TECH Note

Shelf Life Analysis of Beer Using an Automated Lag-Time EPR System

David Barr
Arthur Heiss
Bruker BioSpin USA
Andreas Kamlowski
Diether Maier
Juergen Erstling
Herbert Meling
Bruker BioSpin GmbH

This article describes a new method for measuring and controlling the oxidative stability of beer using an electron paramagnetic resonance system.

The cardboard-like flavor that occurs in stale beer is thought to arise from the free radical–mediated oxidation of various constituents in beer. The characteristic odor and taste are caused by decomposition products from the free-radical process. Similar processes occur in many foods, but in beer, these off-flavor products can be detected by consumers even at very low concentrations.

The environment in which beer is stored is critical for minimizing oxidative staling. If beer is stored at cooler temperatures, the oxidation process will occur very slowly; and, of course, raising the temperature will increase the rate of oxidation. However, as production volumes and distribution distances increase, the ability to carefully control the storage environment for beer is compromised. Therefore, methods for measuring and controlling the oxidative stability of beer have become vital.

Several reports (1–6) have shown that electron paramagnetic resonance (EPR, also known as ESR) spin trapping provides a useful technique for measuring the resistance of beer to free-radical oxidation. All beers have a certain amount of naturally occurring antioxidants that protect their flavor by terminating oxidative free-radical reactions. Beers with a higher antioxidant activity can resist the oxidation for longer periods and, thus, have better shelf life stability.

The so-called lag-time assay uses forced oxidation combined with EPR spin trapping to effectively measure the antioxidant activity of a beer and to even predict a beer's shelf life. It has been well established that the time in minutes (that is, lag time) before a dramatic EPR signal increase occurs correlates with the time in days required for a sensory panel to detect the characteristic cardboard off-flavor (1, 2–5).

Although the information that the lag-time assay provides is undoubtedly powerful, the assay itself has been hindered by a lack of automation and low throughput. Here, we demonstrate the use of a new EPR system from Bruker that simpli-

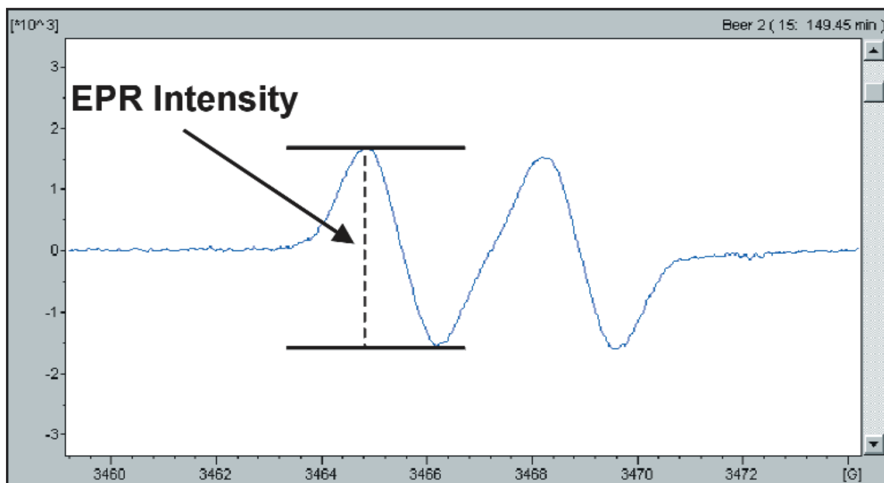

Figure 1. The individual EPR spectra from 210 independent field scans are contained in one file.

fies, automates, and increases throughput for the lag-time assay. Using an automatic sample changer, the brewer can measure the EPR from several samples simultaneously in one time period. The data analysis step is also greatly aided by a software package that was specifically designed for calculating lag times.

BACKGROUND

EPR. EPR is a form of magnetic resonance spectroscopy that measures unpaired free electrons. Samples with free electrons (such as free radicals) are placed in a magnetic field and subjected to a fixed frequency of microwave irradiation. The free electrons have a small magnetic field that will orient mostly parallel to the larger field produced by the spectrometer's magnet. In an EPR experiment, the field of the spectrometer magnet is swept linearly while the sample is exposed to microwave irradiation. At a specific field strength, the microwave irradiation will cause some of the free electrons to flip and orient against the spectrometer's magnetic field. When this occurs, an EPR absorption is detected by the spectrometer.

The spin trapping method. Spin trapping allows us to detect very short-lived free radicals. It involves adding a spin trap to a sample (in this case, beer) that you suspect will contain free radicals. If free radicals are produced, the spin trap (which, itself, is not EPR detectable) will react with the free radical and form a stable chemical bond between the two. This complex of the radical and the spin trap is still a free radical, but is significantly more stable than the initial free radical. The complex, often called a spin adduct or a radical adduct, is then detected by EPR. The general reaction scheme for a spin trapping experiment is

ST + R ⟶ . ST-R

(spin trap) (free radical) (spin adduct).

The lag-time EPR assay. To study free-radical formation in a beer sample we add the spin trapping agent to the beer and incubate at 60 °C. This accelerates the free-radical oxidation process to a rate that is measurable within a relatively short time period (for example, 1–2 h). As free radicals form, they are trapped by the spin trap, and spin adducts will begin to accumulate. The sample is introduced to the spectrometer at specific time intervals and the EPR of the spin adducts is measured. At the end of the assay we have a time course for free-radical formation in the beer sample.

EXPERIMENTAL PROTOCOL

Equipment. The EMX lag-time EPR system consists of:

- the Bruker EMX EPR spectrometer
- an autosampler equipped with a peristaltic pump
- an AquaX (Bruker BioSpin USA) sample cell
- a sample rack and variable temperature water bath
- autosampler interface software with automatic EPR intensity calculation
- Origin graphics program (OriginLab [formerly Microcal Software] Northhampton, MA) with custom lag-time calculation routines.

Sample preparation and data acquisition. The assay involves forcing the oxidation of beer samples in the presence of the spin trap *tert*-butyl-phenylnitrone concen-

Circle 7

tration. We have run 14 beer samples all in one automated assay period. Following is the method we used for preparing the assay: Decant 40 mL of each beer sample into 50-mL centrifuge tubes and centrifuge at 2500 *g* for 15 min. This will degas the beer.

While the samples are spinning, set up the spectrometer and autosampler configurations. Default settings can be configured so this needs to be done only once. The spectrometer's parameters are configured to perform a magnetic field sweep and are optimized for rapid yet sensitive measurements.

Place the beer samples into the rack, add 0.24 mL of *tert*-butyl-phenylnitrone to the first sample (final *tert*-butylphenyl nitrone concentration, 50 mM), and start the acquisition. After the spin trap has been added to the last beer sample, no further user intervention is required. As the assay progresses, the EPR spectra and EPR intensities from each sample at each successive time point are recorded automatically (the total assay time is ~2.5 h). Two files are automatically saved — one containing the EPR spectra, and one with the EPR intensities. Each individual spectrum can be viewed in the spectrum window when the assay has finished (Figure 1).

The time course profiles for each sample can be viewed together or separately from within the acquisition software (Figure 2).

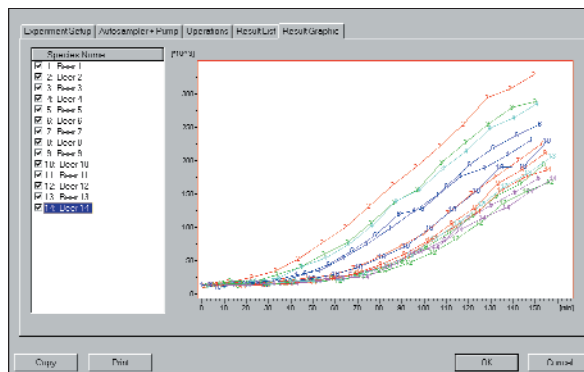

Figure 2. EPR intensity plots for all 14 samples.

Lag-time analysis. After the data have been acquired, the lag time for each sample must be determined. Previously, this step was very tedious and time consuming. It involved determining the EPR intensity manually and inputting the values from each sample into a graphics program. For example, in our experiment, the user would have needed to make 210 EPR intensity measurements (14 samples × 15 time points) and then would transfer these measurements to a graphics program. The EMX software automatically measures and stores the EPR intensity as each EPR spectrum is recorded. A file with a ".lag" extension is created that contains all the sample names, time points, and EPR intensities. The user then loads this file into one of the two following Origin templates to calculate the lag times.

The dual linear regression template. In previous reports, the lag time has been calculated by fitting the data with a pair of linear regression lines (1, 3–5) — one containing the data points before the inflection point, and one containing the points after the inflection point.

The custom Origin plotting routine (shown in Figure 3) applies this method to rapidly calculate lag times for the samples. Load the lag file, select the sample, set the regions for the two linear regression lines, and click the "Calculate lag-time" button.

The sigmoidal fitting template. Although the dual linear regression method has been used in many previous reports, we have found that this fitting routine is somewhat subjective as the user must decide which points to include for the linear regression lines. We have found that the same lag-time values can be obtained (without user input) by using a sigmoidal-type fitting function of the form: $y = A_2 + (A_1 - A_2)/(1 + \exp((x - x_0)/dx))$.

This function (also known as the Boltzmann function) very nicely fits the lag-time data. Comparing Figures 3 and 4 you can see that we get almost identical lag-time values using the sigmoidal fitting routine. The advantage of the sigmoidal fitting routine is that the calculation does not require any user input. (Simply select the sample and click the "Calculate Lag time" button.) The sigmoidal fitting routine also automatically performs an estimated error analysis.

DISCUSSION

On heating, the free-radical oxidation of the beer is accelerated. The process is thought to involve the accumulation of hydrogen peroxide as the beer is heated (7), which then reacts with trace transition metals such as iron or copper. This will occur normally over time, but much more slowly when the beer is kept at cooler temperatures.

The oxidation process is a free radical–mediated chain reaction that is initiated by one molecule, but it can be propagated to involve many molecules. That is, oxygen- and carbon-based radicals will form, react with other molecules, and then reform at a very rapid rate.

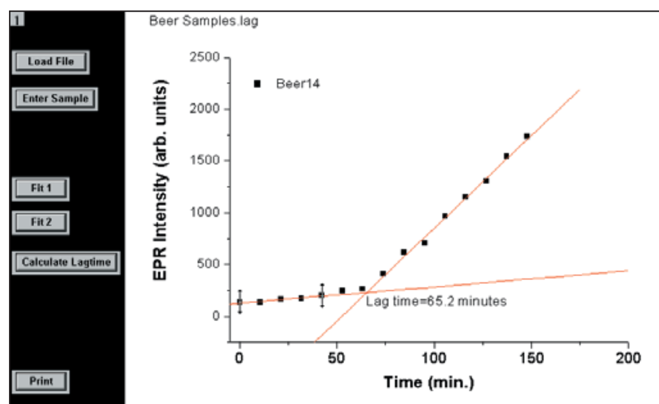

Figure 3. Lag-time calculation using the dual linear regression template.

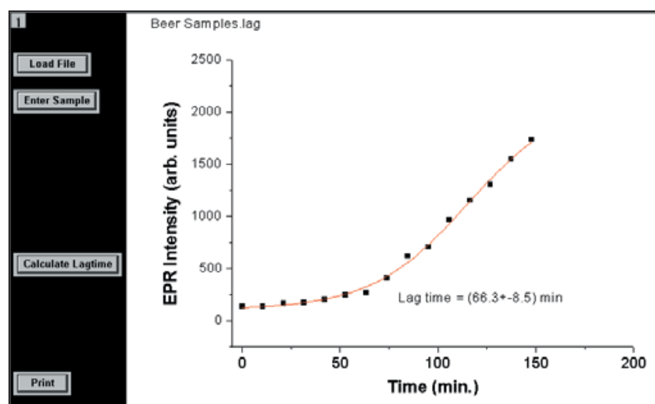

Figure 4. Lag-time calculation using the sigmoidal fitting template.

The spin trap is just one of the molecules in the milieu with which these free radicals can react. However, it appears that other constituents of the beer (the so-called endogenous antioxidants) react more favorably. Thus, we can say the endogenous antioxidants in the beer are quenching free-radical formation during the lag-time period. When these antioxidants become depleted we start seeing a marked ingrowth of the EPR signal due to the trapping of the free radicals by the spin trap. The longer the lag time, the greater will be the endogenous antioxidant activity and also the shelf life of the beer.

The lag-time information is extremely useful for a brewery. It is not only used as a quality control check for beer as it is packaged, but it can be used as a research tool to improve the shelf life of a beer. Several process changes can be made in a full-scale or pilot brewing plant. The efficacy of these process changes can be rapidly monitored using the EPR technique.

Previous EPR instrumentation was not specifically designed for these experiments. As with any quality control assay, simplicity and high sample throughput are necessary. With Bruker's lag-time EPR system, we have incorporated autosampling capability (minimizing user intervention and greatly increasing throughput) with a software package that greatly simplifies data acquisition and lag-time calculation. In the example experiment shown here, 14 beer samples were easily run in a 2.5-h period, thus making it quite reasonable to obtain lag-time values from 45 samples in one 8-h work day. Once the assay has been initiated, the user can leave the instrument unattended and is free to perform other tasks. With previous equipment it was an arduous task to complete even 10 samples in one day, requiring the undivided attention of at least one technician. For these reasons, this lag-time EPR system will greatly advance the usage of the lag-time assay.

REFERENCES

(1) M. Uchida and M. Ono, *J. Am. Soc. Brew. Chem.* **54**, 198–204 (1996).
(2) M.L. Andersen and L.F. Skibstead, *J. Agric. Food Chem.* **46**, 1272–1275 (1998).
(3) S. Takaoka, H. Kondo, M. Uchida, and Y. Kawasaki, *Tech. Q. Master Brew. Assoc. Amer.* **35**, 157–161 (1998).
(4) C. Forster, J. Schweiger, L. Narziss, W. Back, M. Uchida, and M. Ono, *Monatsschrift Fuer Brauwirtschaft*. **5**(6), 86–93 (1999).
(5) M. Uchida and M. Ono, *J. Am. Soc. Brew. Chem.* **58**, 8–13 (2000).
(6) M. Uchida and M. Ono, *J. Am. Soc. Brew. Chem.* **58**, 30–37 (2000).
(7) M. Uchida and M. Ono, *J. Am. Soc. Brew. Chem.* **57**,145–150 (1999).

David Barr *is an application scientist in, and* **Arthur Heiss** *is vice president and product manager for, the EPR division at Bruker BioSpin, Manning Park, Billerica, MA 01821, (978) 663-7406.* **Andreas Kamlowski** *is an application scientist,* **Diether Maier** *is head of development, and* **Juergen Erstling** *and* **Herbert Meling** *are software programmers in the EPR division at Bruker BioSpin GmbH, Rheinstetten, Germany, +49 721-5161.* ◆

Circle 8