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Characterization of Paramagnetic Species in Seeds by Electron Paramagnetic Resonance (EPR)

V. M. Barbana, C. L. B. Guedes and E. Di Mauro

Laboratory of Fluorescence and Electron Paramagnetic Resonance (LAFLURPE),
CCE, State University of Londrina, Londrina, PR, 86051-990 (Brazil)
Phone/Fax number: +55 43 33714684, e-mail: carmen@uel.br, dimauro@uel.br

Abstract. Paramagnetic species were characterized in the seeds of radish wild, rice, mustard, wheat, oats, sorghum, sunflower, soybean, cotton, beans, maize and barley by Electron Paramagnetic Resonance (EPR). Some iron complexes such as goethite, hematite, magnetite, and ferrihydrite, normally present in the soil, were also investigated by EPR, since their signals can, *a priori*, be present in EPR spectra of seeds. The EPR experiments were performed at X-band microwave frequency (9.3 GHz) on the JEOL spectrometer (JES-PE-3X) from 77 to 385 K and on the BRUKER spectrometer (Elexsys E-580) in a temperature range from 30 to 70 K. In the EPR spectra of the seeds, it was detected the same complex of Fe^{3+} found in goethite, with g=2.0±0.1 in all the investigated seeds. In addition, free radicals have also been detected with g=2.004±0.002, on all seeds, and with g=2.013 only in sorghum seeds. The sunflower seeds showed the highest signal intensity of the free radical. During the temperature variation, changes were observed in the spectra, in a manner that at low temperature (30 K), beyond the higher intensity on signal of the goethite and free radical, the spectra of the seeds also showed signal of hematite and traces of Mn^{2+}.

Key words

Biomass, Fe^{3+}, free radical, goethite, hematite.

1. Introduction

Since the 1970's, programs have been developed for renewable fuels in order to replace petroleum. A program that has been exhaustively discussed and encouraged is the biodiesel, which would replace the diesel, a fossil fuel, for biodiesel, a renewable fuel. Biodiesel is a fatty acid ester, renewable and biodegradable, which can be obtained by transesterification, a chemical process that uses animal fat and/or vegetal oils with alcohol in the presence of a catalyst [1]. Plant seed oils such as soybean, maize, and sunflower, among others are used in the production of biodiesel. As seeds are the basis for production of oil and consequently processed into biodiesel, the goal of this project is to characterize and compare paramagnetic species present in the seeds by Electron Paramagnetic Resonance (EPR). In the near future biodiesel can be used in large scale. These paramagnetic species are of great importance, once they may be present in the seed's oils, in all the industrial process of the biodiesel and possibly in the air as pollutants.

Paramagnetic species were characterized in the seeds of wild radish, rice, mustard, wheat, oats, sorghum, sunflower, soybean, cotton, beans, maize and barley by EPR. Some iron complexes such as goethite (FeOOH), hematite (Fe_2O_3), magnetite (Fe_3O_4) and ferrihydrite ($Fe_5HO_8.4H_2O$), normally present in the soil, were also investigated by EPR, since their species can, *a priori*, be present in appreciable quantities in the soil, and therefore in EPR spectra of seeds.

2. Experimental

EPR experiments were performed at the X-band microwave frequency (9.3 GHz) and a magnetic field modulation of 100 kHz on the JEOL (JES-PE-3X) equipment in a temperature range from 77 to 385 K, using variable temperature controller (JES-VT-3A), with a flow of nitrogen, and on the BRUKER spectrometer (Elexsys E-580) in a temperature range from 30 to 70 K. A standard sample of $MgO:Mn^{2+}$ (g =1.981 of the fourth line) was maintained in the EPR cavity of the JEOL spectrometer, the data were recorded simultaneously with the sample. Samples were introduced into 4 mm quartz EPR tubes that were previously checked for the absence of any spurious signal. In addition to the factor g, the line width peak to peak and the EPR signal intensity were used to determine the relative quantity of paramagnetic species in each sample, measuring the mass of the seeds and normalizing their intensities for 1 g of sample. Afterwards, it was compared with the signal intensity of the $MgO:Mn^{2+}$.

3. Results and Discussion

In the EPR spectra of the seeds, we detected the same complex of Fe^{3+} found in goethite (Fig. 1), with g~2 in all the investigated seeds. In addition, free radicals have also been detected with g=2.004±0.002, on all seeds, and with

Table 1 - Summary of the EPR parameters of the seeds

Seeds	Fe^{3+}			Organic free radical		
	g	ΔH (Gauss)	RI	g	ΔH (Gauss)	RI*
Radish wild	2.1 ± 0.2	899 ± 50	7.4	2.004 ± 0.003	10 ± 2	2.0
Rice	2.1 ± 0.2	880 ± 50	3.5	2.004 ± 0.003	10 ± 2	2.1
Mustard	2.1 ± 0.2	744 ± 50	3.3	2.005 ± 0.002	10 ± 1	6.9
Wheat	2.0 ± 0.2	789 ± 50	2.3	2.005 ± 0.003	10 ± 2	1.0
Oats	2.0 ± 0.2	749 ± 50	2.1	2.005 ± 0.003	10 ± 2	1.2
Sorghum	2.1 ± 0.2	873 ± 50	1.4	2.004 ± 0.003	10 ± 2	1.8
Sunflower	2.0 ± 0.2	812 ± 50	1.3	2.003 ± 0.001	7 ± 1	45.7
Soybean	2.0 ± 0.2	778 ± 50	1.3	–	–	–
Cotton	2.1 ± 0.2	804 ± 50	1.1	2.004 ± 0.003	7 ± 2	3.3
Beans	2.1 ± 0.2	792 ± 50	1.0	2.004 ± 0.003	10 ± 2	1.0
Maize	2.1 ± 0.2	852 ± 50	1.0	2.003 ± 0.003	10 ± 2	1.5
Barley	2.0 ± 0.2	826 ± 50	1.0	2.005 ± 0.003	10 ± 2	2.0
Goethite	2.0 ± 0.2	740 ± 50	-	-	-	-

* Relative intensity of the EPR signals

g=2.013 only in sorghum seeds at room temperature. The sunflower seeds showed the highest signal intensity of the free radical with g=2.003±0.001 (Table 1). Saab and Martin-Neto [2] attributed to semiquinone, the organic free radical signal with g=2.004 found in soil organic matter. Saifutdinov et al. [3] determined values of the factor g for several free radicals. In the seeds, the organic free radicals with g=2.003 were due to the unpaired electrons interacting with the semiquinone and in the radicals with g=2.013, the interactions were with the peroxides. All the others seeds investigated showed EPR spectra similar to the spectra of Fig 1, differing only in intensities (Table 1).

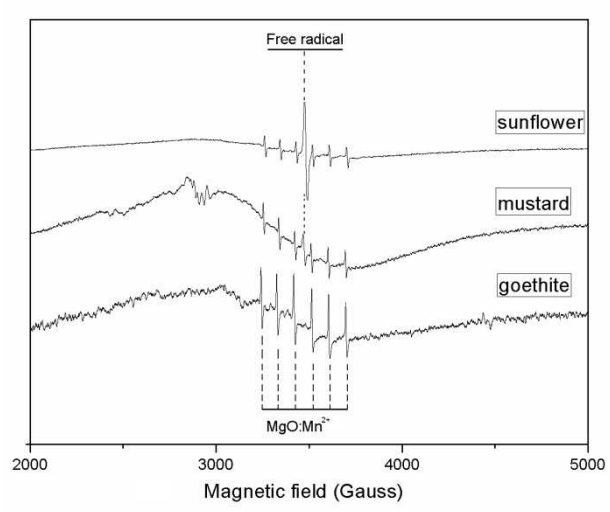

Fig. 1. Spectra EPR of seeds and goethite.

Fig. 2 shows the EPR spectra of the mustard seed and goethite at 30 K. The spectrum of the mustard changed and showed an aspect a little different to spectra found for the seeds at room temperature (Fig. 2), *i. e.* , beyond the higher intensity on the signal of the goethite (g=2.062 and ΔH=317) and free radical (g=2.0044 and ΔH=10), besides that, the spectrum of the mustard also showed hematite, normally present in soil samples [4] with a overlapping line (g=2.0 and ΔH=526) to goethite line (g=2.062 and ΔH=317 Gauss), and two lines (g=5.8 and g= 4.3) on the left of the spectrum (Fig. 2). The spectrum showed also traces of Mn^{2+}, six lines (g=2.022 and A=91 Gauss) overlapping to goethite line (g=2.062 and ΔH=317 Gauss), that is not the standard MgO:Mn^{2+}, because in this experiment it did not use the standard. Manganese is usually present in seeds samples [5-10], but it was not detected in our samples in the spectra at room temperature. This could be due to the fact that the Fe^{3+} showed an intense and wide EPR line which is superposed by the manganese lines [11], since Mn^{2+} associated with organic matter has interaction that broadens the signal and, consequently, the EPR line disappears [12]. Mcbride [13] verified the loss of the EPR signal of Mn^{2+} due to the considerable linewidth enlargement caused by very stable bonds of Mn^{2+} with carboxylic acids. All the others seeds investigated showed EPR spectra at 30 K similar to the spectrum of the mustard. Thus, these results confirm that the seeds showed structures of Fe^{3+} similar to goethite, in addition to small quantities of other species (hematite and Mn^{2+}).

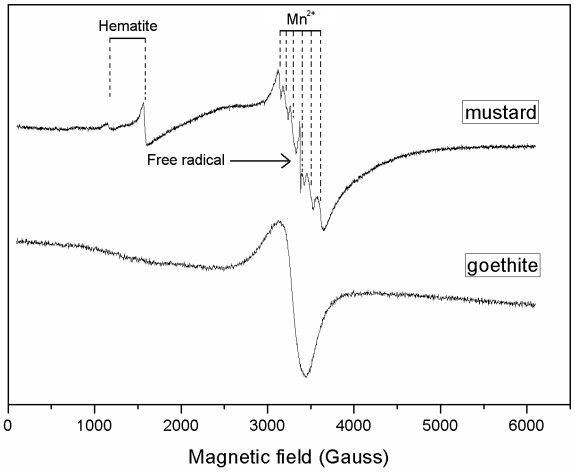

Fig. 2. Spectra of the mustard and goethite at 30 K.

With the purpose of examining the relationship between the soil type and the EPR signal exhibited by the paramagnetic species in the seeds, the research may continue in the future study with the seeds of plants grown in other soils. It observes the behaviour of the EPR signal, if it is similar or different than signals already studied in the present project.

4. Conclusion

According to the EPR spectra, the seeds showed signals of organic free radicals and Fe^{3+}, in addition to traces of Mn^{2+}. The free radical present in the seeds of wild radish, rice, mustard, wheat, oats, sorghum, sunflower, soybean, cotton, beans, maize and barley, with g=2.003±0.002, is due to the unpaired electron interacting with the semiquinone. The sorghum seed showed, beyond this, organic free radical with factor the g=2.013 which is due to the peroxyl radical. The Fe^{3+} signals exhibited by seeds are due to goethite, but hematite was also detected in small quantities in all the samples.

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