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Free radical reactions involving coffee

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Instant coffee solutions were shown by electron paramagnetic resonance (EPR) spectroscopy to act as prooxidants by enhancing the production of ascorbyl radicals from ascorbic acid. The coffee solutions were also shown to act as scavengers of superoxide radicals in competition with a spin trap. The radical scavenging capabilities of several components of coffee were similarly investigated. There was little difference between the radical scavenging abilities of regular and decaffeinated coffee solutions.

INTRODUCTION

The formation of stable free radicals during the roasting of coffee beans is well known (Troup *et al.*, 1989); some of these free radical centres are found in green beans, but the majority are formed during the roasting process. Their electron paramagnetic resonance (EPR) spectrum yields a single line typical of charred polysaccharides resulting from a complex mixture of carbon and oxygen-centred free radicals. These free radicals are, however, considered to be of little biological relevance due to their great stability.

One potentially beneficial property of free radicals is their ability to scavenge other, perhaps more reactive and therefore potentially toxic, free radicals through annihilation reactions of the type:

$$A\cdot + B\cdot \rightarrow AB$$

Examples of such reactive free radicals are superoxide ($O_2\cdot^-$) and hydroxyl ($HO\cdot$) both of which have been linked to degenerative processes in humans (Halliwell, 1987). Such free radicals are generated during the digestion of food and are then scavenged by interaction with other dietary components and the gut lumen. A potentially detrimental property of the stable free radicals in coffee and other charred polysaccharides could be to 'use up' such dietary free radical scavengers, thus reducing the ability of the body to control the reactions of metabolically generated free radicals.

The present study investigates the properties of the soluble solids of coffee as free radical scavengers or as deactivators of other free radical scavengers. The free radical scavenging capacity of coffee was investigated by using electron paramagnetic resonance (EPR) spectroscopy to measure the extent to which the coffee competes with a chemical spin trap for the superoxide radical anion. In an attempt to elucidate which component or components of coffee might be responsible for any radical scavenging ability, decaffeinated coffee was also investigated as were caffeine, theobromine, caffeic acid and chlorogenic acid (3-caffeoyl quinic acid). In addition, the ability of coffee to deactivate other free radical scavengers was monitored by using EPR to compare the quantities of ascorbyl radical in solutions of ascorbic acid, one of the major dietary antioxidant molecules, with and without added coffee.

MATERIALS AND METHODS

Coffee

As the system of interest concerned aqueous extracts of coffee, solutions of instant coffee (Nescafé and Nescafé decaffeinated) were used in order to facilitate the preparation of standard extracts.

EPR Spectroscopy

Free radicals were detected directly in a Bruker ESP300E electron paramagnetic resonance spectrometer with a variable temperature accessory operating at 295 K unless otherwise stated. Sample solutions were contained in a reduced-volume flat quartz cell (Wilmad, Fluorochem, Old Glossop, Derbyshire, UK). Instrumental parameters were: microwave power 10 mW; modulation amplitude 0·1 mT; modulation frequency 100 kHz; sweep width 2 mT for the ascorbyl experiments and 10 mT for the radical scavenging investigations.

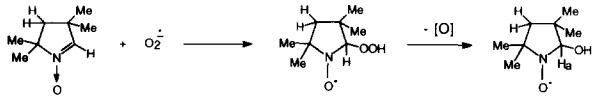
Prooxidant activity

Thermal generation of the ascorbyl radical in the temperature range 300–350 K was monitored by recording the EPR spectrum of 0·5 M solutions of ascorbic acid (Aldrich, Gillingham, Dorset, UK) in K_2HPO_4/KH_2PO_4

buffer at pH 7·4 in the presence of varying concentrations (0–10 mg.ml^{-1}) of instant coffee aqueous solutions.

Spin trapping

Reactive free radicals such as hydroxyl have too short a lifetime in aqueous solutions to be detected directly by EPR; others such as superoxide have relaxation properties which render them undetectable at ambient temperatures and above. Both types of radical can be detected by spin trapping. The technique of spin trapping consists of the addition to experimental solutions of a spin trap, in this case 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (TMPO), which reacts with the superoxide to form a radical which can be monitored by EPR.



The superoxide adduct of TMPO is not persistent and rearranges rapidly to the HO adduct (Janzen *et al.*, 1981). The EPR spectrum of this species has a characteristic 4 line appearance (Fig. 2a) with the central two lines split resulting from two nearly overlapping 1 : 1 : 1 triplets split by the ^{14}N coupling of 1·532 mT and the coupling to the proton H_a of 1·660 mT (*cf* Janzen *et al.* average values of 1·529 and 1·681 mT respectively for the hydroxyl adduct as opposed to 1·567 and 2·002 mT for the peroxyl adduct).

Antioxidant activity

Antioxidant activity was investigated as the ability of the test substance to compete with 0·06 M TMPO in 0·05 M phosphate buffer at pH 7·8 for superoxide radicals generated by a xanthine/xanthine oxidase system (Rice-Evans *et al.*, 1991). The presence of TMPO spin adducts was monitored by EPR spectroscopy. Xanthine (99–100%), xanthine oxidase (buttermilk, 0·5 I.U. mg^{-1}), caffeine, theobromine, caffeic acid and chlorogenic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-[3,4-dihydroxycinnamate]) were purchased from Sigma, Fancy Road, Poole, Dorset, UK).

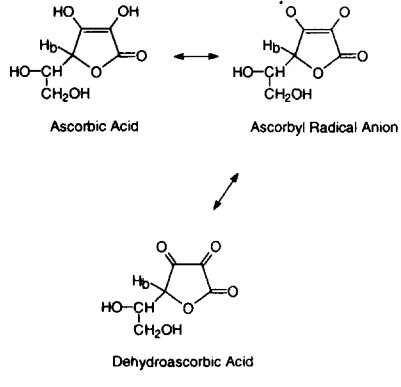
Data handling

TMPO concentrations were kept low to minimise interference with the enzyme; the concomitant low signal: noise ratios of the spectra make it difficult to measure signal intensities directly from the spectra with any accuracy. Spectra were analysed using a quantified maximum entropy method (MaxEnt Systems Ltd, Ely, Cambs, UK). In an ideal world, EPR spectral absorptions would be sharp lines whose height represented the intensity of absorption. In the real world, such lines are broadened by molecular and instrumental effects. By using a single line in a spectrum to represent such 'spread' of the data, MaxEnt uses probability theory to calculate the most probable set of positions and intensities of such lineshapes consistent with the experimental data. Hence in these experiments, a single line from the control spectrum was used to define the lineshape and deconvolute that spectrum into its two overlapping 1 : 1 : 1 triplets. A triplet lineshape was then used wherever possible, to analyse the other spectra and the intensity of the resulting doublets was calculated for each spectrum. In the spectra where the concentration of other species was greatly in excess of that of the TMPO-hydroxyl adduct, single lines were used for the analysis. Thus MaxEnt allowed the determination of the concentration and error estimates, of a known signal (and hence lineshape) in dilute solutions where derivation of the result by measurement of peak heights would have been impossible due to the low signal:noise ratio. Although spectral accumulation would have improved the signal-to-noise to allow peak height measurement, there are potential problems if the spin adducts are of limited stability and MaxEnt's capability to calculate the errors associated with both line position and intensity could only be attained by several accumulated runs to allow calculation of means and standard deviations. Further details of MaxEnt applied to EPR spectra are contained in Goodman *et al.* (1994).

RESULTS AND DISCUSSION

Prooxidant activity

Ascorbic acid is oxidised by dissolved oxygen in water to form the ascorbyl radical



which has a characteristic EPR spectrum of a 0·18 mT doublet resulting from the proton (H_b) splitting. The effect of instant coffee on the formation of the ascorbyl radical is shown in Fig. 1.

It can be seen that the amount of ascorbyl radical remains constant below 320 K and rises to around three times the initial level by 350 K. In the presence of 10 mg

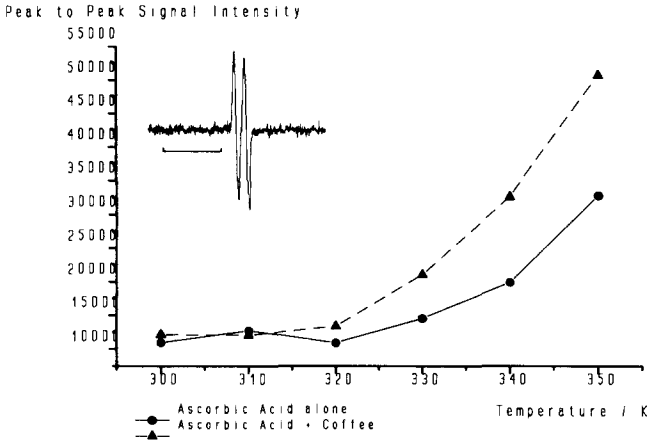


Fig. 1. Formation of the ascorbyl radical as a function of temperature in the presence and absence of 10 mg ml^{-1} instant coffee. Inset: EPR spectrum of the ascorbyl radical. Bar = 1 mT.

ml^{-1} instant coffee, a slight increase in free radical formation is seen at 320 K and ascorbyl radical formation continues to increase at a faster rate than the control reaching an approximate 5-fold enhancement over the 300 K level at 350 K, an increase of around 50% over the control. Thus coffee is acting as a pro-oxidant; it is a source of free radicals which are oxidising the ascorbic acid molecule to its radical ion which in turn will be oxidised to dehydroascorbate. These coffee free radicals would not in themselves pose a problem as they can obviously be scavenged effectively; they would, however have the effect of reducing the antioxidant capacity of the system but only at elevated temperatures; coffee is not commonly drunk at temperatures much above 330 K.

Antioxidant activity

The ability of a solution of coffee to compete with spin trap for superoxide free radicals is shown in Fig. 2 where it can be seen that the amount of TMPO adduct decreases as the concentration of coffee increases. Thus the coffee is acting as a free radical scavenger.

Decaffeinated instant coffee and various components of coffee were then investigated in the same manner for their abilities as radical scavengers.

The results are summarised in Figs 3 and 4. From Fig. 3 it can be seen that regular coffee and decaffeinated coffee followed similar trends. Caffeine, however, exhibits scavenging activity (Fig. 4), thus indicating that the antioxidant activity of coffee must come from more than one component. The related dimethylxanthine, theobromine, was also investigated and brought about a rapid reduction of the spin trap signal and the appearance of another radical species with the extensive hyperfine structure shown in Fig. 5(a).

A similar but less intense signal is obtained from the hydroxyphenols, caffeic acid and chlorogenic acid (Fig. 5(b),(c)), which were also investigated for free radical scavenging properties (Fig. 4) since they are both thought to be antioxidants (Pratt, 1965, 1976); the levels of chlorogenic acid in coffee are much reduced by

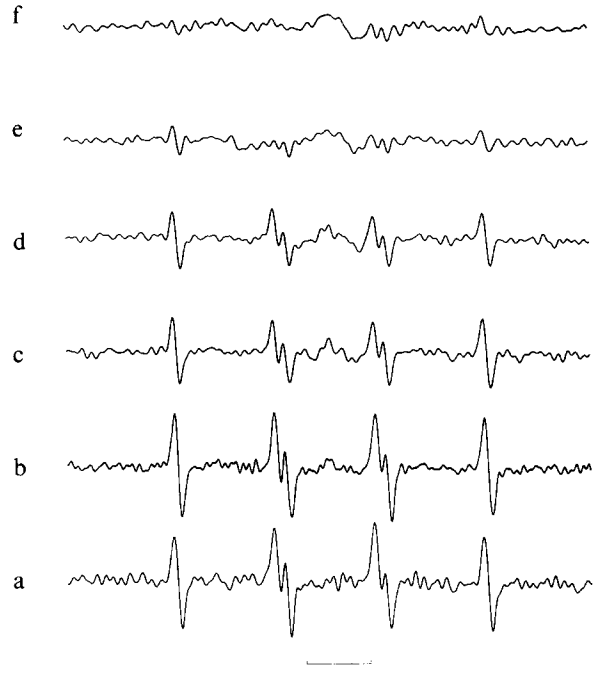
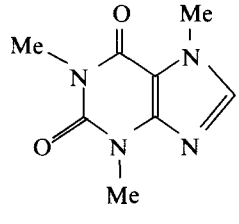
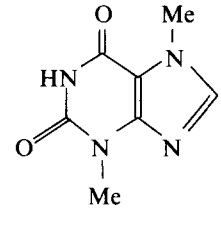


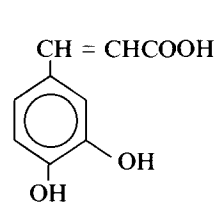
Fig. 2. EPR spectrum of TMPO/xanthine/xanthine oxidase in the presence of different concentrations of instant coffee. (a) 0%; (b) 0·2%; (c) 0·4%; (d) 0·6%; (e) 0·8%; (f) 1·0% coffee. Bar = 1 mT.



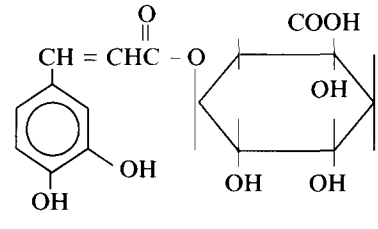
Caffeine



Theobromine



Caffeic acid



Chlorogenic acid

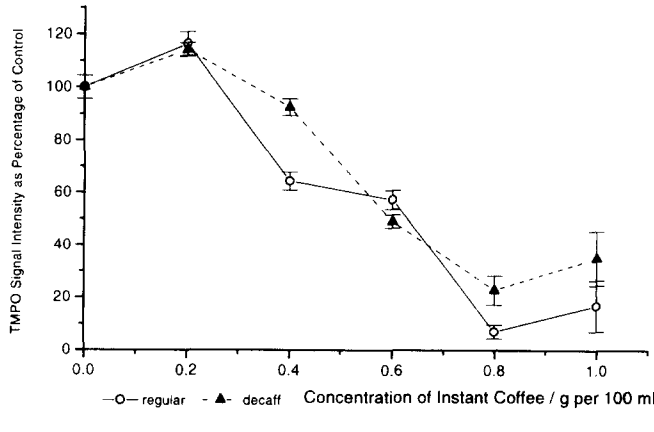


Fig. 3. Coffee as a competitor with TMPO.

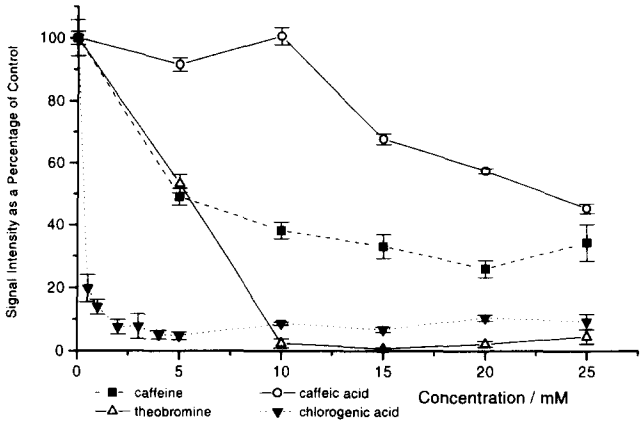


Fig. 4. Components of coffee as competitor with TMPO.

roasting (Merritt & Procter, 1959; Häuserman & Brandenberger, 1961; Clifford & Wight, 1976), to the extent that their measurement can be used to estimate the severity of roast for a given type of bean or blend (Clifford, 1979).

When the signal intensity of the spin adduct is plotted as a function of the concentration of coffee, or the components investigated (Figs 3, 4), it can be seen that theobromine and chlorogenic acid reduce the TMPO adduct signal to low levels at lower concentrations than do caffeine and caffeic acid, which both exhibit a more gradual decrease in TMPO adduct concentration to no less than one-third of the control value. This suggests that superoxide itself rather than the TMPO adduct is scavenged by these latter components and that an equilibrium of superoxide between spin trap and caffeine or caffeic acid is attained. The highest concentration of coffee used reduced the TMPO adduct to 16% of control, whereas the decaffeinated coffee appeared to be a less effective competitor with TMPO, with a reduction to 35% of control. This last result is of interest in that many studies of the effect of caffeine in coffee on various aspects of health have shown that there is no difference in dyspepsia induction by regular or decaffeinated coffee (Elta *et al.*, 1990), and no effect of caffeine on blood lipids (MacDonald *et al.*, 1991), blood pressure (Bak & Grobbee, 1991) and blood clotting (Bak *et al.*, 1990), on lipoprotein and serum cholesterol (Dusseldorp *et al.*, 1990) and an increase in LDL cholesterol and apolipoprotein B when subjects changed from regular to decaffeinated coffee (Superko *et al.*, 1991). Reports of the effects of caffeine intake on fertility are conflicting (Wilcox *et al.*, 1988; Joesof *et al.*, 1990) although the more recent study which found no effect, used a much larger sample size. Caffeine was found to be the only non-mutagenic compound of six coffee components tested with the *Salmonella* Ara test (Arizia *et al.*, 1988). Of the mutagenic activity, 40–60% was found to be due to hydrogen peroxide with chlorogenic acid the weakest of the mutagens tested. Beneficial effects of the antioxidants in coffee were suggested by the finding (Jacobson *et al.*, 1983) that subjects given a diet artificially low in phenolics including caffeic acid experienced symptoms of malaise.

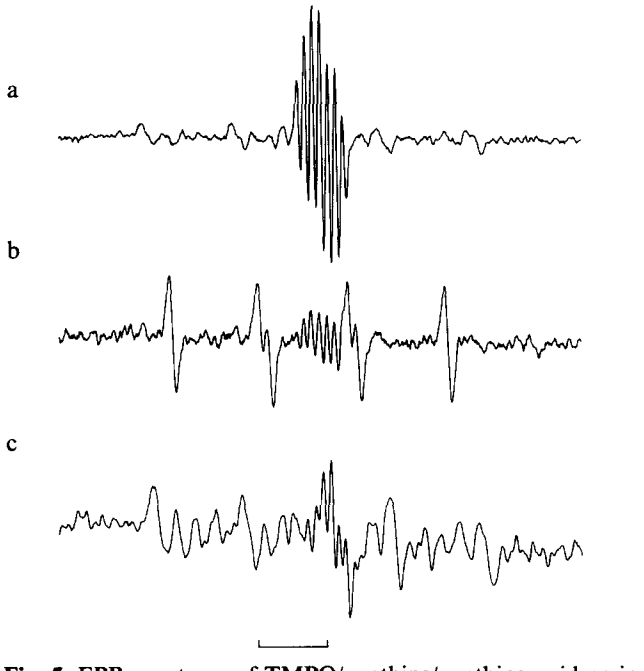


Fig. 5. EPR spectrum of TMPO/xanthine/xanthine oxidase in the presence of 10 mM of (a) theobromine; (b) caffeic acid; (c) chlorogenic acid Bar = 1 mT.

CONCLUSIONS

Instant coffee solutions can act both as source and sink for free radicals. Several of the components of coffee show radical scavenging properties which could account for at least part of coffee's scavenging capacity; the similarity of the results from regular and decaffeinated coffees indicates that caffeine, although possessing radical scavenging ability, is not the principal source of this activity in instant coffee.

REFERENCES

Arizia, R. R., Dorado, G., Barbancho, M. & Pueyo, C. (1988). Study of the causes of direct-acting mutagenicity in coffee and tea using the Ara test in *Salmonella typhimurium*. *Mutation Res.*, **201**, 89–96.

Bak, A. A. A., & Grobbee, D. E. (1991). Caffeine, blood pressure and serum lipids. *Am. J. Clin. Nutr.*, **53**, 971–5.

Bak, A. A. A., Van Vliet, H. H. D. M. & Grobbee, D. E. (1990). Coffee, caffeine and hemostasis: results from two randomized studies. *Atherosclerosis (Shannon, Ireland)*, **8**, 249–55.

Clifford, M. N. (1979). Chlorogenic acids — their complex nature and routine determination in coffee beans. *Food Chem.*, **4**, 63–71.

Clifford, M. N. & Wight, J. (1976). Measurement of feruoylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans. *J. Sci. Food Agric.*, **27**, 73–84.

Dusseldorp, M. van, Kata, M. B., Demacker, P. N. M. & Van Dusseldorp, M. (1990). Effect of decaffeinated versus regular coffee on serum lipoproteins. A 12-week double-blind trial. *Am. J. Epidem.*, **132**, 33–40.

Elta, G. H., Behler, E. M. & Colturi, T. .J. (1990). Comparison of coffee intake and coffee-induced symptoms in patients

with duodenal ulcer, nonulcer dyspepsia and normal controls. *Amer. J. Gastroent.*, **85**, 1339–42.

Goodman, B. A., Glidewell, S. M. & Skilling, J. (1994). The use of quantified maximum entropy methods for optimising information from electron paramagnetic resonance spectroscopy. *Free Rad. Res.*, in press.

Halliwell, B. (1987). Oxidants and human disease — some new concepts. *FASEB J.*, 1,358–74.

Häuserman, M. & Brandenberger, H. (1961). Plant phenols IV. Spectrophotometric methods for determination of chlorogenic acids and their application to plant extracts. *Z. Lebensm. Unters. Forsch.*, **115**, 516–27.

Jacobson, E.A., Newmark, H., Baptista, J. & Bruce, W.R. (1983). A preliminary investigation of the metabolism of dietary phenolics in humans. *Nutrition Reports — International*, **28**, 1409–17.

Janzen, E. G., Shetty, R. V. & Kunanec, S. M. (1981). Spin trapping chemistry of 3,3,5,5-tetramethylpyrroline-N-oxide: an improved cyclic spin trap. *Can. J. Chem.*, **59**, 756–8.

Joesoef, M. R. Beral, V., Rolfs, R. T., Aral, S. O. & Cramer, D. W. (1990). Are caffeinated beverages risk factors for delayed conception? *Lancet*, **335:8682**, 136–7.

MacDonald, T. M., Sharpe, K., Fowler, G., Lyons, D., Freestone, S., Lovell, H. G., Webster, J. & Petrie, J. C. (1991). Caffeine restriction: effect on mild hypertension. *Brit. Med. J. Clin. Res. Ed.*, **303:6812**, 1235–8.

Merritt, M. C. & Proctor, B. E. (1959). Effect of temperature during the roasting cycle of selected components of different types of whole bean coffee. *Food Res.*, **24**, 672–80.

Pratt, D. E. (1965). Lipid antioxidants in plant tissue. *J. Food Sci.*, **30**, 737–41.

Pratt, D. E. (1976). Role of flavones and related compounds in retarding lipid-oxidation flavour changes in foods. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*, ed. G. Charalambous & I. Katz. ACS Symposium Series No. 26, American Chemical Society, Washington, DC, Ch. 1, pp. 1–13.

Rice-Evans, C. A., Diplock, A. T., & Symons, M. C. R. (1991). Techniques in Free Radical Research. In *Laboratory Techniques in Biochemistry and Molecular Biology*, ed. R. H. Burdon & P. H. van Knippenberg. Elsevier, Amsterdam, p. 202.

Superko, H. R., Bortz, W. Jr, Williams, P. T., Albers, J. J. & Wood, P. D. (1991). Caffeinated and decaffeinated coffee effects on plasma lipoprotein cholesterol, apolipoproteins and lipase activity: a controlled, randomized trial. *Am. J. Clin. Nutr.*, *54*, 599–605.

Troup, G. J., Pilbrow, J. R., Hutton, D. R., Hunter, C. R. & Wilson, G. L. (1989). EPR detection of free radicals in (I) coffee and (II) gamma-ray irradiated foodstuffs. *Appl. Rad. Isotop. Int. J. Rad. Appl. Instr. A*, **40**, 1223–6.