An EPR Study on Tea: Identification of Paramagnetic Species, Effect of Heat and Sweeteners

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ABSTRACT

Tea (Camellia Sinensis) is the most widely consumed beverage in the world, and is known to be having therapeutic, antioxidant and nutritional effects. Electron paramagnetic resonance (EPR) spectral studies made on the tea cultivated along the shore of Black Sea, Turkey, show Mn2+ and Fe3+ centers in green tea leaves and in black tea extract. Dry black tea flakes and dry extract show additional sharp line attributed to semiquinone radical. The origins of the paramagnetic species in black tea are defined and discussed. Effect of humidity and heat are investigated. It is observed that dry extract of black tea melts at 100 ◦C and the semiquinone radical lives up to 140 ◦C while Mn2+ sextet disappears just above 100 ◦C in tea extract. Natural and synthetics sweeteners have different effects on the paramagnetic centers. White sugar (sucrose) quenches the Mn2+ and semiquinone lines in black tea EPR spectrum, and glucose, fructose, lactose and maltose quench Fe3+ line while synthetic sweeteners acesulfam potassium, aspartame and sodium saccharine do not have any effect on paramagnetic species in tea.

Key words: Tea; EPR; Catechin; Semiquinone; Sweeteners.

1. 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 [1], but the scientific community has intensified the works on its therapeutic effects in recent years. Tea is produced over 30 countries in the world and

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consumed in three forms: green (non-fermented) tea, black (fermented) tea and oolong (semi fermented) tea. All of the forms are derived from the Camellia Sinensis plants. Among them, black tea has the highest consumption ratio with 75–80%. All forms are known to be having nutritional, antioxidant and antimutagenic effect, and therefore innumerable works are conducted and published in the last decade. The ingredients, antioxidant and other pharmacological properties of both green and black tea have reviewed and discussed [1–10].

Dry green tea leaves include 36% polyphenolic compounds, 25% carbohydrates, 15% proteins and the rest are lignins, ash, chlorophylls, carotenoids and some volatile substances. In the beginning of fermentation process quinones and consecutively catechin quinones are produced after reaction of polyphenols with atmospheric oxygen. These quinones react with each otherin various ways and produce other components. The resulting composition and therefore the taste and quality of the black tea depend on the conditions like temperature and time of fermentation process, and humidity and temperature during storage period. Different manufacturing process changes the profile ofthese compounds considerably [11,12]. The black tea, supplied for human consumption in the market 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% methylxantines, 15% carbohydrates, protein minerals and some volatiles [13–16]. Fig. 1 shows the structures of the main catechin components in the black tea from which the polyphenols (theaflavins, thearugibins, flavanols and phenolic acids. . .) are formed.

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EPR spectroscopy has been widely used as a powerfultool in nutraceutical and food research owing to capability of detecting free radicals and paramagnetic centers,identification of irradiated foodstuffs, chelating properties of antioxidant samples and kinetic physical changes in some food [17]. The antioxidant property of tea are studied by means of various techniques, among them EPR spectroscopy is the most efficient one for monitoring the chemical radicals and other paramagnetic species existing naturally and forming during various reactions in tea catechins [18–21]. This research has aimed at identifying paramagnetic species and observing the effects of heat, humidity and sweeteners on the tea cultivated along the shore of Black Sea, Turkey.

2. Experimental

Fresh green tea leaves are obtained from producers and black tea samples are bought from market including the products of North Eastern Part of Turkey where the climate is suitable for tea cultivation along the shore of Black Sea. Chemicals are obtained commercially from Merck. All glassware is cleaned up by soaking in dilute HNO3 and is rinsed with distilled water prior to use. EPR spectra are taken at room temperature unless stated otherwise. The amounts and concentrations of water extract of black tea and sweetener contents are prepared in the similar way as for daily consumption by taking care of ISO3103 standard; 2 g dry black tea leaves are put in 100 ml boiled water, waited for 6min and than the water extract is filtered out. The EPR spectra of water solutions of black tea are taken freezing the sample at −40 ◦C because of the fact that liquid water absorbs all microwave. In order to determine the sources of paramagnetic species existing naturally, additional MnSO4 (0.02% by weight) and FeCl3 (0.10% by weight) are included into

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separate tea extracts at 70 ◦C. To obtain dry extract of the black tea, water extracts are kept at 40 ◦C to evaporate water content. Distilled water is used in all extraction processes.

The X-band EPR spectra are 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 are kept at 9.52GHz and 2mW, respectively. Temperature is controlled with a Varian temperature control unit. The spectra of tea samples are recorded by putting the sample in quartz sample tube. The spectrometer frequency is corrected using the DPPH (dihenylpicrylhydrazyl, g = 2.0036) sample. Spectrum simulations are made using Bruker's WINEPR software.

3. Results and Discussion

The mineral contents of tea samples from various countriesdetermined by atomic absorption apparatus are reported [15,21,22]. All samples show relatively high mineral contents, among them the paramagnetic transition metal ions are the subject to EPR investigations. The elements Mn, Fe, K, Mg, Na, Ca, Al are in higher concentration when compared to Cu, Zn, Cr, Pb, Cd, therefore paramagnetic Mn2+, Fe3+ and probably Al3+ can be observed clearly while others such as Cu2+, cannot be seen because of that the Cu2+ concentration seems to be about 99% lower in magnitude relative to Mn and Fe; very weak Cu2+ lines, if exist, lies most likely beneath the spectra of other species in higher concentrations.Hot and cold water extracts of black tea give the same EPR spectra as dry black tea flakes, Fig. 2a. A weak shoulder (A), a broad envelope at g≈2.00 (B), and aMn2+ sextet (C), superimposed on it, a single sharp line at g = 2.002 (D) raising from semiquinone radical, and weak line at g = 4.32 (E) are easily resolvable. The mean hyper-

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fine splitting value of Mn2+ (C) sextet is measured to be 9.4 mT.The spectrum is similar to previously observed spectra of black tea from various countries [13,14,18,20]. The relative intensities of the components in the spectra may be slightly different depending on the fermentation processes, storage conditions, age of leaves, geographic region and the climate [1,11,12,14].

When green tea leaves are dried rapidly in dark nitrogen chamber at temperatures between 40 and 50 ◦C, the spectrum is the same as that of black tea leaves except the sharp semiquinone line shown in Fig. 2a. Similarly, the EPR spectra of fresh green tea leaves and water solution of both green and black tea extract do not contain sharp semiquinone line as well. When green tea leaves are dried in normal ambient light (but not in direct sunlight), and in normal atmosphere at room temperature, it takes about one week to dry completely and the color gets darker due to partial oxidizing processes. This time EPR spectra show very weak semiquinone line. In fact, fermentation of green tea leaves takes this oxidizing process to further definite levels to produce black tea by steaming, rolling, drying and firing tea flakes [14]. The chemical composition changes during this process as given in Section 1 above and as a result, the EPR spectra show highly intensive semiquinone line (D) as shown in Fig. 2a.

EPR spectra of riverine materials from tropical ecosystems and municipal solid waste composts, interestingly enough, Show almost the same paramagnetic components as those of black tea [23–26]. Therefore it is not true to claim that the spectrum in Fig. 2a is unique characteristic to black tea.

3.1. Mn2+ Complexes

When 0.01% (by weight) of MnSO4 is added into tea extract, the color of the extract does not change but the intensities of Mn2+lines and envelope around g≈2.00 abruptly increase

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(Fig. 2c), the intensities of other components and especially the shoulder (Fig. 2a,Line A) on the low field side ofMn2+ sextet, however, do not change.Addition of higher MnSO4 amount causes the broadening of Mn2+ sextet due to intensive dipolar interactions (Fig. 2c). This behavior shows that Mn2+ ion in octahedral environment is not responsible from the other components, namely the weak line at g = 4.32 (E), the low field side of the spectrum [27,28]. The cause of broadening of Mn2+ sextet in original dilute samples, Fig. 2a, with slightly unequal separation and the broad envelope on which the sextet superimposes is probably due to the anisotropy in hyperfine and g values of various overlapping lines from Mn2+ complexes formed with catechins or polyphenolic ingredients as ligands. The structures formed with Mn2+ together with some transition metalswill be discussed in a separate paper.

3.2. Fe3+ complex

Paramagnetic 57Fe3+ isotope (nuclear spin I = 1/2) has 2.12% natural abundance, therefore complex will be dilute and the linearising from this complex will be weak. In order to get detectablespectrum and to see the change in intensity more clearly, higheramount of FeCl3 is added into the extract (0.10% by weight). In thiscase the spectrum shows that the intensity of weak line at g = 4.3 (E)increases several times (Fig. 2d). Therefore it is obvious that this linearises from Fe3+ complex in rhombically distorted environment,where the ligands are phenolic groups of the tea. Paramagnetic Fe3+ion exits in high spin (S = 5/2) and low spin (S = 1/2) and sometimes in intermediate states. It isshownthat the line at g = 4.32 arises from high spin Fe3+ complex with rhombically distorted symmetry; the ligands of the complexes are catechins or polyphenolic ingredients

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of the extract. Addition of FeCl3 also increases the intensity of the broad envelope (B) at g≈2.00 to some extent (Fig. 2a). These types of Fe3+ spectra are observed for iron oxides and iron hydroxides, therefore some weak iron oxide spectra may exist underneath the envelope [23–29]. The weak shoulder (A) at low field side of Mn2+ envelope and sextet does not change for samples with additional Mn2+ and Fe3+, therefore it cannot be attributed toMn2+ or Fe3+. The source is probably some superparamagnetic oxides and oxyhydroxides of some metals in trace amounts.

3.3. Effect of humidity and heat on black tea

Since tea is prepared in hot water for consumption and sometimes is kept for relatively longer time before drinking, it has seemed to be necessary to observe what happens to paramagnetic species during these periods. For this purpose, wet black tea flakes are kept in separate pots for several days in different temperatures. When the wet sample is kept at room temperature for one day, green colored fungi cover all over the flakes and deterioration starts afterwards. EPR spectra of wet flakes taken by freezing rapidly below −40 ◦C show Mn2+ sextet and Fe3+ line but not semiquinone line, meaning that semiquinone radical cannot live in water solution, therefore it seems necessary to dry the flakes to see the radical. When wet black tea flakes are dried rapidly by blowing air or nitrogen gas at room temperature, it is seen that the semiquinone line increases to several order of magnitude. The absolute rates of increase cannot be measured because of the instabilities in drying process, but several measurements have shown that the wet flakes kept for 24 h at room and higher temperatures, the intensities of semiquinone line have increased approximately three times. But the increase is too slow for the samples kept

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below 15 ◦C. In longer periods, the semiquinone radical starts decaying in wet flakes because of deterioration.

When dry tea flakes are heated at various temperatures, EPR spectra do not show any appreciable change below 70 ◦C even forlonger periods, which means that the structures in the leaves are stable up to 70 ◦C. When the temperature is increased above 70 ◦C,the intensity of the semiquinone line increases due to the radical produced by heat via loosing one of the hydrogen atoms of hydroxylgroups bonded to B ring, Fig. 1. But after one and more hours the intensity starts decreasing because of evaporation and because of that polyphenols dissociate by loosing both of the hydrogen atoms and producing quinonic ion, ring B of Fig. 1.

It is reported that gamma ray increases, but this line decays rapidly on storage at room temperature and the original intensity is recovered [5,6,18,30]. But heating the dry leaves above 90 ◦C for several hours instead the intensity of semiquinone line also increases but original intensity is not recovered for about four weeks, indicating that the species produced by heat are permanent for longer periods as much as the flakes kept totally dry. The spectrum of frozen water solution of the extract does not contain semiquinone line as in the case of frozen wet flakes. Cold and hot water extracts, after filtering out the flakes and evaporating the water, however give the same spectra as dry flakes (Fig. 2a). The intensity of the semiquinone line does not change with temperature in dry extract. Dry extract starts boiling and evaporating above 100 ◦C, but the semiquinone radical survives even in molten tea extract until about 140 ◦C while Mn2+ sextet and the broad envelope decay rapidly. The probable reason of decay is oxidization of Mn2+ ion by oxides and hydroxides of polyphenols on heating, the result is non-paramagnetic Mn3+. Fe3+ line on the other hand, does not change until 140 ◦C in dry extract.

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3.4. Sweeteners and tea

Tea is consumed mostly together with white sugar, which is the common sweetener all over the world and is a high calorie carbohydrate. It is chemically known as sucrose or disaccharide and is composed of glucose and fructose bonded via oxygen. Closely related other common and biologically important natural sweeteners are glucose, maltose, lactose and fructose, which are also carbohydrates. Some of synthetic sweeteners in common use are sodium saccharine, aspartame and acesulfame potassium which are, on the other hand, known to be low calorie sweeteners and some consumers, especially diabetes use them to substitute White sugar. The effects of sweeteners on tea extract are collected in Table 1. White sugar (sucrose) quenches Mn2+ sextet, the broad envelope, and the semiquinone line, but Fe3+ line at g = 4.32 survive. When pure glucose and fructose, which are the components of White sugar, maltose and lactose, are added to tea extract separately, only Fe3+ line is quenched and other lines survive. The synthetic sweeteners sodium saccharine, acesulfame potassium and aspartame, however, have no detectable effect on the paramagnetic species. When white sugar added in black tea, semiquinone radical may be converted to non-paramagnetic species by removing second hydrogen from phenolic ring, Fig. 1, to produce quinonic ion. In this case, the extract would become too active and the taste of tea would be worse for drinking. The second case, which is the most probable one, hydrogen deficiency of one of the $-\dot{O}$ ion of ring B is fulfilled by a hydrogen atom of related sweeteners and semiquinone radical is quenched completely. The relation between white sugar and quinone ions is reported by Chowdhury and Samita [31]. The study reveals the differences between reduction of electron transfer and hydrogen abstraction

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reactivity when sugar added, which is similar to our supposition about quenching of semiquinone radical. Meanwhile Mn^{2+} is oxidized to non-paramagnetic Mn^{3+} by oxide and hydroxide groups of sweeteners.

Natural and synthetic sweeteners may have different intrinsic thermo-chemical properties. In a recent report, it is shown that aspartame as artificial sweetener and glucose as a natural sweetener exhibit different metal ion affinity, but in the vicinity of divalent cations in the other hand, some sweeteners changes their structures completely [32]. The EPR spectra of the black tea taken in this study after adding glucose, fructose, maltose and lactose separately quench only Fe^{3+} line at $g = 4.32$ by reducing Fe^{3+} to diamagnetic Fe^{2+} by transferring or sharing an electron from a hydroxyl group of the sweeteners, which contrast to white sugar case. Other paramagnetic species are not affected by these sweeteners.

After all, we come to ask the question that how we should consume black tea to be more beneficial. The answer of this question is not completely clear according to the EPR outcomes, because of the uncertainty whether semiquinone radicals or polyphenols are beneficial. The dihydroxy group is required for the participation of a polyphenols in the redox process and in its antioxidant activity. On the other side, the semiquinone radical may accept hydrogen and participate in the mechanism of prooxidation, acting as an oxidant agent. The prooxidant activity appears to be responsible for the harmful biological effects of polyphenols [33]. Other results obtained from various studies suggest that there are associations between consumption of polyphenol-rich food and beverages and the antioxidant activity, namely their ability to scavenge hydroxyl radicals [34]. In general, people believe that polyphenols are more advantageous rather than being disadvantageous. The dual behavior of the polyphenolic compounds prevents us to speak certainly. If we

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think that polyphenols are useful, and to increase their antioxidant effects, then it is suitable to consume tea sugarless or with the least sugar possible. In spite of the fact that EPR spectra of black tea with artificial sweeteners has no any change, there are lots of clinical studies which have shown that usage of excess artificial sweeteners may cause various health problems [32].

4. Conclusion

EPR spectra of black tea flakes and the dry water extract include a Fe3+ complex line (rhombically distorted symmetry) at g = 4.32, a sextet from Mn2+ in octahedral environment, a broad envelope at g≈2.00 and a weak shoulder from superparamagnetic oxides and hydroxides of some metals with trace amounts, and a single sharp line at g = 2.002 atributed to semiquinone radical. Semiquinone line cannot be seen in the spectra of dry green tea, in wet tea flakes and water solution of black tea extract. Dry extract of black tea melts just above 100 ◦C and the semiquinone radical lives up to 140 ◦C where the extract starts boiling rigorously. The Mn2+ sextet and the envelope disappear above 100 ◦C in tea extract. White sugar (sucrose) quenches the Mn2+ and semiquinone lines of black tea. Glucose, fructose, lactose and maltose quench Fe3+ line while synthetic sweeteners acesulfam potassium, aspartame and sodium saccharine do not have any effect on paramagnetic species in tea.

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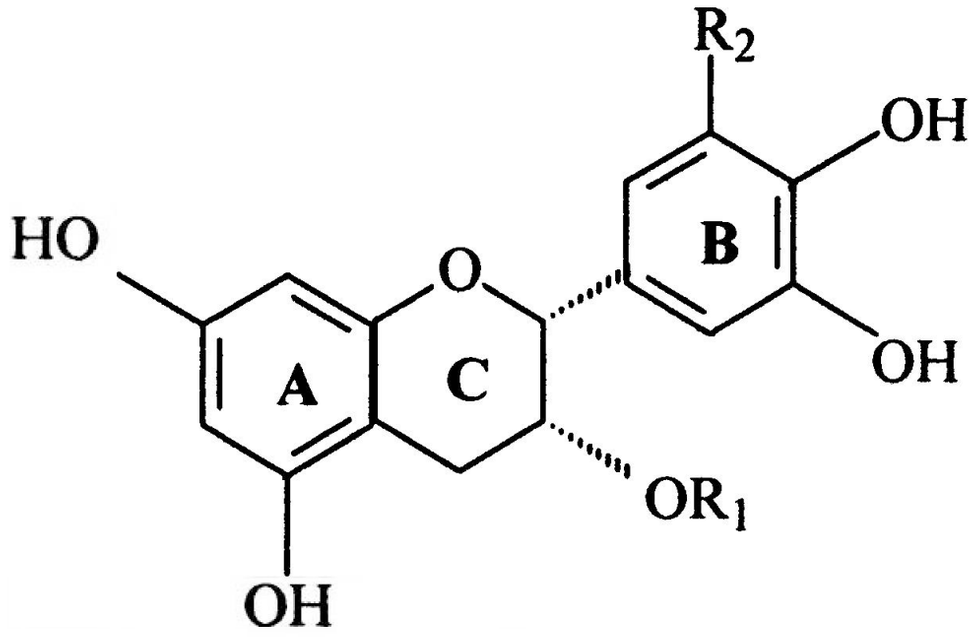
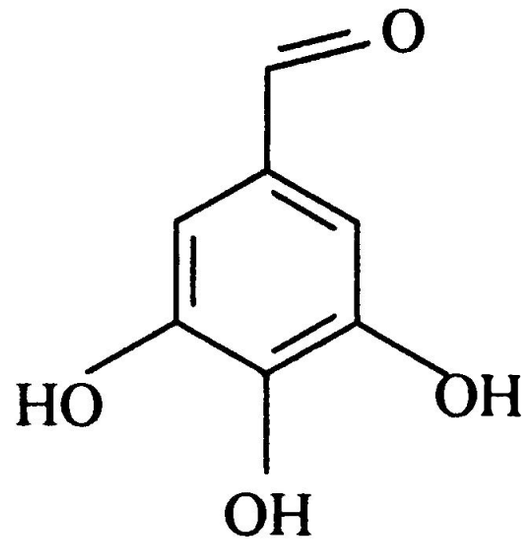
Figures Captions

Fig. 1. The main structures in black tea from which the polyphenols are formed.

Fig. 2. (a) The EPR spectrum of black tea extract; (b) reproduced components of the spectrum by using Bruker's WINEPR software, (c) Mn^{2+} and (d) Fe^{3+} doped extracts.

Figure 3. Structures of common sweeteners.





G (Galloyl)

Epicatechin [EC]: $R_1=R_2=H$
Epigallocatechin [EGC]: $R_1=H$; $R_2=OH$
Epicatechin-3-gallate [ECG]: R_1=Galloyl; $R_2=H$
Epigallocatechin-3-galiate [EGCG]: R_1= Galloyl; $R_2=OH$

Fig. 1.
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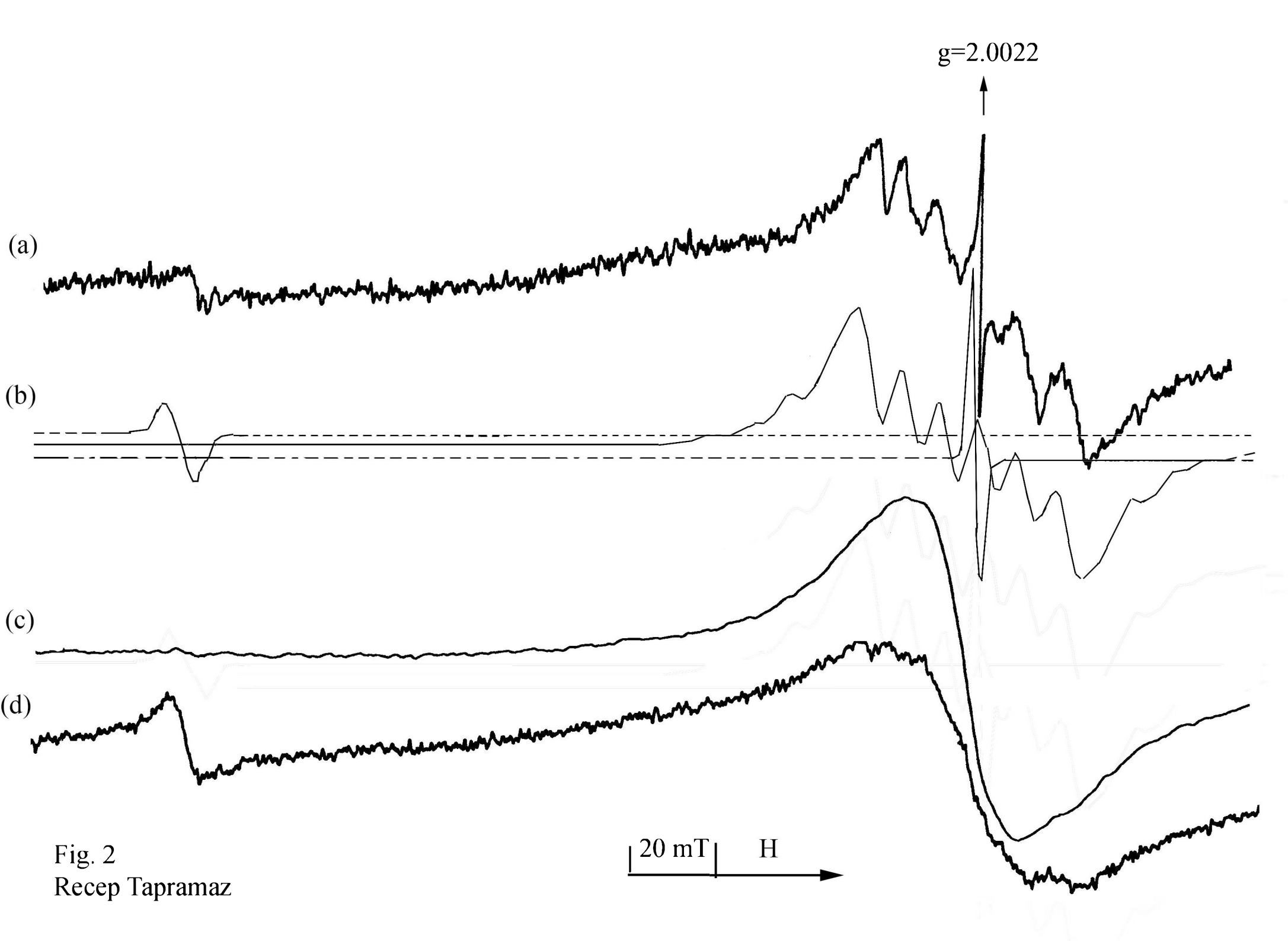

Fig. 2
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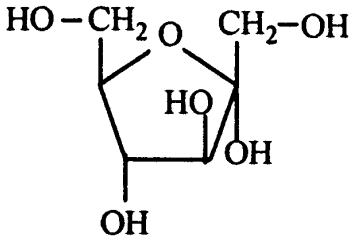
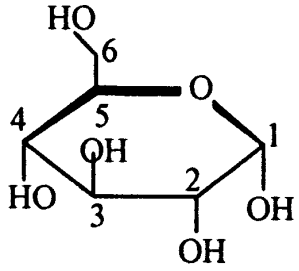
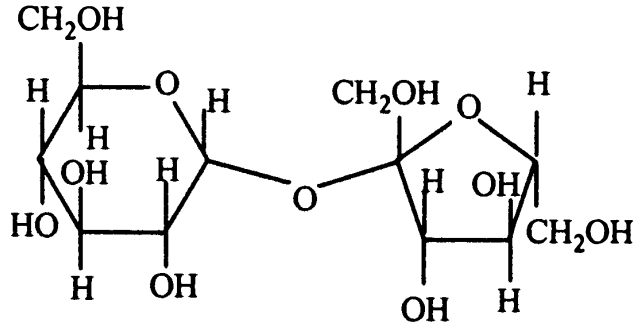


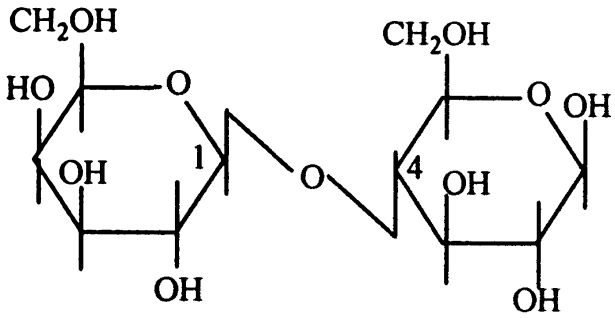
α-D-Fructose



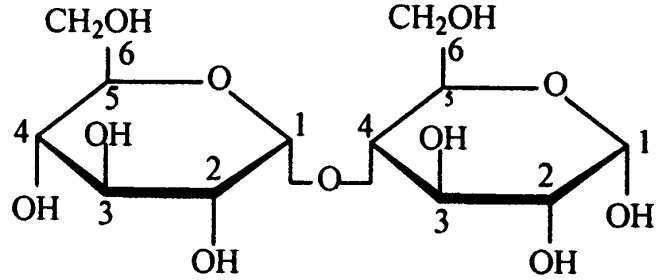
Glucose



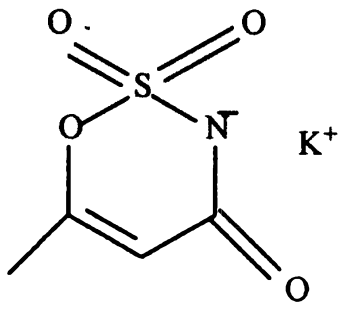
White Sugar (disaccharide)



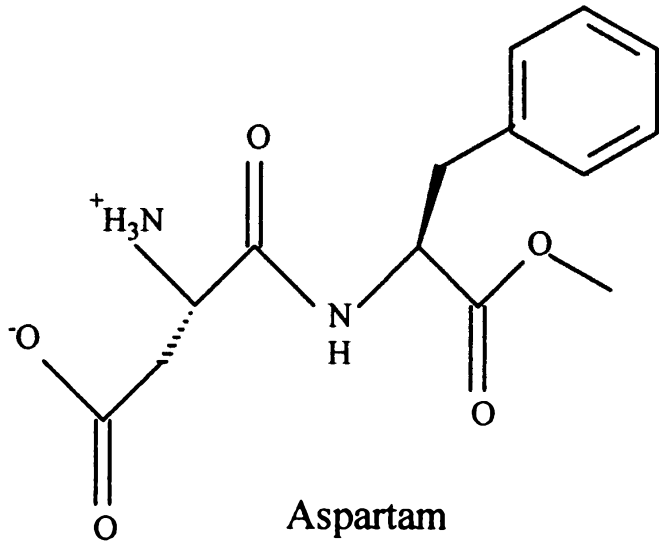
Lactose



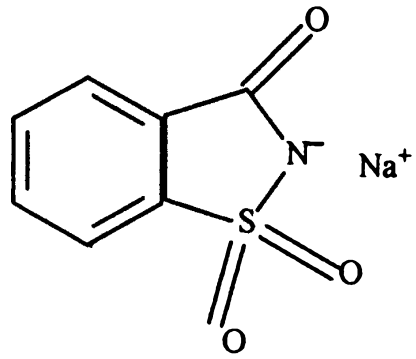
α-Maltose



Acesulfam Potasyum



Aspartam



Sodium Saccharine

Fig. 3.
Recep Tapramaz

Table 1.

The effects of sweeteners on the paramagnetic species in black tea extract

(✓: exists; X: quenched).

	Mn^{2+} sextet and envelope	Semiquinone line	Fe^{3+} line
White sugar	X	X	✓
Glucose	✓	✓	X
Fructose	✓	✓	X
Maltose	✓	✓	X
Lactose	✓	✓	✓
Acesulfam potassium	✓	✓	✓
Sodium saccharine	✓	✓	✓
Aspartam	✓	✓	✓