EDULAB FOR INSTRUCTORS: MAGNETTECH ESR5000

EPR Guide to Beer Freshness

EPR of Beer

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Experiment Hashtag: #beer, #shelf-life, #antioxidants #Educate2Resonate

Keywords:

Beer, free radicals, antioxidants, shelf-life

Target group:

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

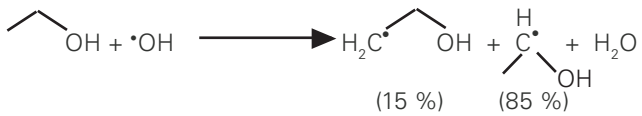
Objectives:

This laboratory experiment utilizes an exploration of beer's storage stability to introduce basic aspects of Electron Paramagnetic Resonance (EPR) spectroscopy. Radicals formed upon aerobic forced aging of beer samples are detected by using a spin trap. Students are introduced to basic principles of EPR spectroscopy as well as food and radical chemistry with a simple example taken from everyday life. The methodology presented provides students with invaluable insights into EPR spectroscopy and the role of free radicals in food chemistry.

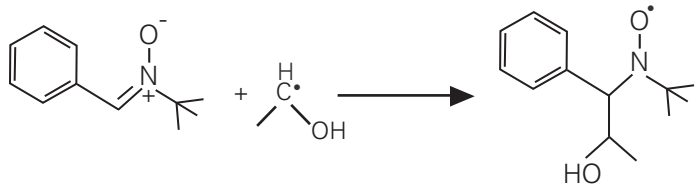
Background of the Experiment:

EPR spectroscopy detects free radicals in the degradation processes of beer. The role of oxidation processes in the aging of beer and the accompanying degradation in flavor is well known. EPR spectroscopy has been employed to verify that short-lived radicals are present as intermediates during the aging of beer. It has been shown that Fenton-like reactions can occur in beer and that oxygen acts as a precursor for the formation of hydrogen peroxide. This hydrogen peroxide, in a subsequent reaction, reacts with metal ions to form the highly reactive hydroxyl radical (•OH).

The hydroxyl radical reacts with several compounds in beer and forms an array of radical follow-up products. The most important of these is the reaction between the hydroxyl radical and ethanol.



Radical follow-up products such as the 2-hydoxyethyl-radical can be "captured" and detected using spin trapping reagents.



Even though the hydroxyl radical is highly reactive, the oxidation of beer still is a slow process. This is attributed to the low concentrations of hydrogen peroxide and metal ions in beer. Therefore, for a positive control, additional Fe(II) and hydrogen peroxide is added to a beer sample to observe immediately the effect of hydroxyl radical burst on beer oxidation. This "lag-time measurement" provides direct information on the amount of antioxidants in beer – the more antioxidants present, the longer oxidation processes are prevented. This time is called the "endogenous antioxidative potential". Only after all antioxidants in the beer are consumed, the signal of the radical adduct is detected. To additionally accelerate this process, "aerobic forced aging" is often employed. Here, the beer is heated under ambient atmosphere to consume the antioxidants in the sample faster.

Students are going to determine the "endogenous antioxidative potential" of beer using EPR spectroscopy. Students' task is to evaluate the time after which all antioxidants in beer are consumed using the method of "aerobic forced aging". Additionally, students determine the influence of adding Fenton reagent (hydrogen peroxide + iron(II)sulphate) to the sample.

Preparation:

The instructor needs to remove the carbon dioxide from the beer by placing the beer in an ultrasonication bath for 15 min and subsequently decanting it the day before the experiment. The removal of CO_2 is necessary because bubbles in the capillaries could disturb the EPR measurements. The experiment is designed to be carried out by students working in pairs for approximately three hours. In addition to protective eyewear, the use of nitrile gloves is required. The spin trap *N*-*tert*-Butyl-α-phenylnitrone (PBN) is an irritant. The laboratory experiment should be carried out in a well-ventilated lab space. 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.

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.

Spin trapping: An analytical technique employed in chemistry and biology for detection and identification of short-lived free radicals through the use of EPR spectroscopy.

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

To perform the measurements, an installed Magnettech ESR5000 spectrometer is required.

Experimental Setup:

Materials:

- *N-tert*-Butyl-α-phenylnitrone (PBN) (MW = 177.24 g/mol)
- Hydrogen peroxide (30 % in H_2O) (MW = 34.01 g/mol)
- Iron(II)sulphate heptahydrate (MW = 278.01 g/mol)
- Lager beer (Bud Light, Modelo, Budweiser) – 1 bottle or 1 can
- Pipettors and tips
- 50 ml beakers
- 50 µL capillaries
- Capillary sealant
- Eppendorf self-lock tubes
- Small glass vials (4 – 5 ml) with screw caps
- Drying oven

Sample Preparation:

1. The following stock solutions need to be prepared:
    - 50 mM PBN in beer (2 ml)
    - 5 mM $FeSO_4$ in beer (2 ml)
2. The PBN stock solution is stored at 60 °C in the drying oven. The $FeSO_4$ solution is stored at room temperature.
3. Every 15 minutes, PBN solution is measured by EPR to evaluate the endogenous antioxidative potential over time (10 points in total).
4. The peak-to-peak amplitude is plotted versus time in minutes using Excel (or similar software) to evaluate the "lag-time".
5. In addition, the following solution is prepared and measured:
    - 0.2 ml PBN stock solution + 0.2 ml $FeSO_4$ stock solution + 0.6 ml beer + 2 µl H_2O_2 (30 %)

Abbreviations

EPR:
Electron paramagnetic resonance

Experimental Procedure:

The students are provided with the degassed beer and PBN. They prepare a 0.05 M solution of PBN in 4 mL of beer, using a volumetric pipette and screw-cap glass vials. The sample undergo aerobic forced aging in a drying oven at 60 °C. Every 15 min, students prepare a sample for EPR measurements: a ring cap capillary pipette is used to take up 50 µL of the PBN solution and subsequently sealed using a capillary-tube sealing kit. The EPR measurements are performed on a Magnettech ESR5000 benchtop spectrometer. The spectrometer is switched on by pressing the power switch on the back. The following parameters must be entered into the corresponding dialog boxes prior to the experiments:

- B0 – 337 mT
- Sweep – 7 mT
- Sweep time – 60 sec
- Modulation – 0.2 mT
- Accumulations – 1
- Microwave power – 10 mW

Create a new container in ESRStudio and name it 'Beer lag time'. All kinetic measurements should be collected in this container.

Data Processing:

1. In ESRStudio, peak-to-peak amplitude is automatically calculated for each spectrum. Write down the values.

2. Evaluate the endogenous antioxidative potential by plotting the amplitude of the EPR spectra versus time. In Excel (or similar software), create a plot of Amplitude = f(time).

3. Determine the g-factor and the hyperfine coupling constants of the PBN-radical adduct

Results & Discussion

The lag time of beer under forced-aging conditions is determined in a straightforward manner by using spin trapping and EPR detection. An example of the data obtained by students is shown in Figure 1. The obtained curves show the typical behavior of an initial slow increase, followed by a fast linear increase of the EPR signal intensity. The intersection between the slow-increase regime and the fast-increase regime represents the lag time of the beer sample (the time at which all antioxidants in the beer sample are consumed by free radicals). Employing this approach, the majority of the students are able to determine the lag time of their beer sample, which is around 80 min in the example displayed in Figure 1.

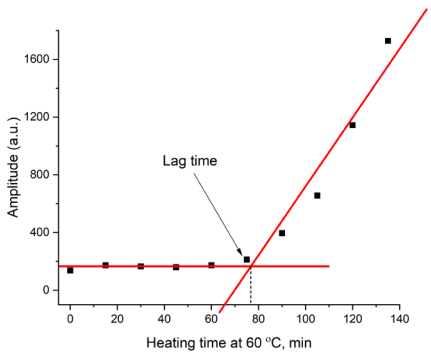


Figure 1 Example of a lag-time measurement of beer obtained by students during the laboratory experiment.

Notes

In addition, the students manage to extract the g-factor and hyperfine coupling constants from the experimental spectra. Figure 2 shows an experimental spectrum obtained by students during the laboratory exercise. From the experimental six-line spectrum, it is obvious that two hyperfine coupling constants are detected in the PBN spin-adduct. One stems from the nitrogen next to the radical center. Nitrogen has a nuclear spin of I = 1, leading to a splitting into three equidistant lines. The additional splitting stems from the H-atom located on the carbon neighboring the N atom with a nuclear spin I = 1/2 (two lines), resulting in a triplet of doublets (six lines). For these two respective nuclei, the students obtain hyperfine coupling constants, a, of a_N ~ 1.58 mT and a_H ~ 0.34 mT, which are in very good agreement with published literature data.

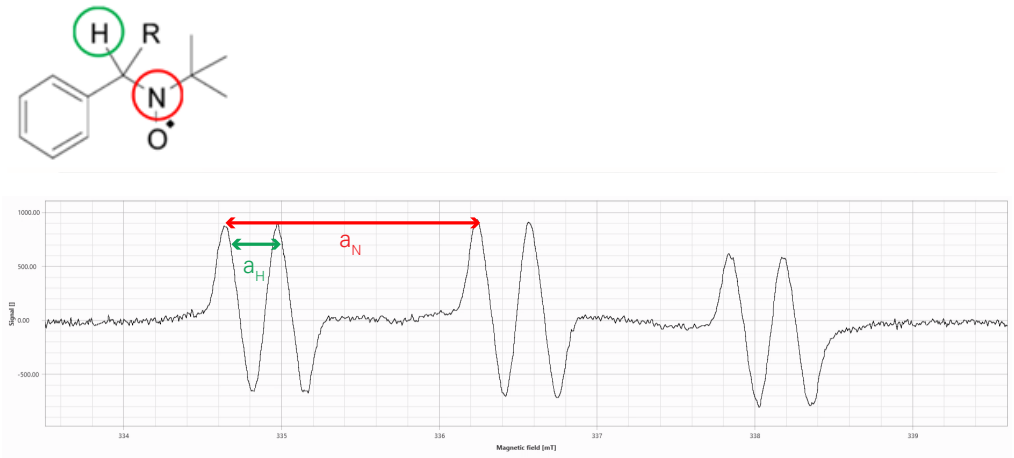


Figure 2 Example of an EPR spectrum of the PBN-radical adduct obtained by students. It was recorded after 150 min of aerobic forced aging at 60°C and was used for the determination of the hyperfine coupling constants.

The g-factor is determined by changing the X-axis from B (mT) to g-factor, placing the mouse cursor at the zero crossover in the middle of the spectrum, and reading out the value on the X-axis (Figure 3).

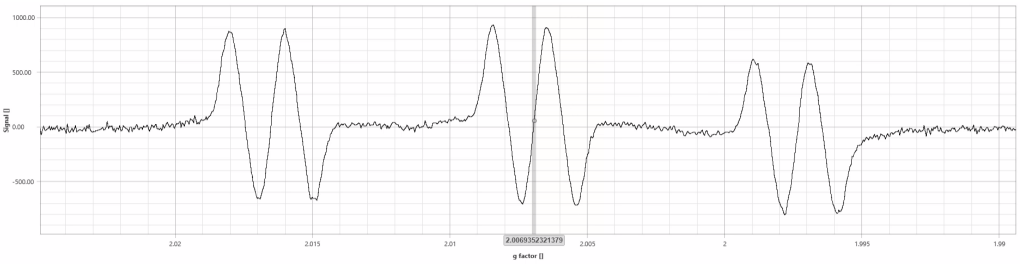


Figure 3 g-factor determination.

Notes

Questions to be answered by students

1. **What is the cause for beer oxidation?**

   It is well established that beer oxidation undergoes via radical mechanisms.

2. **Why do we use a spin trap? What kind of radical is mainly detected in the experiment?**

   Spin-trapping indirectly detects short-lived radicals by forming stable spin adducts in detectable quantities, enabling both characterization and quantification of the trapped radical. The main radical captured in this experiment is 2-hydoxyethyl-radical.

3. **What metric is used to determine the antioxidative potential in beer?**

   Lag time – this is the period it takes until all the antioxidants in beer are consumed and radicals can be detected by EPR. Longer lag time = higher antioxidative potential = longer shelf-life.

Notes

Key Take Home Messages:

- EPR can be used to detect free radicals in degradation processes of food and beverages.
- Short-lived radicals can be captured using the spin-trapping technique.

References:

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