INTRODUCTION

In the last decades the world community has been investing in the development of renewable fuel programs, in order to find suitable fuels capable of replacing the diesel derivative ones (Hassan and Kalam, 2013; Kimura et al., 2019), since the high use of fossil substances has expressively contributed to environmental problems (Ma and Hanna, 1999; Saluja et al., 2016).

Among these fuels, biodiesel has stood out. It is generally produced through a transesterification process (de Almeida et al., 2015; Liang et al., 2006) of vegetable oils (Jain and Sharma, 2010) and animal fats (Knothe, 2005). Several vegetable oils can be used to produce biodiesel such as: soybean (Cremonez et al., 2015), sunflower (Ramadan 2013; Raymon et al., 2013), corn (Baştürk et al., 2018), cotton (Souza et al., 2014), etc.

Biodiesel stability is totally correlated to the raw material used for its production, since unsaturated fatty acids are more likely to oxidize when compared to saturated compounds (Chendynski et al., 2019; Knothe, 2007; Knothe and Steidley, 2018; Mantovani et al., 2018). The biodiesel oxidative stability is one of the main concerns about this biofuel. Several studies have been developed in order to analyze the biodiesel oxidation reaction (Chendynski et al., 2020; Devi et al., 2019; Mantovani, Chendynski, Galvan, Borsato and Di Mauro, 2020; Mantovani, Chendynski, Galvan, de Macedo Júnior, et al., 2020).

Due to the high importance of biodiesel and oils worldwide, the study of different raw materials is necessary. Furthermore, the soil that the raw material is grown, can also add important characteristics to the seed (Ma and Hanna, 1999; Saluja et al., 2016).

As one of the raw materials of biodiesel production, seeds have been the subject of several studies. Nakagawa and Hara (2015) investigated the radical location in sesame seeds by Continuous-Wave (CW) X-band Electron Paramagnetic Resonance (EPR). They observed that the paramagnetic species present in the sesame seeds were located in the seed coat and in the hilum region. In addition, they detected that free radicals were located in the seed as a whole (Nakagawa and Hara, 2015). Hamadou et al. (2020) studied the influence of physicochemical characteristics of neem seeds in the biodiesel production. Gaffney (2020) studied via EPR spectroscopy the lipoxygenases, an important and abundant component in soybean seeds. Chakraborty and Bhattacharjee (2020) investigated substances in mustard seeds via EPR spectroscopy. They observed the radical scavenging activities and also antioxidants properties in the seeds. Additionally, Barbana et al. (2015) showed evidence of the presence of iron complexes and free radicals in a series of oilseeds. Besides the free radical species, iron (Fe) complex is another specie with unpaired spins found in seeds and...
detectable with EPR. Due to its physiological importance at vegetal development, Fe can be found in several plant structures, from leaf mesophyll as well as in its seeds. As are presented next, recent papers with the purpose of tracking down the iron path in plants from its absorption demonstrates the existence of iron in seed. The majority of this Fe is found in the form of ferritin, a hollow protein with quaternary structure, composed of 24 subunits that can store ferric iron (Fe$^{3+}$) in its core (Zielińska-Dawidziak, 2015), working as an iron resource, avoiding the toxic potential of excess iron to generate reactive oxygen species. Although vacuoles are sites of iron reserve in cells, in the legume seeds, it was shown for different stages of pea seeds development (*P. sativum* L.) that ferritin could store up to 4500 iron atoms in its hollow core (Moore et al., 2018).

Iron is a micronutrient of diminished mobility although highly demanded on plants. In most plant species iron is a micronutrient absorbed in greater quantity, being important to enzymatic process, photosynthesis, adenosine triphosphate (ATP) production, among others (Taiz et al., 2017). In spite of Fe importance, it is well known that it is not rare to find researches explaining that absorption, adsorption, mobility and storage in plants still lacks better description.

The present study is motivated by the importance of biodiesel as a source of renewable fuel, and the fundamental role that seeds have in its production. Considering this, it is important to better understand the form of storage and other processes involving Fe ions in plants and seeds. Taking into account the lack of studies in this area using the EPR technique, the present study aims to identify the different magnetic species present in oilseed samples using X-band EPR spectroscopy, comparing their main parameters. This study may assist future investigations in related matters.

**MATERIALS AND METHODS**

**Samples Preparation**

In the present work were analyzed seed samples of sorghum, cotton, oats, mustard, rice, sunflower and radish wild. All seed samples were provided by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), a Brazilian agricultural research corporation. They were cleaned individually using acetone (≥99.9%) by mechanical friction with the aid of cotton swabs. The process was made at room temperature, taking care to completely remove the impurities.

**EPR Analysis**

EPR experiments at room temperature were performed at an X-band JEOL spectrometer (IES-PE-3X) of the Electron Paramagnetic Resonance Laboratory, State University of Londrina. Analyses with temperature variation, in a range from 50 to 70 K, were performed with an X-band BRUKER spectrometer (Elexys E-580) of the Molecular Biophysics Sérgio Mascarenhas Group, Physics Institute, University of São Paulo - São Carlos campus (IFSC – USP). The samples were put into 4 mm quartz EPR tubes, previously checked for the absence of any EPR signal. To obtain the g values, a field marker of MgO:Mn$^{2+}$ (g = 1.981 of the fourth line) was maintained in the EPR cavity of the JEOL spectrometer in selected experiments. The field marker data were recorded simultaneously with the samples.

**RESULTS AND DISCUSSIONS**

EPR of seed samples at room temperature showed a combination of two resonances, besides six lines due to the field marker MgO:Mn$^{2+}$. Three selected seeds spectra (sunflower, mustard and sorghum) are shown in Figure 1. The other seeds spectra showed similar characteristics and its parameters are shown in Tables 1 and 2. The seeds were also submitted to EPR measures without the coat and no difference was observed.

The wide resonance line (g ≈ 2.1) shows parameters (Table 1) similar to iron oxides, as goethite (Valezi et al., 2016, 2019) and ferrihydrite (Siqueira et al., 2011). The resonance in the center of the six field marker lines (g varying between 2.011 and 2.015), which can be found in some seed’s spectra, is identified as a free radical signal.

Despite the similarity of the seed samples’ resonances with goethite and ferrihydrite, it is well known that the most common form of iron storage in organic compounds as seeds is the ferritin (Moore et al., 2018). This similarity is expected, since ferritin structure is similar to ferrihydrite (Chasteen D. and Harrison, 1999; Tatur and Hagen, 2005). The presence of ferritin in the samples can also be seen when we compare their

![Figure 1. EPR spectra of sunflower, mustard and sorghum seeds at room temperature. The six lines shown in the spectra are due to the field marker MgO:Mn$^{2+}$](image)

Table 1. Summary of the EPR parameters of the seeds for Fe$^{3+}$ resonance (goethite)

| Sample   | g   | ΔH$_{pp}$ (mT) | RI* |
|----------|-----|---------------|-----|
| Sunflower| 2.1 | 76.2 ± 5.0    | 0.29|
| Mustard  | 2.1 | 79.0 ± 5.0    | 2.14|
| Sorghum  | 2.1 | 84.5 ± 5.0    | 0.29|
| Cotton   | 2.1 | 75.5 ± 5.0    | 0.23|
| Rice     | 2.1 | 80.2 ± 5.0    | 0.67|
| Oat      | 2.1 | 78.0 ± 5.0    | 0.33|
| Radish wild | 2.1 | 86.6 ± 5.0   | 10.0|

Relative intensity of the EPR signals
EPR spectra parameters with other ferritin spectra found in several systems, such horse spleen ferritin (Aime et al., 1997; Wajnberg et al., 2001), splenic ferritin and hemosiderin (Weir et al., 1985).

From Table 2 it can be noticed that the sunflower seeds showed the highest signal intensity of free radical, with \( g = 2.011 \pm 0.002 \), being higher than the other seeds. The origin of this singlet signal is not obvious. This free radical signal has been attributed to semiquinones radicals (Saab and Martin-Neto, 2008; Sajfutdinov et al., 2001), lignin, phenols, oxidation of fatty acids detectable in some vegetables (Yarbasi et al., 2011) as interactions with peroxides. The semiquinone free radicals are directly associated with the humification of some substances as well as with soil-organic minerals (Moreira et al., 2019; Saab and Martin-Neto, 2008).

It is known that the levels of semiquinone resulting from the quinone reduction has an inverse correlation with Fe\(^{3+}\) species. The higher the semiquinone level, the lower the Fe\(^{3+}\) species concentration is (Mangrich et al., 2009), since the possible oxidation – redox reaction - carried out by the Fe\(^{3+}\) produces semiquinones radicals (Sotomatsu et al., 1990; Wang et al., 2020).

In order to analyze possible changes in the magnetic species characteristics of the seeds, EPR analysis at a lower temperature was employed. Figure 2 shows EPR spectra of selected mustard and sorghum seeds at 30 K. The other samples analyzed showed similar spectra.

At low temperature (30K), the seeds spectra showed differences when compared to their room temperature spectra. Firstly, it can be seen a wide central resonance line, which is composed of two components: \( g \approx 2.3 \) and \( \Delta H_{pp} \approx 140 \) mT; and another one with \( g \approx 2.1 \) and \( \Delta H_{pp} \approx 50 \) mT. These signals, especially the one identified as \( g \approx 2.3 \), are poorly resolved, and their parameters are difficult to estimate accurately. In low fields it is also possible to note resonances at \( g \approx 4.3 \) and \( g \approx 6.0 \).

The signals observed at 30 K can be compared with resonances due to ferritin observed in different biological systems, observed in the data at room temperature as well. EPR spectra with similar characteristics of those showed for seeds at 30 K (Figure 2) are found in EPR spectra of horse spleen at 20 K, which shows a central resonance with \( g \approx 2.0 \), overlaid by a poorly resolved shoulder at lower fields, in addition of a smaller resonance at \( g \approx 4.3 \) (Aime et al., 1997). These signals are attributed to ferritin and the superimposed resonances near \( g \approx 2.0 \) are related to ferricydrite cores, while \( g \approx 4.3 \) are related to rhombohedral F(III) sites (Aime et al., 1997). Low temperatures EPR spectra of neuromelanin extracted from human midbrains, which has polynuclear oxy-hydroxy ferric aggregates and isolated Fe III centers (Aime et al., 1997), shows similarities with the seed’s spectra, especially at 120 K. Another example of EPR spectra with similar parameters are found in Pyrococcus furiosus ferritin at 20 K (Tatur and Hagen, 2005).

EPR signal with \( g \approx 6.0 \) was found in some cases as a shoulder of the signal at \( g \approx 4.5 \) (Castner et al., 1960; Loveridge and Parke, 1971; Sreekanth Chakradhar et al., 2006; Yahiaoui et al., 1994) in experiments done at room temperature in glasses doped with the iron oxide hematite. These resonance lines also appear in EPR experiments at room temperature due to Fe\(^{2+}\) complexes that could be associated with hematite (Bensimon et al., 1999; Jahagirdar et al., 2013).

Besides the characteristic signal of ferritin, it can also be noted in Figure 2 the presence of a free radical in mustard and sorghum spectra, with \( g \approx 2.004 \) and \( \Delta H_{pp} \approx 1.0 \) mT. The observed alteration in radical parameters is caused by changes in the electron neighborhood due to temperature decreasing.

Furthermore, it can be observed in Figure 2 the presence of a sextet of lines, concentrated near the range of \( g \approx 2 \). These lines are characteristic of Mn\(^{2+}\), due to the hyperfine interaction (I = 5/2). These Mn\(^{2+}\) lines are not caused by the field marker, because it was not used in these measurements, indicating that the samples contain some traces of manganese, which is common in seeds (Kikuchi et al., 2010; Ukai et al., 2008). These signals are shown only at very low temperatures. One condition contributing to this may be the fact that, at low temperature, the number of spins in the lower energy level is increased, thus enabling the detection of some species that would be impossible at room temperature. Dixon and Weed (1989) 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.

With the exception of the relative resonance intensity variation of the studied seeds spectra, all seeds analyzed in this study showed EPR spectra similar to those observed in mustard and sorghum samples, both at room temperature and at 30 K.

| Sample     | g      | \( \Delta H_{pp} \) (mT) | RI* |
|------------|--------|--------------------------|-----|
| Sunflower  | 2.011 ± 0.002 | 1.46 ± 0.2 | 10  |
| Mustard    | 2.012 ± 0.002 | 1.09 ± 0.2 | 5.843 |
| Sorghum    | 2.013 ± 0.002 | 0.98 ± 0.2 | 0.57 |
| Cotton     | 2.013 ± 0.002 | 1.20 ± 0.2 | 0.85 |
| Rice       | 2.013 ± 0.002 | 1.33 ± 0.2 | 0.50 |
| Oat        | 2.013 ± 0.002 | 0.64 ± 0.2 | 0.31 |
| Radish wild | 2.013 ± 0.002 | 1.12 ± 0.2 | 3.40 |

*Relative intensity of the EPR signals
Thus, it was possible to demonstrate that the seeds have structures of Fe$^{3+}$ that are similar to those present in ferritin, besides species characteristics of Mn$^{2+}$.

**CONCLUSIONS**

According to the EPR spectra, it was detected in the seed’s samples presence of free radical and iron oxides species, the latter due to the presence of ferritin. The free radical showed spectroscopy factor g varying from 2.011 to 2.013, which was identified as a semiquinone radical. For the iron oxides temperature and low temperature, indicated the presence Fe$^{3+}$ ferritin. The free radical showed resonances, the comparison with literature data at room temperature and low temperature, indicated the presence Fe$^{3+}$ ferritin. Furthermore, it was found traces of Mn$^{2+}$ in sorghum samples, as revealed by the 50 K resonance experiments.

The characterization of paramagnetic species in seeds can potentially assist future studies about biodiesel and other products that have seeds as raw material, as well as the study of pollutants due to the burning of fuels derived from biodiesel.

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**Declaration of interest:** The authors declare that they have no competing interests.

**Ethics approval and consent to participate:** Not applicable.

**Availability of data and materials:** All data generated or analyzed during this study are available for sharing when appropriate request is directed to corresponding author.

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