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Locations of radical species in black pepper seeds investigated by CW EPR and 9 GHz EPR imaging

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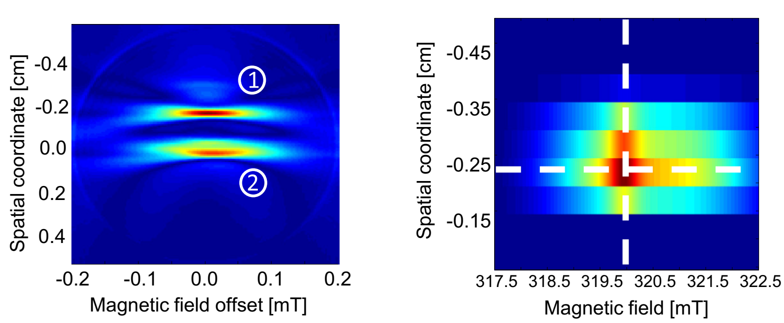
HIGHLIGHTS

- Locations of radical species in black pepper seeds were investigated by continuous wave (CW) EPR and 9 GHz EPR imaging.
- Lithium phthalocyanine (LiPC) phantom was used to examine 9 GHz EPR imaging capabilities.
- Radical species were mostly located at the seed surface.
- CW EPR and EPR imaging were useful for determination of the spatial distribution of paramagnetic species in various seeds.

GRAPHICAL ABSTRACT

Spectral–spatial images of lithium phthalocyanine (LiPC) phantom (left-hand) and a pepper seed (right-hand).



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ABSTRACT

In this study, noninvasive 9 GHz electron paramagnetic resonance (EPR)-imaging and continuous wave (CW) EPR were used to investigate the locations of paramagnetic species in black pepper seeds without further irradiation. First, lithium phthalocyanine (LiPC) phantom was used to examine 9 GHz EPR imaging capabilities. The 9 GHz EPR-imager easily resolved the LiPC samples at a distance of ∼2 mm. Then, commercially available black pepper seeds were measured. We observed signatures from three different radical species, which were assigned to stable organic radicals, Fe^{3+}, and Mn^{2+} complexes. In addition, no EPR spectral change in the seed was observed after it was submerged in distilled H_2O for 1 h. The EPR and spectral–spatial EPR imaging results suggested that the three paramagnetic species were mostly located at the seed surface. Fewer radicals were found inside the seed. We demonstrated that the CW EPR and 9 GHz EPR imaging were useful for the determination of the spatial distribution of paramagnetic species in various seeds.

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, a molecule, or a compound. EPR is capable of noninvasively measuring samples. The majority of the EPR research in food has concentrated on free radicals in irradiated and/or powdered foodstuff [1–4] rather than endogenous species. EPR imaging is a powerful noninvasive technique for measuring the spatial distribution of paramagnetic species [5–7]. Two-dimensional (2D) spectral–spatial imaging provides information on both the distribution and

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line-width of paramagnetic species. Thus, the noninvasive EPR imaging and continuous wave (CW) EPR can provide quantitative information about detailed paramagnetic species.

In the areas of food science and research, identification of localized paramagnetic species in foodstuffs is an important subject. Knowledge of the location (region) of paramagnetic (or free radicals induced) species can guide the further research on the biochemistry or chemistry of the species. The EPR imaging of the radical species may give information about the location of the species. The information may provide important insights for improving of the regarding food processing, conservation strategy, and shell life.

Current applications of *in vivo* EPR imaging mostly concentrate on small animal imaging at frequencies below 1 GHz [8,9]. These EPR frequencies are dictated by the reduction in the microwave irradiation penetration depth in sample tissues at higher frequencies. The imaging of small specimens does not have to be restricted to low frequencies. Generally, the 9 GHz EPR imager is 1–200 times more sensitive than the 1 GHz EPR imager [10]. The sensitivity of the 9 GHz EPR allows it to detect paramagnetic species in materials, such as ascorbic acid in blood [11] and carbon-centered organic radicals in food [1].

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

Materials and methods

Samples

Commercially bottled black pepper seeds were purchased from 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, Wilmad-LabGlass, USA) for measurements.

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

EPR measurements

For 9 GHz EPR imaging, one set of gradient coils with an anti-Helmholtz coil configuration was used. To avoid overheating, the gradient coils were cooled using water. A Techron 7570 power supply (Indiana, USA) was used. The maximum available field gradient along the *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; and sweep width, 15 mT. All measurements were performed at ambient temperature.

Imaging data processing

The first-derivative EPR spectra were numerically integrated to obtain the corresponding absorption spectra. For 2D spectral–spatial EPR imaging, we used 16 projections obtained with gradients

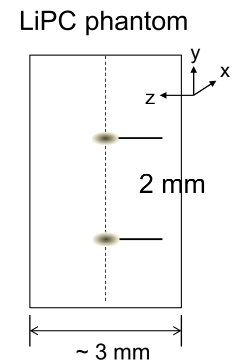

Fig. 1. Schematic of the used LiPC phantom. The coordination is indicated.

(G_i) from 0 to ~6.5 mT/cm. The projections were centered on the crossover magnetic field truncated to

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

where $\Theta = \tan^{-1}(G_i \times dL/dB)$, scaled by $\cos(\Theta_i)$ and resampled to 100 points [13]. The magnetic field support (dB) was 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. The data were processed in the MathWorks MATLAB™ environment, and the "iradon" function was used for back-projection. The details of EPR and data processing are also described previously [5].

Results and discussion

EPR imaging of LiPC (phantom)

For the initial test, the LiPC phantom was imaged. Fig. 1 shows a schematic of the LiPC phantom. The glued LiPC crystals were ~2 mm apart. Fig. 2 shows the EPR spectra at different field gradient strengths. The top spectrum in Fig. 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 a ~6.5 mT/cm field-gradient. The spectrum in Fig. 2 clearly shows a minimum of two separate peaks. Thus, the EPR imager could resolve paramagnetic species separated by 2 mm.

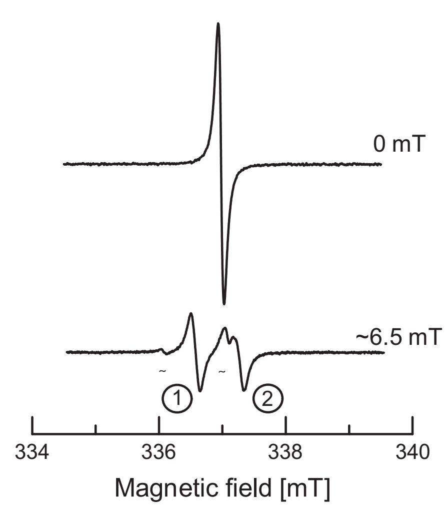

Fig. 2. The EPR spectra observed by the EPR imager with 0 mT/cm (top spectrum) and 6.5 mT/cm (middle spectrum). The filled triangles indicate a small amount of LiPC away from the main points due to the strong static repulsion of the crystals.