Antioxidant properties of liqueurs - an EPR study

ANDREJ STAŠKO - VLASTA BREZOVÁ - STANISLAV BISKUPIČ - PETER RAPTA

Summary
Antioxidant capacities of eleven commercially available liqueurs were investigated by electron paramagnetic resonance (EPR) spectroscopy using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and a cation radical of ABTS (2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid salt)) as oxidants. The determined antioxidant capacities of liqueurs evaluated as Trolox-equivalent antioxidant capacity (TEAC) were compared with those found for wine samples. Using DPPH, most of the liqueurs (with one exception) exhibited $TEAC_{DPPH}$ values in the range of 0.04–0.58 mmol.l^{-1}, while $TEAC_{DPPH}$ values for red wines were in the range of 7.7–8.8 mmol.l^{-1}. Closely analogous results to those found with DPPH were also obtained using ABTS$^{\bullet+}$, as evidenced by the linear dependence between $TEAC_{DPPH}$ and $TEAC_{ABTS}$, having a correlation coefficient $r_{DPPH/ABTS^{\bullet+}} = 0.9938$.

Keywords
liqueur; antioxidants; DPPH; ABTS; TEAC; EPR; radical scavenging

Functional foods and drinks are subjects of intensive investigation, and their health benefits are frequently correlated with their antioxidant properties [1-7]. There is a particular interest in natural antioxidants and free radical scavengers in foodstuffs due to their ability to reduce or prevent various pathological states and ageing [1, 2, 6, 7]. Numerous reports have been published on antioxidant properties of drinks of everyday consumption such as coffee [8, 9], tea [10, 11], beer [12, 13] and wine [14-18]. Only limited data are available on the antioxidant activity of liqueurs [19], which are prepared from various plant and fruit materials. Therefore, we have investigated the antioxidant properties of commercially available liqueurs containing components extracted from numerous plants, from which health benefits may be expected, possibly in connection with the contents of antioxidants. The antioxidant properties were determined using paramagnetic species DPPH and ABTS$^{\bullet+}$ as oxidants, and the antioxidant action of liqueurs was followed by electron paramagnetic resonance (EPR) spectroscopy, which is accepted as the most appropriate technique for such experiments [20]. Additionally, the measured antioxidant capacities were compared to those of several common alcoholic drinks such as cognac, white and red wines.

MATERIALS AND METHODS

Materials

Free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt) were obtained from Fluka (Buchs, Switzerland). Ethanol for UV-VIS spectroscopy was purchased from Mikrochem (Pezinok, Slovakia).

Samples of liqueurs Becherovka (1), Czech Republic; Becherovka (2), Czech Republic; Demänovka bitter, Slovakia; Demänovka, Slovakia; Milder, Slovakia; Bylinovka, Slovakia; Armilar Amaretto, Italy; Stará myslivecká, Czech Republic; Alchymista, Slovakia; Fernet Amaro citrus, Slovakia; Noble Cherry, Germany were investigated along with the cognac (Georgian Cognac, Georgia) and wine samples (Veltlín zelený, 2005 - white wine, Slovakia; Frankovka modrá, 2005 - red wine, Slovakia; Cabernet Sauvignon, 2004 - red wine, Slovakia) used in comparative experiments.

Methods

Characterization of antioxidant capacity by means of DPPH and ABTS$^{\bullet+}$ is based on monitoring the decrease in radical concentration upon the addition of the drinks, because antioxidants present in the samples terminate DPPH and

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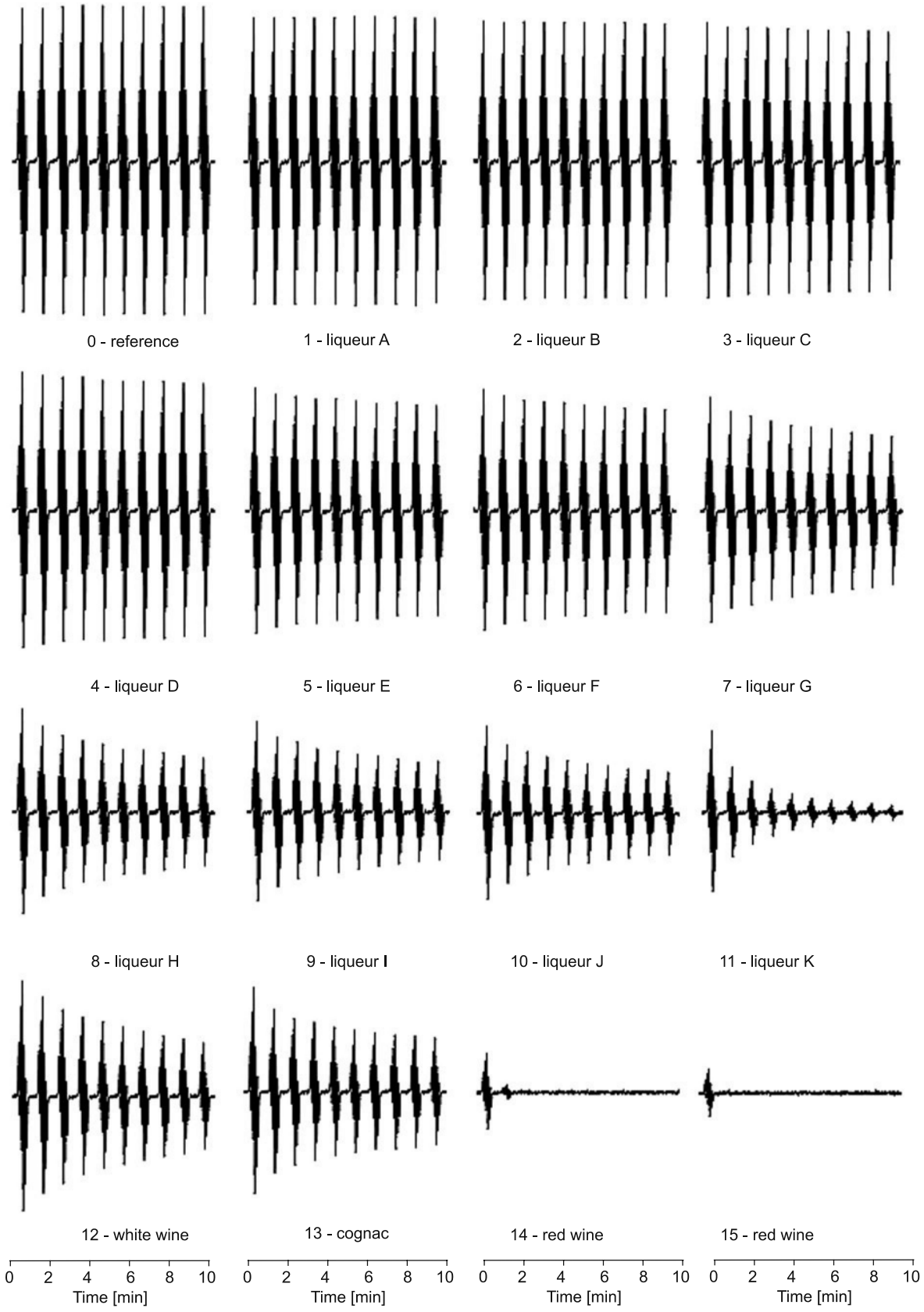

Fig. 1. EPR spectra of DPPH radical in the investigated samples.
Spectra followed for 10 min, investigating the reference (0; the sample without drinks) and 1:19 water-diluted drinks such as: liqueurs (1-11), white wine (12), Georgian cognac (13) and red wines (14, 15).

$ABTS^{\bullet+}$ radicals. The decrease in radical concentration after a given reaction time (10 min in our experiments) is then a direct measure for the antioxidant capacity of the investigated sample. The Trolox-equivalent antioxidant capacity (TEAC) assay was used to quantify the antioxidant capability of the investigated samples [21, 22]. This assay compares the capability of the samples to eliminate $ABTS^{\bullet+}$ (or DPPH) oxidant with a water-soluble compound analogous to vitamin E, namely Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The TEAC values determined for both oxidants ($TEAC_{ABTS}$ and $TEAC_{DPPH}$) are expressed in mmol of Trolox per liter of the investigated sample.

Experimental technique

In experiments with DPPH, one syringe was filled with 100 μmol.l^{-1} DPPH solution in ethanol, and a second one was filled with a sample of the drink diluted with distilled water (drink : water = 1 : 19 v/v; or only distilled water, in the case of the reference sample). Analogously, in experiments with $ABTS^{\bullet+}$ cation radical, a 70 μmol.l^{-1} $ABTS^{\bullet+}$ aqueous solution, prepared according to [23], was filled in one syringe, and the second one was filled with the sample of the drink diluted with ethanol (drink : ethanol = 1 : 39 v/v; or ethanol, in the case of the reference sample). Then simultaneously, equal volumes (1 ml) from each syringe were passed through a small mixing chamber and entered a flat cell fixed in the cavity of the EPR spectrometer (Bruker EMX, Rheinstetten, Germany). The acquisition of EPR spectra started immediately after mixing both solutions, and then individual EPR spectra were monitored over a period of 10 min. The experiments were carried out in triplicate and the relative deviation among the individual samples was less than 5%.

RESULTS AND DISCUSSION

A series of experiments was undertaken, starting with the reference sample (Fig. 1.0), where DPPH solution is in one syringe and distilled water in another. No changes in DPPH concentration over time were evident in 10 measured spectra of liqueur-free solution containing no antioxidants. Then followed a set of measurements in which diluted liqueurs were mixed with DPPH solutions. Since antioxidants present in the samples terminate free radicals, a decrease in the concentration of DPPH radicals was evident over a reaction time of 10 min (Fig. 1.1-11). The liqueur samples (1–11) in Fig. 1 were ordered according to their decreasing spectral amplitude, which indicates an increasing radical-scavenging capacity.

Values of $TEAC_{DPPH}$ were determined according to the following procedure. A quantitative measure for the concentration of radicals is the double integral of the EPR spectra. The double integrals of spectra monitored over 10 min were evaluated; subsequently, the DPPH concentrations for individual reaction times were determined. The concentration of DPPH radical eliminated by the liqueur sample solution after 10 min was used for the calculation of $TEAC_{DPPH}$ values. The $TEAC_{DPPH}$ value was very low in samples 1–3 (0.04–0.11 mmol.l^{-1}), more pronounced in samples 4–10 (0.14–0.58 mmol.l^{-1}), and exceptionally high in sample 11 (0.82 mmol.l^{-1}). Their antioxidant capacities were compared to common alcoholic drinks, namely, white wine (12) having a $TEAC_{DPPH}$ value of 0.49 mmol.l^{-1}, one sample of Georgian cognac (13) with 0.54 mmol.l^{-1}, and finally two samples of red wines (14, 15) with $TEAC_{DPPH}$ values of 7.7 and 8.2 mmol.l^{-1}, respectively. It should be noted here that the experimental results shown in Fig. 1 were measured under strictly identical conditions for the liqueur and the wine samples. In experiments with red wine sam-

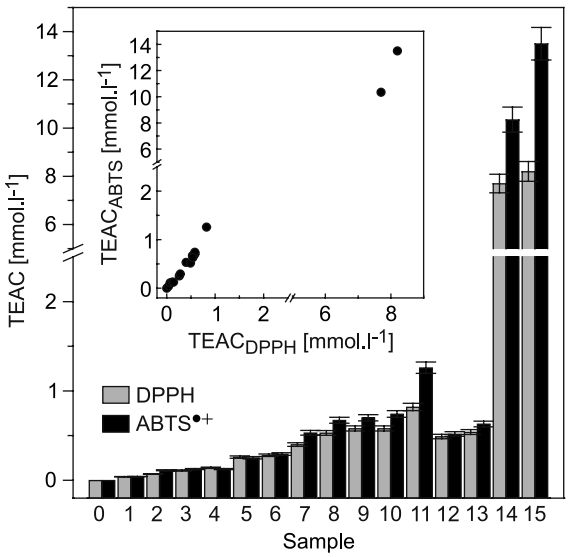

Fig. 2. Antioxidant capacities of the investigated samples.
Antioxidant capacities are expressed as Trolox-equivalent antioxidant capacities (TEAC in mmol.l^{-1}) of the investigated samples such as liqueur drinks (1–11), white wine (12), Georgian cognac (13) and red wines (14, 15) using DPPH and $ABTS^{\bullet+}$ radicals as oxidants. The inset presents a linear correlation between the TEAC values found with $ABTS^{\bullet+}$ and the DPPH oxidant radicals, which has a correlation coefficient $r_{DPPH/ABTS^{\bullet+}} = 0.9938$.

ples possessing high antioxidant capacities, DPPH radical was completely eliminated after only 1 min, as evident from Fig. 1.14 and Fig. 1.15. Therefore, for more precise quantitative calculations of $TEAC_{DPPH}$ values for red wines, data from additional experiments using more diluted wine samples (1 : 40, v/v) were evaluated.

Experiments analogous to those described with DPPH were also carried out using $ABTS^{\bullet+}$ cation radical as the oxidant. The monitored scavenging capacities evaluated in TEAC assays for all investigated samples (1-15) using $ABTS^{\bullet+}$ as well as DPPH radicals are presented in Fig. 2. Very similar results were obtained with both oxidants, as the inset in Fig. 2 also shows, where a linear correlation between the antioxidant capacities of $ABTS^{\bullet+}$ and DPPH is found, with a correlation coefficient $r_{DPPH/ABTS^{\bullet+}} = 0.9938$. The slightly higher antioxidant capacities observed with $ABTS^{\bullet+}$ in comparison to DPPH may be explained by the higher $ABTS^{\bullet+}$ redox potential [24], which means that a portion of the antioxidants with higher oxidation potentials might have been included in electron transfer reactions with $ABTS^{\bullet+}$ but not with DPPH [25].

The comparison of the antioxidant capacities of liqueurs with other drinks (e.g. wines) reveals surprisingly low levels of antioxidant capacities in numerous liqueurs compared to red wines.

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REFERENCES

1. Stanner, S. A. - Hughes, J. - Kelly, C. N. M. - Buttriss, J.: A review of the epidemiological evidence for the "antioxidant hypothesis". Public Health Nutrition, *7*, 2004, pp. 407-422.
2. Vitaglione, P. - Fogliano, V.: Use of antioxidants to minimise the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. Journal of Chromatography B - Analytical Technologies in the Biomedical Life Sciences, *802*, 2004, pp. 189-199.
3. Aruoma, O. I.: Methodological considerations for characterizing potential antioxidant action of bioactive components in plant foods. Mutation Research, *523-524,* 2003, pp. 9-20.
4. Becker, E. M. - Nissen, L. R. - Skibsted, L. H.: Antioxidant evaluation protocols: Food quality or health effects. European Food Research and Technology, *219*, 2004, pp. 561-571.
5. Henn, T. - Stehle, P.: Total phenolics and antioxidant activity of commercial wines, teas and fruit juices. Ernährung-Umschau, *45*, 1998, pp. 308.
6. Kay, C. D. - Kris-Etherton, P. M.: West, S. G.: Effects of antioxidant-rich foods on vascular reactivity: Review of the clinical evidence. Current Atherosclerosis Reports, *8*, 2006, pp. 510-522.
7. Fang, Y. Z. - Yang, S. - Wu, G.: Free radicals, antioxidants, and nutrition. Nutrition, *18*, 2002, pp. 872-879.
8. Dorea, J. G. - Da Costa, T. H. M.: Is coffee a functional food? British Journal of Nutrition, *93*, 2005, pp. 773-782.
9. Anese, M. - Nicoli, M. C.: Antioxidant properties of ready-to-drink coffee brews. Journal of Agricultural and Food Chemistry, *51*, 2003, pp. 942-946.
10. Serafini, M. - Ghiselli, A. - Ferro-Luzzi, A.: In vivo antioxidant effect of green and black tea in man. European Journal of Clinical Nutrition, *50*, 1996, pp. 28-32.
11. Polovka, M. - Brezová, V. - Staško, A.: Antioxidant properties of teas investigated by EPR spectroscopy. Biophysical Chemistry, *106*, 2003, pp. 39-56.
12. Andersen, M. L. - Outtrup, H. - Skibsted, L. H.: Potential antioxidants in beer assessed by ESR spin trapping. Journal of Agricultural and Food Chemistry, *48*, 2000, pp. 3106-3111.
13. Staško, A. - Rapta, P. - Malík, F.: Characterization of beer stability by means of spin trapping (an EPR study). Monatsschrift für Brauwissenschaft, *53,* 2000, pp. 4-7.
14. Urquiaga, I. - Leighton, F.: Wine and health: evidence and mechanisms. World Review of Nutrition and Dietetics, *95*, 2005, pp. 122-139.
15. Staško, A. - Liptáková, M. - Malík, F. - Mišík, V.: Free radical scavenging activities of white and red wines. An EPR spin trapping study. Applied Magnetic Resonance, *22,* 2002, pp. 101-113.
16. Tsang, C. - Higgins, S. - Duthie, G. G. - Duthie, S. J. - Howie, M. - Mullen, W. - Lean, M. E. J. - Crozier, A.: The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. British Journal of Nutrition, *93*, 2005, pp. 233-240.
17. Simonetti, P. - Pietta, P. - Testolin, G.: Polyphenol content and total antioxidant potential of selected italian wines. Journal of Agricultural and Food Chemistry, *45*, 1997, pp. 1152-1155.
18. Landrault, N. - Poucheret, P. - Ravel, P. - Gasc, F. - Cros, G. - Teissedre, P.-L.: Antioxidant capacities and phenolics levels of French wines from different varieties and vintages: Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 3341-3348.
19. Ezaki, N. - Niizawa, S. I. - Yanagisawa, K. - Yahikozawa, Y. - Hatanaka, F. - Kojima, S.: Medicinal tonic liqueur (Yomeishu®) has oxygen radical absorbing activity, and improves the endothelial dysfunction in streptozotocin-induced diabetic rats. Japanese Pharmacology and Therapeutics, *33*, 2005, pp. 1013-1019.
20. Polovka, M.: EPR spectroscopy: A tool to characterize stability and antioxidant properties of foods. Journal of Food and Nutrition Research, *45*, 2006, pp. 1-11.
21. Miller, N. J. - Rice-Evans, C. A.: Factors influencing

the antioxidant activity determined by the ABTS$^{\bullet+}$ radical cation assay. Free Radical Research, *26*, 1997, pp. 195-199.

22. Pellegrini, N. - Serafini, M. - Colombi, B. - Del Rio, D. - Salvatore, S. - Bianchi, M. - Brighenti, F.: Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. Journal of Nutrition, *133*, 2003, pp. 2812-2819.
23. Re, R. - Pellegrini, N. - Proteggente, A. - Pannala, A. - Yang, M. - Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radicals in Biology and Medicine, *26*, 1999, pp. 1231-1237.
24. Scott, S. L. - Chen, W. J. - Bakac, A. - Espenson, J. H.: Spectroscopic parameters, electrode-potentials, acids ionization-constants, and electron-exchange rates of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) radicals and ions. Journal of Physical Chemistry, *97*, 1993, pp. 6710-6714.
25. Zhuang, Q.-K. - Scholz, F. - Pragst, F.: The voltammetric behaviour of solid 2,2-diphenyl-1-picrylhydrazyl (DPPH) microparticles. Electrochemistry Communications, *1*, 1999, pp. 406-410.

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