Bruker BioSpin

Measuring Oxidation of Cooking oil using EPR Spin Trapping

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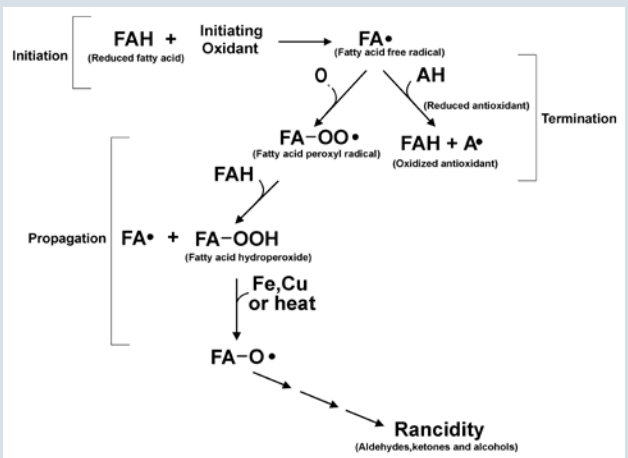
The staling of vegetable oil is a major problem in several food related industries. Rancidity is caused by a free radical process that is both oxygen and temperature dependent. The result is the degradation of long chain free fatty acids to the smaller aldehydes, ketones and alcohols that give rancid foods their characteristic foul odor and flavor. Efforts to decrease the rate of rancidity formation and methods to measure rancidity are of great importance to the food industry.

Figure 1. provides an example of a free radical chain reaction that results in rancidity. It starts with the oxidation of fatty acids to various fatty acid free radicals. These free radicals are usually carbon centered, and react rapidly with oxygen to form peroxyl radicals. Peroxyl radicals propagate further free radical formation by abstracting hydrogen atoms from other fatty acids. The peroxyl radicals are converted to hydroperoxides that either undergo thermolysis or react with metals (e.g., iron or copper) to form alkoxyl, peroxyl and carbon centered free radicals. This free radical chain reaction proceeds until it is terminated by an antioxidant. Antioxidants "intercept" a propagating free radical by donating an electron. A "good" antioxidant is oxidized to a stable non-reactive form. If the free radical process is not terminated in its early stages, bad flavor products (i.e., aldehydes, ketones, and alcohols) will form. See references 1-3 for further review of free radical reactions and antioxidants.

Figure 1.



Simplified scheme for the free radical degradation of a fatty acid.

Various methods exist to measure the end products of rancidity. However, many of these methods require cumbersome HPLC separations and can be very non-specific. Fatty acid hydroperoxides (intermediates in the rancidity process) can be measured using iodometric titration. However, this method is time consuming, lacks sensitivity and because these hydroperoxides are unstable, can underestimate the true extent of rancidity. References 4-5 describe the various techniques commonly used for measuring rancidity. There has also been some previous work using EPR to measure oxidation in vegetable oil (references 6-7). Here, we describe a simple "forced oxidation" assay that uses EPR spin trapping to measure the fatty acid free radicals, themselves.

EPR (electron paramagnetic resonance, also called ESR, electron spin resonance) is a spectroscopic technique that measures samples with unpaired electrons and thus, is very useful for measuring free radicals. At the same time the technique is completely "blind" to molecules without unpaired electrons, which makes it very specific. Free radicals are "short-lived", so to increase our ability to detect them, we add a compound known as a spin trap. The spin trap reacts with the free radical to form a "spin adduct". Spin adducts are also free radicals, but they are more stable than the original radical which allows us to measure them using an EPR spectrometer. The general procedure for the EPR spin trapping assay is depicted below. In this note we describe how to use the EPR technique to measure rancidity in cooking oils. We also used the technique to demonstrate the "protective" effect that the commercially available antioxidant HerboloxTM (Kalsec, Inc.) has on preventing rancidity.

Experimental protocol:

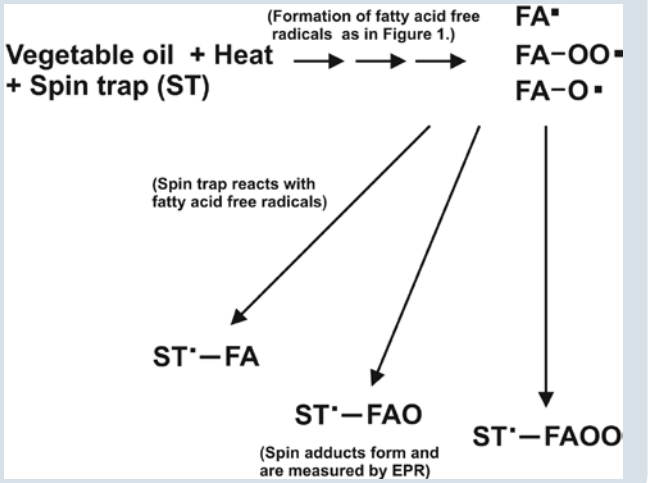
Materials needed:

1. Pipette pumps, pipette tips.
2. Test tubes.
3. Oil sample.
4. Spin trap (N-tert--butylphenyl nitrone (PBN).
5. Bruker EMX spectrometer equipped with ER4131VT temperature controller.
6. Sample cell and holder assembly.

Samples:

Two sample sets were studied. The control sample was obtained from processed soybean oil. The second sample was from the same soybean oil except Herbolox™ was added (0.1 %) on day 0. The samples were incubated in a Schaal oven at 50° C for 17 days. Peroxide value and EPR data were collected on days 0, 5, 10, 14 and 17. Peroxide values were measured using AOCS method CD8-53 in which dichloromethane replaced chloroform as the extraction solvent.

Figure 2.



Spin trapping in vegetable oil.

EPR Assay:

25 uL of a 2.5 M PBN (dissolved in ethanol) solution was added to 0.5 mL of the respective vegetable oil (final concentration 125 mM). The samples were mixed using a vortex and put into a flat quartz sample cell. Samples were then introduced to the EPR probehead which was pre-equilibrated at 70°C using Bruker's ER4131VT variable temperature controller. After approximately 1 minute, the EPR measurements were started. Measurements were made every 5 minutes for a two hour period. The relative quantity of spin adduct was measured from the peak-to-trough intensity of the first EPR line in Figure 2 Spin trapping in vegetable oil. each of the spectra. These values were plotted versus the time of measurement to show the respective level of oxidation that occurred in each vegetable oil sample. The following spectrometer parameters were used: modulation frequency, 100 kHz; modulation amplitude, 2 gauss; microwave power, 40 mW; time constant, 164 msec, scan time, 167 sec.; sweep width, 100 gauss.

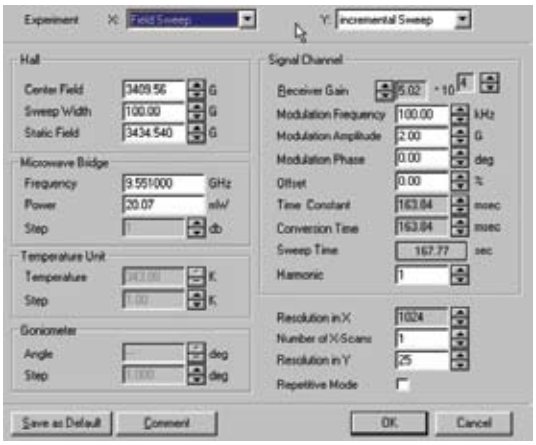
Results and Discussion

Performing the EPR assay was greatly simplified using the automated acquisition routine of Bruker's EMX spectrometer. Figure 3A and 3B show the experimental parameters window and a stack plot view from a typical 2 hour vegetable oil assay. The assay was repeated for each of the samples described in the experimental section.

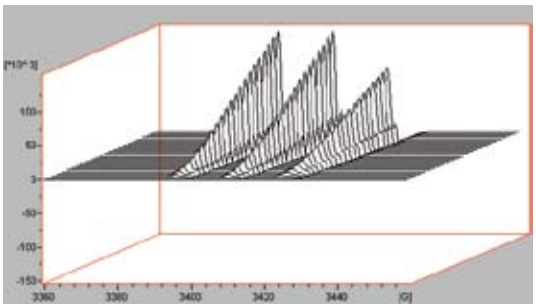
Vegetable oils that were stored for longer periods (i.e., 14 and 17 days) had both a faster rate and greater total extent of spin adduct formation. The addition of HerboloxTM greatly reduced the rate of increase and total extent of the spin adduct formation, Figure 4A and 4B. The data in figure 4A and 4B are plotted using the same intensity range to demonstrate how effectively Herbolox™ inhibited spin

Figure 3.

A



B



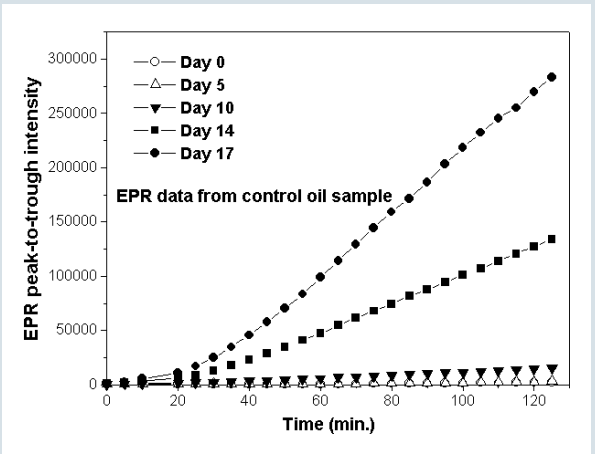
Experimental parameters (A) and a stack plot from a typical vegetable oil spin trapping assay (B).

adduct formation in these oil samples. The rate, of EPR signal increase closely correlated the peroxide value data for the respective oil samples (Figure 5.). Similar results were obtained from the Herbolox™ containing sample (data not shown.). The rate of EPR signal formation and peroxide value data for each sample are plotted for comparison in Figures 6A and 6B. Measuring the rate of EPR signal growth has advantages in developing a standard EPR assay for rancidity measurement because it eliminates the need to compare "absolute" measurements. Thus, errors due to variation between instruments or experimental conditions

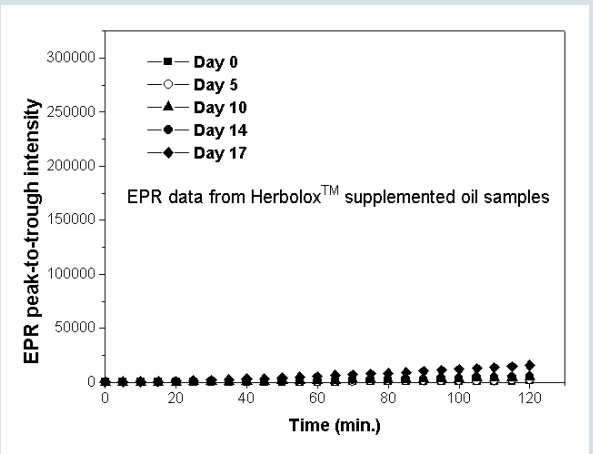
Summary

Figure 4.

A

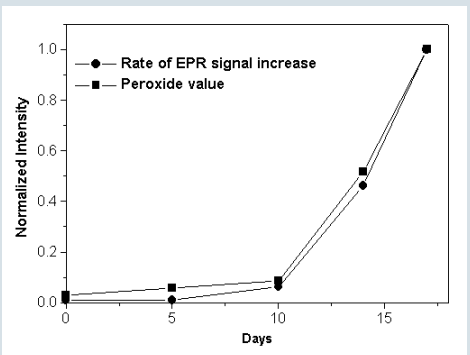


B



Formation of spin adducts in the control vegetable oil (A), and the Herbolox supplemented oil samples (B).

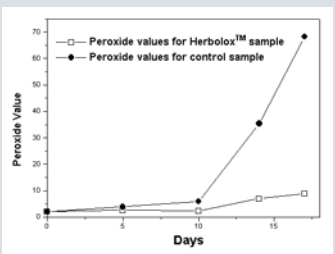
Figure 5.



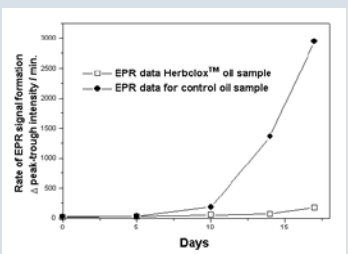
Comparison of EPR data with peroxide value data for the control oil sample.

Figure 6.

A



B



Peroxide value and EPR spin adduct formation data for the control oil sample (A) and the Herbolox containing Sample (B).

Summary

EPR spin trapping provides a very simple and useful analytical tool for measuring rancidity in vegetable oils. The sample handling is minimal and Bruker's fully automated EPR spectrometer made data acquisition and analysis both facile and reliable. These features give EPR an advantage over other techniques that are more labor intensive and prone to uncontrollable variation. We found that the rate of EPR signal increase for various samples correlated very well with peroxide value data from these same samples. We also found that adding antioxidants such as Herbolox™ had an inhibitory effect on the formation of spin adducts in vegetable oil. We believe these preliminary findings justify the further development of EPR methods for measuring and improving the resistance of vegetable oils to rancidity formation.

References

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