Free Rad. Res., Vol. 23, No. 4, pp. 393–399
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THE CHEMICAL ORIGIN OF FREE RADICALS IN COFFEE AND OTHER BEVERAGES

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(Received September 31st, 1994; in revised form, February 2nd, 1995)

Sugars or carbohydrates are identified as the source of free radicals in coffees, ersatz coffees, a number of other food flavouring and colouring agents formed by processes involving heating, and in beers and stouts. The radicals are not derived from phenolic constituents, in contrast to those in wine, and are unlikely to be due solely to the occurrence of Maillard reactions.

KEY WORDS: Coffee, ersatz coffee, Electron Spin Resonance

INTRODUCTION

The presence of free radicals in food and in living organisms has been known for many years[1] and has attracted very considerable recent interest.[2,3] It is important to establish the chemical nature of the radicals present in foods in order to be able to understand their physiological effects, claimed to include both good and bad effects on cancer and heart disease. We recently reported that the radicals in wine could be almost completely removed by treatment with poly(vinyl pyrrolidone) (PVPP) – a resin capable of selectively extracting phenolic components. This correlated with inhibition of lipid oxidation by red wine phenolics[4] and led us to wonder whether the radicals already found in coffee and other beverages would also have the same chemical origin and possibly similar physiological effects. So we have investigated, using Electron Spin Resonance (ESR) spectroscopy, the effect of some selective extracting reagents on the free radical signals from a number of different food products.

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. g-Values were determined by measuring the magnetic field strength using proton resonance, and measuring the microwave frequency directly with an electronic counter.

We investigated the free radical signals from Moccona® coffee, Cooper's® stout,

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Kraft® Bonox, Gravox®, Soy sauce, Worcestershire sauce and Parisian Essence which were all obtained commercially and in caramel beer colouring which was donated by Carlton and United Breweries.

Standard extraction procedure

An aqueous suspension of instant coffee (20.0 g) in water (200 ml) was stirred with Amberlite® XAD-2 (50.0 g – previously washed with deionised water to remove chloride) for 30 min. The resin was collected and washed thoroughly with water. It was then filtered and washed with 80:20 methanol:water (3 × 50 ml). The aqueous methanol was evaporated under reduced pressure to provide a brown crude coffee extract. This was further separated into a soluble fraction (2.9 g) showing essentially no ESR absorption and an insoluble fraction (2.12 g) which was a light brown powder (2.12 g) with a strong ESR signal, $g = 2.0040 \pm 0.0006$.

This general procedure was used to isolate radical fractions from Caro, Ecco, Cooper's Stout (350 ml), Kraft Bonox (35.0 g), Gravox (20.0 g), Parisian Essence (100 ml), Caramel beer colouring (30 ml), and caramelised glucose (25.0 g).

Use of polyvinylpyrrolidone

A sample of instant coffee (20 g) in water (200 ml) was treated with polyvinylpyrrolidone (50 g). After 30 min the suspension was filtered and the filtrate concentrated to ca. 5 ml under reduced pressure. The solution showed a strong ESR signal, $g = 2.0040 \pm 0.0006$.

Caramelisation of glucose

A slurry of (+)-glucose in water was heated at ~150° until a caramelisation reaction had occurred to give a dark brown product. The solid which formed was treated with XAD-2 resin as described above. The soluble fraction showed a very weak ESR signal ($g = 2.0043 \pm 0.0006$) and the insoluble fraction a strong signal ($g = 2.0038 \pm 0.0006$).

DISCUSSION

Troup *et al.*[5,6] have established the presence of free radicals in both roasted and instant coffees as well as in various types of wine.[7] Although green coffee beans from a variety of locations showed no electron spin resonance (ESR) signal, roasted beans and instant coffee gave a strong signal ($g = 2.0040 \pm 0.0006$).[5] The signal persisted in solution for several weeks and showed no evidence of hyperfine splitting. Subsequent work showed that imitation coffees (such as Ecco and Caro) and stouts, all of which are based on roasted barley derivatives, also showed similar paramagnetic properties in solution.[8] Decaffeinated coffees give a similar signal.[5] Of all the beverages so far examined, only teas, produced by low temperature fermentation and subsequent dehydration, showed no ESR signal.[5] In the case of wine the radicals appear to be associated with the tannins and can be removed almost completely, along with the colour, by treatment with polyvinylpyrrolidone. No evidence has been obtained yet regarding the chemical structure(s) responsible for the radical signals in the brewed beverages.

Coffee beans are roasted, and analysis of the chemistry of a number of food products has revealed that "browning" due to cooking may be a source of free radical activity.[9]

In this process sugars react with amino-acids to produce soluble free radicals, in a process known as the Maillard reaction. This reaction produces an enormous range of products, because of the range of amino-acids and sugars found in natural foods, but many are based on 1,4-disubstituted pyrazines.[10] The lifetime of the radicals, first indicated by a narrow line with hyperfine structure which rapidly loses structure and becomes broader, is much shorter than those we have found.

It has been known since at least 1849[11] that coffee contains phenolic compounds which are obviously capable of conversion into free radicals. In view of our discovery that the radicals in wine were based on phenols it seemed reasonable to speculate that the polyphenolic compounds of coffee may have been at least in part responsible for the ESR signals. Previous workers have given a detailed procedure for the isolation of feruloyl quinides from coffee involving adsorption on XAD-2 resin followed by chromatography on silica.[12] These compounds represent part of the phenol fraction and could cause an ESR signal. However, using this procedure we quickly found that the ESR signal was not associated with the quinide fraction although it was present in the material extracted by the XAD-2 resin.

The lack of involvement of any other phenolic component was then established by treatment of the crude coffee extract with polyvinylpyrrolidone (PVPP). PVPP is a basic resin which is a very effective adsorbent for aromatic acids, aldehydes and phenols.[13] This removed some colour from the extracts, but, in contrast to the experience with wine,[5] failed to remove the radical signal (Figure 1).

We further extended the investigation to a number of food flavourings and additives which share a key feature during their preparation. Thus coffee is prepared by roasting coffee beans and Ecco and Caro are derived from roasted barley. The production of Parisian Essence, Caramel, Bonox, Gravox, Worcestershire sauce and Soy sauce all

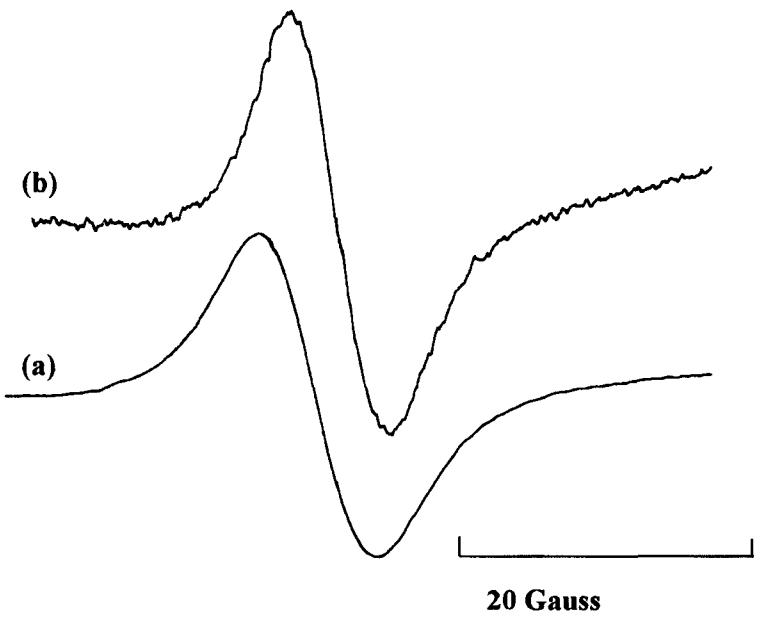

FIGURE 1 ESR signal from methanol-insoluble coffee fraction (a) before and (b) after treatment with PVPP.

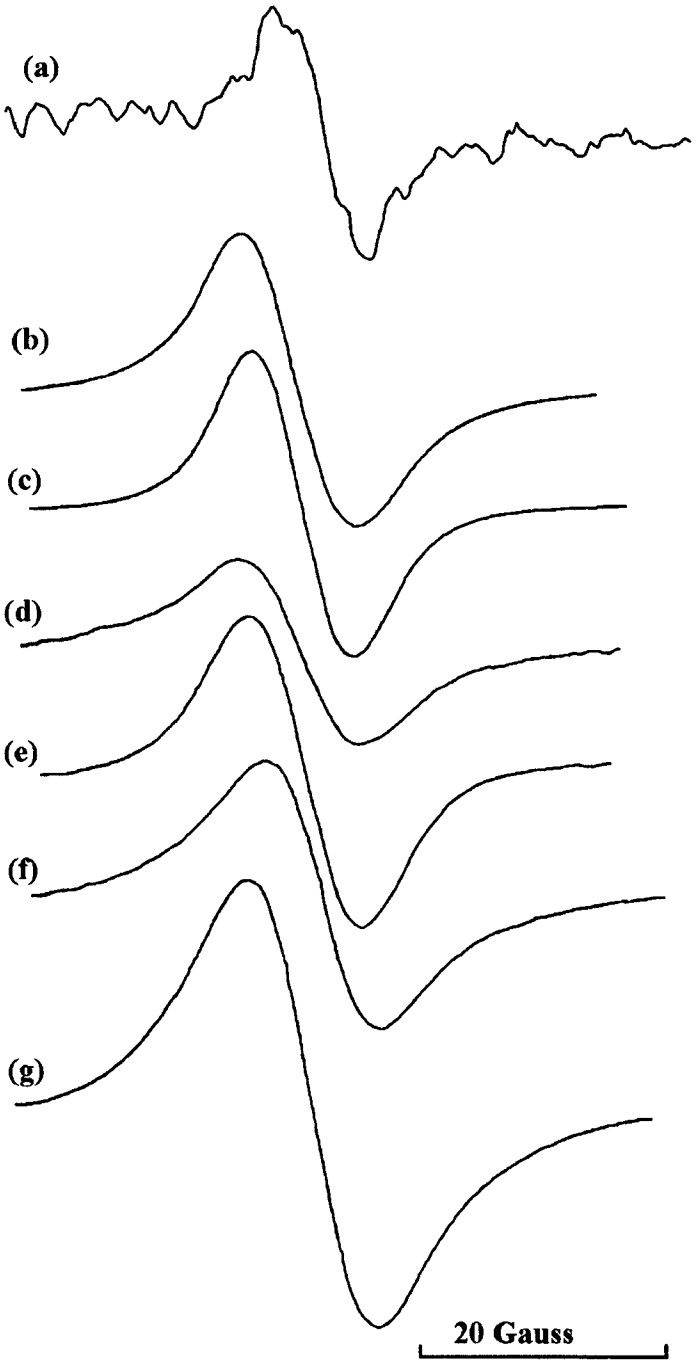

FIGURE 2 ESR signals derived from (a) weak pitch (reference sample) and methanol-insoluble fractions from (b) Gravox, (c) Coffee, (d) Bonox, (e) Stout, (f) Parisian Essence and (g) Caramel.

involve the heating of grains or other carbohydrate containing material. Bonox and Gravox are meat extracts which both contain caramel food colouring. Parisian Essence and Caramel are derived by heating sugars in the presence of ammonia (ie. the Maillard reaction) and are used for colouring of beverages such as beer and stout.

These beverages and flavouring materials were extracted using a simplified procedure. The sample, either suspended in water, or used as the supplied liquid was treated with XAD-2 resin to adsorb most of the organic material. The filtered resin was then washed with water and the organic materials desorbed from it with an aqueous methanol solution. This was evaporated to leave a solid residue which was separated into methanol soluble and insoluble fractions which were examined spectroscopically. In all cases the methanol insoluble fraction was responsible for the bulk of the ESR signal. Typical examples of the ESR spectra obtained are shown in Figure 2. The intensity of the ESR signals was not affected by treatment with PVPP resin.

Other spectroscopic analysis of the insoluble fractions using infrared and nuclear magnetic resonance spectroscopy showed the material to be mainly carbohydrate with spectra indicative of sugars and showing little sign of signals from aromatic groups.

The radicals are remarkably stable and withstand a number of vigorous chemical treatments such as acetylation, oxidation and reduction, and treatment with strong acids and bases.[5] In the case of the signals extracted from wine the signals are not attenuated by treatment with a good radical scavenging system (superoxide generated by xanthine/xanthine oxidise and trapped by TMPO).[14] Microanalysis showed a very low nitrogen content (< 2%) in the radical-containing extracts implying that most of

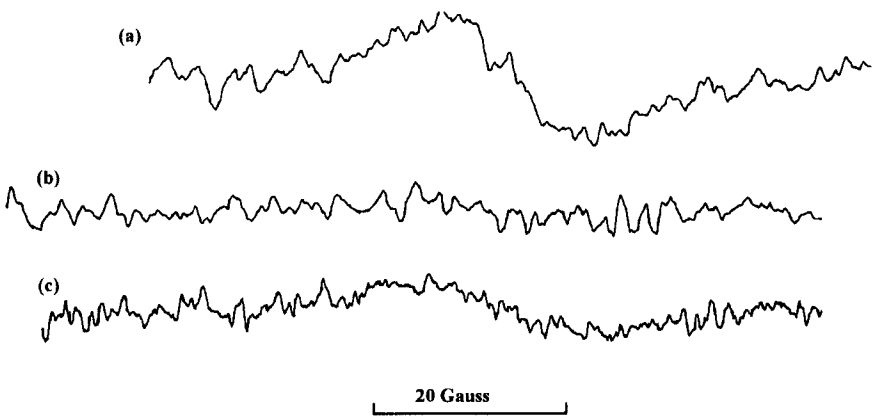

FIGURE 3 ESR spectra of sugar. (a) Heated aqueous slurry, (b) and (c) briefly melted in ESR tube.

the material was simply carbohydrate and little, if any, was derived from Maillard reactions. It therefore was necessary to ask if the radical was derived from sugar alone. Sucrose in an ESR tube was heated briefly to its melting point. This produced a very pale yellow melt which on cooling showed a strong ESR signal ($g = 2.0043$), very similar to that from coffee. It was not possible to produce an entirely colourless product and completely prevent caramelisation which sets in at the melting point. In another experiment an aqueous slurry of glucose was heated at ~ 150° until a dark brown product had formed. This could be separated into methanol soluble and insoluble fractions. The insoluble fraction showed a strong ESR signal and infrared and ultraviolet absorption very similar to the other extracts (Figure 3).

Hence the formation of a radical signal in solution requires no amino-acid or other source of nitrogen and the signal-generating product could be derived solely from carbohydrates such as sugar, starch or even cellulose. This would explain the ubiquity of free radicals in roasted beverages. The signal is probably absent from tea in solution because its production involves ambient temperature fermentation rather than roasting, and the drying temperature is comparatively low (110–120°C) in comparison to coffee (~ 220°C) and dark roasted barley (~ 220°C).

CONCLUSION

ESR studies supported by simple chemical fractionation shows that radicals in coffee and a number of other foods prepared by a roasting process are derived from sugars or other carbohydrates and not from phenols. This is in contradistinction to the situation in wines where the radicals are clearly formed from phenolic tannins.

TABLE
ESR signals of XAD-2 extracts (Methanol insoluble fractions)

Sample	E.s.r.g-value
Coffee	2.0040
Cooper's stout	2.0039
Bonox	2.0041
Gravox	2.0043
Parisian Essence	2.0039
Caramel	2.0042
Heated glucose	2.0038
Worcestershire sauce	2.0038
Soy sauce	2.0038

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Accepted by Dr B Kalyanaraman