prune juice extracts could inhibit oxidation of LDL by up to 98%. Kulišić-Bilušić *et al.* [9] studied antioxidant activity versus cytotoxic and nuclear factor-kappa B regulatory activities on HT-29 cells by natural fruit juices. The DPPH test and β-carotene bleaching method indicated that grape, strawberry, cherry and sour cherry natural juices at a concentration of 2.5% showed a very strong free radical scavenging effect (75 - 92%) and antioxidant activity (71 - 94%). Phenolic profiles in common fruits were investigated by Sun *et al.* [14]. Total oxyradical scavenging capacity (TOSC) assay experiments showed cranberry to have the highest total phenolic content, followed by apple, red grape, strawberry and the others. Cranberry also had the highest inhibitory effect with regard to $HepG_2$ human liver cancer cells. The authors also proposed a bioactivity index (BI) for dietary cancer prevention as a new alternative biomarker for future epidemiological studies in the field.

Alonso *et al.* [1] studied the antioxidant activity of wine by-products (marc, stalks and dregs) using electrochemical methods. They determined the correlation of total antioxidant activity with the polyphenolic content of both white and red grape cultivars. The polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in the Adriatic region of Croatia were investigated by Katalinić *et al.* [7]. Their results indicated that white grape skins could be an interesting and inexpensive material for the production of new food products or food additives. The antioxidant, hypolipidemic and anti-inflammatory effects of concentrated red grape juice were studied in both haemodialysis patients and healthy subjects as reported by Castilla *et al.* [2]. The authors found that dietary supplementation with concentrated red grape juice improved lipoprotein profile, reduced the plasma concentrations of inflammatory biomarkers and oxidized low-density lipoproteins, it may also favour a reduction in cardiovascular disease risk.

Electron paramagnetic resonance spectroscopy (EPR) has the unique ability of direct monitoring of free radicals and antioxidants, as well as their interactions, in natural environments and laboratory systems. Tzika *et al.* [15] investigated commercially available fruit and vegetable juices with regard to their scavenging activity against the stable nitroxide 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPOL). Their results indicated that the natural antioxidant compounds contained in those commercially available juices were not eliminated or inactivated when the juices were kept refrigerated according to the instructions of the manufacturers. They also found that freshly prepared orange juice had double the antioxidant activity compared to the commercial ones. The EPR spin trapping method and a colorimetric assay were used by Leonard *et al.* [10], who proved that the antioxidant activities of several fruits and vegetables were dependent on many different compounds, not only on ascorbic acid (vitamin C).

The aim of this work was to compare the total antioxidant activity of three white grape cultivars (Afus Ali, White Chasselas and Chardonnay) and four red grape cultivars (Red Chasselas, Muscat Hamburg, Cardinal and Blue Frankish) conventionally farmed in Croatia using EPR as a rarely used but precise and sensitive technique for determination of free radicals and antioxidants. Possible differences in antioxidant activity in table grape and wine grape cultivars could be envisaged. The results obtained by the time-dependent experiments may propose a model describing the dynamic properties of interactions between free radicals and antioxidants contained in fresh grape juice.

Material and Methods

Grape cultivars and origin: The white grape cultivars used in this work were Afus Ali, White Chasselas and Chardonnay. The red grape cultivars were Red Chasselas, Muscat Hamburg, Cardinal and Blue Frankish. Afus Ali, White Chasselas, Red Chasselas, Muscat Hamburg and Cardinal are table grape cultivars, whereas Chardonnay and Blue Frankish are wine grape cultivars. The grapes were grown in the conventional manner and originated from the region of Slavonia (the area of Brodsko vinogorje) situated in the continental (eastern) part of Croatia. All grapes used in this work were picked in early September 2010.

Spin label: 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) was purchased from Sigma Aldrich (St. Louis, MO, USA). Its EPR spectrum consists of three peaks and can reveal both qualitative and quantitative properties of the systems examined. The middle peak of each nitroxide spin label EPR spectrum is very often the one with the largest intensity (magnitude) and the area below it is suitable for quantitative determinations. The relative content of the spin label can be monitored either by double integration of the first derivative EPR spectrum or by measuring the maximum to minimum points of intensity of the middle peak (MPI, Fig. 1) in comparison to the initial content. Since the first method is highly dependent on the signal-to-noise ratio of the spectra, it is not suitable for low spin label concentration experiments, and the second method was chosen accordingly.

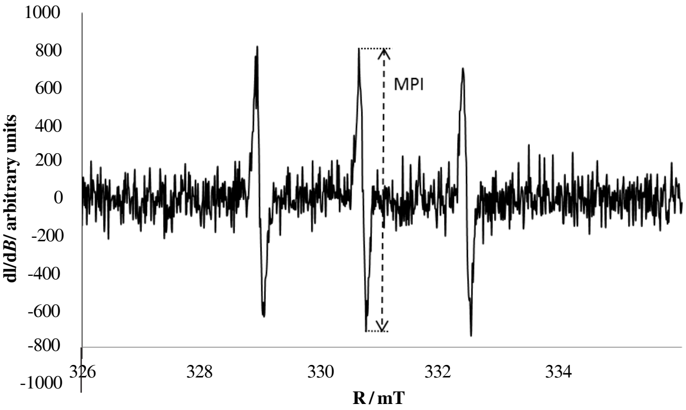

Figure 1. EPR spectrum of TEMPO in Red Chasselas juice. Medium peak intensity (MPI) parameter is denoted.

Sample preparation: Grapes of each examined cultivar were mechanically squeezed (in a juicer) and their liquid phase (juice) was separated. TEMPO spin label was dissolved in acetone to the concentration of $3.6 \cdot 10^{-4}$ M. Each juice sample was a properly stirred mixture of 10 µl of spin label and 1 ml of grape juice.

EPR measurements: EPR measurements were done on a Varian E-9 spectrometer (10 GHz). The spectra were recorded with digital acquisition, EW-ESR ware software, at room temperature [12]. Sample capillaries were inserted into the standard 4-mm diameter EPR quartz tubes and centred in a TE102 EPR cavity. At each time point, four EPR spectra were recorded and their average values were obtained.

Experimental data fitting: A non-linear curve fit for exponential first-order decay based on the Levenberg-Marquardt method was used for each cultivar (Equation 1):

$$y = y_0 + A_1 \cdot e^{-x/t_1} \qquad (1)$$

The fitting of experimental data was done with analysis tools provided within the Origin software (produced by OriginLab Corporation). Parameter R^2 indicates correlation between the experimental data and the proposed mathematical model.

Results and Discussion

Antioxidants contained in fruits are well known as free radical sweepers. Because of the opposite oxidative activity of spin labels and antioxidants, higher concentrations of antioxidants in grape juice would cause a faster decrease of TEMPO spin label signal in the sample. For each grape cultivar, the decrease of TEMPO spin label signal due to the presence of antioxidants was monitored at several time points.

Time dependence of MPI decay in EPR spectra for all three white grape cultivars is compared in Fig. 2. All MPI parameters are shown relative to the initial value and expressed in percentages (%) relative to the initial 100% value.

Since MPI decay is clearly the fastest for the Afus Ali cultivar, antioxidant activity in those grapes is greater and the concentration of antioxidants higher than in the White Chasselas and Chardonnay cultivars. After half an hour, antioxidants in Afus

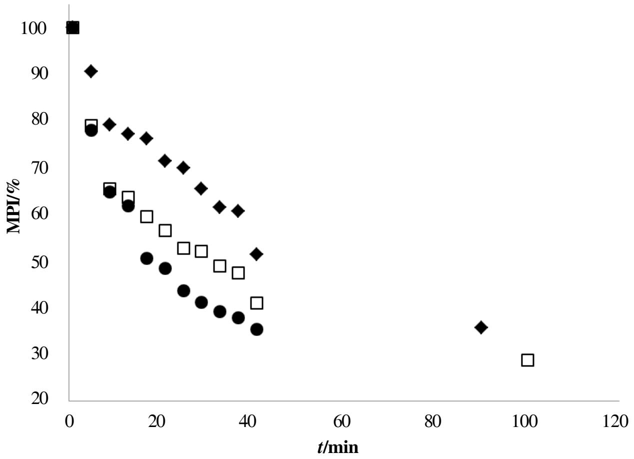

Figure 2. Time dependence of relative MPI decay in EPR spectra for white grape cultivars (*Vitis vinifera* L.): White Chasselas (squares), Afus Ali (circles) and Chardonnay (rhombs).

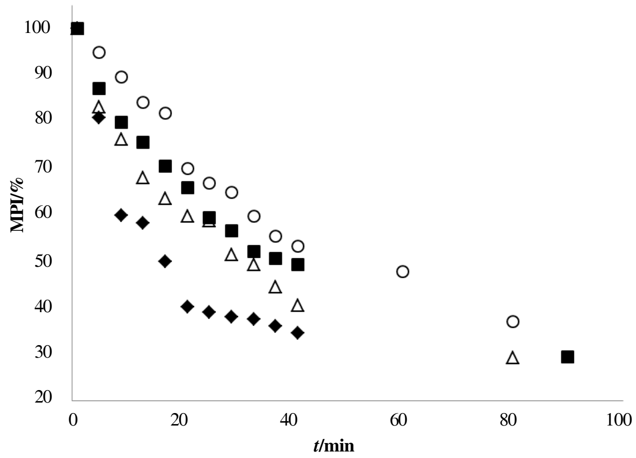

Figure 3. Time dependence of relative MPI decay in EPR spectra for red grape cultivars (*Vitis vinifera* L.): Red Chasselas (squares), Blue Frankish (circles), Cardinal (triangles) and Muscat Hamburg (rhombs).

Ali reduced the spin label concentration to 41.1% of the initial value, while at the same time antioxidants in White Chasselas and Chardonnay reduced the spin label concentrations to 52.1% and 65.5% of the initial value, respectively.

For each cultivar a non-linear exponential first-order decay curve fit based on the Levenberg-Marquardt method was used. In the first step, all three parameters from Equation 1 (y_0, A_1 and t_1) were variable. The results indicated the greatest antioxidant activity for the Afus Ali cultivar, followed by White Chasselas and Chardonnay. For the second step, y_0 and A_1 were fixed to values obtained in the first step fitting for the Afus Ali cultivar (y_0= 34.5232; A_1 = 69.2667) as the Afus Ali fit was the best one (R^2 = 0.99124). Parameter t_1 was the only one variable. The results of fitting shown in Table 1 clearly confirmed that the t_1 parameter, describing the time scale of decay, is the smallest for Afus Ali (t_1 = 12.1226 min), while White Chasselas and Chardonnay have larger values (16.8624 min and 32.5063 min, respectively). It means that the Afus Ali grape has greater antioxidant activity than White Chasselas and Chardonnay. Comparing the t_1 parameters for the table and wine grape cultivars, it can be seen from Table 1 that the table grape cultivars (Afus Ali and White Chasselas) had greater antioxidant activity than the wine grape cultivar (Chardonnay).

Table 1. Fitting parameters obtained by non-linear curve fit for exponential first-order decay based on the Levenberg-Marquardt method (y_0= 34.5232; A_1 = 69.2667).

Cultivar	t_1 (min)	R^2
Afus Ali	12.1226 ± 1.1497	0.99124
White Chasselas	16.8624 ± 1.2563	0.92649
Chardonnay	32.5063 ± 1.6819	0.95444
Muscat Hamburg	10.4405 ± 0.4842	0.98497
Cardinal	19.4557 ± 0.9048	0.97313
Red Chasselas	24.5356 ± 0.9260	0.98059
Blue Frankish	33.7421 ± 1.1589	0.98287

The high values of R^2 parameters obtained for all examined cultivars indicated a very good correlation between the experimental data and the proposed mathematical model of exponential first-order decay described by Equation (1).

Time dependence of MPI decay in EPR spectra for all four red grape cultivars is compared in Fig. 3. All MPI parameters are shown relative to the initial values and expressed in percentages (%) relative to the initial 100% value.

Among the four red grape cultivars examined in this work, MPI decay was the fastest for the Muscat Hamburg cultivar. Antioxidant activity in those grapes was greater and the concentration of antioxidants higher than in the other three cultivars examined. After half an hour, antioxidants in the Muscat Hamburg cultivar reduced the spin label concentration to 38.0% of the initial value, while at the same time antioxidants in the Cardinal, Red Chasselas and Blue Frankish cultivars reduced the spin label concentrations to 51.4%, 56.6% and 64.8% of the initial values, respectively.

For each cultivar, a non-linear exponential first-order decay curve was fitted using the Levenberg-Marquardt method in the same way as for the white grape cultivars. In the first step fitting, the results indicated the greatest antioxidant activity for the Muscat Hamburg cultivar, followed by Cardinal, Red Chasselas and Blue Frankish. The results of fitting in the second step (presented in Table 1) confirmed that the t_1 parameter was smallest for Muscat Hamburg (t_1 = 10.4405 min), while Cardinal, Red Chasselas and Blue Frankish had higher values (19.4557 min, 24.5356 min and 33.7421 min, respectively). That means that the Muscat Hamburg grape has greater antioxidant activity than any of the other three cultivars. Results presented in Table 1 reveal that the table grape cultivars (Muscat Hamburg, Cardinal and Red Chasselas) had greater antioxidant activity than the wine grape cultivar (Blue Frankish), as seen with the white grape cultivars, too.

The results on the antioxidant activity of both white and red grapes are in accordance with previous findings by several authors who experimented on the antioxidative properties of fruits[9, 11, 14, 16].

By combining the fitting results of all seven grape cultivars examined in this work, it can be seen that the Muscat Hamburg cultivar had the greatest antioxidant activity, very narrowly followed by the Afus Ali cultivar. White Chasselas, Cardinal and Red Chasselas followed with moderate antioxidant activity and Chardonnay and Blue Frankish cultivars were at the end of the list. Generally speaking, no significant difference in the antioxidant activity of white and red grape cultivars was found, but the table grape cultivars showed greater antioxidant activity than the wine grape cultivars.

Conclusions

The study contains EPR investigations of antioxidant activity in three white grape cultivars (Afus Ali, White Chasselas and Chardonnay) and four red grape cultivars (Red Chasselas, Muscat Hamburg, Cardinal and Blue Frankish) conventionally farmed in Croatia. The results indicated differences among the examined cultivars. Muscat Hamburg had the greatest antioxidant activity, very narrowly followed by Afus Ali. The antioxidant activity of the White Chasselas, Cardinal and Red Chasselas cultivars was shown to be moderate, albeit greater than that of the Chardonnay and Blue Frankish cultivars, which were on the low end of the scale. The table grape cultivars showed greater antioxidant activity than the wine grape cultivars. High values of R^2 parameters obtained for the fitting of all experimental data confirmed the proposition of exponential first-order decay as a mathematical model for explaining the dynamic properties of interactions between antioxidants and free radicals.

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