EDULAB FOR STUDENTS: MAGNETTECH ESR5000

Chocoholic? Indulge in the sweet science of EPR!

EPR of Dark Chocolate

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Experiment Hashtag: #darkchocolate, #Educate2Resonate

Keywords:

Cocoa, dark chocolate, free radicals, antioxidants, food analysis

Target group:

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

Objectives:

Electron Paramagnetic Resonance (EPR) spectroscopy is a sensitive and versatile technique for analyzing molecules that contain unpaired electrons, such as paramagnetic metal ions and free radicals. The formation of free radicals in foods is an indication of food oxidation mainly due to redox chemistry reactions. In this exercise, the students will learn what information can be extracted from dark chocolate samples using EPR. The goal of this exercise is to teach the students how to detect free radical signals coming from the cocoa and how to correlate the radical concentration with the percentage of cocoa content in dark chocolate. In addition, the students will learn how to run EPR experiments on a bench-top Magnettech ESR5000 spectrometer, optimize experimental parameters, and create a calibration curve using ESRStudio software.

Background of the Experiment:

Chocolate is one of life's most decadent treats. No matter how you enjoy it – as a candy bar, in a hot drink, drizzled over ice cream – chocolate brings joy. Having a healthy relationship with all foods is important for your mind and your body. But beginning or creating a balanced relationship particularly with dark chocolate, may have a significantly positive impact on your overall health. The percentage of cocoa listed on the dark chocolate refers to the percentage of all ingredients that the cacao plant contributes. That means a dark chocolate product with a higher percentage of cocoa may have a larger amount of the nutrients that deliver its benefits.

Cocoa beans are the seeds of the tropical tree *Theobroma* cacao. Because of the high concentration of bioactive compounds, including antioxidants (polyphenols, tocopherols, flavonoids), they are valued not only in the food industry, but also in the pharmaceutical and cosmetic industries. In recent years, interest in these cocoa components has greatly increased because of their potentially beneficial effects on human health. Cocoa antioxidants can inhibit or delay cellular damage, either by quenching free radicals or through chelation of transition metal ions, which reduces their capability to form reactive oxygen species. They also exhibit a wide range of physiological properties, resulting in protection against diseases, including coronary heart diseases, cancer, or neurodegenerative disorders. The most important antioxidants of cocoa beans are polyphenols. Their concentration is around 12 – 18 % of a raw cocoa bean's dry weight.

Roasting is the principal technological production step affecting the quality of both processed cocoa and their derived products. The thermal processing of cocoa beans plays an important role in formation of the mild aroma and characteristic taste of cocoa beans. Some studies reveal that the temperature and duration of roasting affect the antioxidant activity of cocoa beans, mainly due to degradation of phenolic compounds, especially flavonoids. It is also known that polyphenols can be oxidized into stable free radicals that are easily detected by EPR.

In this exercise, students will analyze dark chocolates containing various known cocoa content (78, 85, 90, and 95%), as well as white chocolate (0% cocoa). Students will look for a free radical EPR signal coming from oxidized polyphenols due to roasting and then will characterize the signal from each sample (g-factor and peak-to-peak amplitude). They will also analyze a sample with unknown polyphenol radical concentration.

For the quantitative analysis, students will create a calibration curve, EPR amplitude$_{\text{peak-to-peak}}$ = f (% cocoa content), and perform a linear fit. Students will then use the calibration curve to measure the cocoa content of a chocolate sample of unknown content.

Preparation:

To ensure efficient completion of the experiments, it is recommended to form groups with a maximum of three students. The estimated time for sample preparation is approximately 1 hour. The EPR experiments are expected to take approximately 2 hours in total (including 4 samples of dark chocolate and 1 sample of white chocolate). After completing the experiments, an additional 1 – 1.5 hours will be needed to write a report. It is assumed that students have already covered introductory concepts of EPR and have a basic understanding of instrumental parameters.

For comprehensive information on EPR basics and optimizing instrumental parameters, students can consult the Magnettech ESR5000 educational kit, which is provided with the bench-top EPR spectrometer.

To perform these experiments, an installed Magnettech ESR5000 spectrometer is required. In addition, a balance, filter paper, and a mortar and pestle should be available.

Glossary

EPR: Electron paramagnetic resonance or electron spin resonance spectroscopy is a method for studying materials that have unpaired electrons. The basic concepts of EPR are analogous to those of nuclear magnetic resonance, but the spins excited are those of the electrons instead of the atomic nuclei.

Free radicals: An atom, molecule, or ion that has at least one unpaired valence electron.

Polyphenols: Reducing agents, together with other dietary reducing agents, such as vitamin C, vitamin E and carotenoids, referred to as antioxidants, protect the body's tissues against oxidative stress and associated pathologies such as cancers, coronary heart disease, and inflammation.

Experimental Setup:

Materials:

- 4 samples of dark chocolate with different cocoa content
- 1 sample of white chocolate
- 1 sample with unknown cocoa content (mixture of dark and white chocolates in a ratio of the instructor choice)
- 5 mm OD tubes – 6 pieces (tubes can also be borosilicate and not quartz)
- Mortar and pestle

Sample Preparation:

1. Take a small section of each chocolate bar and grind it with the mortar and pestle until it reaches a homogeneous powder consistency. (Tip: keep the chocolate relatively cool to achieve good powder consistency after grinding. You can also use a 3 mm OD tube or another tool to push the chocolate powder towards the bottom of the tube).

2. Fill each 5 mm tube about 3 cm high with your material and insert it into the spectrometer resonator. You can scoop the material through the open end of the tube and easily fill the tube (Figure 1). A filling height of 3 cm is suggested since the entire cavity is then filled with sample. This leads to consistent results and an increase of signal intensity since the EPR signal is directly proportional to the spins inside the sample. More volume means higher signals.

3. Clean the mortar after each new sample to avoid cross-contamination.

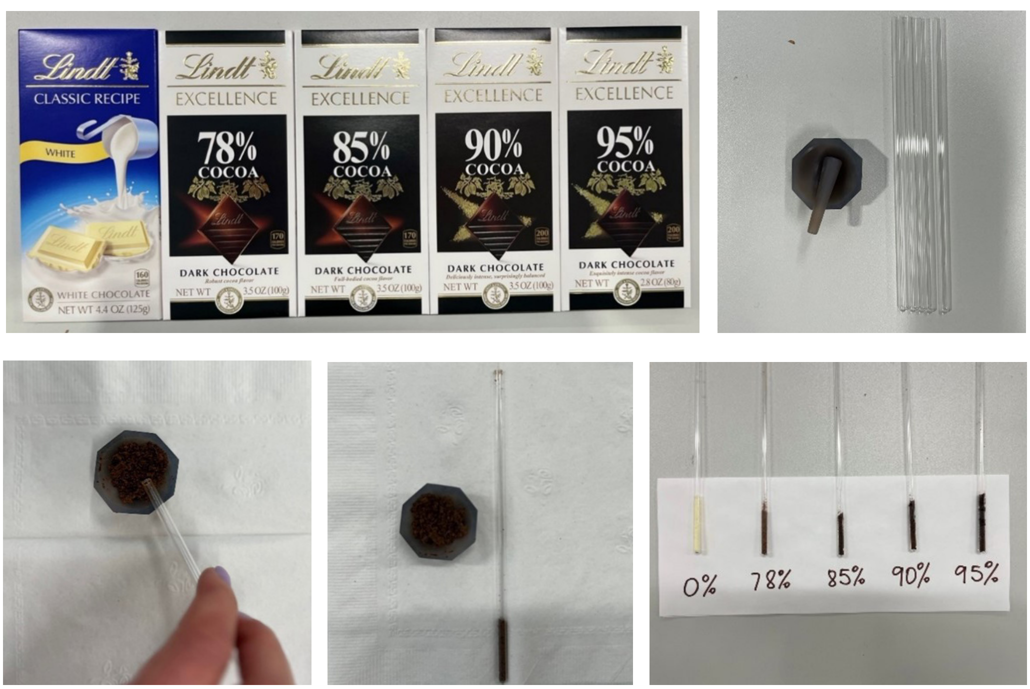

Figure 1 Sample preparation.

Abbreviations

EPR:
Electron paramagnetic resonance

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 dark chocolate 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 default Alanine recipe from the recipe list and acquire a spectrum by clicking on the Start button. The spectrum will be automatically saved in the folder of your choice.

3. Optimize the center field and sweep width. Change the recipe name to 'Dark chocolate' and save the new recipe.

4. Create a new container in ESRStudio and name it 'Dark chocolate'.

5. Collect the spectra from the chocolates including the one with unknown cocoa content in the container 'Dark chocolate'.

Data Processing:

1. Determine the g-factors for each spectrum in ESRStudio and/or manually.

2. In ESRStudio, peak-to-peak amplitude is automatically calculated for each spectrum. Simply highlight the spectrum of interest and read the amplitude value displayed in arbitrary units above the spectrum window. Write down the values.

3. In Excel (or similar software), create a plot of Amplitude = f(% cocoa content). For white chocolate the cocoa content is zero. Fit a line to the data using simple linear regression.

4. Use the equation from the linear fit to calculate the cocoa content in the sample with unknown concentration.

Results & Discussion

1. How does the measured EPR spectrum look like? Discuss the instrument parameters (center field and sweep) optimization. How are these parameters different from the original 'Alanine' recipe?

2. What are the g-values? Are they similar for each spectrum? What information can you get from the g-values?

Notes

3. Is there a correlation between EPR amplitude and cocoa content? Explain.

4. What is the result for the sample with unknown cocoa concentration? How did you calculate it?

Notes

Key Take Home Messages:

- Free radicals are very common and not all free radicals are bad.
- Learn how to optimize center field and sweep, and creating and saving new recipes.
- Understand the concept of EPR intensity and what quantitative information we can get from it.

References:

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