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Location of Radical Species in a Black Pepper Seed Investigated by CW EPR and 9 GHz EPR-Imaging

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Abstract

The noninvasive 9 GHz Electron paramagnetic resonance (EPR)-imaging and continuous wave (CW) EPR were used to investigate locations of paramagnetic species in black pepper seeds without further irradiation. First, Lithium phthalocyanine (LiPC) phantom was used to examine 9 GHz EPR imager capabilities. The 9 GHz EPR-imager easily resolved LiPC samples spaced at ~ 2 mm distance. Then, commercially available black pepper seeds were measured. We observed signatures of three different radical species, which can be stable organic radical, Fe^{3+} , and Mn^{2+} complexes. In addition, no EPR spectral change of the seed was observed after submerged in distilled H_2O for one hour. The EPR and spectral-spatial EPR imaging results suggested that the three paramagnetic species are located mostly at the seed surface. Fewer radicals were found inside the seed. We demonstrated that CW EPR and 9 GHz EPR-imaging can be useful for determination of spatial distribution of paramagnetic species in various seeds.

Keywords

EPR; imaging; free radical; LiPC; pepper; ESR

1. Introduction

Electron paramagnetic resonance (EPR) or Electron spin resonance (ESR) spectroscopy utilizes the electron-spin resonance phenomenon and measures the resonant microwave power absorption spectra of unpaired electrons subjected to a constant magnetic field in an atom, molecule, or compound. EPR is capable to noninvasively measure samples. Majority of the EPR research in food was concentrated on free radicals of the irradiated and/or grind foodstuff [1-4] rather than on the endogenous species. EPR imaging is a powerful noninvasive technique for measuring of the spatial distribution of paramagnetic species [5-7]. A two-dimensional (2D) spectral-spatial imaging provides information both distribution and the line-width of paramagnetic species. Thus, noninvasive EPR imaging and

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continuous wave (CW) EPR can provide quantitative information about the detailed paramagnetic species.

In the areas of food science and research, identification of localized radical species in foodstuffs is an important subject. Knowledge of location (region) of paramagnetic (or free radicals induced) species can guide for further biochemistry or chemistry of paramagnetic species. EPR imaging of the radical species might give information about location of the species. The information will provide important ideas regarding food processing, conservation strategy, and shelf-lives.

Current applications of *in vivo* EPR imaging are mostly concentrated on small animal imaging at frequencies below 1 GHz [8, 9]. This choice of EPR frequencies is dictated by the reduction of microwave irradiation penetration depth in samples at higher frequencies. These measurements have relatively low sensitivity because of the low frequency at which they operated. Generally, the 9 GHz EPR imager is one to two-hundred times more sensitive than that of 1 GHz [10]. The sensitivity of the 9 GHz EPR is able to detect paramagnetic species existing in materials such as ascorbic acid in blood [11] and carbon centered organic radicals in foods [1].

We investigated location of paramagnetic species in black pepper seeds without further irradiation and chemical treatment using CW 9 GHz EPR and the EPR imaging. We also studied LiPC crystals spaced at 2 mm apart. In addition, we analyzed the distribution of the paramagnetic species in pepper seed. The application of EPR imaging and CW EPR techniques for the food monitoring are also discussed.

2. Material and methods

2.1 Samples

The bottled black pepper seeds were purchased at a local super market about five years ago. The seeds were used as purchased. One seed (~0.02 g) was inserted into an EPR tube (o.d., 5.0 mm, i.d. 4.0 mm, Wildmat-LabGlass, USA) for measurements.

Lithium phthalocyanine (LiPC) crystals were a gift from Prof. H.M. Swartz (Dartmouth Medical College) and used as given [12]. LiPC is useful for EPR oximetry due to sensitivity to oxygen of its line-width. In our measurements, the LiPC crystals were attached to a plastic plate (~3 mm width) using commercially available synthetic rubber type glue. The phantom was composed of 2 LiPC point-samples with the layout as depicted in Figure 1. The first and the second LiPC crystals were 2 mm apart. For the measurements, the LiPC phantom was inserted into an EPR tube.

2.2 EPR measurements

For 9 GHz EPR imaging, one set of gradient coils with an anti-Helmholtz coil configuration were used. The gradient coils were cooled by water to avoid overheating. Techtron 7570 power supply (Indiana, USA) was used. The available maximum field gradient along z-axis was approximately 9 mT/cm. All CW EPR spectra were obtained with a single scan.

Typical EPR imaging settings were as follows: microwave power, 5 mW; time constant, 1 s; sweep time, 2 min; magnetic field modulation, 0.3 mT; sweep width, 15 mT. All measurements were performed at ambient temperature.

2.3 Imaging data processing

The first-derivative EPR spectra were numerically integrated to obtain the corresponding absorption spectra. For two dimensional (2D) spectral-spatial EPR imaging, we used 16 projections obtained with gradients, G_i , from 0 to ~6.5 mT/cm. The projections were centered on the crossover magnetic field, truncated to

$$SWEEP_i = \sqrt{2dB} / \cos(\Theta_i), \quad (1)$$

where $\Theta_i = \tan^{-1}(G_i \times dL/dB)$, scaled by $\cos(\Theta_i)$ and resampled to 100 points [13]. The magnetic field support, dB, was chosen to be 2 mT, and the spatial support of the image was 1 cm. Then, the data were back-projected to obtain a 2D spectral-spatial image using a filtered back-projection algorithm. Data were processed in the Mathworks MATLAB™ environment, 'iradon' function was used for back-projection. The details of the EPR and data processing are also described elsewhere [5].

3. Results and Discussion

3.1 EPR imaging of LiPC (Phantom)

For the initial test, the LiPC phantom was imaged. Figure 1 shows that schematic illustration of the LiPC phantom. The glued LiPC crystals were ~2 mm apart. Figure 2 shows EPR spectra at different field gradient strengths. The top spectrum in Figure 2 shows the phantom measured without the field gradient. The EPR spectrum has a strong single EPR line. The bottom spectrum shows the phantom measured with the ~6.5 mT/cm field-gradient. The spectrum in Figure 2 clearly shows at least two separate peaks. Thus, the EPR imager is able to resolve objects of the paramagnetic species separated by 2 mm.

It is noted that the small additional peaks indicated by filled triangles were due to small LiPC crystals beside the two main crystals. As mentioned in the method section, the LiPC crystals repel each other due to strong static electrical charges.

The left panel of Figure 3 shows 2D (spectral-spatial) image of the LiPC phantoms. The two areas indicated by the marks ① and ② correspond to the two peaks in Figure 2. The middle panel of Figure 3 shows the spatial dimension at the maximum of EPR lines. Two strong peaks were obtained after the data processing. The right panel of Figure 3 shows EPR signal as a function of the magnetic field. Two signals of the EPR spectrum at spatial positions ① and ② are displayed. Thus, 9 GHz EPR imaging of the phantom prove two paramagnetic species in approximately 2 mm apart.

3.2 EPR imaging of Pepper (Seeds)

Figure 4 shows a picture of a pepper seed. The pepper seed does not have a smooth surface. We measured the whole pepper seed inserted into an EPR tube. There are three distinguishable species. First, one is a relatively sharp feature, which can be the stable

organic radicals such as carbon-centered radicals [1]. The line-width (H_{pp}) is approximately 1.0 mT.

To verify the paramagnetic species of the pepper seed, we broke it up into two parts, outside and inner side of the pepper. Figure 5 shows the EPR spectrum of a whole pepper seed. The spectrum was taken with 200 mT scan-width. There are three species in the spectrum of the whole seed. The first one is a broad feature indicated by filled diamonds. The second one is characteristic Mn^{2+} related sextet. The sextet is Mn^{2+} 6 lines ($I = 5/2$, natural abundance is 100%) [14].

The third one can be Fe^{3+} . The two filled diamonds indicate field positions corresponding to $g = 2.50$ and $g = 1.98$, respectively. The g -values of the spectrum in Figure 5 were calculated by the following:

$$H\gamma = g\beta H, \quad (2)$$

where γ is the microwave frequency, g is the g -value, β is Bohr magneton, and H is the applied magnetic field. Based on the g -values of the peaks, the paramagnetic species can be iron complexes [15]. It is noted that the magnetic field of the EPR spectrometer is controlled by the current in order to perform EPR imaging (field gradient). The g -values may not be very accurate because the magnetic field is not monitored by the NMR probe which EPR spectrometer ordinarily does.

The EPR spectrum composed of the sextet are attributed to Mn^{2+} peaks. The hyperfine couplings are larger at a higher field due to overlapping with other broad features. A similar EPR spectrum was observed in other black pepper (not seed), previously [1]. The relatively sharp single-peak can be due to stable organic carbon-centered radicals or transition metal ions [15].

The EPR spectrum with 30 mT scan is shown in Figure 6 (top spectrum). The peak-to-peak width of the relatively sharp feature is an approximately 0.68 mT. EPR spectrum of the inner side of the pepper is shown in Figure 6 (bottom spectrum). After removal of the surface area of the pepper, the rest was inserted into an EPR tube. The relatively sharp feature was observed.

In addition, we effect of humidity on paramagnetic species in the pepper seed. The sample was soaked in distilled water (1 ml) for 1 hr and dried it for 5 hrs at ambient temperature. It is noted that the solution became brown color after submerge. Then, EPR measurements were performed. The EPR intensities are the same that was not soaked as shown in Figure 7. We found no difference in spectrum between the two samples.

We attempted to reveal the distribution of paramagnetic species in the seed using 9 GHz EPR imager. The 2D (spectral-spatial) image of the whole pepper seed is shown in Figure 8. The relatively sharp peak at the central region was used for the 2D imaging. To overcome broad background signals that position filtered back-projection reconstructions we used iterative parametric reconstruction. The spectral shape of the signals was estimated from zero gradient spectra. The spatial distribution was the optimization parameter. The spatial

coordinate of Y-axis shows that the signals are within 2 mm size in the left-hand panel of Figure 8. Image shows signals from all areas because of the low image resolution and paramagnetic species distributed all over the surface area of the pepper.

For proper reconstruction of image using filtered back-projection, complete support of an object in spatial and spectral domains is required. This is hard to achieve for the relatively sharp peak overlapped with very broad signal observed in the pepper. The development of region of interest (ROI) imaging in spectral domain can potentially be instrumental. ROI reconstruction of spatial image was demonstrated elsewhere [16]. The present EPR imaging may not resolve the inner core of the seed. To achieve a better resolution we may need to perform 2D or 3D imaging with higher applied magnetic field gradients.

Furthermore, the 9 GHz EPR imaging may provide noninvasive information regarding environmental damage (e.g. UV irradiation, radiation, corruption) and/or physical damages inside the materials. The location (region) of paramagnetic (or free radicals induced) species can guide for further biochemistry or chemistry of paramagnetic species.

4. Conclusions

The noninvasive 9 GHz EPR imager and CW EPR were used to investigate paramagnetic species in the whole seed of black pepper. CW EPR measured at least three distinguish paramagnetic species. The EPR imaging was able to resolve samples spaced at approximately 2 mm. The EPR imager is also capable of the 2D (spectral-spatial) imaging of pepper. The present results suggest that paramagnetic species are located on the surface region of the pepper seed. The 9 GHz EPR imaging and CW EPR can be useful to detect and identify the location of paramagnetic species in foodstuffs and other small materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LiPC Phantom

Figure 1.
Schematic illustration of LiPC phantom used. The coordination is indicated.

Figure 2.

EPR spectra observed by the EPR imager with 0 mT/cm (top spectrum) and 6.5 mT/cm (middle spectrum) are shown. The filled triangles indicate a small amount of LiPC away from the main points due to strong static repulsion of the crystals.

Figure 3.

The 2D (spectral-spatial) imaging of the LiPC phantom is shown. Two compositional diagrams of the 2D imaging in Fig. 2 are presented. The middle and right-hand panel indicate absorption mode of EPR spectra (peaks ① and ②) in the spatial and magnetic field coordination, respectively.

Figure 4.
A picture of a black pepper seed. The scale is also indicated.

Figure 5.

Wide range scan of the EPR spectrum for a black pepper seed is presented. The spectrum was taken with 200 mT sweep width. There are three species. The first is Fe^{2+} related signals (two filled diamonds) indicating $g = 2.50$ and $g = 1.98$, respectively. The second is Mn^{2+} related signals (6 lines). The third (arrow) is a relatively sharp signal related to organic radical.

Figure 6.
EPR spectra of the whole seed of black pepper. Top spectrum is a whole black pepper seed.
The bottom spectrum is inner portion of the pepper seed.

Figure 7.

EPR spectrum was taken before (A) and after the soak (B). The sample was soaked in distilled water (1 ml) for 1 hr and dried for 5 hrs at ambient temperature.

Figure 8.

The 2D (spectral-spatial) images of the black pepper seed are presented. It is noted that the resolution is not good because of the strong background signal (Mn^{2+} related signals), which is truncated by the acquisition window.