EPR spectroscopy: A tool to characterize stability and antioxidant properties of foods

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Summary

The antioxidant status of foods and plant products is nowadays widely characterized by means of various analytical and spectroscopic methods. In the present work, the thermal stability of lager beers and the influence of ascorbic acid addition on their stability, as well as the antioxidant properties of commercial teas and wines have been studied by means of EPR spin trapping technique, using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO); α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) and α-phenyl-*N*-*tert*-butylnitrone (PBN) as spin traps in various, mostly oxygen-centred radicals producing systems; as well as by means of stable free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPOL).

The thermal stability of lager beer can be well characterized by means of spin trapping agent PBN. The addition of L-ascorbic acid (ASC) to the beer samples accelerated the radical processes and a lower stability was found.

The highest antioxidant potential to terminate superoxide radicals in tea solutions was found in green tea, followed by black and fruity teas. The influence of Mn^{2+} and ascorbic acid presence on the antioxidant properties of tea samples was also tested. Various sources of free radicals used in the antioxidant tests, due to their specific action, show different termination rates in the presence of the individual tea samples.

The radical scavenging abilities of 30 samples of Tokay wines from the Slovak region were compared with 10 samples of red and 10 samples of white wines originating from various regions. The results showed that the Tokay wines have a very good scavenging ability, positioned between the white and red wines.

Keywords

antioxidants; foods stability; EPR; spin trap; free radicals; beer; tea; wine

In the past decade, considerable attention has been focused on the improving of human well-being by consumption of foods beneficial to human health [1-9], since they can serve as a source of antioxidants, able to scavenge and eliminate free radicals, which otherwise may cause oxidative damage to biomolecules, and initiate various illnesses. The investigations are focussed on foods and drinks, containing vitamins, flavonoids and other natural antioxidants, which evidently can prevent from diseases. Furthermore, radical reactions and antioxidant properties are also considered to be decisive for the quality and flavour stability of foodstuffs. Various methods have been used to monitor and to compare the antioxidant activity of foods [10-18], from which EPR spectroscopy is considered to be one of the most efficient. It was formerly successfully applied to determination of radical scavenging activity of catechins and their epimers [19-22], for monitoring antioxidant behaviour of selected tea components [23], wines [24, 25], cognacs [26] and fruits [27]. In the EPR laboratory at the Department of Physical Chemistry, Faculty of Food and Chemical Technology, Slovak University of Technology in Bratislava, the EPR spin trapping technique was successfully used to investigate the beer stability [28, 29], antioxidant properties of teas [30], wines [31, 32], 1,3-β-D-glucans [33], or carotenoids producing yeasts [34].

The EPR spin trapping method involves trapping of reactive short-lived free radicals, (produced in the experimental system via chemical reaction, thermal decomposition, or by photochemical excitation) by a diamagnetic EPR silent compound (spin trap) via addition to a spin trap double bond to produce a more stable radical product (spin adduct). Spin adducts are paramagnetic, and have EPR spectra with hyperfine splitting constants and g-value characteristic of the type of free radical trapped [35]. Nitrone spin traps, such as α-(4-pyri-

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dyl-1-oxide)-*N-tert*-butylnitrone (POBN) or α-phenyl-*N-tert*-butylnitrone (PBN) scavenge free radical species via addition to a carbon located in a alpha position relative to the nitrogen (Fig. 1) [35].

On the other hand, the antioxidant status of food samples can be effectively monitored through the elimination of stable free radicals added to the sample. This involves also the characterization of kinetics aspects of antioxidant's action. Stable free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) or 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPOL) (Fig. 2) were effectively used in our previous experiments [28-32, 36, 37].

In the present work, the application of EPR spin trapping technique, as well as of several stable free radicals on the investigation of the lager beer stability and on the characterization of antioxidant properties of several commercial green, black and mixed fruity teas as well as of several red, Tokay and white wines has been shown.

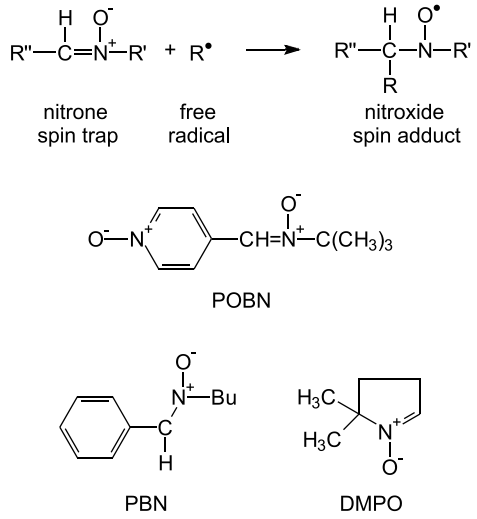

Fig. 1. The structures of spin trapping agents POBN, PBN and DMPO and the key reaction of spin trapping technique.

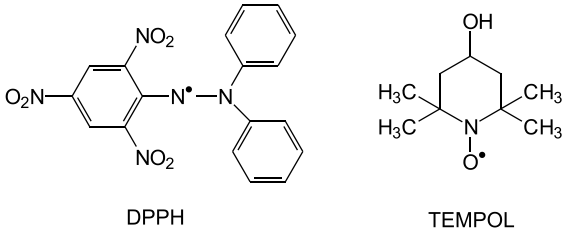

Fig. 2. The structures and characteristic EPR spectrum (magnetic sweep width 6 mT) of DPPH and TEMPOL free radicals.

MATERIAL AND METHODS

Samples characterization

The thermal stability of 6 commercially available lager beer samples of Slovak and Czech production (lager 12 %, minimal ethanol content 4.1 vol.%) has been studied using 5% (v/v) aqueous ethanol solution as a reference. Commercially available samples of 5 green (*g1-g5*), 5 black (*b1-b5*) and 5 mixed (fruit) (*m1-m5*) teas of Slovak, Czech and Ukrainian distributors have been used for the monitoring of their antioxidant properties. The radical scavenging abilities of 30 samples of Tokay wines from the Slovak region were compared with 10 samples of red and 10 samples of white wines originating from various regions, using a 12 % (v/v) aqueous ethanol solution as a reference.

Chemicals

Spin trapping agents DMPO, freshly distilled before use and stored at –18 °C under argon (Sigma Aldrich, Milwaukee, WI, USA); POBN (Fluka Chemical, Derbyshire, Great Britain) and PBN (Sigma Aldrich); stable free radicals TEMPOL (Sigma Aldrich) and DPPH (Fluka); as well as dimethyl sulphoxide (DMSO, Sigma Aldrich), 2,2'-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) salt (ABTS), 2,2'-azo-bis(2-methylpropionamidine) hydrochloride (AAPH, Polysciences, Warrington, PA, USA) $K_2S_2O_8$ (Merck, Darmstadt, Germany), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma Aldrich), ascorbic acid (ASC, analytical grade), H_2O_2, NaOH, DMSO (Lachema Brno, Czech Republic); redistilled water and ethanol of spectroscopic grade were used in the experiments.

EPR measurements

X-band EPR spectrometer Bruker 200D (after the innovation of the EPR laboratory, Bruker EMX), equipped with Bruker temperature control unit ER 4111 VT was used for all the experiments. The g-values were determined with uncertainty of ±0.0001 by simultaneous measurements of DPPH standard. Experimental EPR spectra were simulated using *WinEPR* and *SimFonia* programs (Bruker, Bruker BioSpin, Karlsruhe, Germany).

Further details about the sample characterization, preparation and experimental conditions and specifications of the studied systems can be found in [28, 30, 32].

RESULTS AND DISCUSSION

Lager beer stability

The method is based on the monitoring of radical processes during the thermally-accelerated ageing of beer samples, which leads to the breakdown of beer stability. The basic idea is that beer sample has its own endogenous antioxidant capacity preventing the breakdown of its stability. After this capacity is exhausted a dramatic breakout of free radicals can be indicated by means of the EPR spin trapping technique. The time needed for the breakdown of stability (lag time) during a thermally-accelerated ageing test is a measure of the beer's stability [28].

A representative time course of EPR spectra is shown in Fig. 3A. After an induction period (lag time), which varies according to the quality of the beer sample, a pronounced rise of the relative intensity in the six-line spectra of PBN-adducts is observed. The induction period was evaluated plotting the integral intensity of the spectra versus time of accelerated beer ageing at 333 K, as shown in Fig. 3B. As the linewidth of the spectra remained constant, an analogous dependence was also obtained by quoting the signal heights versus time. The induction period marked with the crossing point is a quantitative measure for beer stability, as generally suggested in previous papers [38-44]. The EPR spectra of the •PBN-adduct obtained can be well fitted with the following splitting constants: a_N = 1.607 mT, a_H = 0.356 mT and a g-value of 2.0055, which are in the region characteristic for carbon-centered radical adducts (•PBN-C) [45]. However, due to the very limited selectivity of the PBN spin adduct parameters [35], a discussion of the type of radical trapped is not entirely appropriate. The adduct •PBN-CH(OH)CH_3 originating from ethanol by hydrogen abstraction by generated hydroxyl radicals, were previously identified during the accelerated beer ageing [41]. Our supplementary investigations using DMPO spin trap in thermally-accelerated beer ageing confirmed this assignment, as we obtained the spin adduct characterized by a_N = 1.588 mT, a_H = 2.270 mT and g = 2.0057 attributable to the •DMPO-CH-(OH)CH_3 radical [46]. Typical time course of EPR spectra (6 mT scan) of four beer samples showing various stabilities during their accelerated thermal ageing at 333 K in the presence of PBN spin trapping agent is depicted in Fig. 4.

One question frequently raised is, whether this ageing process is, or may be, altered by some specific substances - antioxidants such as vitamins, or other substances [44]. Then, one of beer samples was chosen for a further series of experiments,

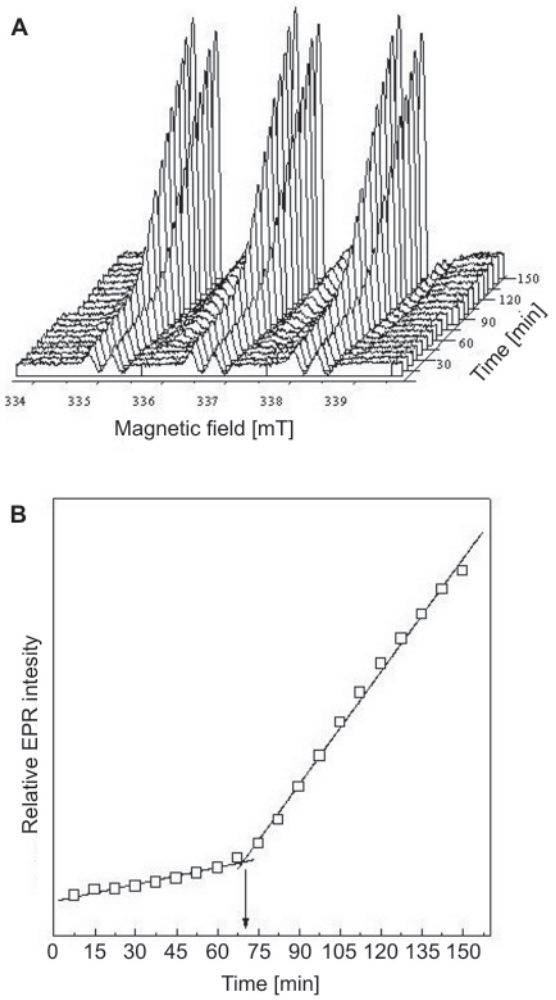

Fig. 3.
A. An illustration of a typical time course of EPR spectra measured during a thermally-accelerated ageing of beer sample at 333 K in the presence of PBN spin trapping agent.
B. The evaluation of induction period of a thermally-accelerated ageing of beer sample via the time dependence of the relative EPR intensity of •PBN-adducts shown in A. Details on the experimental conditions can be found in [28].

oriented on the investigation of the influence of ASC on beer stability. The behavior of this system is relatively complex.

The obtained results suggest that the free radicals generated in the presence of ASC possess a relatively high reactivity in the beer medium under the given experimental conditions. They can be visualized only by means of PBN trapping agent, and consequently, due to their high reactivity they are probably involved in consecutive reactions with the beer components. With the increased ASC concentrations, the relative intensity of the six-line

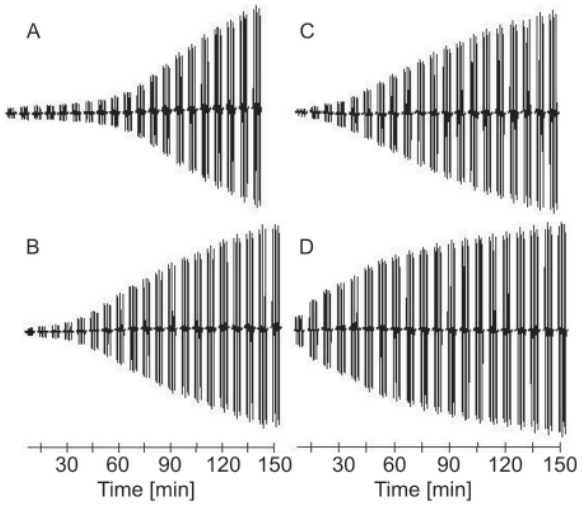

Fig. 4. The time course of EPR spectra (6 mT scan) of four beer samples (A–D) showing various stabilities during their accelerated thermal ageing at 333 K in the presence of PBN spin trapping agent. Details on the experimental conditions can be found in [28].

signal of the •PBN-adducts is increasing. This is due to the additional formation of free radicals originating from ASC.

Consequently, at least a part of the radicals originating from ASC participates in the initiation of the radical reactions in the beer samples, and so ascorbic acid works as a pro-oxidant in the degradation of beer. It can be concluded, that the addition of ascorbic acid to beer may be not a desired route for the enhancement of beer stability. An analogous behavior of beer samples during forced thermal decomposition in the presence of PBN and ascorbate additions (0.2 or 0.5 mM) was also evidenced by ANDERSEN et al. [44].

Antioxidant properties of teas

Tea, originally prepared from the leaves of *Camellia sinensis*, became one of the most popular beverages, and it has been re-investigated in many studies for its pharmaceutical properties [3, 4, 47].

Antioxidant properties of tea solutions were studied in various experimental system, containing H_2O_2/NaOH/DMSO in the presence of DMPO or POBN; or DPPH and TEMPOL free radicals, respectively.

H_2O_2/NaOH/DMSO system was established as a non-enzymatic and non-Fenton type system generating reactive radical species ($O_2^{-\bullet}$, •OH, •CH_3) suitable for evaluation of radical scavenging activity [48]. Spin traps DMPO and POBN were used in our experiments to evaluate the effect of tea solution on the radical formation [30].

Figure 5A shows typical EPR spectra obtained in reference samples using spin trapping agents DMPO (Fig. 5A1) or POBN (Fig. 5A2) in H_2O_2/NaOH/DMSO system. Only formation of superoxide anion-radical was evidenced with both spin traps, as •OH and •CH_3 intermediates are under the given reactant concentrations involved in rapid consecutive reactions leading to superoxide anion-radical formation as described below. Dramatic changes in EPR spectra are found if an analogous radical generation proceeds in the presence of tea solutions. Characteristic spectra of such systems are shown in Fig. 5B, where both spin traps evidenced practically only carbon-centered adducts (Fig. 5B1, 2). The formation of superoxide adducts is in accordance with the mechanism proposed by Polyakov for systems containing DMSO and high hydrogen peroxide concentration [49, 50].

Figure 6A shows a representative time course of EPR spectra measured in H_2O_2/NaOH/DMSO/DMPO system in the presence of mixed fruit tea *m1*. A simulation of experimental spectra revealed the formation of two radical species, namely ascorbyl radical (a_H = 0.18 mT; g = 2.0053)

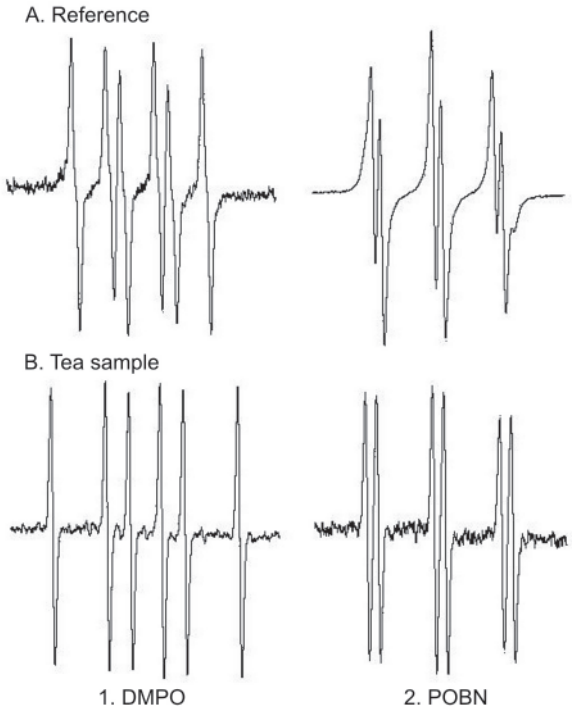

Fig. 5. Experimental and simulated EPR spectra of reference (water) and tea sample *g4* in H_2O_2/NaOH/DMSO using DMPO (scan 8 mT) and POBN (scan 6 mT) spin traps illustrate the elimination of superoxide anion adduct observed in the reference (A) with carbon-centered adducts found in tea sample (B).

and •DMPO-CH_3 adduct (a_N = 2.31 mT, a_H = = 1.615 mT; g = 2.0057). No signals matching •DMPO-OH or •DMPO-O_2^- adducts formation were detected. Relative concentrations of ascorbyl radical and •DMPO-CH_3 adduct were evaluated from the corresponding EPR spectra simulations. From this simulations, the relative concentration decrease of ascorbyl radical, accompanied with a simultaneous increase of •DMPO-CH_3 adduct relative concentration have been calculated. YOSHIMURA and co-workers [48] previously observed the proportional decrease of •DMPO-OH or •DMPO-O_2^- signal heights after addition of various concentration of ascorbic acid to H_2O_2/NaOH/DMSO/DMPO solutions. Conversely, the signal amplitude of •DMPO-CH_3 increased with increasing ascorbic acid concentration. Consequently they proposed a mechanism of methyl radical generation via reaction with ascorbic acid [48].

The time course of EPR spectra measured in H_2O_2/NaOH/DMSO/POBN system after addition of *m1* tea solution is depicted in Fig. 6B. The POBN molecule is a very effective scavenger of radical species produced under the given experimental conditions. The experimental EPR spectra were simulated as a mixture of two individual spectra corresponding to •POBN-CH_3 (a_N = 1.585 mT, a_H = = 0.261 mT, $a_{^{13}C}$(6 ^{13}C) = 0.51 mT; g = 2.0058) and •POBN-OH adduct (a_N = 1.448 mT, a_H = = 0.215 mT, $a_{^{13}C}$(6 ^{13}C) = 0.51 mT; g = 2.0059).

The ability of green (*g1-g5*), black (*b1-b5*) and mixed (fruit) (*m1-m5*) tea drinks to decrease the spin adduct yield was evaluated using both spin traps. The EPR spectra measured after 37 minutes of hydrogen peroxide addition were compared. The addition of green or black tea solutions to the radical-producing system fully eliminates formation of •DMPO-OH and •DMPO-O_2^- adducts, and consequently, in series of EPR spectra measured in the presence of green tea samples *g1-g3* dominates a six-line EPR signal attributable to •DMPO-CH_3. The three-line EPR signal of low intensity also evident in the spectra was attributed to DMPO decomposition product (a_N = 1.71 mT; g = 2.0059).

The intensity of EPR spectra measured in H_2O_2/NaOH/DMSO/DMPO solutions in the presence of mixed fruit teas was significantly lower compared to green or black tea. Also here, the six-line EPR signal corresponding to the •DMPO-CH_3 radical formation predominates in all spectra. But besides the formation of ascorbyl radical found in *m1*, in mixed teas *m2* and *m3* we confirmed the presence of a typical four-line signal matching •DMPO-OH adduct (a_N = 1.485 mT, a_H = 1.455 mT, g = 2.0059). This suggests that the efficiency to terminate reactive •OH radicals by fruit teas is lower than in the case of green or black teas.

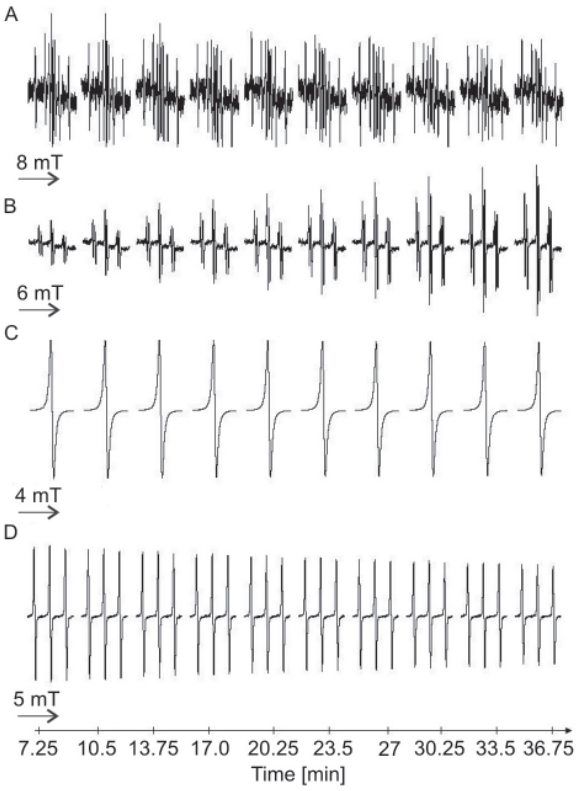

Fig. 6. Time course of EPR spectra in tea drink *m1* using various experimental systems: A - H_2O_2/NaOH/DMSO/DMPO; B - H_2O_2/NaOH/DMSO/POBN; C - DPPH; D - TEMPOL. Details on the spectrometer settings can be found in [28].

Results of EPR measurements obtained in H_2O_2/NaOH/DMSO/POBN system in the presence of POBN spin trap are fully compatible to those described above for DMPO. The six-line EPR signal dominating in all EPR spectra was assigned to •POBN-CH_3 adduct, and only in the presence of *m1* and *m4* tea solutions the formation of hydroxyl radical adduct was evidenced.

The prepared tea drinks represent complex systems, as they contain various concentrations of polyphenols, phenolic acids, flavonoids, ascorbic acid, as well as traces of metal ions (Mn^{2+}, Fe^{3+}, Cu^{2+}) [51-53]. As it was discussed more detaily in [30], the influence of pH changes, resulting from the tea solutions added, on the radical formation is minimal. But the simultaneous presence of Mn^{2+} ions and ascorbic acid in tea samples can considerably influence reaction pathways of superoxide anion-radical and hydroxyl radical in H_2O_2/NaOH/

DMSO/spin trap system [30]. Ascorbic acid in the presence of even very small amounts of transition metals can act as a pro-oxidant. This action of ascorbate was evidenced as an increase of methyl radical adducts formation. The highest intensities of $^{\bullet}$DMPO-CH_3 and $^{\bullet}$POBN-CH_3 EPR signals in H_2O_2/NaOH/DMSO solutions were observed using tea beverages with high concentration of ascorbic acid coupled with presence of Mn^{2+} ions [30].

DPPH and TEMPOL free radicals

In these experiments, the antioxidative properties of tea drinks were tested using stable free radicals DPPH and TEMPOL, respectively (Fig. 2). The solutions of DPPH or TEMPOL were mixed with solutions of prepared tea drinks and the decline of EPR signal was monitored, as it is depicted on Fig. 6C and Fig. 6D for mixed fruit tea *m1*.

Under the given experimental conditions, the EPR spectrum of DPPH free radical represents a singlet (ΔH_{pp} = 0.4 mT; g = 2.0036). The EPR spectrum of TEMPOL is a triplet (a_N = 1.70 mT; g = 2.0060). The concentrations of DPPH and TEMPOL free radicals was evaluated using the double integrated EPR signal and a calibration curve, with the DPPH solutions of known concentrations as the standards. To compare the scavenging activity of all tea beverages, relative concentrations were determined after 37 minutes of reagents mixing (Fig. 7). The highest DPPH scavenging activity (Fig. 7A) was found for green teas *g2, g3* and for black tea *b2*, whereas in the presence of mixed fruit teas only a 10 % loss of initial free radical concentration was observed. As it was discussed in [30], the DPPH scavenging activity is influenced by the structure, the position and degree of hydroxylation on the ring structure as well as the electron and hydrogen donating activity of polyphenols, present in tea drinks. The lower antioxidative action of mixed fruit teas reflected the presence of different organic acids, previously characterized with lower capability to quench free radical species. Additionally, a very rapid reaction of ascorbic acid with DPPH free radical [54, 55], has to be taken into account by the evaluation of antioxidant activity.

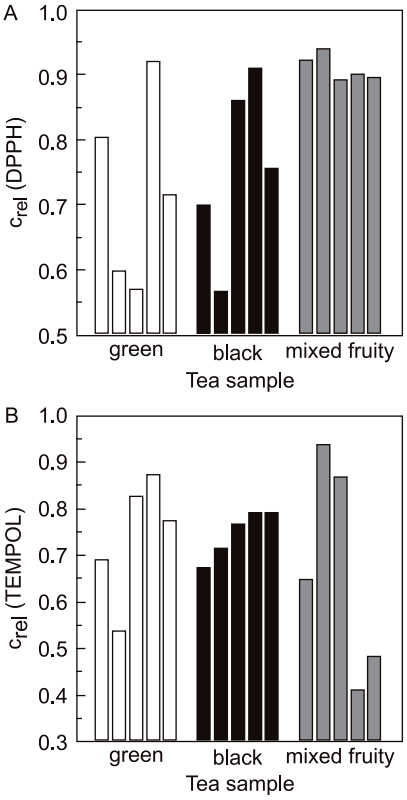

Fig. 7. The comparison of relative DPPH (A) and TEMPOL (B) concentrations, evaluated for all investigated tea solutions 37 minutes after reagents mixing.

The behaviour of individual tea samples in the presence of TEMPOL free radical is different comparing to the DPPH systems. An excellent antioxidant activity was observed for green tea *g2*, as well as for mixed teas *m1, m4* and *m5* containing *Hibiscus* flowers. It should be noted here, that the role of TEMPOL in our experiments could be affected by the redox potential of the tea sample components in a similar way, as we assumed in the case of DPPH [30].

Antioxidant properties of wines

In the previous studies, the scavenging abilities of red and white wine samples [31] using EPR spin trapping technique were characterized. Here, these experiments were spreaded, and the antioxidant properties (radical scavenging ability) of Slovak Tokay wines was investigated and compared with that one found with selected red and white wine samples originating from various sources.

Four different experimental systems were used for the monitoring of antioxidant status of wines. In two series of experiments free radicals were generated in the thermal decomposition of radical initiators $K_2S_2O_8$ or azo compound AAPH at 60 °C in the presence of DMPO spin trap; in two other series, ABTS$^{\bullet+}$ and DPPH free radicals were used [32].

Thermal decomposition of $K_2S_2O_8$ or azo compound AAPH

A representative time course of EPR spectra from nine selected samples observed using ther-

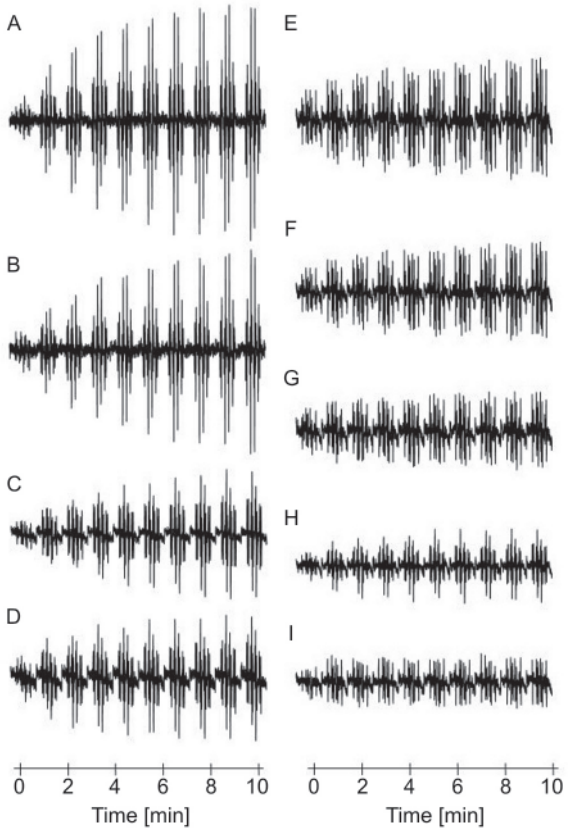

Fig. 8. The time course of EPR spectra of control (A), white (B-C), Tokay (D-G) and red (H-I) wine samples monitored for 10 minutes at 60 °C in the presence of $K_2S_2O_8$ radical initiator, DMPO spin trap and phosphate buffer (pH = 7). Details on the spectrometer settings can be found in [32].

mally decomposed $K_2S_2O_8$ radical initiator in the presence of DMPO is shown in Fig. 8. The maximal amplitudes of EPR spectra were found in the reference sample (Fig. 8A). Replacing the ethanol solution with the white wine samples, the amplitudes of EPR spectra decreased considerably (Fig. 8B,C). The amplitudes on average were still lower if Tokay wines were used, as illustrated in Fig. 8D-G. The lowest amplitudes, indicating the maximal scavenging activity, were found in the red wine samples, as shown in Fig. 8H,I.

The thermal decomposition of $K_2S_2O_8$ under the used experimental conditions leads to the formation of dominantly two types of spin adducts, as illustrates the EPR spectrum in the inset of Fig. 9A. A quartet (marked with solid circles) characteristic of •DMPO-OH adduct dominates there. As a minor by-product, evidenced with relatively low amplitude, there is a sextet characteristic of carbon-centred radicals added to DMPO. As it was previously described in [56], the DMPO-adduct of the primary $SO_4^{\bullet-}$ radical formed is very unstable (half life of 21 s). Additionally, radical $SO_4^{\bullet-}$ is also rapidly terminated with water or wine antioxidants, or, in addition, it can also oxidize DMPO to its cation radical DMPO$^{\bullet+}$ [57, 58], which hydrolyses in aquatic media, forming dominant •DMPO-OH adduct [59].

The relative scavenging activity of each wine sample (RS_{rel}) was evaluated from the experimental spectra after the double-integration of their time dependence, as the difference between the double integrals of the reference and the wine samples, as mentioned in more details at [32]. RS_{rel} value characterizes the amount of radicals scavenged by the antioxidants present in wine. The relative double integrals of the radicals scavenged by the investigated samples using $K_2S_2O_8$ radical initiator are summarised in Fig. 9A. On average, the red wines showed the highest value (1.00 ± 0.12) and therewith the highest scavenging activities. Tokay wines have a comparable scavenging activity (0.86 ± 0.15), and on average, the white wine samples show the lowest radical scavenging activities (0.60 ± 0.15).

Analogously as described with $K_2S_2O_8$, azo compounds AAPH (R-N=N-R) was used as the radical initiator. The carbon centered radicals R•, generated by thermal decomposition of AAPH undergo the consecutive reactions as discussed in [32], finally forming carbon (R•) and oxygen-centred (RO•, HO•) spin adducts in the presence of DMPO. A characteristic EPR spectrum observed in the experiments with AAPH is shown in the inset of Fig. 9B. The time course of EPR spectra monitored during the thermal decomposition of AAPH was similar to those presented in Fig. 8 using $K_2S_2O_8$ initiator. The relative scavenging activities (RS_{rel}) were obtained in the same way as in the case of $K_2S_2O_8$. Here again, the average scavenging ability of red wines is the highest (1.00 ± 0.11), followed by Tokay wines (0.71 ± 0.16), and the white wines showed the lowest relative scavenging capacity (0.37 ± 0.19).

Free radicals ABTS$^{\bullet+}$ and DPPH

Whereas in experiments with $K_2S_2O_8$ and AAPH the reactive free radicals were generated through their thermal decomposition, ABTS after its oxidation with persulfate forms its cation radical stable at room temperature [60]. After mixing ABTS$^{\bullet+}$ solution with 12 % aqueous ethanol (serving as reference) or with a wine sample, the time course of EPR spectra was monitored for 10 minutes in the same way as already demonstrated in Fig. 8. Again, the relative value of

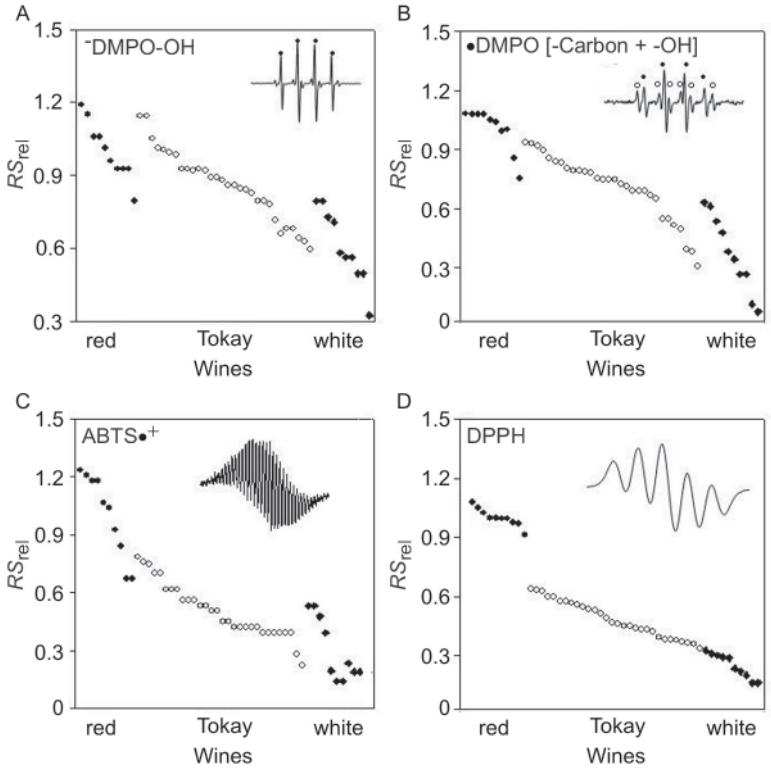

Fig. 9. The relative amounts of radicals scavenged (RS_{rel}) in experiments with $K_2S_2O_8$ (A), AAPH (B) radical initiators and with ABTS$^{\bullet+}$ (C) and DPPH (D) free radicals in the investigations of red (●), Tokay (○) and white (◆) wine samples. Insets represent characteristic EPR spectra of radicals observed.

radicals scavenged RS_{rel} was evaluated. Fig. 9C shows an overview with the relative activities for red (1.00 ± 0.11), Tokay (0.55 ± 0.23) and white wines (0.22 ± 0.11), confirming the trends demonstrated in Fig. 9A,B. Identical experiment as with the ABTS$^{\bullet+}$ was repeated by means of DPPH free radical. The amount of radical scavenged are presented in Fig. 9D with relative scavenging activity 1.00 ± 0.05 for red, 0.43 ± 0.13 Tokay and 0.25 ± 0.07 for white wines.

Comparing the relative amounts of radicals scavenged, summarized in Fig. 9, it is evident that using all four radical sources, the highest relative scavenging activity was found for the red wines, followed by Tokay and then the white wines. But, identically as in the case of above described experiments with tea samples, the radical scavenging activity of wines can be influenced by the composition of polyphenols, presence of transient metal ions, organic acids and by other factors [30]. In the previous paper [31], taking a limited number of samples from the Bratislava region, the higher Mn^{2+} concentrations were found in the red wines, comparing the white wines, correlating with their scavenging activity (a higher one by the red and a lower one by the white wines). Taking into account here a wide range of samples from various regions, there is no a significant correlation between the Mn^{2+} content and antioxidant activity of wines.

In order survey better the scavenging activity obtained with all four radical initiators, a relative comparison was chosen, setting the scavenging values of red wines (which are the highest) to one and relatively to them are given the scavenging activities of Tokay and white wines. From such comparison it is evident, that the Tokay wines show a relatively high scavenging ability if compared to the red wines from 0.43 using DPPH up to 0.86 with $K_2S_2O_8$ radical initiator, and the white wines a relatively lower one from 0.25 to 0.60 respectively [32].

The increased scavenging activity from DPPH towards $K_2S_2O_8$ radical sources correlates well with their rising redox potentials with approximate values *vs.* NHE: DPPH$^{\bullet}$/DPPH^{-} = 0.43 V [61], ABTS$^{\bullet+}$/ABTS = 0.68 V [62], AAPH-(ROO$^{\bullet}$, RO$^{\bullet}$, OH$^{\bullet}$) = 1.0 V to 2.3 V [63], $SO_4^{\bullet-}/SO_4^{2-}$ = = 2.5 V to 3.1 V [64].

CONCLUSIONS

The results presented in this work demonstrated the wide range of the application of EPR spectroscopy on the characterization of stability and antioxidant properties of food samples.

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