Antioxidant Activity of Wines and Related Matters Studied by EPR Spectroscopy

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Abstract: Antioxidant activity and free radicals were studied in various parts of *Vitis vinifera* plant *in vivo* and in wines using EPR spectroscopy. Antioxidative properties of polyphenolic substances play an important role for the evaluation of quality of natural products. Determination of antioxidant activity of experimental samples by EPR method was based on measuring the changes of EPR spectrum of stable nitroxide radicals as a result of their interaction with antioxidants. In the leaves of *Vitis vinifera* vine varieties for the production of red wines there was observed a higher decrease in free Tempol radicals as compared to the leaves of varieties used for the production of white wines. For all monitored wines there was established a clear decrease in free Tempol radicals to the average value of 75.5 ± 15.6% as compared to the original EPR spectrum. A higher antioxidant activity was observed in yeast sediments from the production of red wines than in those from white ones.

Keywords: antioxidant activity; EPR; wine; vine grape; vine leaf; yeast sediment

Common foods of plant origin contain many flavonoids and other phenolics at level ranging from traces to several grams per kilogram (Burns *et al*. 2000). The strong antioxidant properties of wine are mainly due to the presence of a large amount of polyphenols and anthocyanins as major families of the compounds having antioxidative properties in red wine. (Rice-Evans *et al*. 1997; Dugo *et al*. 2003). The group of phenolic substance involves a number of compounds ranging from simple phenolic compounds, phenolic acids and their derivatives to coumarins, flavonoids and stilbenes, tannins and lignins (Merken & Beecher 2006). Over 4000 different naturally occurring flavonoids have been described (Middleton & Kandaswami 1994) and the list is still growing. Polyphenolic compounds present in grapevine and in products made of them (grape juice, wine) can gain ground in the mechanism of mutagenesis and carcinogenesis as chemopreventive factors. In grapes, *trans*-resveratrol is considered to be the main stilbene derivative. It is present above all in skins of berries and also in leaves and grape stalks (Mikeš *et al*. 2008) and during the process of maceration it passes into wine. It is known that after the infestation with fungal diseases (*Botryotinia fuckeliana* Whetzel anamorph *Botrytis cinerea* Pers.) the grapevine plants can increase the synthesis of *trans*-resveratrol as a natural means of protection in the neighbourhood of infested spot

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so that a further spread of infection is prevented (Langcake 1981).

It is absolutely necessary for the successful research in this area to measure free radicals and antioxidant activity of grapevine and wine. Reactive oxygen species (ROS) are constantly formed in the human body and are removed by antioxidant defences. Antioxidants can act by scavenging biologically important reactive oxygen species. Many methods have been used to study antioxidant activity of wines (Rossetto *et al*. 2004; Rocha-Guzmán *et al*. 2007), more recently electron paramagnetic resonance has been applied to evaluate the antioxidant activity of different food matters (Pedersen *et al*. 2002; Polovka *et al*. 2003).

Free radicals in different parts of the *Vitis vinifera* L. plants were studied *in vivo* using EPR spectroscopy (Electron Paramagnetic Resonance). One of the most important aspects of EPR is the possibility to determine the concentration of radical species, particularly in biological systems. Antioxidant activity is the expression of quantity or concentration of antioxidants which are available for the degradation of free radicals and antioxidants. The EPR method is based on measuring the decline of EPR spectrum of relatively stable radicals as a result of their interaction with antioxidants.

The study of the wines antioxidant activity has been carried out by *in vitro* methods (Rivero *et al*. 2005). Very important additional information will be the antioxidant activity and free radicals measurements (*in vivo* at the *Vitis vinifera* plants and in wine samples) and antimutagenic activity of the wine samples (Staško *et al*. 2006). The principle of antioxidant capacity determination is the addition of oxidising agent in surplus, which will oxidise present antioxidants quantitatively. Antioxidant activity (AA) is calculated from the difference between the content of the oxidising agent before and after the reaction with antioxidants. The main analytical methods for the determination of antioxidant activity are spectrophotometric, electrochemical and EPR spectroscopic procedures. Growth or decline in AA reflects the listed methods reliably. AA values measured by different methods, however, usually differ. This is due to the fact that the reactivity of various oxidising substances differs from the reactivity of various antioxidants (Fernández-Pachón *et al*. 2004; Polovka 2006).

Methods for the determination of AA can be reliably calibrated using defined antioxidants, which are applied in the exact quantities. This cannot be reliably ensured in real wine samples and part of the vine, as well as for other food and drinks. The AC values depend also on the modification of technology procedures of cultivation and harvest of grapevines, technology of juices, musts and wines as well as ways of storing, packaging, distribution, and consumption (Garcia-Alonso *et al*. 2005; Otreba *et al*. 2006; Staško *et al*. 2006). Four different types of Chilean wines were studied (Espinosa *et al*. 2008), where Cabernet Sauvignon was the one with the highest activity against all radical tested.

The determination of AA by the mentioned methods has been used also in human medicine at the patients with suspected vitamin deficiency and other metabolic disorders. Nevertheless, similar analytical problems occur. AA determination methods thus continue to develop, since AA has been very useful information on the properties of plants, products and health status of patients (Stopka & Křížová 2006).

MATERIALS AND METHODS

Samples. Only wines and plants parts from south Moravia winemaking area and sub-area Mikulov were used. Seventeen Moravian wines of the 2006 or 2007 vintage were made from eleven red and white grapevine varieties (Dornfelder, Pinot noir, Blaufrankisch, Blauer Portugieser, Saint Laurent, André and Pálava, Grüner Veltliner, Welschriesling, Chardonnay, Pinot gris). The samples of wines from 2007 were made by different winemaking technologies as barrique, vinifitank, cryomaceration, rotary tank or pulp cap dipping. Healthy and infected leaves and shoots (young lateral leaves) and healthy berries of six grapevine varieties (Andre, Saint Laurent, Blaufrankisch, Grüner Veltliner, Sauvignon, Pinot gris) were sampled. Nine yeasts sediments were sampled after fermentation of wines (Blaufrankisch, André, Saint Laurent, Grüner Veltliner, Palava, Sauvignon and Pinot gris).

Standards, spin traps, calibration. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) (Sigma-Aldrich, Prague, Czech Republic) was used as a radical trapping agent for oxygen and nitrogen radicals. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol) (Sigma-Aldrich, Prague, Czech Republic) was used as a reactant with antioxidative substances for determination of antioxidant activities. Calibration standards were Mn^{2+}/ZnS and Cr^{3+}/MgO (Magnettech, Berlin, Germany).

Electron paramagnetic resonance (EPR) measurements. EPR spectra were recorded with an E-540 Spectrometer X-Band (Bruker-Biospin, Germany). The following conditions were used while recording the spectra: microwave power 20 mW, magnetic modulation amplitude 0.2 mT, attenuation 20 dB, time constant 0.5 s, scan speed 0.3 mT/min, calibration standards Mn^{2+}/ZnS and Cr^{3+}/MgO at 25°C. WinEPR and Bruker (Bruker-Biospin, Germany) programs were used for spectra recording, handling and evaluation. The reaction system of the EPR experiments is described in the legend to Figure 1. UV irradiation of liquid sample in resonator was used in the study of effect of irradiation on free radicals generation.

Measurement of antioxidant activity by EPR spectroscopy. Aqueous solution of 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol) was used as a source of relatively stable free radical and its EPR spectrum was measured. The resulted spectrum shows nitroxide triplet. This standard was measured for some of its concentration. Studied extract was then added. The decreasing intensity of nitroxide radical's spectrum is value of antioxidant capacity of the studied sample. The samples were prepared from natural materials by extraction with methanol and filtration according Mikeš *et al*. (2008).

RESULTS AND DISCUSSION

The optimal conditions for the study of antioxidant activity of Moravian red and white wine samples were studied. They depend on antioxidative properties of studied samples. The results are very useful for evaluation of the quality of wines. The changes of antioxidant activity were studied as a function of temperature, oxygen concentration and irradiation (UV and visible light). Antioxidant activity of grapes depends also on the use of copper substances and their doses for the protection of grapes. Antioxidant activity of yeast sediment samples from wines was also studied. A source of relatively stable radicals was Tempol solution and a course of its EPR spectrum is shown in Figure 1. Determination of antioxidant activity of experimental samples by EPR method is based on measuring the changes of EPR spectrum of radicals as a result of their interaction with antioxidants. EPR spectrum of the wine containing complex of $Fe^{(III)}$ and complex of high-spin $Mn^{(II)}$ is depicted on the Figure 2.

In the leaves of vine varieties for the production of red wines, a higher average decrease in free Tempol radicals was observed as compared to the leaves of vine varieties used for the production of white wines. The amount of Tempol free radicals decreased to the value of 89.0 ± 0.3% or 91.0 ± 11.3% respectively. Similar results were observed also in the young lateral shoots. The large fall of EPR spectrum (to 71%) was found in the leaves of variety Blaufrankisch infected with *Uncinula necator* (Schw.) Burr. or *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni. Generally, the opposite trend with a higher reduction in free Tempol radicals was showed in healthy young leaves or young lateral shoots as compared to the microbially contaminated samples, however, without a clear statistical significance. In the case of grapes infected with *Botryotinia*

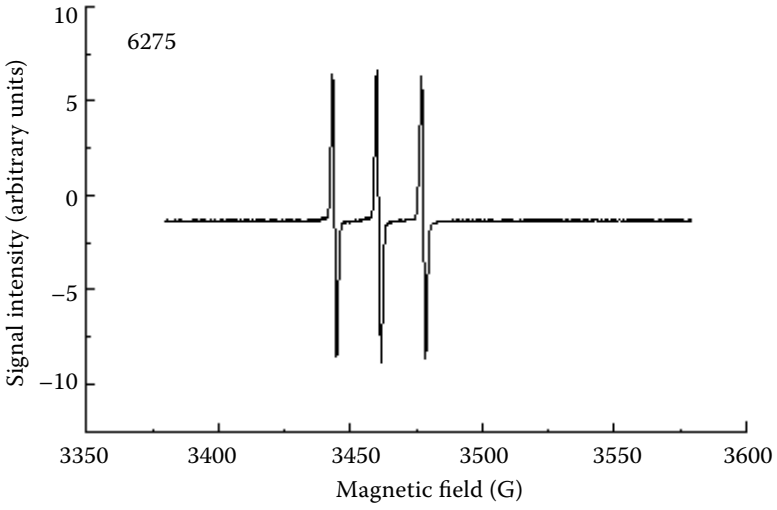

Figure 1. EPR spectrum of stable radical Tempol (2,2,6,6-tetramethylpiperidine-1-oxyl) (aqueous solution), temperature 25°C

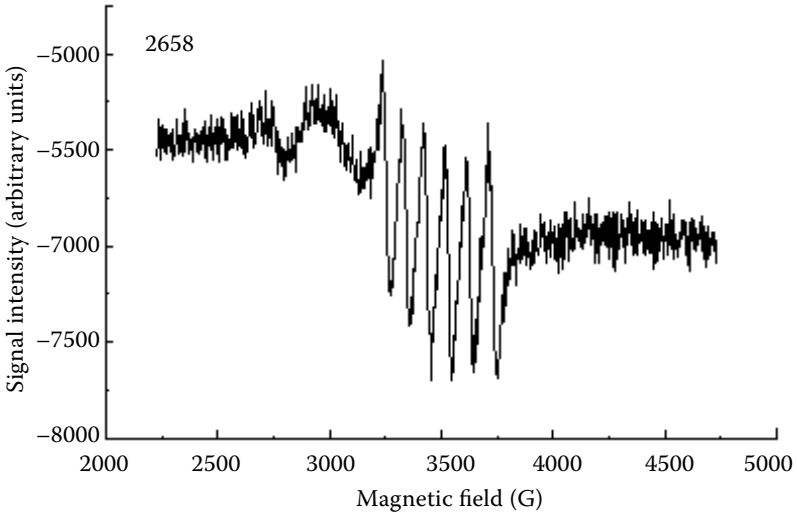

Figure 2. EPR spectrum of wine containing complex of $Fe^{(III)}$ and complex of high-spin $Mn^{(II)}$

fuckeliana Whetzel anamorph *Botrytis cinerea* Pers. the increase rather than decrease of radicals was observed due to the presence of antioxidants (99.4 ± 11.0%). The observed results may be associated with an increased content of the active copper as a result of the treatment of the leaves with copper containing fungicides (Table 1).

By contrast, a clear decrease in free Tempol radicals to the average value of 75.5 ± 15.6% was found for all investigated wines as compared to the original EPR spectrum. Statistically significant difference was found neither between the white and red wines, nor in EPR spectrum changes in dependence on the technology used in vinification or vintage (Table 2). Decreases of free Tempol radicals were also observed for yeast fermentation sediment (81.2 ± 8.7%). Higher antioxidant activity was observed in yeast sediment from the production of red wines. It is likely that this is related to larger quantities of adsorbed polyphenols, in particular tannins and anthocyanin dyes (Table 3).

CONCLUSIONS

Antioxidant activity and free radicals in various parts of *Vitis vinifera* plant were studied *in vivo* using EPR spectroscopy. The antioxidant activity of different red and white wine samples was also determined. EPR method for the determination of

Table 1. The changes of Tempol free radicals by effect of vine leaves and grapes in different healthy state

Variety	Tempol free radicals (%)				
	leaves (A)	leaves (B)	*shoots (A)	*shoots (B)	berries (C)
André	89.4	88.4	92.5	84.5	107.3
Saint Laurent	88.9	101.9	87.9	98.9	108.2
Blaufrankisch	88.9	71.0	82.8	101.3	103.9
Mean(red)[1]	89.0 ± 0.3	87.1 ± 15.5	87.7 ± 4.9	94.9 ± 9.1	106.5 ± 2.3
Grüner Veltliner	101.9	94.6	90.0	97.9	79.4
Sauvignon	91.8	119.2	108.0	102.3	103.1
Pinot gris	79.3	105.3	97.1	95.1	94.4
Mean(white)[2]	91.0 ± 11.3	106.4 ± 12.3	98.4 ± 9.1	98.4 ± 3.6	92.3 ± 12.0
Mean(total)	90.0 ± 7.2	96.7 ± 16.4	93.1 ± 8.7	96.7 ± 6.5	99.4 ± 11.0

[1,2]mean and standard deviation for varieties for production of red or white wine, respectively

(A) – healthy; (B) – infection with *Uncinula necator* (Schw.) Burr. or *Plasmopara viticola* (Berk. & M.A. Curtis) Berl & De Toni; (C) – infection with *Botryotinia fuckeliana* anamorph *Botrytis cinerea* Pers.; *young lateral shoots

Table 2. The changes of Tempol free radicals by effect of red and white wines

Abb.	Variety	Vintage/technology note	Tempol free radicals (%)
RW1	Dornfelder	2007/barique	64.1
RW2	Dornfelder	2007/non-barique	82.1
RW3	Pinot noir	2007/barique	84.2
RW4	Pinot noir	2007/non-barique	89.1
RW5	Pinot noir	2007/vinifitank	56.2
RW6	Pinot noir	2007/cryomaceration	78.6
RW7	Blaufrankisch	2007/pulp cap dipping	83.3
RW8	Blaufrankisch	2007/rotary tank	76.2
RW9	Blaufrankisch	2007/vinifitank	82.8
RW10	Blauer Portugieser	2006/vinifitank	64.2
RW11	Saint Laurent	2006/vinifitank	87.4
RW12	André	2006/vinifitank	81.9
Mean (red)[1]			77.5 ± 10.4
WW1	Pálava	2007	76.1
WW2	Grüner Veltliner	2007	90.0
WW3	Welschriesling	2006	27.5
WW4	Chardonnay	2006	72.6
WW5	Pinot gris	2006	87.5
Mean (white)[2]			70.7 ± 25.3
Mean (total)			75.5 ± 15.6

[1,2]mean and standard deviation for red or white wine, respectively

Table 3. The changes of Tempol free radicals by effect of yeast sediment from red and white wines

Abb.	Variety	Tempol free radicals (%)
RS1	Blaufrankisch	73.7
RS2	Blaufrankisch	91.7
RS3	André	65.4
RS4	Saint Laurent	77.1
Mean (red)		77.0 ± 11.0
WS1	Grüner Veltliner	89.7
WS2	Grüner Veltliner	89.5
WS3	Pálava	85.3
WS4	Sauvignon	80.4
WS5	Pinot gris	77.7
Mean (white)		84.5 ± 5.4
Mean (total)		81.2 ± 8.7

[1,2]mean and standard deviation for sediment from production of red or white wine, respectively

antioxidant activity is useful method for evaluation of the quality of wines.

The evaluation of antioxidant capacity of experimental samples by EPR method is based on measuring the changes of EPR spectrum of radicals as a result of their interaction with antioxidants. In the leaves of the vine varieties for the production of red wines, a higher mean decrease in free Tempol radicals was observed as compared to the leaves of the vine varieties used for the production of white wines. For all monitored wines a clear decrease in free Tempol radicals to the average value of 75.5 ± 15.6% was observed as compared to the original EPR spectrum. A higher antioxidant activity was observed in yeast sediments from the production of red wines than in those from white ones.

References

Burns J., Gardner P.T., O´Neil J., Crawford S. Morecroft L., McPhail D.B., Lister C., Matthews D.,

MacLean M.R., Lean M.E.J., Duthie G.G., Crozier A. (2000): Relationship among antioxidant activity, vasodilatation and phenolic content of wines. Journal of Agricultural and Food Chemistry, **48**: 220–230.

Dugo G., Salvo F., La Torre G.L., Mondello L. (2003): Antioxidants in sicilian wines: Analytic and compositive aspects. Drugs under Experimental and Clinical Research, **29**: 198–202.

Espinoza M., Azar C.O., Massiff G., Villa A. (2008): Development of a reference material for copper and iron in wine. Journal of Food Composition and Analysis, **21**: 684–688.

Fernández-Pachón M.S., Villaño D., García-Parrilla M.C., Tronsoco A.M. (2004): Antioxidant activity of wines and relation with their polyphenolic composition. Analytica Chimica Acta, **513**: 113–118.

Garcia-Alonso M., Rimbach G., Sasai M., Nakahara M., Matsugo S., Uchida Y., Rivas-Gonzalo J.C., de Pascual-Teresa S. (2005): Electron spin resonance spectroscopy studies on the free radical scavenging activity of wine anthocyanins and pyranoanthocyanins. Molecular Nutrition & Food Research, **49**: 1112–1119.

Langcake P. (1981): Physiology. Plant Pathology, **18**: 213.

Merken H.N., Beecher G.R. (2000): Liquid chromatographic method fort the separation and quantification of prominent flavonoid aglycones. Journal of Chromatography A, **897**: 177–184.

Middleton E., Kandaswami C. (1994): The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne J.B. (ed.): The Flavonoids: Advances in Research Science 1986. Chapman & Hall/CRC, London: 619–652.

Mikeš O., Vrchotová N., Tříska J., Kyseláková M., Šmidrkal J. (2008): Distribution of major polyphenolic compounds in vine grapes of different cultivars growing in south Moravian vineyards. Czech Journal of Food Sciences, **26**: 182–189.

Otreba J.B., Berhofer E., Wendelin S., Eder R. (2006): Polyphenole und antioxidative Kapazität in österreichischen Weinen aus konventioneller und biologischer Traubenproduktion. Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Früchteverwertung, **56**: 22–32.

Pedersen J.A.(2002): On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols. Spectrochimica Acta A, **58**: 1257–1270.

Polovka M., Brezová V., Stasko A. (2003): Antioxidant properties of tea investigated by EPR. Biophysical Chemistry, **106**: 39–56.

Polovka M. (2006): EPR spectroscopy: A tool to characterize stability and antioxidant properties of foods. Journal of Food and Nutrition Research, **45**: 1–11.

Rice-Evans C.A., Miller N.J., Paganga G. (1997): Antioxidant properties of phenolics compounds. Trends Plant Science, **2**: 152–159.

Rivero D., Pérez-Magarino S., Gonzáles-Sanjosé M.L., Valls-Belles V., Codonez, P., Muniz P. (2005): Inhibition of induced DNA oxidative damage by beers: Correlation with the content of polyphenols and melanoidins. Journal of Agricultural and Food Chemistry, **53**: 3637–3642.

Rocha-Guzmán N.E., Herzog A., González-Laredo R.F., Ibarra-Pérez F.J., Zambrano-Galván G., Gallegos-Infante A. (2007): Antioxidant and antimitagenic activity of phenolic compounds in three different colour groups of common bean cultivars (*Phaseolus vulgaris*), **103**: 521–527.

Rossetto M., Vanzani P., Zennaro L., Mattivi F., Vrhovsek U., Scarpa M., Rigo A. (2004): Stable free radicals and peroxyl radical trapping capacity in red wines. Journal of Agricultural and Food Chemistry, **52**: 6151–6155.

Staško A., Polovka M., Brezinová V., Biskupič S., Malík F. (2006): Tokay wines as scavengers of free radicals (an EPR study). Food Chemistry, **96**: 185–196.

Stopka P., Křížová J. (2006): Antioxidants and free radicals in natural products. Chemicke Listy, **100**: 261–265.

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