

Transition metal ions in black tea: an electron paramagnetic resonance study

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Abstract Tea (*Camellia Sinensis*) is the most widely consumed beverage in the world and is known to have therapeutic, antioxidant and nutritional effects. It contains dimeric flavanols and polyphenols which are known as the most important organic compounds in tea infusions, and can make strong and stable complexes with metal ions. In this study, we carried out a series of electron paramagnetic resonance experiments on well-known paramagnetic transition metal ions, namely Mn^{2+} , Fe^{3+} , Cu^{2+} , VO^{2+} , and Cr^{3+} doped in black tea cultivated along the shore of Black Sea, Turkey, to see the effects and structures formed.

Introduction

Tea (*Camellia Sinensis*) is the most widely consumed beverage after drinking water all over the world. Green tea has been used as crude medicine in China and Japan for thousands of years and therefore the scientific community has intensified work on its therapeutic effects in recent years [1]. Tea is produced by over thirty countries in the world and consumed mainly in two forms: green (or non-fermented) tea and black (or fermented) tea. A semi fermented form, known as Oolong tea is also consumed in some Asian countries. Black tea has the highest consumption ratio at 75–80%. All forms are known to have nutritional, antimutagenic and antioxidant effects. Antioxidants are also known to play an important role in metal poisoning, as excellent scavengers of free radicals and ions, and chelators of heavy metals [2]. The antioxidant,

pharmacological, nutritional, and related properties of ingredients of both green and black tea have drawn the attention of scientists, and the work done so far has been reviewed and discussed [1–5].

Black tea supplied for human consumption contains approximately 10–12% catechins, 3–6% theaflavins, 12–18% thearugibins, 6–8% flavanols, 10–12% phenolic acid and depsides, 13–15% amino acids, 8–11% methylxanthines, 15% carbohydrates, protein minerals and some volatiles [6–9]. Figure 1 shows the structures of the main catechin components in black tea, from which the polyphenols, namely theaflavins, thearugibins, flavanols and phenolic acids, etc., are formed. Polyphenols are the most important ingredients among organic compounds in tea infusions and make strong and stable complexes with metal ions [10]. In this study, we report EPR studies on some transition metal ions doped into black tea infusions. The structures of the complexes are discussed.

Experimental

Black tea samples were bought from local markets including the products of the northeastern part of Turkey where the climate is suitable for tea cultivation along the shore of Black Sea. Chemicals were purchased from Merck.

All glassware was cleaned by soaking in dilute HNO_3 and rinsed with distilled water prior to use. EPR spectra were taken at room temperature. The amounts and concentrations of water extracts of black tea were prepared in a similar way as for daily consumption by following the ISO3103 standard; 2 g of dry black tea leaves were put in 100 mL boiled water, kept for 6 min and then filtered. Distilled water was used in all extraction processes.

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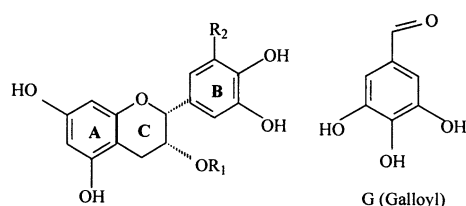


Fig. 1 The main polyphenol structures in black tea. Epicatechin [EC]: $R_1 = R_2 = H$. Epigallocatechin [EGC]: $R_1 = H$; $R_2 = OH$. Epicatechin-3-gallate [ECG]: $R_1 = \text{Galloyl}$; $R_2 = H$. Epigallocatechin-3-gallate [EGCG]: $R_1 = \text{Galloyl}$

Water extracts of black tea divided into separate cups with equal amounts. MnSO_4 , FeCl_3 , CuSO_4 , VO_4 and CrCl_3 was added to each cup with molarities changing between 0.01 M and 0.2 M at 70 °C, the average temperature of preparation for consumption. The samples were then left for slow evaporation in a clean oven operating at 40 °C. The dry extracts were put into quartz tubes, and EPR spectra were recorded. Pure extract was also dried for spectroscopic analysis.

The X-band EPR spectra were recorded using a Varian E-109 Line Century Series spectrometer equipped with a Varian E-231 TE-102 rectangular cavity. The microwave frequency and power were 9.52 GHz and 2 Mw, respectively. Temperature was controlled with a Varian temperature control unit. The spectra of tea samples were recorded in quartz sample tubes. The spectrometer frequency was corrected using DPPH (dihenylpicrylhydrazyl, $g = 2.0036$) as standard. Spectrum simulations were made using Bruker's WINEPR software.

Results and discussion

Figure 2a shows the EPR spectrum of black tea extract. The spectrum contains a broad envelope at $g = 2.00$ and a sextet superimposed on it with average hyperfine splitting of 9.4 mT, originating from a slightly distorted octahedral Mn^{2+} complex, a relatively weak broad shoulder at the low field side of the Mn^{2+} sextet, a weak line at $g = 4.32$, and a single sharp line at $g = 2.0022$. The components of the spectrum are similar to the previously reported structures [11–13]. The sharp and intense line at $g = 2.0022$ is attributed to the semiquinone radical. The two weak lines, one on the low field side of the sextet and the other as at $g = 4.32$, are attributed to different Mn^{2+} complexes with distorted tetrahedral symmetry as in previous work [11, 12]. The relatively weak line at $g = 4.32$, however, originates mainly from a different paramagnetic center and will be discussed below. The relative intensities of the components in the spectra may vary depending on the fermentation processes, storage conditions, age of leaves, geographic region and the climate [1, 7, 14, 15].

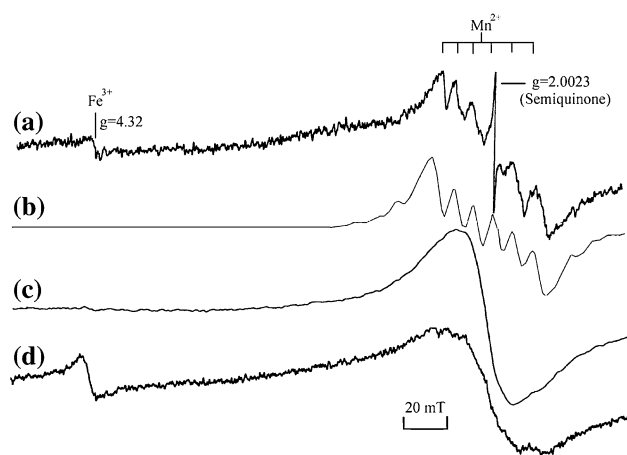


Fig. 2 **a** The EPR spectrum of black tea extract, **b** the simulation of the Mn^{2+} components of the black tea extract, **c** the EPR spectrum of Mn^{2+} and **d** Fe^{3+} doped black tea extracts

Ferrara et al. have determined the metal contents of tea samples taken from various regions by chromatography, and appreciable amounts of Mn, Fe, Al, Zn, Mg, Ni, Ca and Na were observed, whereas Cu was detected in relatively small amounts [8]. EPR spectra of tea show only paramagnetic Mn^{2+} and Fe^{3+} clearly; the transition lines of other paramagnetic metals, like Cu^{2+} , as complex structures and oxides or hydroxides are relatively weak and are probably lying under the broad envelope.

In the following paragraphs, the results of the introduction of small amounts of excess paramagnetic metals, namely Mn^{2+} , Fe^{3+} , Cu^{2+} , VO^{2+} and Cr^{3+} as dopants in the tea catechin will be discussed.

Mn^{2+} ions in black tea

Mn^{2+} with $3d^5$ ($^6S_{5/2}$) configuration exists in high spin state ($S = 5/2$). Therefore, the EPR spectra must fit to the standard Hamiltonian for Mn^{2+} complexes given in references [16–20].

Since the samples were in glassy state, the anisotropic values of g , hyperfine and zero field splitting are seen in the spectra, Fig. 2a. The average g and hyperfine values are typical for Mn^{2+} complexes in slightly distorted octahedral symmetry. For such complexes, the g value is relatively anisotropic, changing between 2.02 and 1.98 and averaging to $g = 2.00$. The anisotropy in hyperfine splitting, on the other hand, is small compared to the hyperfine value itself, changing around 8.5 ± 1.5 mT [7, 11, 12, 19, 20]. In the tea samples used in the present study, it was measured as approximately 9.4 mT. The zero field splitting parameters D and E for the complex under study, however, are relatively small to fit to slightly distorted octahedral structure of the complex [7, 11, 12, 16, 19, 20]. In other words, the

Table 1 Measured EPR parameters from the metal ion doped black tea extracts

<i>Mn²⁺ doped tea extract</i>			
$g_{\text{avg}} = 2.00$	$a_{\text{avg}} = -9.4 \text{ mT}$	$D = -9 \text{ mT}$	$E = 4 \text{ mT}$
<i>Cu²⁺ doped tea extract</i>			
$g_{//} = 2.26$	$g_{\perp} = 2.05$	$A_{//} = 15 \text{ mT}$	$A_{\perp} = 3 \text{ mT}$
<i>VO²⁺ doped tea extract</i>			
$g_{//} = 1.93$	$g_{\perp} = 1.98$	$A_{//} = 18 \text{ mT}$	$A_{\perp} = 8 \text{ mT}$

The calculated parameters are also given

absolute value of D must be of the order of the hyperfine splitting and that of E must be smaller.

High-spin Mn^{2+} being an S-state ion, the hyperfine coupling is nearly isotropic. Theoretic unrestricted Hartree–Fock calculations on high spin Mn^{2+} complexes have shown that this isotropic coupling constant arises via polarization of inner electrons with negative spin, so in general A is presumed negative [19]. We also suppose A to be negative. If the separations between the sextets decrease on the high-field side, then D/A will be positive so one can estimate the relative sign of D . In the present work, simulation of the dilute Mn^{2+} with $D = -9 \text{ mT}$, $E = 4 \text{ mT}$ values gives successful result Fig. 2b. Since the samples are in glassy state, we cannot carry out detailed analysis on the sextets on high field. But from the simulation values we suppose that separations between the sextets decrease on high field.

The sign and magnitude of D depends on the symmetry of the metal ion. Negative values of D are related to tetragonal elongation or trigonal compression [19, 20]. The values determined are given in Table 1. The D and E values are smaller compared to those of most Mn^{2+} complexes, and D is greater than E , which reflects that the distortion is also smaller and mainly axial.

The broad envelope belonging to the Mn^{2+} ion seems to be symmetric for both pure tea, Fig. 2a, and MnSO_4 doped tea extracts, Fig. 2c. Spectra of MnSO_4 doped extracts with various molarities show that the intensity of the Mn^{2+} envelope at $g = 2.00$ increases for all Mn^{2+} concentrations. The intensities of other components and especially the broad shoulder on the low field side of the Mn^{2+} sextet, however, do not change, while the sextet disappears because of broadening due to dipolar interactions as the Mn^{2+} concentration is increased, Fig. 2c. The other components of the spectrum, including the weak line at $g = 4.32$ remain unaffected after this process.

Fe^{3+} ions in black tea

Paramagnetic $^{57}\text{Fe}^{3+}$ isotope (nuclear spin $I = 1/2$) has natural abundance of 2.12%. Therefore, the paramagnetic complex will be dilute, and the spectrum will be weak.

In order to obtain detectable spectra and to see the changes in intensity more clearly, 0.1 M or higher amounts of FeCl_3 were added into the extracts. The extracts were dried rapidly at about 40 °C and the EPR spectrum obtained is given in Fig. 2d. The weak line at $g = 4.32$ and broad envelope at $g = 2.00$ have increased appreciably compared to the untreated extract. The increase of the weak line at $g = 4.32$ shows that this line arises mainly from Fe^{3+} complexes and seems to be inconsistent with the results of some previous work which claimed that it arises from Mn^{2+} complexes [21–24], and is consistent with the results obtained from atomic absorption spectroscopy [8]. The increase of the broad envelope, which overlaps with Mn^{2+} and the semiquinone line, can be attributed to various iron compounds including mainly free FeCl_3 and probably oxides and hydroxides of iron in trace amounts formed after the introduction of FeCl_3 . The Mn^{2+} lines persist but the semiquinone line diminishes as the semiquinone radical is scavenged by Fe^{3+} ion, Fig. 2d. Paramagnetic Fe^{3+} ion exists in high spin ($S = 5/2$) and low spin ($S = 1/2$) and sometimes in intermediate states. It is shown that the line at $g = 4.32$ arises from high spin Fe^{3+} complexes with rhombically distorted symmetry; the ligands are phenolic groups in the tea extract, which coordinate via oxygen atoms [17, 18, 21–25].

The shoulder at low field side of the Mn^{2+} envelope does not increase in any of the samples therefore it cannot be attributed to components or complexes of Mn^{2+} or Fe^{3+} . The source is probably trace amounts of superparamagnetic oxides and hydroxides of some metals. Further analysis is impossible because of lack of information which can be obtained from EPR spectra.

Cu^{2+} ions in black tea

Water extracts of black tea were divided into separate cups in equal amounts and CuSO_4 was added in each cup with molarities changing between 0.01 and 0.1 M at 70 °C. Samples were then left for slow evaporation in a clean oven operating at 40 °C. The colors of the extracts changed to dark green immediately after addition of CuSO_4 . Dry extracts were put in sample tubes, and EPR spectra were recorded. The EPR spectra show that 0.05 M of Cu^{2+} is enough to suppress the existing spectroscopic lines shown in Fig. 2a, except the sharp line belonging to the semiquinone radical, Fig. 3a. Higher Cu^{2+} concentrations produce a very broad envelope due to dipolar interactions.

The spectra belong to Cu^{2+} ion with $S = 1/2$ and $I = 3/2$, and can be explained according to the well-known Hamiltonian for Cu^{2+} complexes, [26–28]. Measured EPR parameters show axial structure with values given in Table 1. The ligands are phenolic ingredients, coordinated to Cu^{2+} ion in a bidentate mode via oxygen atom and

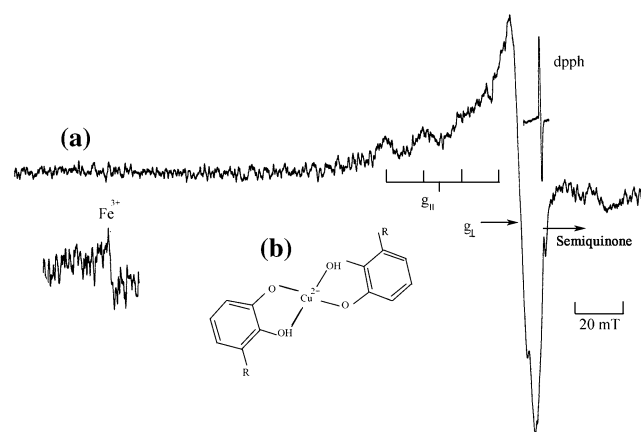


Fig. 3 **a** The EPR spectrum of the Cu^{2+} doped tea extracts, **b** phenolic ingredients coordinated to Cu^{2+}

hydroxide group of the phenol ring, which forms the equatorial plane of the octahedron. It is likely that two other phenolic groups are coordinated at apex positions [26], as shown in Fig. 3b. The structure formed is axially symmetric as observed from the spectrum and common to most Cu^{2+} complexes.

VO^{2+} ions in black tea

When small amounts of VOSO_4 , from 0.01 to 0.1 M, were added into extracts of black tea at 70 °C prepared similarly to the samples above, the solutions became black immediately, which means that the dopant react vigorously with the ingredients of the tea infusion. The solutions were left for slow evaporation at 40 °C. Figure 4 shows the EPR spectrum of 0.05 M VOSO_4 -doped black tea extract. The spectrum belongs obviously to the VO^{2+} ion with $S = 1/2$ and $I = 7/2$ in glassy state and can be explained according to the common Hamiltonian for VO^{2+} complexes, [27, 28]. Perpendicular and parallel components of the VO^{2+} ions are resolved and given in Table 1. The parameters fit to VO^{2+} ions in slightly distorted octahedral environment where the phenolic groups are coordinated via oxygen atoms.

Cr^{3+} ions in black tea

Tea extracts with Cr^{3+} dopant (CrCl_3) were prepared similar to the samples above. The solutions were left for slow evaporation at 40 °C, and the EPR spectra were then recorded, Fig. 5. Cr^{n+} ($n = 1, 2, 3, 4, 5$) ions can form complexes with different symmetries when doped in different host structures. As well as different ionic states, Cr^{1+} ($3d^5$), Cr^{2+} ($3d^4$), Cr^{3+} ($3d^3$), Cr^{4+} ($3d^2$), Cr^{5+} ($3d^1$), there are high- or low-spin states [29, 30]. EPR spectra of Cr^{3+} -doped black tea have a broad envelope at $g = 2.00$ with

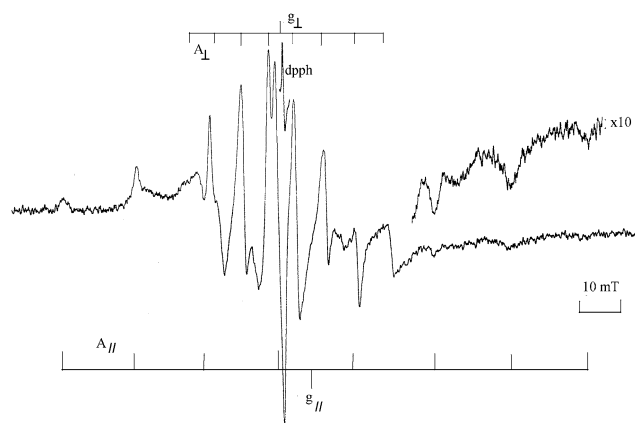


Fig. 4 The EPR spectrum of the VO^{2+} doped tea extracts

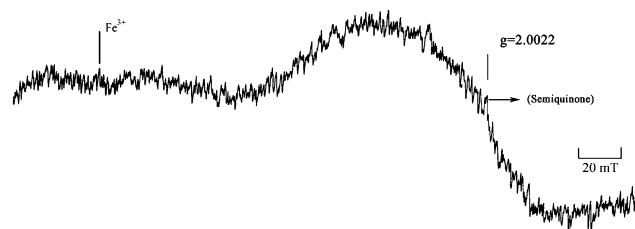


Fig. 5 The EPR spectrum of the Cr^{3+} doped tea extracts

average line width of 100 mT. Cr^{3+} suppresses all other spectroscopic components in the pure tea extract including the semiquinone radical. It is assumed that Cr^{3+} ions in black tea coordinate to phenol groups more strongly and diminish the existing radicals and other paramagnetic species. Further analysis is not possible because of insufficient information due to the samples being in glassy structure and the broad envelope observed.

Conclusion

The interactions between black tea and some commonly encountered paramagnetic transition metal ions have been investigated by EPR. The spectra of pure and dry tea extract has the same natural components as previously reported; a Mn^{2+} sextet superimposed on a broad envelope, a sharp semiquinone line and a weak line at low field which was attributed previously to Mn^{2+} ion. In order to see the effects of excess paramagnetic metal ions, Mn^{2+} , Fe^{3+} , Cu^{2+} , VO^{2+} , and Cr^{3+} were added separately to tea extracts at 70 °C, which is the average temperature during preparation and consumption. Mn^{2+} interacts with catechins rapidly, produces distorted octahedral complex structures and the original sextet disappears due to dipolar interactions. The semiquinone line is suppressed by a broad envelope. The weak line at low field side, however, does

not change for all concentrations indicating that this line does not belong to manganese ion.

The structures formed by excess Cu^{2+} and VO^{2+} ions fit to common structures formed by these ions. Both ions suppress other spectroscopic components, and the weak line at $g = 4.32$ does not change. The addition of excess FeCl_3 diminishes the semiquinone line and suppresses the original Mn^{2+} sextet, but the weak line at $g = 4.32$ increases greatly. This shows that the weak line belongs to the Fe^{3+} structure in octahedral environment. The oxides and hydroxides of iron are also formed. The addition of CrCl_3 gives similar behavior to iron except that the weak line at $g = 4.32$ is not affected.

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