Using Electron Spin Resonance spectroscopy to monitor the brewing process

Marcus Pagenstecher
University of Copenhagen
University of Agriculture in Krakow
Introduction
Beer flavor stability is a result of the whole brewing process.
Introduction - Spontaneous chemical changes of beer quality

**Oxidative reactions**

- The presence of oxygen
- Radical reactions
- Formation of off-flavors
- Loss of bitterness
- Haze formation

**Thermal reactions**

- Maillard chemistry
- Formation of flavor active aldehydes
Introduction – Beer oxidation

Catalyzed by increasing temperature and transition metal ions like iron & copper

Short-lived radical intermediates lead to further oxidative damage

Radicals are important intermediates of beer oxidation. Knowing about them can help assess the extent and rate by which beer flavour can deteriorate.
Introduction – Methods for evaluating beer oxidation

**Flavour Analysis**
Sensory evaluation or quantitative analysis of oxidation marker compounds using GC-MS.

**Oxygen Consumption**
Monitoring the oxygen concentration in a closed sample over several hours. Oxygen is the primary reactant during oxidation.

**ESR**
Monitoring the formation of radicals during a forced ageing experiment.
ESR spectroscopy
• Electron Spin Resonance spectroscopy (ESR)
  • Also called: Electron Paramagnetic spectroscopy (EPR)
• Only detecting unpaired electrons (→ paramagnetic)
  • This includes radicals intermediates during beer oxidation
• Based on transition between energy levels of electron spins placed in strong magnetic fields resonance
  • Constant microwave frequency, varying magnetic field

Field on
\[ m_s = +1/2 \]

Field off
\[ g_s \mu_B B_0 \]
\[ m_s = -1/2 \]

• Example of an ESR spectrometer (0.33 L can for scale) used for manual analysis.
• Instruments for automated analysis are also available.
• Simple ESR spectrum of the stable radical 2,2,6,6-Tetramethylpiperidinyloxyl (TEMPO)
• Amplitude of the middle peak is recorded
• Often used as a standard to normalize the spectra of other samples
  • Measured at the beginning and end of each day, in a known concentration (for example 2 µM)
• Short-lived radicals in beer are difficult to impossible to detect.
  • Spin traps bind to these radicals, resulting in stable spin adducts which accumulate over time to detectable concentrations
  • Spin adduct spectra are characteristic for the spin trap used.
  • Most commonly used in the brewing industry are N-tert-Butyl-α-phenylnitrone (PBN) and α-(4-Pyridyl 1-oxide)-N-tert-butyl nitrone (POBN)
Applying ESR in the brewery
Simple measurement can be either performed manually or automated.

Different sample preparation for wort and beer samples.

Normalization of samples by measuring a standard with a known radical concentration (for example TEMPO).

Single measurements can be as short as 30 s.

Work flow of analysis:

Spin traps: PBN can only be used in beer, POBN in both beer and wort.
Applying ESR in the brewery – Spectra

- Typical ESR spectrum of POBN spin adducts in beer/wort
- Always record the peak-to-peak height of the same peak. Best suitable:
  - 1\textsuperscript{st} peak of 1\textsuperscript{st} doublet
  - 1\textsuperscript{st} peak of 2\textsuperscript{nd} doublet
    (see example)
• Typical radical formation over time in wort samples
• Little to no antioxidant activity leads to immediate increase in spin adduct concentration.
• Possible characteristic number:
  • Amount of spin adducts generated after time \( T_x \)
  • Commonly taken after 150, 300, or 500 min
• Worts with significantly higher \( T_x \) indicate oxidation issues.

**Applying ESR in the brewery - Wort**

![Graph showing spin adduct concentration over time with key points labeled](image)
- Monitoring the oxidative stability of different sweet wort batches.
- Intensity after 90 minutes ($T_{90}$) normalized against TEMPO solution (2 µM)
- Batch #7 displays poor oxidative stability, for example due to high iron concentration
Typical radical formation over time in beer samples

Antioxidants delay initial formation of radicals
  - Antioxidant potential can be quantified as “lag time”
  - Strongly linked to sulphite (SO$_2$) concentration

T$_x$ also possible as characteristic number
  - Advantage: only one measurement necessary
  - Shorter lag times indicate a beer with poor storage stability
• Monitoring the oxidative stability of different finished pilsner beer batches.
• Batches #5 and #7 display poor oxidative stability, for example due to:
  • Low sulphite concentrations
  • High storage temperatures

Applying ESR in the brewery – Example 2
Questions & Solutions
Go back through the lecture and try to answer the following questions. Solutions can be found at the end of all questions.

1. True or false?
   a. Storage at high temperatures leads to faster flavour deterioration.
   b. Increasing amounts or iron in beer slow down the oxidation processes.
   c. Radicals are always easy to detect.
   d. Beer and wort samples have to be treated differently before ESR analysis.
2. Estimate $T_{300}$ in both samples as well as the lag time in the beer sample.
3. Which wort/beer has oxidation problems according to the ESR analysis?
1. True or false?
   a. Storage at high temperatures leads to faster flavour deterioration.
      True
   b. Increasing amounts or iron in beer slow down the oxidation processes.
      False. Iron catalyzes the formation of reactive oxygen species, thus increasing the oxidation rate.
   c. Radicals are always easy to detect.
      False. Only stable radicals like TEMPO can be detected easily. Others are short-lived and require for example spin trapping for detection.
   d. Beer and wort samples have to be prepared in a different way for measuring ESR.
      True. Wort requires centrifugation, beer requires degasification.
2. Estimate $T_{300}$ in both samples as well as the lag time in the beer sample.

**Wort sample**

$Wort: T_{300} \approx 1.0$

**Beer sample**

$Beer: T_{300} \approx 0.6; \text{Lag time } \approx 155 \text{ min}$
3. Which wort/beer has oxidation problems according to the ESR analysis?

Wort 1

Beer 1
MEBAK method: “Endogenous Antioxidative Potential (EAP Value) and Radical Generation (T Value) in Beverages (ESR Spectroscopy)”

- [https://www.flavoractiv.com/instrument/beer-freshness/](https://www.flavoractiv.com/instrument/beer-freshness/)


Thank you for interest!

Marcus Pagenstecher

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