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FREE RADICALS IN RED WINE, BUT NOT IN WHITE?

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By using Electron Spin Resonance (ESR) spectroscopy, we have detected free radicals in red wine, whether fermented on oak or not, and in white wine only when it has been fermented on oak. These radicals would appear to be associated with the phenolics, because the ESR signal from the residue of red wine treated with polyvinyl polypyrrolidone is reduced by ~80%. Any inhibition of lipid oxidation by red wine phenolics *in vitro* will take place in the presence of these radicals, which have a linewidth of 2.0 ± 0.1 gauss and a g-value of 2.0038 ± 0.0001.

KEY WORDS: Red wine, phenolics, Electron Spin Resonance.

INTRODUCTION

On reading the report of the inhibition of lipid oxidation by red wine phenolics[1], we recalled that polyphenolics can stabilise free radicals quite well, so a programme was set up to test for them, using Electron Spin Resonance (ESR) spectroscopy[2].

MATERIALS AND METHODS

A Varian X-band (9.1 GHz), E-12 spectrometer, capable of detecting ~10^{11} spins of 1 gauss linewidth, in a volume of 0.15 mL, working at liquid N_2 temperature to avoid microwave polar losses in water, was employed.

Initially, a red wine (Brown Bros., 12L. cask) and a white (Tisdall, 12L. cask), were used at their normal concentration. No free radical signal was observed. 25 mL samples were then cold vacuum evaporated to ~1/10 of their volume, and the resulting rather syrupy liquids examined. Both samples showed Mn^{2+} signals, but only the red wine sample showed a free radical signal: see Figure 1. The red wine had been fermented on oak, the white had not. However, a red wine not fermented

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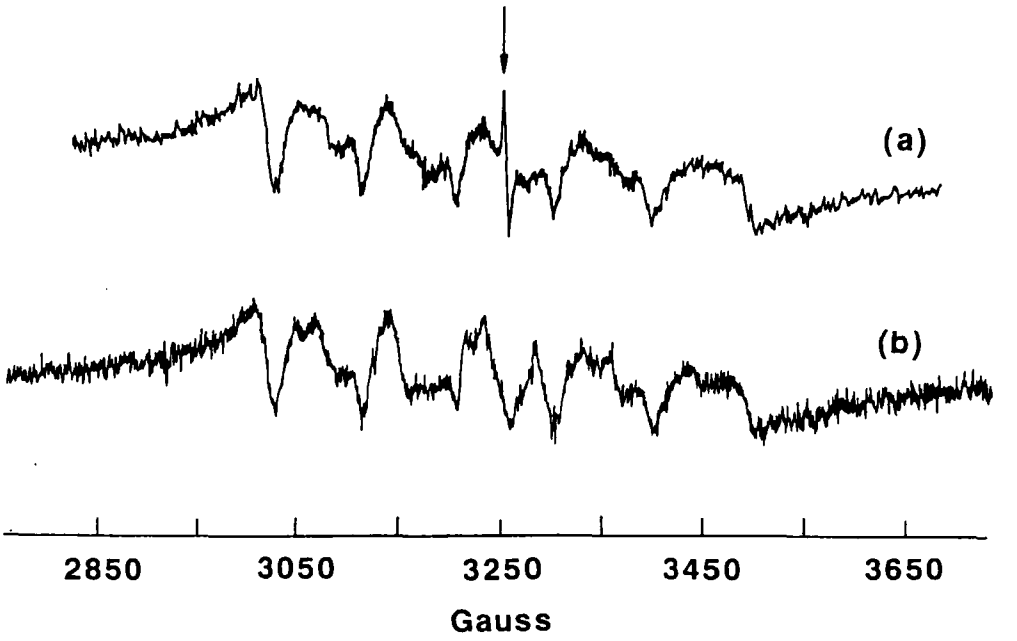

FIGURE 1 Curve (a) free radical (arrow) and Mn^{2+} signals from red wine (Brown Bros.); curve (b) spectrometer trace from Tisdall white wine. 1 gauss = 10^{-4} mT.

on oak (Mitchelton Winery's Cab Mac) gave both Mn^{2+} and free radical signals; a semillon (Yaldara) which had been oak aged also gave both these signals. (Figure 2).

It would appear that all wines probably contain Mn^{2+} to a similar concentration (within an order of magnitude). This is perhaps not surprising, since manganese is known to play an important role in plant physiology[3].

Other Australian red wines, and another white (Tarrawarra chardonnay) which had been fermented under 'minimum oak' conditions, were similarly tried. The reds showed the free radical signal quite clearly; the white showed little, if any, signal. This is consistent with the minimum oak description of the fermentation, where the wine is exposed to only a small quantity of oak during the process. All oak used in wine preparation is 'toasted'; that is, heated for a certain period of time, to a temperature of 200 C, or more.

Concentrated glycoside extracts of both red (shiraz) and white (semillon) grape juices were prepared by a standard technique[4], and examined by ESR. The red extract gave the ESR signal, the white did not (Figure 3). This shows that the free radical is already present in *detectable* amounts in the original red 'berry' juice, but not in white.

Fractionation of Wine Samples

Attempts were made to identify broad fractions of the wine which contained higher concentrations of free radicals. To this end the samples were treated with several adsorbents, each with different specificity. Unfortunately, at this time we are unable

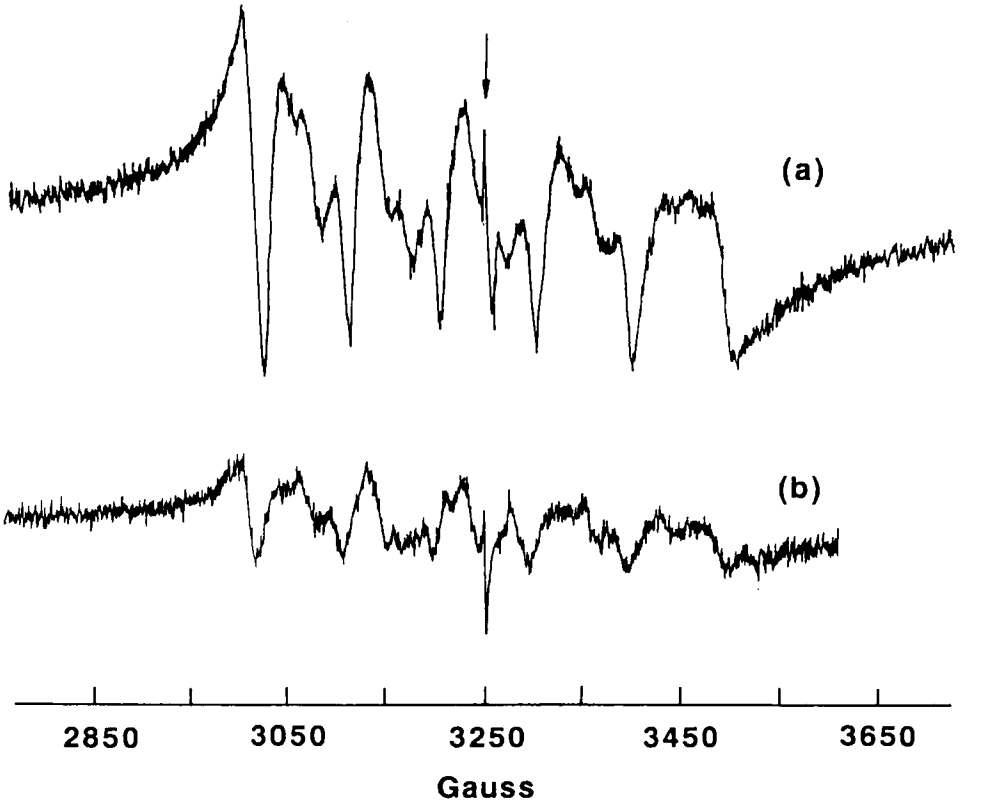

FIGURE 2 Curve (a), free radical (arrow) and Mn^{2+} signals from "Cab Mac" *red* wine (no contact with oak), curve (b), free radical and Mn^{2+} signal from *oak aged* semillon white wine.

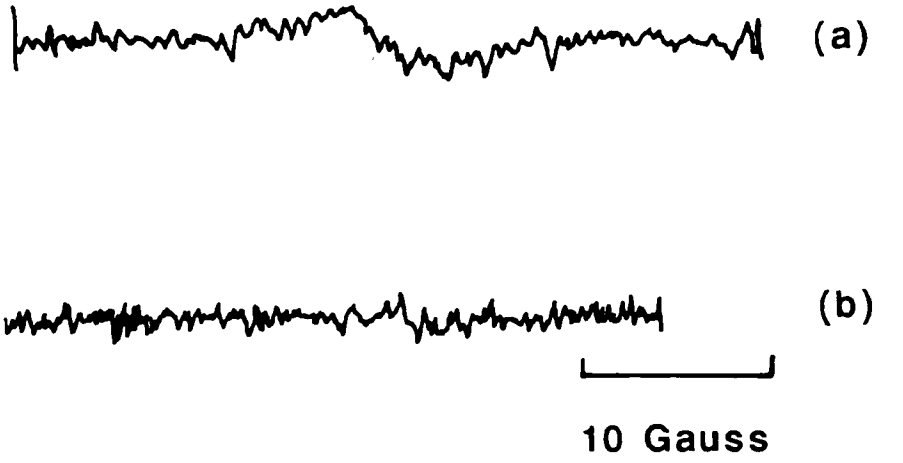

FIGURE 3 (a) Free radical signal from glycoside extract of shiraz grape juice (red); (b) spectrometer trace from glycoside extract of semillon grape juice (white).

to define exactly which classes of compounds might be adsorbed in each case. In order to make reasonable comparisons between the samples, 100 mL of each was treated with 10 g of the appropriate adsorbent. After about 30 minutes the adsorbent was filtered and the filtrate evaporated under reduced pressure, at a temperature of $\leq 50°$. At the same time 100 mL of untreated wine was also evaporated. The residues, usually in the form of sweet-smelling syrups which often partially crystallised on standing, were dissolved in water (~ 5 mL) for ESR examination. The adsorbents used were activated carbon, Amberlite® XAD-2 resin, and cross-linked polyvinyl polypyrrolidine. Amberlite® XAD-2 is a non-ionic polystyrene used for the adsorption of organic materials, and cross-linked polyvinyl pyrrolidine (PVPP) is a basic resin which adsorbs aromatic acids, aldehydes and phenols[5,6]. The most obvious visual effect of this treatment was removal of the red colour. This was most effectively achieved by charcoal and PVPP – the Amberlite® resin left a significant amount of colour in the filtrate.

On most occasions the samples were not evaporated to complete dryness to make it easier to prepare solutions for ESR analysis. However two samples were

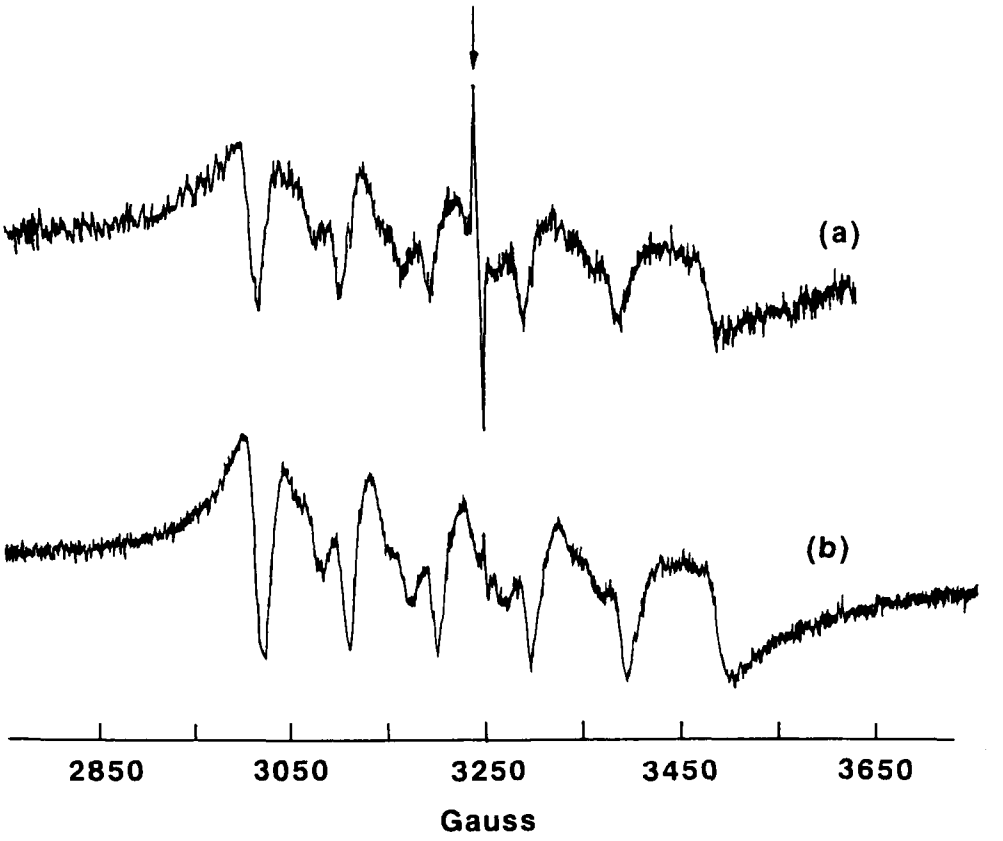

FIGURE 4 Curve (a), free radical (arrow) and Mn^{2+} signals from red wine (Brown Bros.); curve (b), signal from material remaining after treatment with PVPP. The free radical signal is slightly modulation broadened.

evaporated fully in order to estimate the amount of materials removed by PVPP. One sample (Tarrawarra red wine) had 4.0% (w/v) non-volatile material before resin treatment and the 4.0% afterwards – the other changed marginally from 3.2% to 3.1% (w/v) non-volatiles. These results indicate that the combined amount of the red colour, which is probably caused by anthocyanins, other colourless phenols, and the free radical represents no more than 3% of the non-volatile fraction, and no more than 0.12% of the total wine. Most of the non-volatile material remaining after the PVPP treatment consists of sugar(s).

The most profound effect on the free radical signal was achieved by PVPP which reduced the free radical signal by ~80%, while leaving the Mn^{2+} signal effectively the same (Figure 4).

DISCUSSION

Since treatment with PVPP is known to remove most of the phenolics, and also removes most of the ESR signal, this result is consistent with the free radicals being associated with the red wine phenolics. The concentration of phenolic compounds in white wines depends on the methods of grape processing, grape crushing and must preparation[7]. For example PVPP is routinely used to *remove* phenolics from white wines, to prevent the wines turning brown during maturation. No details on the preparations of the white wines used in our experiments were forthcoming from the manufacturers. Since some white wines have been shown to possess the characteristic ESR signal, a number of questions now arise. What is the function, if any, of the free radicals in the *in vitro* experiments on the inhibition of oxidation of human LDL by red wine phenolics reported in (1)? And does the ESR signal in certain white wines indicate the presence of the inhibitatory phenolics in these also? There are many people who have allergic reactions to the histamines in red wines, but enjoy white wines. Such subjects could therefore perhaps be recommended to drink certain white wines for their possible LDL oxidation inhibition; however, Halliwell[8] suggests that further work on the characterisation of the antioxidants should also be done.

Since free radicals can be beneficient, neutral or maleficent in human physiology, further work is in progress to attempt to identify the free radicals, using pulsed ESR; NMR, IR, and UV spectroscopy.

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