



EDULAB FOR INSTRUCTORS: MAGNETTECH ESR5000

# Spice and Spin: The Zesty World of EPR

## The Benefits of Irradiated Spices and Herbs

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**Experiment Hashtag: #spices, #herbs, #irradiation, #Educate2Resonate**

### Keywords:

Spices, Free Radicals, Antioxidants,  $\gamma$ -irradiation, Food Analysis

### Target group:

Advanced Undergraduate or Graduate, General Chemistry, Analytical Chemistry, Food Chemistry, Food Safety and Control Laboratory, General Life Sciences

### Objectives:

- Gain practical experience with the Bruker Magnettech ESR5000 benchtop spectrometer while deepening your understanding of electron paramagnetic resonance (EPR) in a hands-on setting
- Learn about food irradiation and antioxidants
- Learn to collect, process and interpret EPR data effectively

## Background of the Experiment:

Currently, there is growing interest in both industry and scientific research regarding spices and aromatic herbs due to their antioxidant and antimicrobial properties. These benefits stem from various active phytochemicals, such as vitamins, flavonoids, carotenoids, coumarins, etc. Because of these nutrients, spices and aromatic herbs are considered vital in diets and medical therapies for delaying ageing and preventing tissue deterioration caused by free radicals. However, spices and herbs can be a source of microbial contamination in the foods they are added to. During harvesting and storage, spices and herbs are often exposed to high levels of natural contaminants such as mesophilic, sporogenic, and asporogenic bacteria, hyphomycetes, and fecal coliforms. Most spices and herbs are dried in the open air, making them susceptible to contamination from air- and soil-borne bacteria, fungi, and insects. To address this, spices and herbs are treated with ionizing radiation to eliminate microbial contamination. International studies confirm that food irradiation is safe in terms of radiation, microbiology, and toxicology. However, debates continue about nutrient loss, free radicals, radiolytic byproducts, and sensory quality of irradiated food. Radicals can already exist in spices and herbs before irradiation due to natural aging, oxidation of components like antioxidants and phytonutrients, or the presence of transition metals. These radicals can further change when exposed to ionizing radiation. The quality and quantity of radicals can also vary during long-term storage, influenced by temperature, humidity, and other environmental factors and can be determined by EPR spectroscopy. In aqueous environments, radicals quickly undergo termination reactions, resulting in short lifetimes. However, in dry samples, radicals can be trapped within a solid matrix, extending their lifetimes to several months. This makes it possible to detect these radicals using EPR spectroscopy in dry samples, a method now included in international protocols for detecting spice and herb irradiation.

In this exercise, students will examine dry spices and herbs to detect paramagnetic species, such as free radicals and transition metals. They will also attempt to identify the detected signals by analyzing their g-factors and hyperfine splittings, if present.

## Preparation:

Students are encouraged to bring spices and herbs from home. To ensure efficient completion of the experiments, it is recommended to form groups of up to three students. Sample preparation should take about 15-30 minutes, depending on the number of samples (a maximum of 5-6 different spices or herbs is recommended). The EPR experiments will take approximately 2 hours, followed by an additional 1-1.5 hours for data processing and report writing. It is assumed that students have already covered the basics of EPR and understand the instrumental parameters.

For detailed information on EPR basics and optimizing instrumental parameters, students can refer to the Magnettech ESR5000 educational kit, provided on an USB drive with the bench-top EPR spectrometer. An installed Magnettech ESR5000 spectrometer is required to perform these experiments.

## Glossary

**EPR (Electron Paramagnetic Resonance):** Also known as electron spin resonance spectroscopy (ESR), EPR is a technique used to study materials with unpaired electrons. The fundamental principles of EPR are similar to those of nuclear magnetic resonance (NMR), but EPR focuses on the spins of electrons rather than atomic nuclei.

**Free radicals:** These are atoms, molecules, or ions that possess at least one unpaired electron.

**Transition Metal Ions:** Transition metals can become positively charged ions by losing electrons, resulting in ions with varying charges and unpaired electrons.

**Antioxidants:** Antioxidants can be found in many foods, especially fruits, vegetables, nuts, whole grains, and spices. Some well-known antioxidants include vitamins C and E, flavonoids, and carotenoids. They work by neutralizing free radicals, thereby reducing oxidative stress and preventing cell damage.

## Experimental Setup:

### Materials:

- 5-6 different spices or herbs (~ 1 g of each). Examples are oregano, turmeric, rosemary, cinnamon, etc.
- 5 mm OD tubes – 6 pieces or more depending on the number of samples (tubes can also be borosilicate and not quartz)

### Sample Preparation:

Fill each 5 mm tube with your material to a height of about 3 cm and insert it into the spectrometer resonator. You can easily scoop the material through the open end of the tube. A 3 cm filling height is recommended to ensure the entire cavity is filled with the sample. This results in consistent outcomes and increased signal intensity, as the EPR signal is directly proportional to the number of spins in the sample. More volume means stronger signals.

## Experimental Procedure:

1. Start the EPR spectrometer up by turning on the power switch located on the back of the unit. Start the ESRStudio software. Connect to the spectrometer by clicking the Initialize button. Insert one of the samples carefully using the proper size sample holder. Use the positioning tool to ensure the sample is properly centered inside the resonator.
2. Select the following parameters:
  - $P_{MW}$  – 10 mW
  - $B_0$  – 337 mT (optimize the value if needed)
  - Sweep – 10 mT
  - Sweep Time – 120 s
  - Modulation – 0.5 mT
  - Frequency – 100 kHz
  - Accumulations – 1
3. Save these parameters as a new recipe 'Spices'.
4. Create a new container in ESRStudio and name it 'Spices and Herbs'.
5. Collect spectra from all the samples in the container 'Spices and Herbs'.
6. After acquiring data from all samples, change the sweep to 550 mT and the sweep time to 300 s.
7. Create another recipe 'Species and Herbs – 550mT'.
8. Collect spectra from all the samples in the container 'Spices and Herbs – 550 mT'.

## Glossary

### Food irradiation:

This is a process that uses ionizing radiation to improve the safety and extend the shelf life of foods and spices. This technology works by reducing or eliminating microorganisms and insects that can cause foodborne illnesses or spoilage.

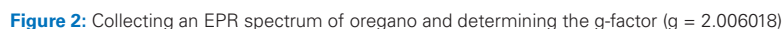
## Abbreviations

**EPR:** Electron  
Paramagnetic  
Resonance

**HFS:** Hyperfine Splitting

## Notes

Figures 1-4 show the EPR spectra of various spices using the instrumental parameters suggested in the experimental procedure. The free radical signals at  $g \sim 2.005$  are typically assigned to quinone radicals. To accurately determine the  $g$ -factor, switch the X-axis from 'B' to the 'g\_Factor' option. Next, choose 'Automatic peak picking' from the processing menu and initiate the operation by clicking the orange funnel icon. Once you enable the 'Show Peak List' option, the  $g$ -factor will be displayed in the peak list on the right side of the EPR spectrum. (Figures 1-4).





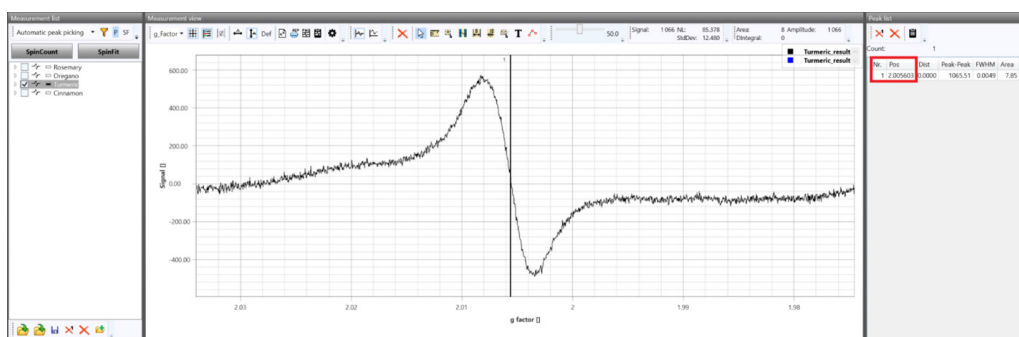


Figure 3: Collecting an EPR spectrum of turmeric and determining the g-factor ( $g = 2.005603$ )

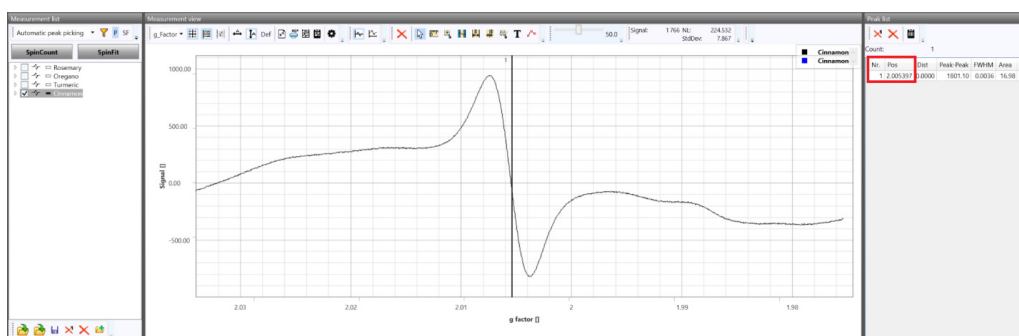


Figure 4: Collecting an EPR spectrum of cinnamon and determining the g-factor ( $g = 2.005397$ )

Adjust the sweep to 550 mT and set the sweep time to 300 s. Then, reacquire the EPR spectra for all samples (Figure 5).

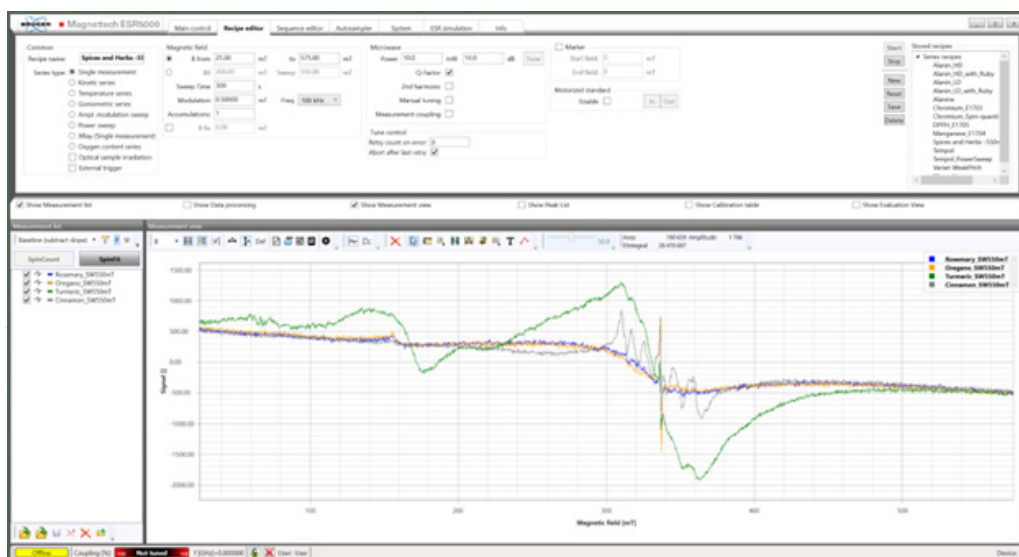
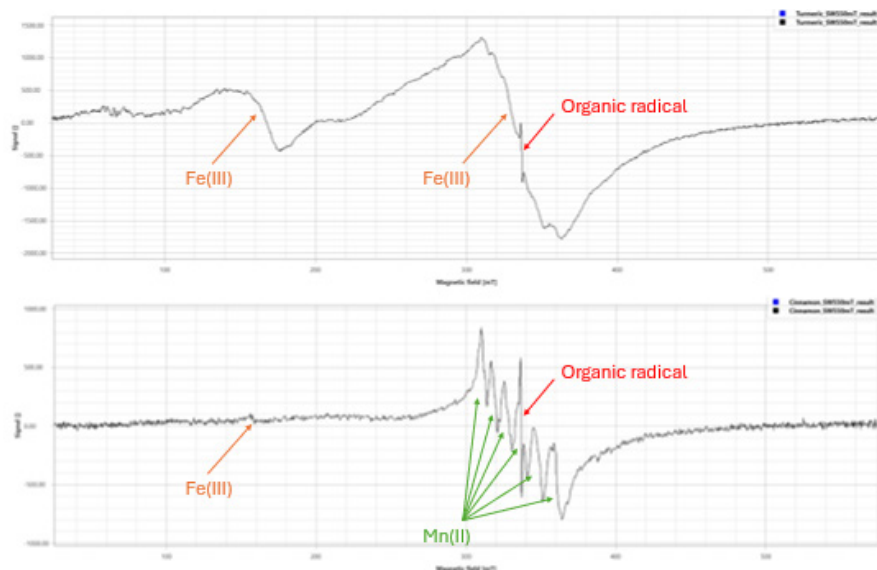


Figure 5: Collecting EPR spectra of all spices with a sweep range of 550 mT

EPR signals due to transition metal ions, such as Fe(III) and Mn(II) were observed. The six-line spectrum of Mn(II) centered at  $g = 2.006$  was easily distinguishable in the spectra of cinnamon and turmeric. The hyperfine splittings (HFS) are well-resolved in the spectrum of cinnamon with  $a_{\text{HFS}} \sim 9$  mT.

Broad signals attributed to Fe(III) ions with signals at  $g = 2.1$  and  $4.3$  were observed for all the samples. They appeared to be remarkably strong in turmeric (Figure 6).



**Figure 6:** Identifying paramagnetic centers in turmeric and cinnamon.

Students should create a table summarizing the paramagnetic species and their characteristics, including the  $g$ -factor and hyperfine splitting constants (if applicable), for all samples.

### Questions for the Students:

**1. What could be the cause of EPR signals in spices?**

Free radicals in spices may result from the natural oxidation of antioxidants and phytonutrients, or in some cases, cellulose. They can also form due to ionizing radiation used to eliminate microbial contamination. Additionally, transition metals, which can naturally occur in spices, can be detected by EPR.

**2. Can you distinguish between signals from organic radicals and transition metals in the spectra you've collected?**

EPR signals with  $g$ -factors near 2 and relatively narrow linewidths (up to 1 mT) are typically associated with free radicals, whereas broader features with  $g$ -factors differing from 2 generally indicate the presence of transition metal ions.

**3. Why are broader sweeps necessary to detect certain EPR signals?**

Transition metals typically have larger orbital angular momentum, causing their  $g$ -values to deviate significantly from  $g = 2$ . The  $d$  and  $f$  orbitals, commonly found in transition metal and rare earth ions, contribute to this substantial orbital angular momentum. Since the  $g$ -value (and thus the resonant magnetic field value) may be unknown beforehand, a broad magnetic field sweep is necessary to locate the signal.

### Notes

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## Key Take-Home Messages:

- Free radicals are prevalent in spices and can originate from various sources.
- Understand the fundamental idea of broad sweeps.
- Recognize how transition metal ions can exhibit different line shapes, hyperfine splittings, and g-factors compared to free radicals.

## References:

1. Galucci L. et al., *Effects of  $\gamma$ -irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices*, J. Agric. Food Chem. 51 (2003) 927-934
2. Pawlowska-Goral K. et al., *Application of EPR spectroscopy to examination of the effect of sterilization process on free radicals in different herbs*, Food Biophys 8 (2013) 60-68
3. Ahn J.J. et al., *Alcoholic extraction enables EPR analysis to characterize radiation-induced cellulosic signals in spices*, J. Agric. Food Chem. 62 (2014) 11089-11098

