BEER FLAVOR STABILITY ASSAYS: ELECTRON SPIN RESONANCE PRINCIPLES

ESR - Assay Mechanism

"**Free radicals** are molecules and/or parts and atoms of same which, characterized by a single electron, are generally extremely reactive and thus cannot be isolated in larger quantities. Reactions in the presence of free radicals generally process instantaneously, completely and irreversibly" (Pschyrembel, 1998).

Antioxidant assays: i) enzymatic oxidation during chemiluminescence peak height suppression ii) hydroxyl radical assay iii) super oxide radical quenching assay iv) non-enzymatic lipid peroxidation assay (auto oxidation of linoleic assay).

Foster: "Iron and copper are known to have a catalytic effect on oxidation, especially at higher beer temperatures and low pH level. In well known Fenton and Haber-Weiss reaction, the iron (II) atoms are oxidized to iron (III) by hydrogen peroxide, forming a hydroxyl radical and a hydroxyl ion. Iron (III) will then react with another H_2O_2 molecule forming the superoxide radical and two protons. Copper (II) then reacts with the superoxide radical to form copper (I) and oxygen. Copper (I) can then cleave another H_2O_2 molecule into a hydroxyl radical & a hydroxyl ion. These free radical species are very reactive, removing hydrogen from any organic molecule because the energy gain in the formation of the OH bond is always higher than the energy required to cleave a CH bond." Easy for you to say Bob!

Pathway of oxygen to free radicals:

O_2 ..... $O_2^{\cdot}$ .....$HO_2^{\cdot}$ ....H_2O_2.....$HO^{\cdot}$

Any of these radicals can trigger oxidation of wort/beer constituents (per the "Good, the Bad & the Ugly"!!!!).

Iron or Copper as Prooxidants: Haber Weiss:

Fe^{2+} or Cu^{+} + H_2O_2 .....Fe^{3+} or Cu^{2+} + $^{\cdot}OH$ + OH^{-}

Sapporo's chemiluminescene (free radical) activity/ potential of beer analysis.

Foster: "EPR is a magnetic resonance technique that measures the transition of unpaired electrons in an applied magnetic field. Like a proton, the electron has **"spin"** called the **"magnetic moment"** (GPC: no comment....too easy). The magnetic moment makes the electron behave like a tiny magnet similar to the one you might put on your refrigerator. When an external, magnetic field is applied, the paramagnetic electrons can either orient in a direction parallel or antiparallel to the direction of the magnetic field.
In wort or beer, this "spin" is trapped with a nitrone compound when free radicals are formed producing a stable radical-spin adduct complex detectable by EPR. In wort and beer there are sufficient supplies of antioxidants so that the radicals being formed are scavenged before the spin trap can react with them. When the natural antioxidants are used up, the spin adduct starts to form in large enough quantities to cause a dramatic *increase in the EPR signal intensity*. On a graph, the EPR signal shows a significant upwards inflection and the **"lag time"** can be measured."

Torline: properties of radical oxidation:
a) are catalytically initiated
b) are fast and non-selective pathways
c) are self-propagating in the absence of antioxidants
d) are triggered by prooxidants.

Predicting/Correlating Flavor Stability

Free radical reactions initiated by active oxygen (-)

EA value corresponds to the lag time needed for OH^{-} free radical formation and the time needed for H_2O_2 generation & accumulation.

Lower beer pH accelerates increase in chemiluminescence increasing the rate of free radical reactions, flavor staling, degradation of IAA's & procyanidins (-)

Bruker unit: assay includes a **"spin-trap reagent (e.g PBN)"**...heat sample to 60^0C to promote oxidation...eventually sample reducing power/natural antioxidants will be consumed and can no longer quench free radicals.....spin-trap captures the free radicals as detected by the **EPR spectrophotometer**. Longer the lag time before spec readings increase, more flavor stable the beer.

Free radicals generating light in luciferin dependent luminescence trials (-)

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ASBC No. Xc
(Casey; June/05)