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An electron paramagnetic resonance study of antioxidant properties of alcoholic beverages

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ABSTRACT

The antioxidant properties of samples of different kinds of commercially available alcoholic beverages were investigated by electron paramagnetic resonance (EPR) spectroscopy. This analytical technique is chiefly designed to enable accurate detection of free radicals. The determination of antioxidant activity by the EPR method is conducted by measuring the changes of the intensity of the EPR spectrum of stable radicals, which results from their interaction with antioxidants. Antioxidant capacity of alcoholic beverages samples was assessed with the use of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·). The highest antioxidant activity was found in red wines, followed by a blend of wine and brandy and then by whiskies, whereas plain vodkas and gin showed negative values of antioxidant activity. From our studies it can be concluded that the value of antioxidant capacity depends on the polyphenolic content, the time and method of ageing, as well as on the flavour and colour additives.

1. Introduction

Many studies have focused on the determination of the antioxidant capacity of red and white wines (Fernández-Pachón, Villaño, Troncoso, & García-Parrilla, 2006; Li, Wang, Li, Li, & Wang, 2009; Stasko et al., 2008; Stasko, Liptakowa, Malik, & Misik, 2002) and Tokay wines (Stasko, Polovka, Brezova, Biskupic, & Malik, 2006). A number of studies concerning antioxidant activity and chemical composition of brandies and cognac are also available in the literature (Alonso, Castro, Rodriguez, Guill, & Barroso, 2004; Canas, Casanova, & Belchior, 2008; Da Porto, Calligaris, Celotti, & Nicoli, 2000; Goldberg, Hoffman, Yang, & Soleas, 1999; Nicoli, Anese, & Parpine, 1999; Schwarz et al., 2009). However, there is a shortage of information on the antioxidant properties of other alcoholic beverages (Goldberg et al., 1999; Umar, Boisseau, Segur, Begaud, & Moore, 2003).

Various methods have been applied to evaluate antioxidant activities; nevertheless, it is worth mentioning that no simple universal method exists by which antioxidant activities can be measured accurately and quantitatively. The most popular screening assays are the following: oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), photochemiluminescence (PCL), chemiluminescence (CL), total oxidant scavenging capacity (TOSC), total antioxidant capacity (TAC), Trolox equivalent antioxidant capacity (TEAC), and total antioxidant potential using Cu(II) (CUPRAC) (Karadag, Ozcelik, & Saner, 2009).

UV/vis spectroscopy has been applied to estimate the antioxidant properties of food and beverages and a number of experimental methods such as HPLC, MS, NMR, and analytical methods have been applied to identify some chemical compounds in food and beverages that can be closely related to the antioxidant activities of these products (Anli, Vural, Demiray, & Ozkan, 2006; Brainina, Ivanova, Sharafutdinova, Lozovskaya, & Shkarina, 2007; Canas, Belchior, Spranger, & Bruno de Sousa, 2003; Da Porto et al., 2000; Fernández-Pachón et al., 2006; Herraiz & Ough, 1992; Mateus, Silva, Santos-Buelga, Rivas-Gonzalo, & De Freitas, 2002).

Electron paramagnetic resonance spectroscopy (EPR) is a rarely applied method, though in the case of free radicals it seems the most adequate. EPR spectrometry is the only analytical technique that can specifically detect free radicals. This technique is based on the measurements of transitions of unpaired electrons in a magnetic field. Determination of antioxidant activity by the EPR method is based on measuring the changes of intensity of the EPR spectrum of stable radicals as a result of their interaction with antioxidants.

DPPH· is a stable free radical adequate for testing antioxidant properties of antioxidants (Yordanov, 1996). DPPH· is an artificial radical and cannot be reproduced *in vivo*; however, it is useful to evaluate the antioxidant activity (Mishra, Ojha, & Chaudhury, 2012; Locatelli et al., 2009). DPPH· in solution is purple in colour and absorbs at 515–520 nm. This assay is based on the principle that DPPH· accepts a hydrogen (H) atom from the scavenger molecule, i.e., antioxidant, and this results in the reduction of DPPH· to $DPPH_2$. Moreover the colour changes from purple to yellow with a concomitant decrease in absorbance. However, in the case of

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antioxidant compounds whose spectra overlap DPPH· at its maximum absorbance, the use of electron paramagnetic resonance (EPR) spectroscopy is the preferred way to assess the DPPH· radical, since it directly measures the free radical concentration (Locatelli et al., 2009).

Moreover, studies of the correlation between the optical and paramagnetic properties of DPPH· solutions show that the decrease of the absorption maximum in the UV/vis spectrum does not always result in the decrease of the concentration of the free radical (Yordanov, 1996). Hence, it seems that the EPR technique can determine the antioxidant activity more effectively.

Only a few research groups have used EPR spectroscopy for monitoring antioxidant behaviour of tea leaves (Morsy & Khaled, 2002; Polovka, Brezova, & Stasko, 2003; Unno, Yayabe, Hayakawa, & Tsuge, 2002), coffee (Brezova, Slebodova, & Stasko, 2009), wine (Stasko et al., 2008, 2002, 2006), honey (Zalibera et al., 2008), beer (Brezova, Polovka, & Stasko, 2002), fruits, and vegetables (Tzika, Papadimitriou, Sotiroudis, & Xenakis, 2008). Therefore the aim of this study is to evaluate the antioxidant activity of various alcoholic beverages with the use of EPR spectroscopy and DPPH· as the source of free radicals.

2. Materials and methods

Samples of different kinds of commercially available alcoholic beverages were investigated (Table 1). Electron paramagnetic resonance (EPR) spectra were obtained with a Bruker EMX EPR spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at X-band frequency at room temperature. The typical instrument parameters were: central field, 3480 G; modulation amplitude, 2.0 G; time constant, 40.96; gain, 1×10^4 G; microwave power, 20.12 mW.

The compound 1,1-diphenyl-2-picrylhydrazyl (DPPH·) (Sigma–Aldrich, Poznań, Poland) was used as the source of free radicals.

To quantify the antioxidant activity of the alcohols, Trolox (Acros Organics, Geel, Belgium) was used. Trolox (molecular formula $C_{14}H_{18}O_4$) is the water-soluble derivative of vitamin E. On increasing the concentration of Trolox, the intensity of the corresponding EPR spectrum decreased and consequently the percent inhibition (%*I*) increased. The regression equation of the linear relationship of the percent inhibition of EPR signal intensity to concentration of Trolox (Fig. 1) was used to calculate the antioxidant activity of the studied samples in units called Trolox Equivalents Antioxidant Capacity (TEAC), e.g. μmolTE/100 mL of the studied alcohol.

The percent inhibition of the EPR spectrum was calculated according to the following equation: % Inhibition = $[(I_0 - I)/I_0] * 100\%$, where I_0 is the intensity of the EPR spectrum of DPPH· (control sample), and I is the intensity of the EPR spectrum of DPPH· with an alcohol sample.

A typical reaction mixture contained 1 mL of 200 μM DPPH· solution in ethanol together with 0.001–0.01 mL of red wine and from 0.02 to 0.24 mL in the case of the remaining samples of commercially available alcohols. For all samples the regression equation of linear relationship of the percent inhibition (%*I*) of the EPR signal intensity to the volume of alcohol sample (*V*) was determined (Table 1). On the basis of this equation (Table 1) %*I* corresponding to 100 mL of the studied alcohol was calculated. Then from the standard curve (Fig. 1) the antioxidant activity in μmol Trolox per 100 mL of alcohol was defined. The presented data are the means of three determinations.

3. Results and discussion

The EPR spectrum of DPPH· dissolved in ethanol is manifested by five lines (Fig. 2) formed by the interaction of the unpaired electron with the two equivalent nitrogen nuclei ($g = 2.0036$). DPPH· is reduced by the electron transfer from the antioxidant AH, according to the reaction (Brand-Williams, Cuvelier, & Berset, 1995; Mishra et al., 2012; Stasko et al., 2002, 2006):

$$AH + DPPH^{\cdot} \rightarrow A^{\cdot} + DPPH - H.$$

$$DPPH^{\cdot} + A^{\cdot} \rightarrow DPPH - A.$$

$$A^{\cdot} + A^{\cdot} \rightarrow A - A.$$

The radicals A· formed in this reaction can sometimes be observed directly by EPR. However in general the concentration of A· is low, due to the disappearance of these radicals *via* recombination or disproportionation and hence cannot be observed by EPR spectroscopy (Stasko et al., 2002).

The antioxidant activity of 21 samples of commercially available alcohols was measured using EPR spectroscopy. The decrease of the intensity of EPR signal of DPPH· after adding alcohol

Table 1
Names of the samples of the studied alcoholic beverages, regression equations and TEAC values obtained for the studied samples.

Name of alcoholic beverage	Sample	TEAC (μmol Trolox/100 mL)	r^2	Regression equation*
Martini Red	S1	91.0 ± 9.02	0.98	$y = 768x + 8.64$
Martini Bianco	S2	36.8 ± 7.14	0.98	$y = 311x + 2.72$
Red Wine Merlot	S3	1114 ± 24.96	0.98	$y = 9393x - 2.03$
Red Wine Cabernet Sauvignon	S4	1400 ± 28.11	0.96	$y = 11805x + 9.56$
Red Wine Chianti	S5	1244 ± 30.61	0.92	$y = 10489x + 15.6$
Whisky Ballantines 12	S6	78.0 ± 6.07	0.94	$y = 658x + 0.81$
Whisky Ballantines	S7	66.6 ± 3.52	0.99	$y = 562x + 1.463$
Whisky Jim Beam	S8	115 ± 9.73	0.96	$y = 969x + 11.58$
Whisky Johnnie Walker	S9	60.6 ± 13.15	0.95	$y = 511x + 5.75$
Campari	S10	72.5 ± 7.33	0.98	$y = 816x + 7.30$
Malibu	S11	0	–	–
Blue Curacao	S12	0	–	–
Gin	S13	0	–	–
Polish Vodka Dębowa	S14	44.2 ± 8.91	0.97	$y = 373x + 4.41$
Vodka Finlandia Redberry Fusion	S15	56.4 ± 3.88	0.98	$y = 476x + 4.96$
Vodka Finlandia Cranberry Fusion	S16	41.2 ± 6.46	0.99	$y = 347x - 1.09$
Polish Vodka Żubrówka	S17	0	–	–
Vodka Finlandia	S18	0	–	–
Vodka Finlandia Blackcurrant	S19	0	–	–
Polish Vodka Wyborowa	S20	0	–	–
Brandy Metaxa	S21	92.83 ± 11.40	0.96	$y = 782.86x + 5.2405$

TEAC are mean values ± SD.
* *y*, Inhibition (%); *x*, volume of sample (ml).