

## Antioxidant properties of wines produced in the Podkarpacie region

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**Abstract.** The wine is famous and popular drink having properties of reactive oxygen species scavenging. In this paper we study the antioxidant activity of the selected wines produced in the south-east region of Poland, in Subcarpathian Voivodeship, in the Podkarpacie region. We have concentrated our studies on the superoxide radical anion  $O_2^{\cdot-}$ . We have used electron spin resonance spectroscopy (ESR) as it is the best method for radical detection. The nitron spin trap  $\alpha$ -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN) has been used in the experiment. The radical scavenging ability of wines were determined from the changing intensity of the spectrum in time.

**Key words:** electron spin resonance (ESR) • POBN spin trap • superoxide radical anion • Polish wines

### Introduction

Sequential reduction of the triplet molecular oxygen leads to the formation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals [1]. With the aim to enhance the defensive power of the human organism against these species many natural antioxidant products should be introduced into foodstuffs. The wine is one of them, as it is popular drink having properties of ROS scavenging [10, 11]. In Poland there is a rapid development of vineyards and wine production in recent time [6].

In this paper we investigate the antioxidant properties of the selected wines from the Subcarpathian region, using a spin trap method in ESR. Among the analytical tools, ESR spectroscopy is the only method that directly detects free radicals in such components of food as wine [4], tea leaves [7], beer [3], oil [14], flavonoids [15], starches [2] etc.

### Superoxide anion radical

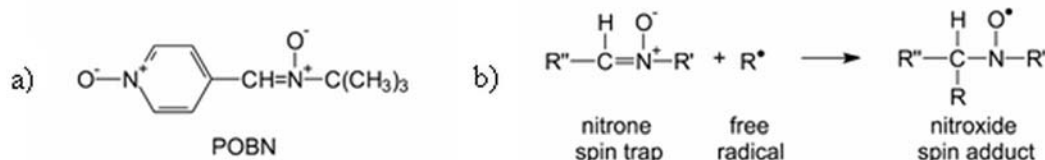
The most important source of oxide radical in the majority of organic cells is an electron transport chain. The leak of electrons from these chain, causes the reduction of molecular oxygen. It also should be noted that the active form of oxygen has an essential influence on defense of organism against bacteria [5].

We concentrate our studies on the superoxide radical anion  $O_2^{\cdot-}$ . The nitron spin trap  $\alpha$ -(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN), manufactured by Sigma-Aldrich, has been used in the experiment (Fig. 1a). The key reaction of adduct formation is shown in Fig. 1b [8].

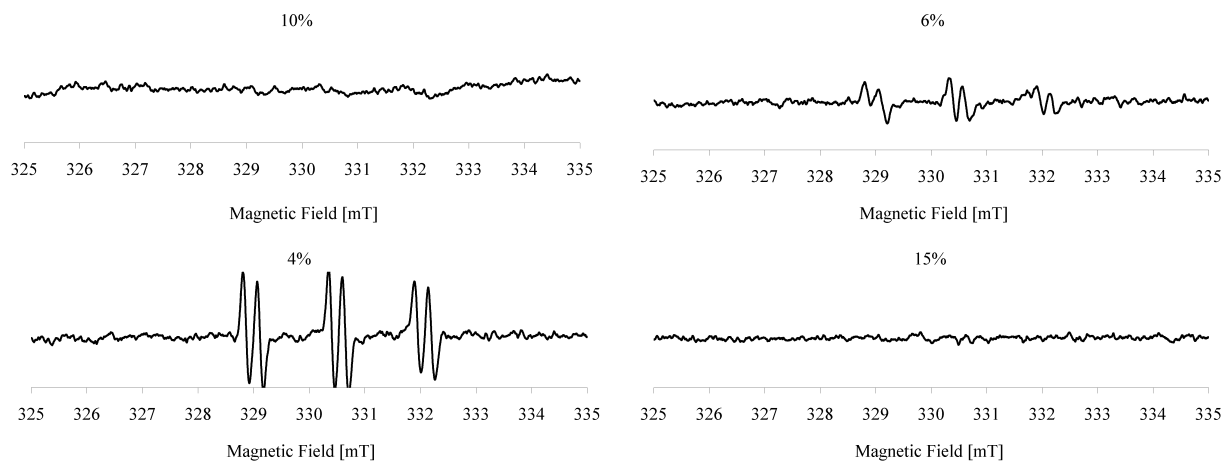
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**Fig. 1.** (a) Spin trap of nitron  $\alpha$ -(4-pyridyl-1-oxide)-*N*-tert-butylnitron (POBN), (b) general scheme of spin trapping.



**Fig. 2.** Effect of concentration of the KOH solution on the ESR spectrum, with a steady 6% concentration of  $\text{H}_2\text{O}_2$ .

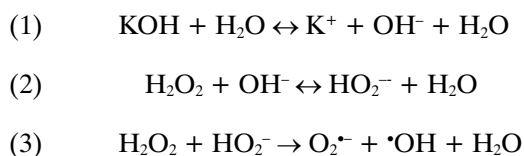
As a result, we have recorded the ESR spectra consisting of six lines forming triplet of doublets arising from hyperfine interaction of the electron spin  $S = 1/2$  with proton spin  $I_{\text{H}} = 1/2$  and nitrogen spin  $I_{\text{N}} = 1$ .

## Experimental

Samples of four wines were tested. Two white wines, from the Plochocki Vineyard, were chosen for study, because they were available in the local market. First one (white wine I) is dry, grape wine “Seyval Blank 2008”, originating from a traditional French-American mix wine graft. The content of alcohol is 11%, acidity 5.5 g/l, residual sugar 1.4 g/l. The crop took place in October 20th, whereas the wine was bottled in November 11th, 2009. This wine is from a vineyard situated at Glinik Polski, near by the Jasło town in the Subcarpathian Voivodeship. The vineyard was established in 2003 and its area is 0.3 hectare. Second white wine (white wine II) is dry, grape wine “Sibera 2008”, originating from a German-Czech mix wine graft. The content of alcohol is 10.5%, acidity 6.9 g/l, residual sugar 3.5 g/l. The crop took place in October 20th, whereas wine was bottled in November 11th, 2009. This wine is from a vineyard situated at Daromin in the district of Sandomierz. The vineyard was established in 2005 and its area is 1.7 hectare.

Two red wines were home-made, based on mixed grafts in the household vineyards. Both wines are from 2011 vintage. One grape vine is cultivated at Radymno in the Jarosław district, while the second one at ŻoŹylnia in the Łańcut district.

The  $\text{O}_2^{\cdot-}$  radicals were created in the non-Fenton reaction:



Finally we have obtained both  $\text{O}_2^{\cdot-}$  and  $\text{OH}^{\cdot}$  radicals, however the number of former is significantly prevalent [9]. Experimentally, we have established that the ESR spectrum strongly depends on the concentration of the substrates in the free radical generation and trapping processes (Fig. 2). The solution of KOH/ $\text{H}_2\text{O}_2$ /DMSO – 6%/6%/6% was chosen as the best option [13]. DMSO was used as POBN solvent. Concentration of POBN was 0.18 mol/dm<sup>3</sup>.

The radical scavenging ability of wines studied were determined from the changing in time of the intensity of the spectrum.

ESR spectra were recorded using an X-band ESR spectrometer, manufactured by the Wrocław University of Technology, connected with a PC computer equipped with a LabVIEW system [12]. Measurements were performed in various intervals of time. In the beginning of the recording it was 30 min. Then, the ESR spectra were measured after few or dozen hours. Between measurements samples were stored in a fridge. The spectra were integrated computationally in the whole range of the magnetic field.

## Results and discussion

The ESR spectra obtained from a reference sample (ethyl alcohol 96% diluted to 11% with DMSO was used instead of the wine) is presented in Fig. 3a. The spectra of wine samples are shown in Figs. 3b–3e. The  $g$ -factor and hyperfine structure ( $a_{\text{N}}$  and  $a_{\text{H}}$ ) constants were calculated and are collected in Table 1 for reference system and for each sample. The constants  $a_{\text{N}}$  and  $a_{\text{H}}$  are stable for each sample and they increase in respect to the reference system.

In fact, the non-Fenton system generates reactive radical species  $\text{O}_2^{\cdot-}$ ,  $\cdot\text{OH}$ ,  $\cdot\text{CH}_3$ . However, the shape of spectra obtained (almost the same intensity of each lines) points to the fact that only superoxide anion radical was trapped by POBN. The  $\cdot\text{OH}$ ,  $\cdot\text{CH}_3$  species were submit-

**Table 1.** Hyperfine constants and  $g$ -factors for reference system and for the wine samples

Sampe	$a_N$ (mT)	$a_H$ (mT)	$g$ -factor
Reference sample	$1.487 \pm 0.002$	$0.243 \pm 0.002$	$2.078 \pm 0.002$
White wine I	$1.560 \pm 0.002$	$0.259 \pm 0.002$	$2.078 \pm 0.002$
White wine II	$1.519 \pm 0.002$	$0.259 \pm 0.002$	$2.078 \pm 0.002$
Red wine I	$4.556 \pm 0.002$	$0.259 \pm 0.002$	$2.078 \pm 0.002$
Red wine II	$1.556 \pm 0.002$	$0.259 \pm 0.002$	$2.078 \pm 0.002$

ted to consecutive rapid reactions yielding  $O_2^{\cdot-}$ . The  $H_2O_2/KOH(NaOH)/DMSO/POBN$  system with  $\cdot CH_3$  and  $\cdot OH$  species leads to the spectra having intensity of central doublet considerably grater in respect to the others (Fig. 6a in [8]).

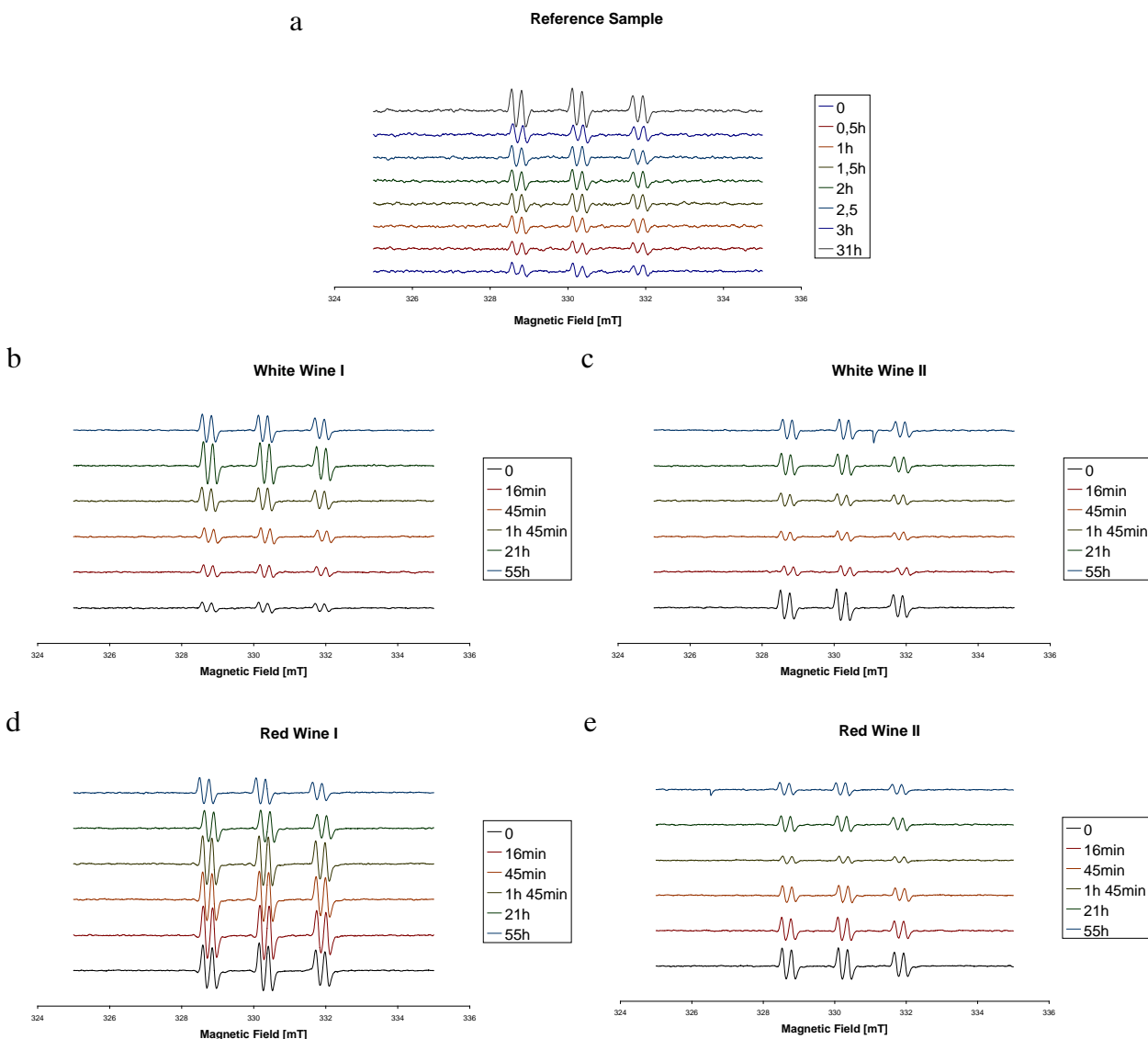
The time changes of the integral area of the spectra are presented in Figs. 4a–4e. The spectra were integrated by summing the integral intensities of each line:

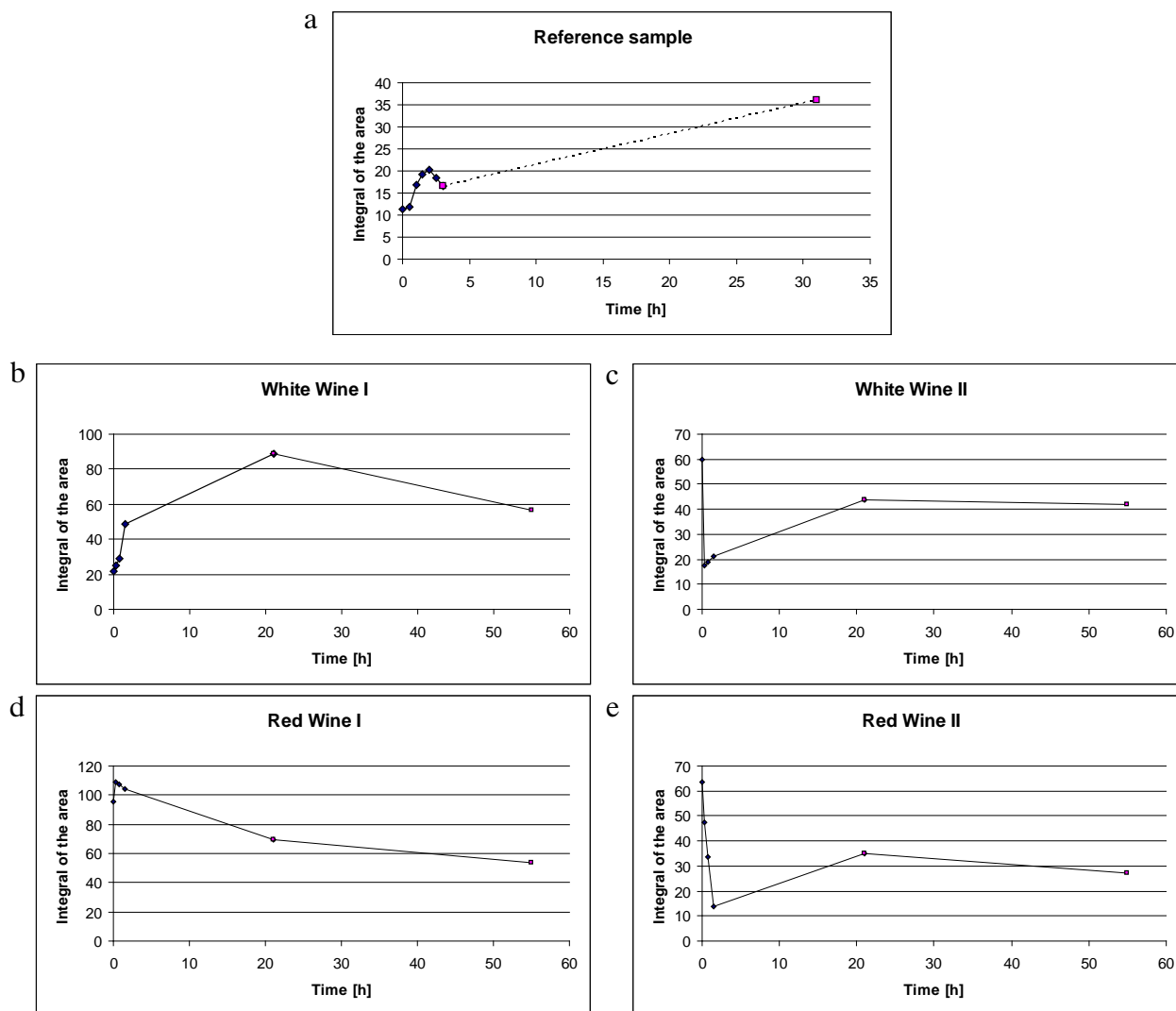
$$(4) \quad I_{\text{int}} = \sum_i I_i \Delta B_i^2$$

where  $I_i \Delta B_i^2$  is the integral intensity of the separated line,  $I_i$ ,  $\Delta B_i$  is the intensity and width of the  $i$ -th line.

## Conclusions

In Fig. 4 graphs of integrals of the area are shown. We can see that at the initial time (up to 1.5 h) a radical amount increases, then decreases. This is due to the nature of the chemical generation of radicals. We can observe a drastic difference in the intensity of the ESR signal for wines in the early stages of our tests (up to few minutes). Although red wines have greater antioxidant properties in the early stages of tests, white wines have a less number of radicals. This is due to the fact that in the studies, in contrast to white wines, non-professional red wines produced by domestic were used.

**Fig. 3.** ESR spectra of samples: (a) reference sample, (b) white wine I, (c) white wine II, (d) red wine I, (e) red wine II.



**Fig. 4.** Graph of the integral of (a) reference sample, (b) white wine I, (c) white wine II, (d) red wine I, (e) red wine II.

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