Investigation of active ingredients in red wines transferred into non-aqueous media

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Summary
A special procedure to transfer the antioxidants of red wines into the non-aqueous media was developed. The selected red wine samples from different regions of Croatia and Slovakia, as well as their non-aqueous analogues, were characterized by electron paramagnetic resonance (EPR) and optical spectroscopy regarding their antioxidant and radical-scavenging activities. Optical spectra of 36 red wine samples were collected and compared to their dimethyl sulfoxide (DMSO) "virtual wine" analogues. The average absorbance calculated using absorbance at three selected wavelengths (278 nm, 315 nm and 510 nm) correlated well with the antioxidant capacity of the mixture of phenolic compounds naturally present in wine for most of the samples. The radical-scavenging activity of the active ingredients of red wines in DMSO was considerably higher in comparison to the corresponding original red wine samples as determined by EPR spectroscopy. The concentration of selected polyphenols responsible for the antioxidant properties of the studied wines was analysed by liquid chromatography.

Keywords
red wine; antioxidant; non-aqueous media; magnetic resonance; radical scavenging; polyphenol

Wine has gained great popularity and is still the subject of a large number of studies [1–5]. The active ingredients of wines, in view of a positive effect on cardiovascular disease, are believed to be alcohol and polyphenolic compounds, in particular their combination [6–8]. The polyphenolic compounds often display strong antioxidant activity and some, such as quercetin, were found to exhibit numerous activities, including anti-inflammatory, antimicrobial and anticarcinogenic properties [9–12]. Phenolic compounds are crucial components of red wines, which strongly affect a series of wine sensorial characteristics, including colour, flavour, palate fullness and body, as well as bitterness and astringency [13, 14]. As an example, anthocyanins are among the most abundant components of red wine [15], being responsible for different grape colours [16]. The profile of antioxidants in wine depends on variety of vine, climate and microclimate conditions, type of growing and treatment of wine, as well as technology of winemaking [17]. Consequently, optical spectra of wine samples differ from each other and represent a simple and fast way to compare a large number of samples concerning the concentration of antioxidants in both red and white wines, as already indicated in our preliminary studies [17].

Wine is a rich source of compounds with antioxidant properties, which act as radical scavengers and which were recently intensively studied using different spectroscopic assays including electron paramagnetic resonance (EPR) and optical spectroscopy [18–20]. In this context, wines are the subject of growing interest and numerous studies were undertaken with a broad range of wine samples. However, much fewer investigations of active ingredients in red wines transferred into the non-aqueous media can be found in the literature (e. g. [21]). In our previous studies devoted to the antioxidant properties of yeasts and various cereals, we extensively used dimethyl sulfoxide (DMSO) as the solvent for extraction of ingredients possessing

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antioxidant properties [22–24]. The main advantage of DMSO was its ability to dissolve polar as well as non-polar compounds. Consequently, both hydrophilic and lipophilic antioxidants could be extracted into one medium, and a comprehensive view on the antioxidant properties of the analysed substrates could be obtained.

In this paper, a simple procedure for preparing non-aqueous solutions of substances present in selected red wine samples in order to transfer the active ingredients of red wines into non-aqueous media is described. Water and ethanol were replaced by DMSO, in which all wine components were soluble. The "virtual wines" prepared in this way in DMSO were characterized by spectroscopic techniques, particularly by EPR and ultra-violet/visible/near-infrared (UV/Vis/NIR) spectroscopy. Antioxidant and radical-scavenging activities of wine samples, applying optical and EPR spectroscopy using monitoring of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) cation radical ($ABTS^{+\bullet}$) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) elimination by selected red wine samples and their DMSO analogues, was studied. Additionally, the concentration of selected polyphenols was analysed by high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Temperature-controlled Reacti-Therm heating module (Thermo Scientific, Rockford, Illinois, USA) was used to remove water and ethanol from the wine samples. Dimethyl sulfoxide (DMSO, purity ≥99.9%, SeccoSolv) purchased from Merck (Darmstadt, Germany) was used as received. Optical spectra were measured using UV/Vis/NIR spectrophotometer Shimadzu 3600 (Shimadzu, Kyoto, Japan). Measurements of original wine samples and their DMSO analogues were carried out in 1 mm quartz cuvette. The reference for red wine samples was a 12% aqueous solution of ethanol (spectrophotometric grade; Mikrochem, Pezinok, Slovakia) and the "red wines" in DMSO were measured with reference to pure DMSO.

The antioxidant activity of samples was assessed by a modified Trolox equivalent antioxidant capacity (*TEAC*) method. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%; Sigma-Aldrich, St. Louis, Missouri, USA) was used as received. Potassium persulphate ($K_2S_2O_8$; p.a. purity) from Merck and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; purum, >99%) from Fluka (Buchs, Switzerland) were used as received. The decolorization assay involved the initial formation of the blue-green $ABTS^{+\bullet}$ cation radical. For the preparation of $ABTS^{+\bullet}$, 17.2 mg of ABTS was added to 3.3 mg $K_2S_2O_8$ in 5 ml distilled water and the final solution was stored in the dark for 12 h. A volume of 1 ml of such solution was diluted with 60 ml of distilled water to prepare the suitable concentration for the ABTS test. Addition of antioxidants led to the reduction of $ABTS^{+\bullet}$ radical to the neutral ABTS. Red wine samples were six times diluted by 12% aqueous solution of ethanol. To 2 ml of $ABTS^{+\bullet}$, 25 μl of diluted red wine sample was added and optical spectra were measured after 1 min, 5 min and 10 min after adding the sample, using a double-beam Shimadzu 3600 spectrometer.

For determination of radical-scavenging capacity (*RSC*) of selected samples, we used a stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, purum; Fluka) as the most suitable oxidation agent because of its good solubility both in ethanol/water mixtures as well as in DMSO. This assay is based on the ability of the antioxidant to react with DPPH radical accompanied with its elimination. The EPR integral intensity in the 30th min, detected for the sample solutions, was compared to that of the reference. The difference between these EPR intensities is proportional to the amount of DPPH radicals scavenged by the scavengers present in the corresponding sample. The value for the virtual conditions, when all radicals were

Tab. 1. Detection wavelengths for selected phenolic compounds.

Standard	Purity, Source	Detection wavelength
Gallic acid	97.5%, Sigma-Aldrich	272 nm
3,4-Dihydroxy-benzoic acid	≥97%, Sigma-Aldrich	272 nm
Catechin	≥96%, Sigma-Aldrich	280 nm
Cinnamic acid	≥99%, Sigma-Aldrich	280 nm
Caffeic acid	≥98%, Sigma-Aldrich	320 nm
Syringic acid	≥95%, Sigma-Aldrich	280 nm
Resveratrol	>99%, Sigma-Aldrich	320 nm
p-Coumaric acid	≥98%, Sigma-Aldrich	360 nm
Ferrulic acid	≥99%, Sigma-Aldrich	320 nm
Polydatin	≥95%, Sigma-Aldrich	320 nm
Rutin	≥94%, Sigma-Aldrich	360 nm
Epicatechin	≥96%, Sigma-Aldrich	280 nm
Vanillic acid	≥97%, Fluka	272 nm
Quercetin	≥98%, Sigma-Aldrich	360 nm

scavenged, was set to 100% of radical-scavenging capacity, and *RSC* of samples was presented as a percentage of DPPH radicals scavenged relative to the reference sample (water/ethanol or DMSO solvent). All EPR measurements were carried out in a single 4 mm flat quartz cell in a Bruker TE102 (ER 4102 ST) cavity using the X-band Bruker EMX EPR spectrometer (Bruker, Rheinstetten, Germany).

In order tom obtain a detailed information on phenolic compounds, samples were analysed by Agilent 1100 Series HPLC (Agilent, Santa Clara, California, USA) with UV detector. Polyphenols were separated by LiChrospher 100 RP-18 columm (5 μm) from Merck Millipore (Darmstadt, Germany) with a constant flow 0.5 ml·min^{-1}. Constitution of mobile phase was changed according to the programme: A (acetic acid : water 1 : 99), B (acetic acid : water 6 : 94), C (acetic acid : acetonitrile : water 65 : 30 : 5). 0–15 min 100% A, 15–30 min 100% B, 30–50 min 90% B + 10% C, 50–60 min 80% B + 20% C, 60–80 min 70% B + 30% C, 80–125 min 100% C, 125–130 min 100% A. Selected detection wavelengths are listed in Tab. 1. All results were described as mean value of triplicate measurements. Standard deviation was calculated using MS Excel (Microsoft Office 2007, Microsoft, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Red wines used for experiments were from different regions of Croatia and Slovakia, and of different varieties. Tab. 2 shows an overview of all investigated samples. A special procedure to transfer the active ingredients of red wines into non-aqueous media was developed. In the first phase, the samples of red wine (1 ml of red wine in 5 ml vials) were inserted into the temperature-controlled reactor, which was heated to 30 °C. This reactor enabled a continuous supply of argon (or nitrogen) over the surface of liquid samples using stainless steel jets. This allowed evaporation of all water and ethanol from wine samples under an inert atmosphere in about 2–3 h. To the dry substance at the bottom of the vial, 1 ml of DMSO was added for each sample. In this way, DMSO-dissolved equivalents for all red wine samples in aprotic environment were prepared, which means that water and ethanol were replaced by DMSO and all components of wine were dissolved in DMSO. In all cases, solutions with no sediments or suspended particles were obtained when fresh. However, in some cases the formation of small transparent crystals in the solution was observed during storage of several days, which indicated a slow precipitation of wine acids.

Tab. 2. Studied wine samples.

Sample	Variety	Year	Country of origin
1	Merlot	2009	Croatia
2	Cabernet Sauvignon	2009	Croatia
3	Zweigeltrebe	2009	Croatia
4	Blaufränkisch	2008	Croatia
5	Zweigeltrebe	2009	Croatia
6	Blaufränkisch	2008	Croatia
7	Zweigeltrebe	2008	Croatia
8	Merlot	2008	Croatia
9	Cabernet Sauvignon	2007	Croatia
10	Pinot Noir	2008	Croatia
11	Merlot	2009	Croatia
12	Cabernet Sauvignon	2009	Croatia
13	Pinot Noir	2009	Croatia
14	Plavac Mili	2005	Croatia
15	Plavac	2008	Croatia
16	Teran	2009	Croatia
17	Plavac	2008	Croatia
18	Dingac	2005	Croatia
19	Babic	2006	Croatia
20	Merlot	2008	Croatia
21	Plavac	2009	Croatia
21a	Plavac	2008	Croatia
22	Blaufränkisch	2008	Slovakia
23	Blaufränkisch	2009	Slovakia
24	Blaufränkisch	2009	Slovakia
25	Blaufränkisch	2009	Slovakia
26	Blaufränkisch	2009	Slovakia
27	Blaufränkisch	2009	Slovakia
28	Blaufränkisch	2009	Slovakia
29	Blaufränkisch	2009	Slovakia
30	Cabernet Sauvignon	2009	Slovakia
31	Cabernet Sauvignon	2009	Slovakia
32	Cabernet Sauvignon	2009	Slovakia
33	Cabernet Sauvignon	2008	Slovakia
34	Merlot	2008	Slovakia
35	Alibernet	2009	Slovakia

In the first stage of experiments, UV/Vis spectra of all red wine samples were collected. Fig. 1 shows an overview of optical spectra of the Cabernet Sauvignon wines (Fig. 1A) and their DMSO analogues (Fig. 1B). A similar trend was found for Blaufränkisch samples (not shown). A very

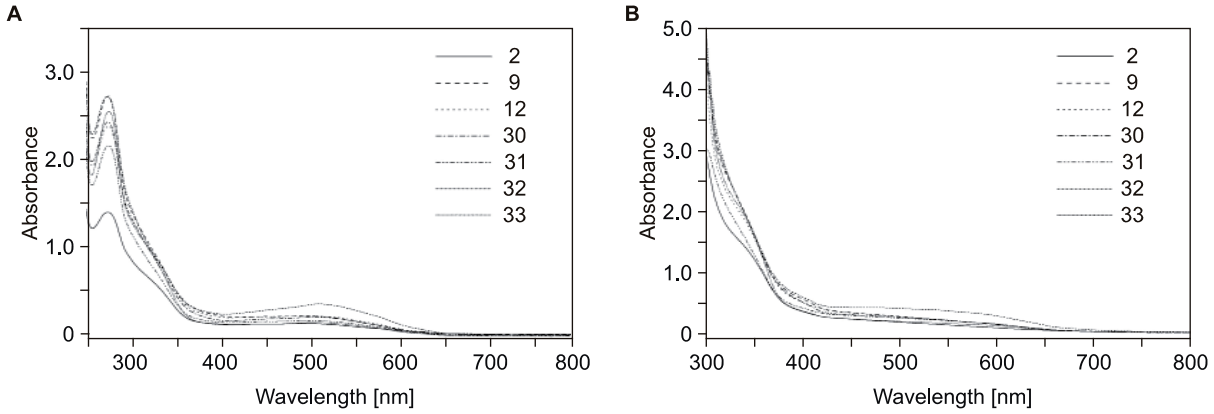

Fig. 1. UV/Vis spectra of Cabernet Sauvignon samples.

A – red wine, **B** – non-aqueous analogues of wine in DMSO.

good correlation between the height of absorption bands in the protic (water/ethanol) environment and in the aprotic (DMSO) one was observed for all samples, although the shape and the height of the corresponding optical bands were different (Fig. 2). A slight batochromic shift in the UV-Vis spectra going from "real" red wine to its "virtual one" in DMSO was observed. The different shape and intensity of UV/Vis bands of active ingredients of red wines in DMSO, compared to the water/ethanol system, reflected different states of polyphenolic compounds in both environments. It

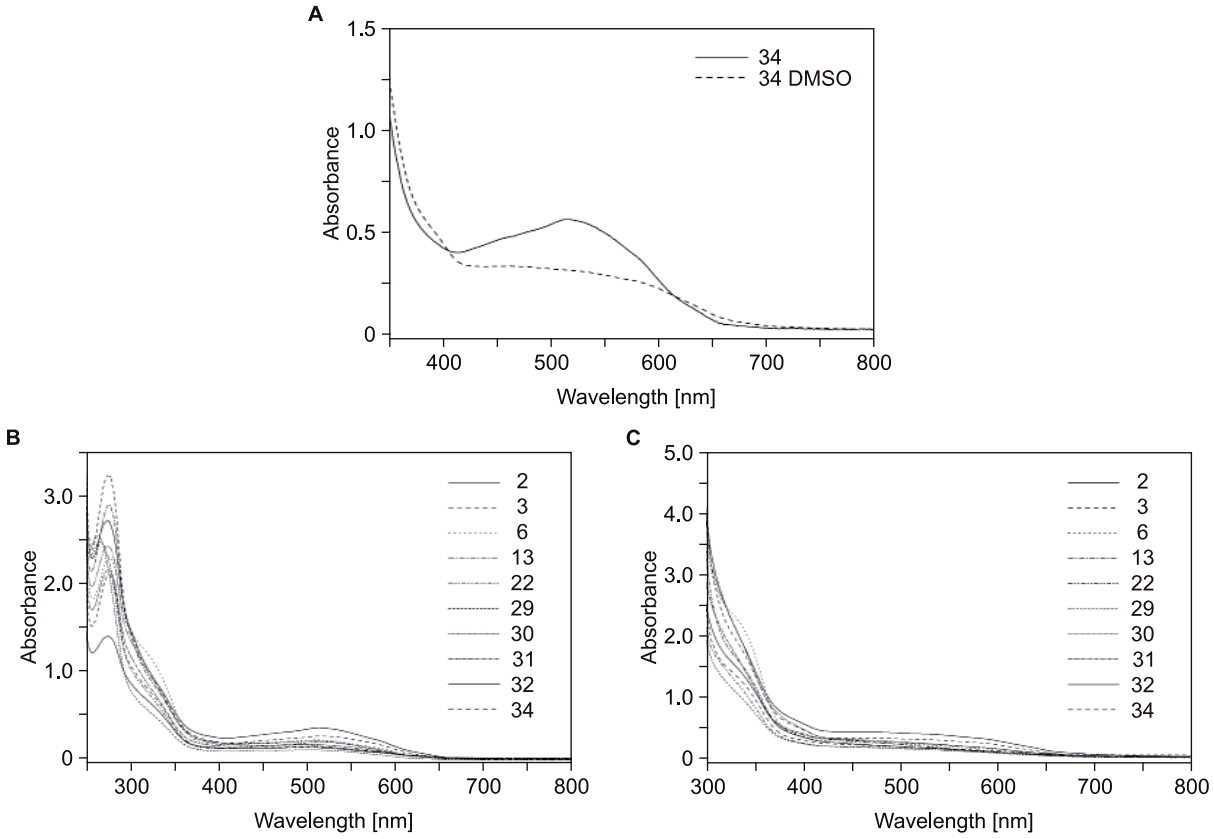

Fig. 2. UV/Vis spectra of wine samples selected for EPR studies.

A – sample 34 and its corresponding analogue in DMSO;
B – red wine; **C** – non-aqueous analogues of wine in DMSO.

is well known that the absorption spectra and extinction coefficients of polyphenols are influenced by the nature of the solvent, intra- and inter-molecular hydrogen bondings and the pH-dependent formation of resonance forms with altered conjugation compared to the parent compounds [25].

The lowest absorbance was observed for rose wine 27, both in DMSO and water/ethanol environment, where the lowest amount of phenolic compounds was confirmed by HPLC. The most intense UV/Vis band in the region below 300 nm was observed for a variety of Blaufränkisch, sample 29, but this sample exhibited a comparatively low UV/Vis band in the region from 450 nm to 600 nm. In this region, the most intense bands were observed for wine samples 25 and 28, which correlated very well with the measurements in DMSO. In the case of Cabernet Sauvignon, the most intense optical transition in the region 450–600 nm was observed for sample 32, with the highest absorbance in the region below 300 nm. The lowest intensity for both UV/Vis regions was exhibited in the sample Cabernet Sauvignon 2. We found that a suitable simple parameter, which could reflect the concentration of antioxidants in red wine as well as its antioxidant activity, seems to be an average absorbance A_{avg} defined as the average of the absorbance at three selected wavelengths, specifically at 278 nm, 315 nm and 510 nm. Fig. 3A shows the average absorbance A_{avg} for all investigated wine samples.

An overview of *TEAC* values determined for all studied samples is shown in Fig. 3B. The lowest *TEAC* value was exhibited by a rose wine sample 27 with the lowest average absorbance, as expected. On the other hand, the highest *TEAC* value was found for sample 20 with the highest average absorbance. Although a very good correlation between the *TEAC* value and A_{avg} was found for most of the tested samples, as shown in Fig. 4, in some cases a small deviation was observed (e.g. for samples 1, 15).

To compare the antioxidant and radical scavenging capacity of active ingredients in red wine samples in both environments (water – ethanol versus DMSO), we selected eleven representative samples for EPR studies. Their corresponding UV/Vis spectra are shown in Fig. 2. In EPR studies,

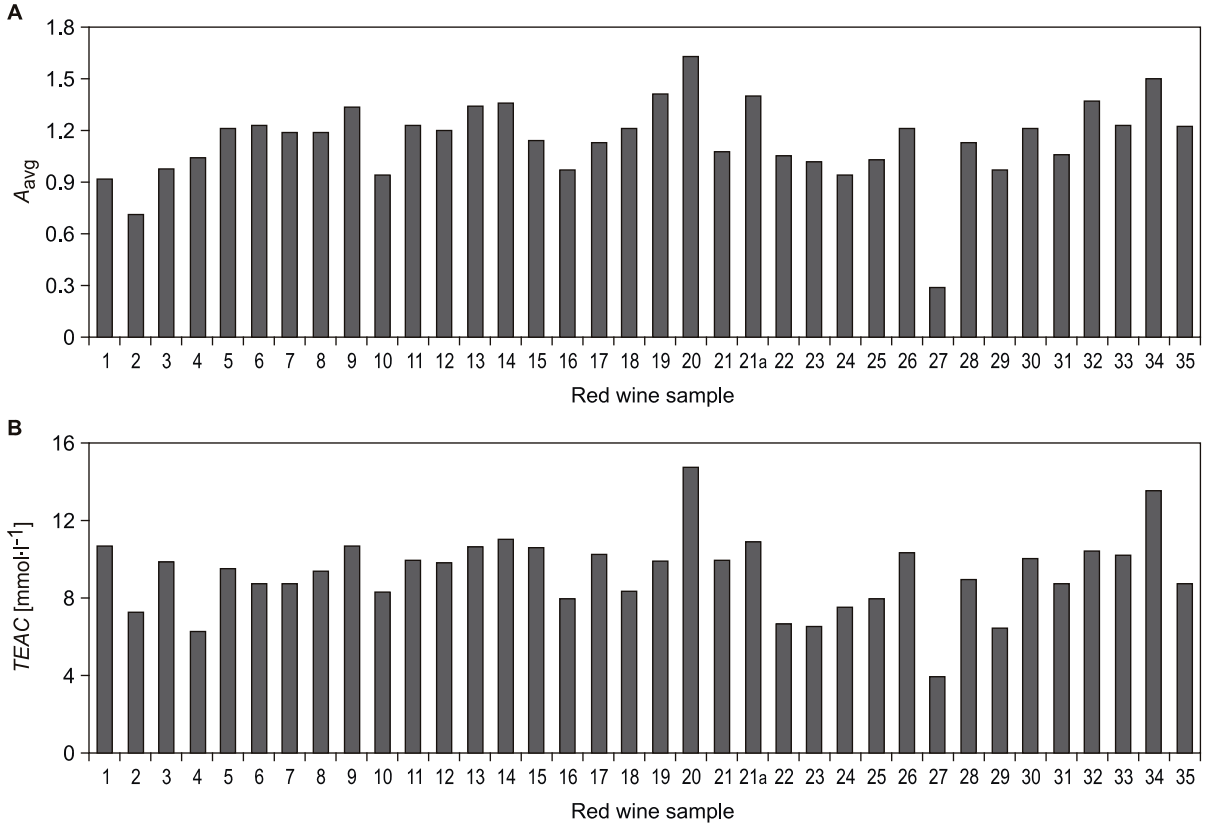

Fig. 3. Summary of average absorbance A_{avg} and *TEAC* values.

A – average absorbance A_{avg}, defined as $(A_{278nm} + A_{315nm} + A_{510nm})/3$);
B – Trolox equivalent antioxidant capacity (*TEAC*).

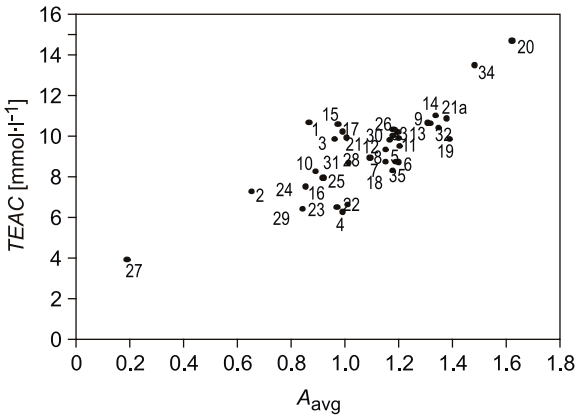

Fig. 4. Total antioxidant capacity as a function of average absorbance.

TEAC – Trolox equivalent antioxidant capacity, A_{avg} – average absorbance.

the radical-scavenging capacity was determined using the DPPH radical assay. DPPH radical was selected as most suitable for this purpose because of its good solubility both in ethanol and in DMSO. Fig. 5A shows the characteristic time dependence of EPR spectra of 0.5 ml 1×10^{-3} mol·l^{-1} DPPH in ethanol measured after addition of 20 μl of red wine sample 4. Under the same experimental conditions, a similar experiment was carried out with 0.5 ml 1×10^{-3} mol·l^{-1} DPPH in DMSO, to which 20 μl of "red wine" 4 analogue in DMSO was added (Fig. 6A). The time dependence of the double integral EPR intensity determined from the corresponding experimental EPR spectra measured in ethanol and in DMSO are shown in Fig. 5B and Fig. 6B, respectively. It should be noted that, although the line broadening of EPR spectra of DPPH was higher in the case of etha-

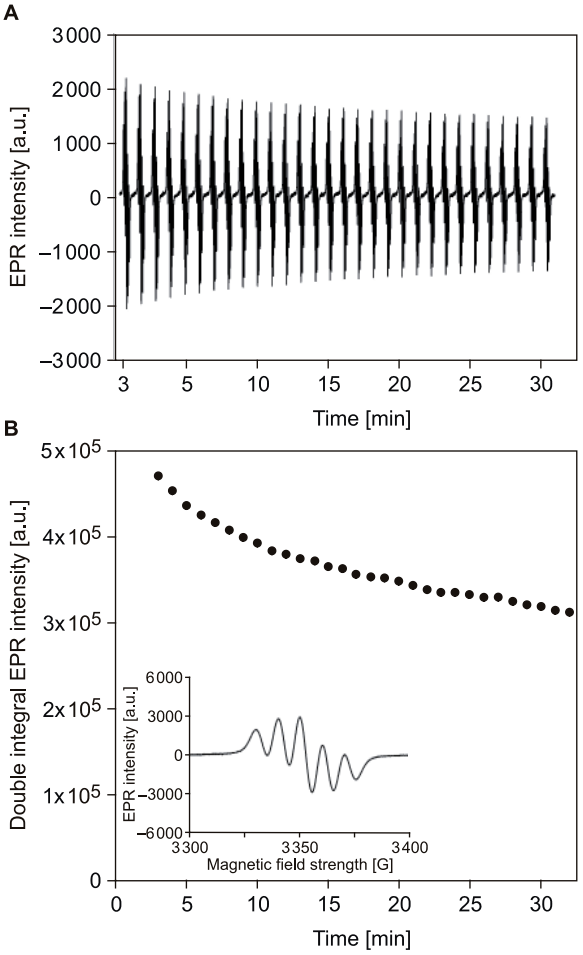

Fig. 5. Scavenging of radicals by wine sample 4 in ethanol.

A – EPR spectra of DPPH in ethanol measured in time after addition of sample 4; **B** – time dependence of the corresponding double integral EPR intensity and the characteristic EPR spectrum of DPPH in ethanol.

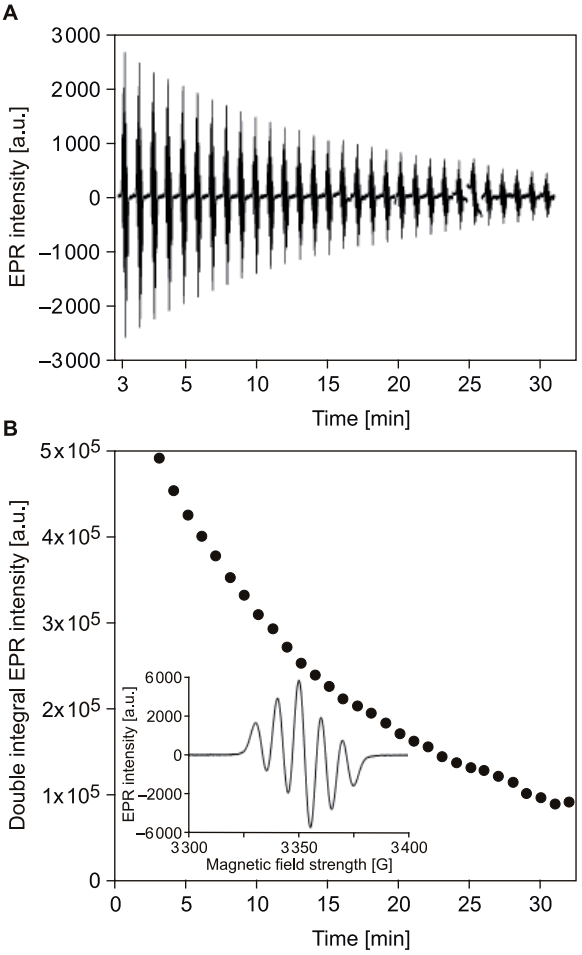

Fig. 6. Scavenging of radicals by wine sample 4 in DMSO.

A – EPR spectra of DPPH in DMSO measured in time after addition of sample 4; **B** – time dependence of the corresponding double integral EPR intensity and the characteristic EPR spectrum of DPPH in DMSO.

nol solutions (see inset in Fig. 5B) compared to DMSO solutions (see inset in Fig. 6B), the double integral EPR intensity of 0.5 ml 1×10^{-3} mol·l^{-1} DPPH solutions (measured in quartz EPR flat cell) was almost the same in both environments. Comparing Fig. 5 and Fig. 6, there is a clear evidence of a higher radical-scavenging activity of the active ingredients in the "wine sample" in DMSO compared to the corresponding original red wine. This difference was much more evidenced when comparing the integral intensity in the 30th minute of the reaction, as summarized in Fig. 7A, using the radical-scavenging capacity calculated for all selected samples. In all cases, much better radical-scavenging ability of DPPH was confirmed for DMSO analogues. For the selected wine samples, a fairly good correlation between the *TEAC* value, as well as *RSC* value, and the average absorbance was found.

Tab. 3 summarizes the detailed quantitative overview of phenolic compounds determined by HPLC using selected detection wavelengths (see Tab. 1) for all investigated wine samples. The dominating phenolics were gallic acid, rutine, catechin and resveratrol. The highest concentrations of gallic acid and rutine were found for samples 20 and 34, along with the highest *TEAC* and A_{avg}. Sample 20, with the highest *TEAC*, also exhibited the highest concentration of caffeic acid and a high concentration of resveratrol. On the other hand, the lowest antioxidant activity was observed for sample 27 with the lowest concentration of gallic acid, catechin and caffeic acid. Generally, a good correlation between concentration of the phenolics and antioxidant activity was confirmed by quantitative HPLC analyses, as is already known from the literature (see e.g. [18, 19]).

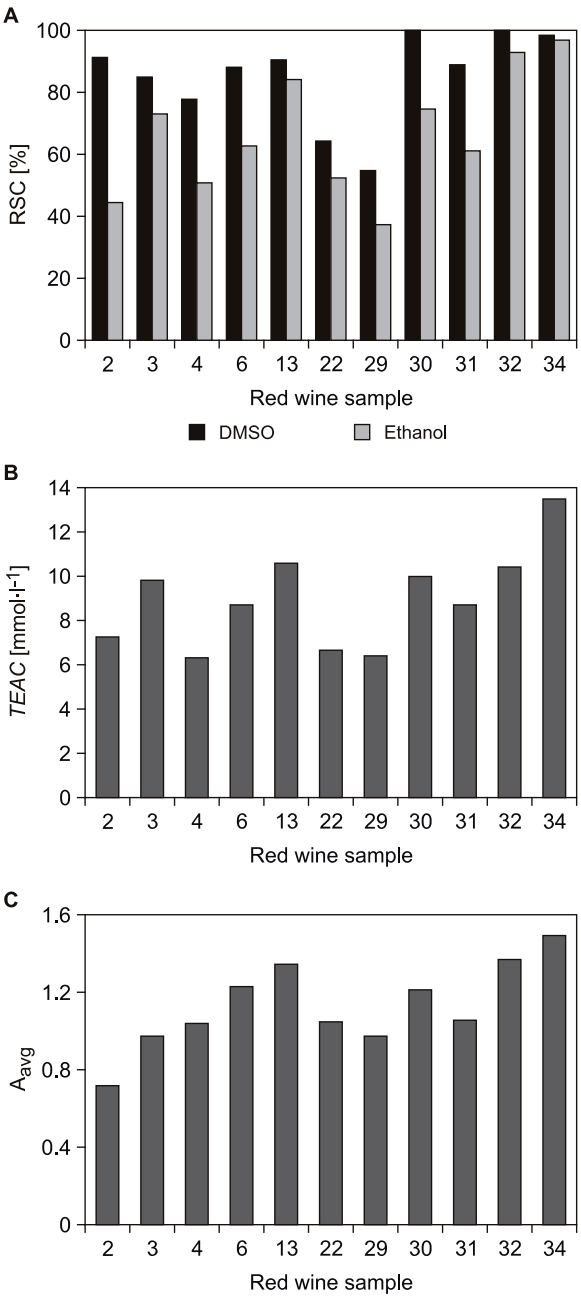

Fig. 7. Summary of *RSC*, *TEAC* and A_{avg} for selected wine samples.

A – Radical-scavenging capacity (*RSC*) determined in DMSO and in water/ethanol; **B** – Trolox equivalent antioxidant capacity (*TEAC*); **C** – average absorbance (A_{avg}).

CONCLUSIONS

A simple procedure to transfer the active ingredients from red wines into non-aqueous media was developed, where the water/ethanol part in wines was replaced by DMSO. In DMSO, both polar as well as non-polar wine components were completely re-dissolved. Comparing UV/Vis spectra of the original red wine samples and their *TEAC* values, determined using ABTS test, we found that the average absorbance defined as mean value of absorbances at 278 nm, 315 nm and 510 nm correlated well with their total antioxidant capacity. Radical-scavenging capacity (*RSC*) of selected wine samples was determined by EPR using scavenging of the stable DPPH radical in both environments (water/ethanol or DMSO). The radical-scavenging capacity of the active ingredients of red wines was considerably higher in DMSO solutions indicating that, in less polar environments, the antioxidant activity of these substances increased. A good correlation between concentration of the phenolics and *TEAC* was confirmed by quantitative HPLC analyses.

Tab. 3. Concentration of phenolics in wine samples.

Sample	Gallic acid	3,4-dihydroxybenzoic acid	Cinnamic acid	Caffeic acid	Chlorogenic acid	Syringic acid	p-coumaric acid	Ferrulic acid
	[mg·l^{-1}]							
1	574.9 ± 14.8	30.5 ± 1.4	55.9 ± 2.6	24.0 ± 1.4	14.9 ± 1.1	29.7 ± 1.4	13.1 ± 1.2	2.1 ± 0.3
2	330.5 ± 7.6	37.1 ± 1.8	68.0 ± 3.5	50.1 ± 2.6	4.8 ± 0.2	36.1 ± 1.9	62.2 ± 3.4	3.8 ± 0.4
3	714.3 ± 21.1	21.9 ± 2.1	40.7 ± 2.0	21.7 ± 1.5	18.0 ± 1.3	24.8 ± 1.4	17.3 ± 2.4	4.0 ± 0.4
4	318.6 ± 8.8	21.7 ± 1.9	44.9 ± 2.1	40.4 ± 2.4	8.8 ± 0.6	38.1 ± 2.1	11.1 ± 1.0	2.4 ± 0.3
5	551.5 ± 13.9	49.8 ± 2.4	44.8 ± 2.6	82.8 ± 5.1	12.6 ± 0.9	34.3 ± 1.5	36.8 ± 2.9	3.3 ± 0.4
6	426.2 ± 10.4	41.0 ± 2.0	13.9 ± 1.1	35.7 ± 2.1	83.4 ± 3.9	18.7 ± 1.0	11.4 ± 0.8	5.5 ± 0.6
7	496.9 ± 14.5	37.0 ± 1.9	11.2 ± 1.2	28.8 ± 1.4	68.7 ± 2.7	23.5 ± 1.1	10.8 ± 0.9	2.2 ± 0.3
8	377.2 ± 11.3	23.4 ± 1.1	18.0 ± 1.4	37.6 ± 2.0	84.4 ± 4.9	19.0 ± 0.9	6.8 ± 0.7	5.7 ± 0.6
9	474.6 ± 12.5	39.1 ± 1.7	42.5 ± 2.0	151.6 ± 8.6	54.5 ± 3.4	27.5 ± 1.2	0.5 ± 0.2	6.1 ± 0.7
10	480.3 ± 16.8	51.6 ± 2.1	73.9 ± 5.4	19.5 ± 1.4	8.9 ± 0.9	25.0 ± 1.1	1.9 ± 0.9	3.0 ± 0.4
11	347.6 ± 12.9	53.6 ± 2.4	51.5 ± 3.9	17.0 ± 1.4	31.4 ± 1.7	13.6 ± 1.3	65.8 ± 3.4	4.9 ± 0.5
12	320.0 ± 13.1	38.7 ± 1.4	57.2 ± 4.2	33.4 ± 1.8	22.2 ± 1.8	20.0 ± 1.7	100.6 ± 6.1	4.3 ± 0.5
13	608.0 ± 15.0	44.5 ± 2.1	84.0 ± 6.7	113.9 ± 7.9	0.7 ± 0.4	36.5 ± 1.7	31.3 ± 1.4	8.1 ± 0.7
14	849.6 ± 17.8	79.3 ± 3.1	52.1 ± 4.7	55.6 ± 3.4	12.0 ± 0.8	15.3 ± 1.4	117.8 ± 7.8	nd
15	684.0 ± 14.8	15.3 ± 0.9	54.3 ± 4.3	12.5 ± 1.4	35.7 ± 1.4	6.4 ± 0.8	15.6 ± 1.1	nd
16	453.7 ± 13.9	44.6 ± 2.0	50.1 ± 5.1	10.9 ± 1.1	20.4 ± 1.1	11.9 ± 0.9	12.6 ± 0.9	10.1 ± 1.0
17	391.5 ± 10.6	34.1 ± 1.8	65.0 ± 4.8	nd	42.0 ± 1.7	12.5 ± 1.0	5.8 ± 0.7	13.2 ± 0.9
18	612.4 ± 15.3	43.2 ± 2.4	59.9 ± 4.9	9.4 ± 1.0	26.4 ± 1.2	9.5 ± 0.7	1.2 ± 0.4	2.4 ± 0.3
19	905.7 ± 24.8	49.0 ± 2.6	69.6 ± 6.0	31.4 ± 1.7	121.5 ± 8.3	13.9 ± 1.2	19.6 ± 1.4	nd
20	1 124.6 ± 30.4	40.7 ± 2.4	37.1 ± 1.9	236.7 ± 20.1	30.9 ± 1.4	23.8 ± 1.4	nd	8.8 ± 0.9
21	254.6 ± 10.8	30.2 ± 1.4	88.3 ± 3.8	92.9 ± 6.7	24.7 ± 1.2	9.1 ± 1.0	4.7 ± 0.5	3.6 ± 0.4
21a	183.3 ± 11.2	17.7 ± 0.9	96.8 ± 5.2	41.6 ± 3.0	33.2 ± 1.8	22.6 ± 1.1	3.3 ± 0.6	4.2 ± 0.5
22	211.9 ± 12.4	96.0 ± 4.8	29.5 ± 1.4	4.7 ± 0.8	29.7 ± 1.5	13.2 ± 1.2	13.4 ± 0.9	14.1 ± 1.2
23	208.7 ± 11.9	101.6 ± 5.4	34.8 ± 1.4	11.2 ± 1.4	0.7 ± 0.5	5.0 ± 0.8	18.2 ± 0.8	4.5 ± 0.5
24	469.1 ± 14.5	42.1 ± 3.4	49.3 ± 2.7	26.4 ± 1.5	15.1 ± 1.2	16.6 ± 1.1	31.5 ± 1.4	6.2 ± 0.7
25	248.7 ± 9.8	127.8 ± 6.2	46.4 ± 2.9	17.1 ± 0.9	0.7 ± 0.3	7.9 ± 0.6	26.5 ± 1.4	5.0 ± 0.4
26	853.4 ± 16.8	45.9 ± 2.3	35.6 ± 1.9	11.6 ± 1.1	12.7 ± 0.8	16.0 ± 1.4	9.4 ± 0.7	4.9 ± 0.5
27	77.4 ± 6.2	32.4 ± 1.4	30.2 ± 2.0	0.2 ± 0.7	13.6 ± 1.0	10.2 ± 1.2	nd	nd
28	283.0 ± 11.5	46.0 ± 1.9	62.9 ± 3.8	3.7 ± 0.9	49.1 ± 3.2	10.5 ± 0.9	7.5 ± 0.9	23.9 ± 1.4
29	309.5 ± 10.8	46.9 ± 2.4	38.5 ± 2.4	22.8 ± 1.9	0.7 ± 0.4	18.2 ± 0.7	10.1 ± 0.7	nd
30	467.4 ± 13.7	34.3 ± 1.7	58.7 ± 3.4	26.2 ± 1.5	15.6 ± 1.1	15.8 ± 0.8	34.2 ± 1.4	7.7 ± 0.8
31	582.8 ± 14.6	50.2 ± 2.2	54.1 ± 3.1	46.7 ± 2.8	21.2 ± 1.4	18.1 ± 1.4	23.2 ± 1.0	nd
32	549.6 ± 16.3	44.5 ± 2.3	69.5 ± 2.8	11.4 ± 1.2	30.4 ± 1.6	21.3 ± 1.5	35.0 ± 1.4	nd
33	824.8 ± 17.1	32.4 ± 1.4	48.2 ± 2.7	76.9 ± 4.3	105.1 ± 5.8	7.2 ± 0.6	43.2 ± 1.9	nd
34	971.1 ± 22.4	44.0 ± 3.1	48.5 ± 2.8	20.4 ± 1.9	22.4 ± 1.2	7.8 ± 0.8	7.2 ± 0.9	2.6 ± 0.3
35	202.0 ± 10.1	46.3 ± 2.7	79.6 ± 5.1	113.0 ± 6.2	28.9 ± 1.4	34.7 ± 1.9	7.1 ± 1.0	4.9 ± 0.5

nd – not detected.

Tab. 3. continued

Sample	Vanillic acid	Resveratrol	Polydatin	Catechin	Rutine	Epicatechin	Quercetin
	[mg·l^{-1}]						
1	7.8 ± 0.3	16.7 ± 1.4	28.4 ± 1.3	112.6 ± 5.4	8.7 ± 0.9	23.6 ± 1.2	37.9 ± 1.9
2	4.5 ± 0.2	6.1 ± 0.4	nd	72.6 ± 4.1	nd	3.5 ± 0.7	17.6 ± 1.7
3	5.2 ± 0.3	54.2 ± 6.4	8.7 ± 0.5	300.4 ± 20.7	207.8 ± 15.6	3.7 ± 0.6	24.3 ± 1.2
4	5.3 ± 0.3	10.3 ± 0.9	23.1 ± 1.4	39.8 ± 1.8	371.2 ± 18.7	2.5 ± 0.4	28.8 ± 1.4
5	7.1 ± 0.4	41.7 ± 2.4	34.8 ± 1.8	55.1 ± 2.9	463.5 ± 23.1	10.4 ± 1.0	46.3 ± 2.2
6	5.7 ± 0.3	17.5 ± 1.4	6.9 ± 0.7	60.6 ± 3.5	187.6 ± 14.5	30.5 ± 1.7	41.4 ± 2.0
7	5.7 ± 0.3	15.4 ± 1.3	10.7 ± 0.9	38.5 ± 2.3	346.4 ± 17.9	5.7 ± 0.6	37.9 ± 1.9
8	6.9 ± 0.1	63.1 ± 3.4	20.6 ± 1.3	82.5 ± 4.8	244.4 ± 14.8	21.2 ± 1.0	58.9 ± 3.1
9	9.3 ± 0.5	211.4 ± 11.4	26.4 ± 1.4	37.0 ± 2.0	156.5 ± 10.4	nd	26.5 ± 1.1
10	4.1 ± 0.3	26.0 ± 1.4	29.0 ± 1.5	204.1 ± 17.4	233.7 ± 16.5	nd	109.8 ± 5.6
11	4.1 ± 0.3	20.3 ± 1.0	38.8 ± 1.9	81.8 ± 4.1	395.1 ± 20.7	nd	50.5 ± 2.6
12	6.9 ± 0.4	15.2 ± 0.8	35.1 ± 1.8	135.4 ± 7.2	nd	nd	40.0 ± 2.2
13	4.3 ± 0.5	51.3 ± 2.5	83.6 ± 4.5	596.5 ± 41.5	762.2 ± 47.8	58.2 ± 3.1	52.2 ± 2.6
14	5.5 ± 0.6	2.5 ± 0.3	132.9 ± 6.7	143.7 ± 6.4	409.2 ± 20.1	10.7 ± 1.1	45.0 ± 2.2
15	5.1 ± 0.5	2.5 ± 0.3	76.7 ± 4.2	79.8 ± 4.3	382.6 ± 18.6	nd	54.2 ± 2.9
16	7.0 ± 0.4	34.7 ± 2.4	56.3 ± 3.0	69.6 ± 3.8	930.4 ± 72.6	94.7 ± 4.6	34.7 ± 1.8
17	4.1 ± 0.3	2.5 ± 0.3	110.6 ± 5.8	63.8 ± 3.5	294.3 ± 17.8	nd	25.9 ± 1.4
18	4.1 ± 0.3	36.6 ± 1.7	9.1 ± 0.9	49.0 ± 2.7	546.7 ± 41.8	9.4 ± 0.8	90.0 ± 4.8
19	6.4 ± 0.4	192.0 ± 10.9	10.2 ± 1.0	58.2 ± 3.1	380.7 ± 18.3	nd	100.0 ± 6.2
20	5.6 ± 0.3	191.0 ± 12.1	4.7 ± 0.5	32.3 ± 1.4	920.9 ± 59.4	3.0 ± 0.6	28.8 ± 1.5
21	5.1 ± 0.3	77.0 ± 4.4	273.8 ± 14.5	30.4 ± 1.6	196.2 ± 15.6	3.0 ± 0.6	63.6 ± 3.4
21a	4.1 ± 0.3	137.8 ± 7.4	10.8 ± 0.9	30.2 ± 1.3	222.2 ± 17.1	27.7 ± 1.4	267.9 ± 12.6
22	6.6 ± 0.3	34.8 ± 2.1	7.7 ± 0.8	57.4 ± 2.7	nd	nd	236.4 ± 14.7
23	6.7 ± 0.4	44.3 ± 2.4	11.4 ± 1.0	75.1 ± 4.2	58.4 ± 4.4	142.8 ± 7.3	307.2 ± 13.4
24	5.6 ± 0.3	61.3 ± 2.9	32.5 ± 1.6	202.8 ± 16.4	69.6 ± 3.8	131.5 ± 6.8	40.2 ± 1.9
25	6.1 ± 0.3	25.8 ± 1.4	46.3 ± 2.6	58.8 ± 2.4	433.3 ± 26.8	nd	37.5 ± 1.7
26	4.9 ± 0.2	44.3 ± 1.9	64.0 ± 3.1	167.2 ± 9.6	236.3 ± 19.2	11.5 ± 1.3	35.5 ± 1.6
27	4.3 ± 0.2	38.6 ± 2.0	20.5 ± 1.1	2.6 ± 0.4	199.1 ± 15.7	0.0 ± 1.4	32.8 ± 1.4
28	12.9 ± 1.0	2.5 ± 0.3	40.9 ± 1.9	91.1 ± 5.3	87.3 ± 4.1	245.0 ± 18.6	284.6 ± 13.8
29	5.2 ± 0.4	15.5 ± 0.6	12.3 ± 1.0	40.8 ± 1.9	36.7 ± 1.8	nd	25.3 ± 1.3
30	6.3 ± 0.4	34.9 ± 1.9	34.3 ± 1.6	165.9 ± 10.2	113.4 ± 5.6	72.1 ± 3.6	67.2 ± 3.2
31	4.8 ± 0.3	14.5 ± 0.8	39.1 ± 2.2	57.1 ± 3.0	nd	23.8 ± 1.3	90.4 ± 4.7
32	5.7 ± 0.3	50.0 ± 2.4	27.9 ± 1.4	83.8 ± 4.6	103.4 ± 4.6	nd	101.4 ± 5.6
33	5.6 ± 0.3	109.0 ± 6.1	90.7 ± 5.3	342.1 ± 19.1	272.9 ± 23.3	161.1 ± 8.7	76.0 ± 4.1
34	4.1 ± 0.2	30.8 ± 1.5	56.8 ± 3.0	88.9 ± 4.7	686.7 ± 41.9	nd	150.7 ± 8.1
35	6.1 ± 0.3	111.2 ± 5.4	46.4 ± 2.8	84.9 ± 4.1	nd	185.3 ± 9.8	142.1 ± 1.4

nd – not detected.

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