Direct Electron Paramagnetic Resonance Study of Tobacco. 1. Manganese(II) as a Marker

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Three categories of tobacco products were studied using electron paramagnetic resonance (EPR) spectroscopy: Cuban cigar brand name Montecristo, four international trademark cigarettes, and three types of Middle Eastern tobacco blends called Al-Moassal or Jurak. The Montecristo Cuban cigar is used as standard of high-quality tobacco. Mainly two EPR signals from all of the studied samples are observed: a very weak sharp EPR signal superimposed on a broad signal. The broad EPR signal is attributed to a manganese(II) complex. The intensity of the manganese(II) EPR signal is found to be related to the quality of the tobacco content. The sharp signal, which is characteristic of semiquinone radicals, is observed at room temperature, and its intensity increases drastically with temperature.

INTRODUCTION

It is well-known that cigarette manufacturing involves the addition of a large number of chemicals to the tobacco leaves, paper, and filter. These include, for instance, glycerol derivatives, many organic solvents and acids, mineral oils and clay, some quaternary ammonium compounds, and aliphatic resin (*1*).

Traditional tobacco blends in the Middle East, called Al-Moassal or Jurak, are finding their ways to the United States. They are gaining global recognition and are widely used. Al-Moassals are composed of a sticky mixture of tobacco−glycerin−sugar. Many types of these blends are, for instance, manufactured in Holland and exported all over the globe. These types contain flavors and other additives in addition to their tobacco−glycerin−sugar base material.

Extensive efforts were carried out to study tobacco plants (*2*−*8*) and tobacco products (*5*, *6*). Trace elemental analyses on a wide variety of the tobacco blends were carried out using inductively coupled plasma mass spectroscopy (*2*), X-ray fluorescence (*3*), instrumental neutron activation analysis (*4*), and extraction techniques (*5*, *6*). Recently, fluorescence emission (*7*) and infrared (IR) (*8*) were used as spectroscopic indicators for the early stress events in plants and quality of the plant part, respectively. Tobacco smoke analysis shows that it, in general, contains high concentrations of particulate matter (soot and tar) in a dynamic aerosol, various noxious compounds (such as carbon monoxide and HCN) in the vapor phase, and stable radicals (*9*−*15*). Electron paramagnetic resonance (EPR) spectroscopy has been utilized to study the radicals generated after pyrolysis by direct EPR and EPR spin-trap methods (*11*). However, a very limited number of EPR investigations were carried out to study directly the properties of the original tobacco itself (*16*, *17*).

This work attempts to characterize a Montecristo Cuban cigar, three Al-Moassal tobacco−glycerin−sugar blends, and four brand name cigarettes using EPR spectroscopy. The Montecristo Cuban cigar is considered as a reference of high-quality tobacco in this work. The study correlates several factors that influence the quality of the tobacco blends, such as industrial treatment to control the contents of tar and nicotine, the market price of the studied systems, and the duration of storage.

Table 1. Tar and Nicotine Contents in the Studied Tobacco Systems

	tar		nicotine	
tobacco type	mg/cig[a]	mg/g[b]	mg/cig[a]	mg/g[b]
cigar				
Montecristo	0.01	0.001	0.122	0.014
cigarettes				
Merit	1	1.8	0.1	0.2
Carlton	4	7.7	0.4	0.8
Marlboro	8	10.9	0.6	1.0
Rothmans	10	16.3	0.6	1.0
Al-Moassal				
all types[c]	NA	NA	NA	NA

[a] Amount in milligrams per cigar or cigarette. [b] Amount in milligrams per gram of tobacco in cigar or cigarette. [c] Not available: no tar or nicotine contents are provided for any type by the manufacturer.

EXPERIMENTAL PROCEDURES

The tobacco−glycerin−sugar blends (three types), Cuban cigar (one type), and different trademarks of cigarettes (four types) were purchased locally. The four types of cigarettes were selected to provide a variation in their contents of nicotine and tar per cigarette according to the manufacturer's label. Table 1 shows the contents in nicotine and tar for the studied tobacco blends. The nicotine contents vary from 0.1 to 0.6 mg per cigarette (0.2−1.0 mg/g of tobacco), and the tar contents vary from 1 to 10 mg per cigarette (1.8−16.3 mg/g of tobacco), whereas the nicotine and tar contents of the Montecristo Cuban cigar are 0.122 and 0.01 mg per cigar (0.014 and 0.001 mg/g of tobacco), respectively. The cigar type used in this study is among the most expensive tobacco types on the market.

All of the EPR measurements were performed on an equivalent amount of the studied tobacco blends as received and without any further treatment. An ER-200-D-SRC series spectrometer was used to obtain the EPR spectra. A cylindrical cavity has been used along with a 9 GHz (X-band) microwave bridge. The magnetic field sweep was calibrated with a Bruker-ER 035 NMR gauss meter with an accuracy of 2 mG. The frequency was measured with a model 5342A Hewlett-Packard frequency counter. Both microwave power and modulation

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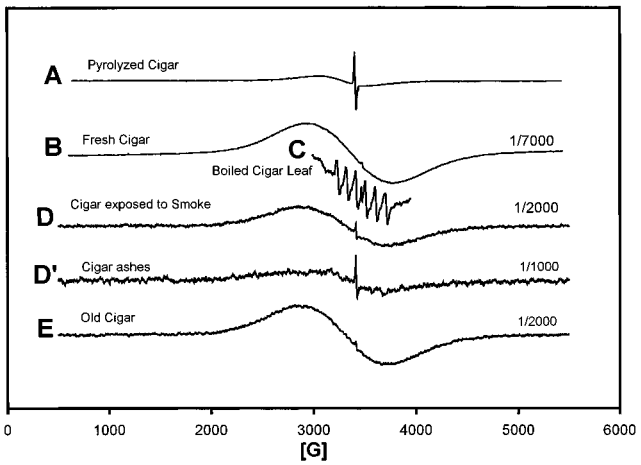

Figure 1. EPR spectra of Montecristo Cuban cigar (A) at 160 °C and (B−E) at room temperature. Numerical fraction above the right side of each spectrum is the normalization factor per each scan.

amplitude were verified to be at least 10 times below the onset of the broadening. The scan speed and time constant were also carefully chosen so as not to introduce any artifact during scanning.

The EPR temperature-dependent experiments were performed using the variable temperature unit from Bruker (ER-4111 VT). The temperature was controlled over a temperature range from 293 to 673 K. An Omega Digicator thermocouple unit was used to determine an accurate temperature setting to ±0.1 °C.

RESULTS AND DISCUSSION

Figure 1 shows the EPR spectra of the high-quality Montecristo cigar purchased on the local market. Spectrum B is recorded at room temperature of the freshly bought Montecristo cigar. The spectrum shows a very strong broad signal that extends over ∼3000 G and has a *g* factor value of ∼2.00434. It also incorporates another very weak sharp signal with a *g* factor value of ∼2.00342.

A temperature-controlled EPR experiment was carried out on the Montecristo tobacco cigar to characterize the sharp signal. The temperature was varied from 20 to 450 °C. EPR spectra were collected at ∼20 °C intervals. It is noted that the magnitude of the sharp signal increases with temperature. At 160 °C the broad signal becomes weak and the sharp signal becomes more remarkable (spectrum A in Figure 1). At higher temperatures the sharp signal increases drastically and dominates totally the spectrum. This sharp signal was attributed to semiquinone radicals in a tar matrix (*12*, *13*). This stable radical has reducing properties and is found to be in equilibrium with quinones and hydroquinones (*14*).

The broad EPR signal shown in Figure 1B exhibits a "powder" line shape typical of macromolecular complexes with Mn(II). To further identify the nature of this signal, a reflux of a sample from the Montecristo cigar leaf in distilled water was carried out for 5 h. EPR measurements were then performed on both the boiled solution and a sample of the boiled cigar leaves. The resulting spectra indicate that the broad signal is composed of six major splitting (spectrum C of Figure 1). The sextet splitting EPR spectrum is a typical EPR spectral line for $^{55}Mn^{2+}$ ($I = {}^5/_2$) in solution. Manganese(II) is an essential element and micronutrient in photosynthetic green plants and cyanobacteria. It plays a fundamental role in the catalysis of water splitting and oxygen evolution in photosystem II, which is a vital part of plant survival (*18*, *19*).

The spectrum of Mn(II) bound to protein becomes different from the isotropic spectrum for the free Mn(II) in aqueous solution (*20*). The rotational freedom of the Mn(II)−protein systems is restricted to the slow tumbling rate of the protein molecule that makes the spectral line shape approach the one for a polycrystalline or powder sample. Hence, the immobilization of Mn(II) by the protein was found to be responsible for the disappearance of the sharp EPR splitting and should result in a broad featureless signal as observed in the Cuban cigar. Several research groups investigated (*21*−*23*) the relationship of the EPR signal of Mn(II)-enzyme/membrane systems. The studies on aqueous solutions of protein−Mn(II) complexes using 35 GHz (Q-band) showed an Mn(II) EPR spectrum with hyperfine splitting similar to the reported one in Figure 1C. On the other hand, they explained that the broadening appearance in 9 GHz (X-band) spectra for these solutions was attributed to inhomogeneous broadening caused by incomplete resolution of the forbidden transitions, resulting from the solid-state character of the studied systems (*22*). Other researchers (*24*−*26*) showed that the enzyme type and high concentrations of inorganic phosphate broaden the hyperfine lines of the X-band Mn(II) spectrum. This is consistent with a change in coordination geometry of the bound Mn(II) or a change in accessibility of the Mn(II) site to solvent or both.

To investigate the effect of hot gaseous flow on the EPR signals, EPR spectral measurements were carried out on an unsmoked portion from an incompletely smoked sample of the Motecristo cigar and on the burned products from the smoked portion (ashes). Spectra D and D′ in Figure 1 show the signal from these samples, respectively. A sharp decrease in the magnitude of the manganese signal is observed from the unburned portion, whereas, in the ashes, the manganese signal almost disappeared. The intensity of the semiquinone radical signal was not relatively affected. These results show that the hot gaseous gradient due to the inhalation flow of the cigar smoke has reduced manganese(II) and leads to a reduction in its corresponding EPR signal.

EPR experiments were conducted on a cigar sample stored under laboratory conditions for several months. A decrease in the EPR intensity of the manganese(II) signal is also observed compared to the original Montecristo cigar (spectrum E in Figure 1). This decrease of the EPR signal can be correlated with the aging of the leaf.

EPR measurements were also performed on four types of cigarette samples. The samples were carefully selected to show significant variation in their tar and nicotine contents. According to the listed values in Table 1, Rothmans cigarette has the greatest content of tar and nicotine, whereas Merit cigarette has the lowest contents. Marlboro cigarette and Carlton cigarette have intermediate values for their nicotine and tar contents. All of the studied samples are export brands from the original manufacturer.

Figure 2 shows the EPR spectra of the four types of international brand names cigarettes, Rothmans, Marlboro, Carlton, and Merit, in addition to the spectrum of the fresh Montecristo Cuban cigar. The EPR spectra for the fresh cigarette samples generally exhibit weaker EPR signals of the manganese(II) relative to the fresh

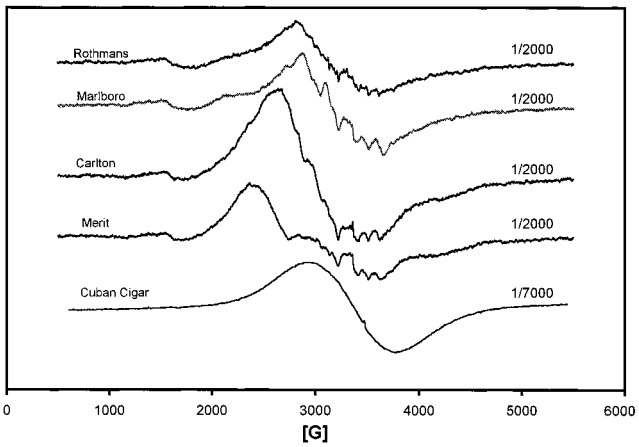

Figure 2. EPR spectra of Montecristo Cuban cigar and different samples of cigarettes at room temperature. Numerical fraction above the right side of each spectrum is the normalization factor per each scan.

Cuban cigar. Carlton and Merit showed a distortion at lower magnetic fields. This distortion at lower magnetic field usually arises whenever there are asymmetries in the electronic environment of the bonded Mn(II) (*21*). Computer simulations suggest that these spectra arise from rhombic distortions of the bound Mn(II) ion (*21*). The Merit cigarette sample exhibits the lowest EPR signal for manganese. Moreover, Merit and Carlton exhibit the highest distortions at lower magnetic fields.

Close examination of the spectra in Figure 2 shows that Mn(II) signals are no longer featureless, and Marlboro has relatively the most resolved hyperfine structure. On the other hand, Rothmans' EPR signal shows the least resolved hyperfine structure. Its EPR profile mimics the Cuban cigar EPR signal but with smaller intensity. The resolved broad hyperfine sextet in the cigarette samples may be attributed to the free tumbling of manganese. It has been shown (*24*, *27*) that the X-band EPR spectrum of the complex of Mn(II)−protein, namely ATPase, exhibits a powder line shape. These experiments showed also that Mn(II) bound to other macromolecules, namely ATP, ADP, ANP−BNP, increased the line broadening of the Mn(II)−protein spectrum. Therefore, one may conclude that Marlboro and Merit cigarettes might have been subjected to treatments that induced structural changes in the Mn-(II) bonded to chlorophyll and hence their spectra approach the spectrum of free tumbling.

Room temperature EPR experiments were also carried out on Al-Moassal. Figure 3 contains their EPR spectra compared to that of the Montecristo Cuban cigar. Spectrum B in Figure 3 shows the signal of the most expensive Al-Moassal brand among the three studied samples. Spectra C and D show the signal of the less expensive and the least expensive blends, respectively. It is noted that the EPR signal magnitude of the manganese decreases drastically with the market price. The signal of the cheap Al-Moassal is significantly less than that of the high-quality cigar and ∼4 times smaller than that of the studied cigarettes. It is even smaller than the observed signal of the long-term-stored cigar in Figure 1D. This very small manganese signal from the less expensive or least expensive Al-Moassal indicates that the original tobacco might have been subjected to very long-term storage or exposed to very high thermal effects or that their ingredients include very low-quality tobacco types or less tobacco.

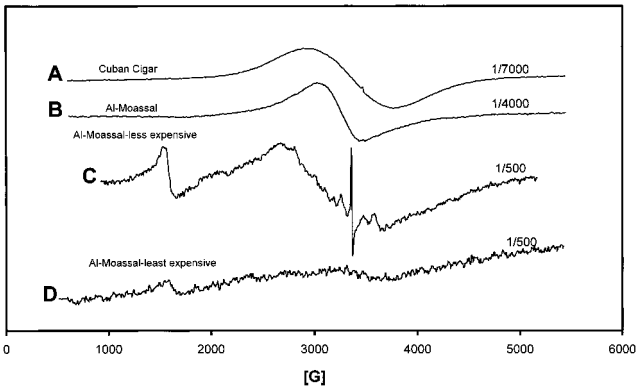

Figure 3. EPR spectra of (A) Montecristo Cuban cigar and (B−D) different types of Al-Moassal samples at room temperature. Numerical fraction above the right side of each spectrum is the normalization factor per each scan.

CONCLUSION

The two characteristic EPR signals from all of the studied tobacco samples are related to the presence of the manganese(II) complex and a stable semiquinone free radical. The Mn(II) signal is found to be characteristic of the storage period and thermal conditions. The signal decreases drastically after long-term storage under laboratory conditions. A noticeable weakness in this signal was also observed for the heat-affected samples, whereas the semiquinone radical signal strengthens. Although it is not clear why the manganese EPR signal is generally weaker in cigarettes and is much weaker in cheap Al-Moassal, the study might suggest that several factors including thermal degradation of the tobacco leaves and/or low tobacco contents may be the cause. Therefore, the manganese(II) EPR signal, according to this study, could be a means of monitoring the freshness and quality of tobacco products.

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