



EPR and Antioxidant Studies of Dark Chocolate, a Red Wine Vinegar and a Cider Vinegar

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Dark chocolate (>70% cocoa) contains Cu and polyphenols. The EPR has not been reported before. From both Swiss and Australian samples, it shows Cu⁺⁺ and a very strong free radical signal, as well as unreported Mn⁺⁺. Cider vinegar has a strong reputation in alternative medicine for healthgiving effects. Some might be due to antioxidants? Red wine vinegar must have some antioxidants. So both were examined by EPR and antioxidant efficiency tests. From a ‘reputable’ sample of each, the EPR spectrum was due to Mn⁺⁺, and there was no free radical signal. The Antioxidant efficiency for cider vinegar was 63%, and that for red wine vinegar, 85%: errors +/- 5%.

1. Introduction

EPR (Electron Paramagnetic Resonance, also ESR, E Spin R) is a well established spectroscopic technique [1] using electron spin precession in a constant magnetic field. It can detect free radicals and certain paramagnetic ions in appropriate valence states: eg, Cu⁺ is not detected, Cu⁺⁺ is. EPR therefore has many uses, including chemical analysis. Properties of the detected spectra can help to understand ionic surroundings, and the activity of free radicals.

Dark chocolate (>70% cocoa) contains Cu and polyphenols. The EPR spectrum has not been reported: it could show a Cu⁺⁺ signal, should show a strong free radical signal, and maybe others. Cider vinegar has an ‘alternative medicine’ reputation for its healthgiving properties [2]. Some of these could be due to antioxidant action. Red wine vinegar should contain some antioxidants, even if only the anthocyanins [3]. Antioxidant efficiency measurements are complementary to EPR. The comparisons are worth while: after all, vinegar is used in cooking and in salad ‘dressing’, and red wine vinegar is certainly part of the ‘Mediterranean diet’.

2. Sample preparation

Dark Swiss (85% cocoa) and Australian (75% cocoa) chocolates were purchased from a local supermarket. Samples were scraped from each with a plastic knife, and placed into standard quartz EPR tubes (Wilmad). The measurements were carried out with a Bruker x-band (~9.4 GHz) EPR spectrometer at room temperature (20C).

A ‘good’ imported Italian wine vinegar, and a ‘good’ imported American unfiltered (cloudy) cider vinegar were purchased from a local supermarket. The cloudy cider product was chosen because it might contain more antioxidants. Recent work published *after* our work was done has verified this surmise [4]. A sample of each was cold evaporated down to 1/10 of its original volume, and a portion of each sample was placed in a standard EPR tube. The preparation for the antioxidant test, as regularly used by this group, as well as the test itself, are described in [5]. In the test, free radicals are generated in a solution at a constant rate, giving an increasing absorption in the UV. The antioxidant will reduce this absorption rate, so

an efficiency can be determined. For the EPR work, it was necessary to freeze the samples, to avoid microwave losses.

3. Results

3.1 EPR results

The Swiss dark chocolate EPR spectrum is shown in Fig 1. The Australian specimen result is almost the same. The truncated signal is from the stable free radicals on the polyphenols [3]. The broad signal is from Cu^{++} , and some of the small narrow lines are from Mn^{++} , previously unreported [1]. A spectrum taken 6 weeks later on the same specimen left exposed in the laboratory showed a reduced Cu^{++} line, and the Mn^{++} and free radical lines had almost vanished. EPR of milk chocolate, under the same procedures, showed the Cu^{++} line, but the free radical line was enormously reduced, and somewhat broadened: milk products give such a line [6].

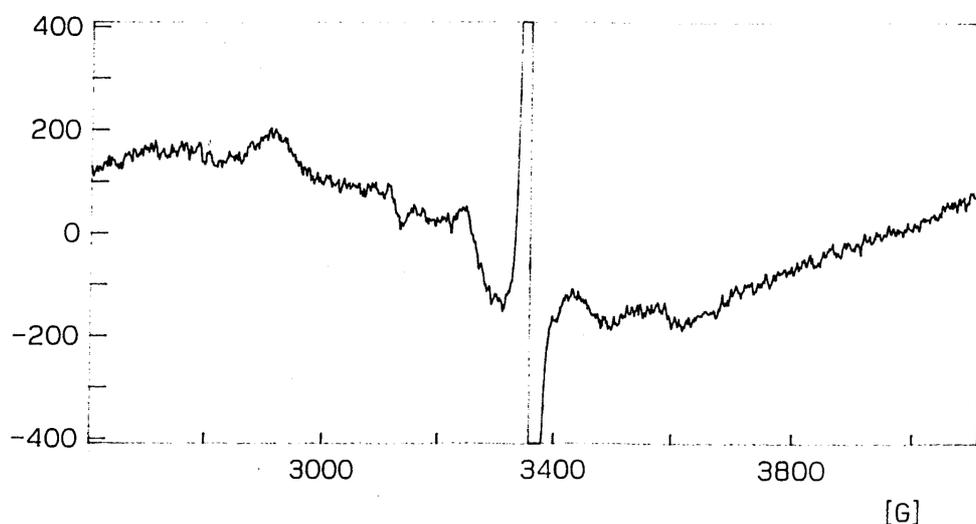


Figure 1. EPR spectrum of Swiss chocolate. Horizontal axis: magnetic induction, Gauss. Vertical axis signal strength, arbitrary units.

The wine and the cider vinegar spectra, taken under the same conditions, are almost identical (except that of the cider vinegar is less intense) so only the red wine vinegar spectrum is shown (Fig.2). The signal is from Mn^{++} , very similar to that from wines [3].

The vinegar spectrum display is phase reversed with respect to the chocolate one. The transition is between the electron spin states ($+1/2$) to ($-1/2$): the six large peaks are due to hyperfine structure, since the Mn nuclear spin is $5/2$. The small lines in between are due to ‘forbidden transitions’, showing that the Mn ion is in a comparatively low symmetry site for both vinegars. A free radical signal would be a single narrow line, placed at the centre of the Mn^{++} spectrum. Note that the free radical signal for chocolate is not at the centre of the broad line, which helps identify it as Cu^{++} .

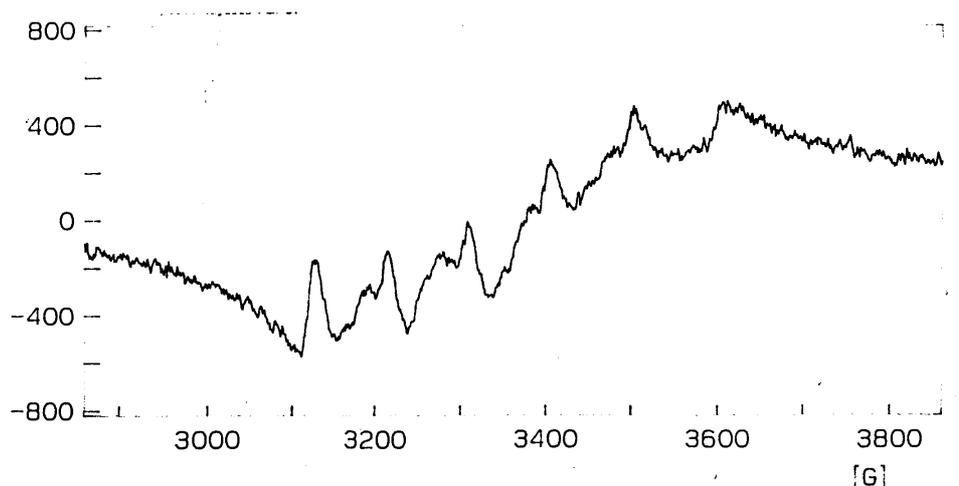


Figure 2. EPR spectrum of red wine vinegar. Horizontal axis: magnetic induction, Gauss. Vertical axis, signal strength (arbitrary units).

3.2 Antioxidant efficiency results.

The antioxidant efficiency for the red wine vinegar was 85% and for the cider vinegar, 63%, both +/- 5%, a significant difference.

4. Discussion

The dark chocolate contains Cu as Cu⁺⁺, as verified. The Mn⁺⁺ has not been previously reported. The diminution in all signal intensities after exposure to the air shows that the antioxidants are at work. The absence of the large free radical signal in the milk chocolate indicates that the inactivation of the polyphenol antioxidant action may already have taken place.

The similarity in the EPR spectra of the vinegars is to be expected, since both wine and cider are fermented fruit juices. The wine vinegar contains anthocyanins, the cider does not. Cu is known to ‘hide’ from EPR as Cu⁺, but is known to occur in wine. There must be fewer remaining polyphenols in the cider vinegar, because there would have been less to start with. So with hindsight, the results are explainable. Of course, further work on further samples needs to be done, but red wine vinegar has been a part of the ‘Mediterranean Diet’ for a long while!

References

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