Link 6: Lipids oxidation and monitoring

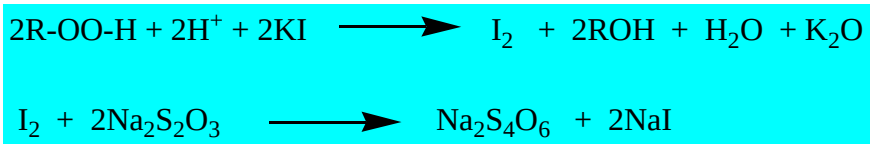
Lipid Oxidation: lipids (fatty acids, triglycerides, etc) undergo redox reaction, reacting with oxidizing agents/oxidants, in general displaying with adding ”oxygen” or loosing ”hydrogen”, and changed into ”oxidized form”. The existence of unsaturated fatty acids in oils and fats is the intrinsic reason for lipids oxidation. Depending product existing form, lipid oxidation includes oxidation of bulky oil (cooking oil) and oxidation of lipids in emulsion products (w/o emulsion like butter and margarine; o/w emulsion like Milk, ice-cream, soup, sauces, maynnase, etc).

Mechanism of lipids oxidation: In general, lipid oxidation includes 3 phases: initiation (unsaturated fatty acid reacting with initiator to for radical), propagation (radical reacting with other fatty acids to propagate as chain reaction) and termination (high concentrated radical interacting each other for non-radical compounds). The primary oxidation product formed in the first stage can be further decomposed (form smaller volatile compounds such as aldehydes, ketones, alcohols etc) or polymerized (form dimmers or oligomers).

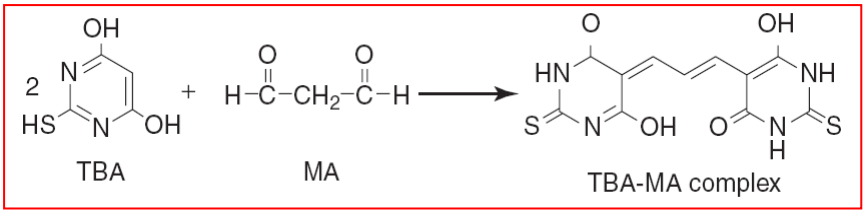
By mechanism, lipid oxidation can be divided into auto-oxidation, photooxidation and enzymatic odidation. Fig. 2 demonstrated the mechanism of autooxidation of linoleic acid. The external factors to influence lipids oxidation include Oxygen, Light (photooxidation), Heat (autoxidation), Sensitizers (pigments, etc photooxidation), Enzymes, (Lipoxygenase oxidation), Metal ions, (all oxidation pathways), Metalloproteins, microorganisms, etc.

Assays of lipids Oxidation: Oxidation of lipids is an important index to denote quality of oils and fats and the products containing lipids because oxidized lipids not only spoil the smells and tastes of products but also generate a lot of harmful biological effects to human health. peroxidation products have been pointed out as harmful to health due to carcinogenic and atherosclerotic evidences, alteration in the composition of cell membranes or reduction in high-density lipoproteins Therefore, detect lipid oxidation and its level, and monitor progress of lipid oxidation are day to day activity of lipids production companies. Developing fast, reliable, facile and accurate advanced techniques is also an important research area in the community of lipids.
Traditional chemical assaying methods for lipids oxidation include

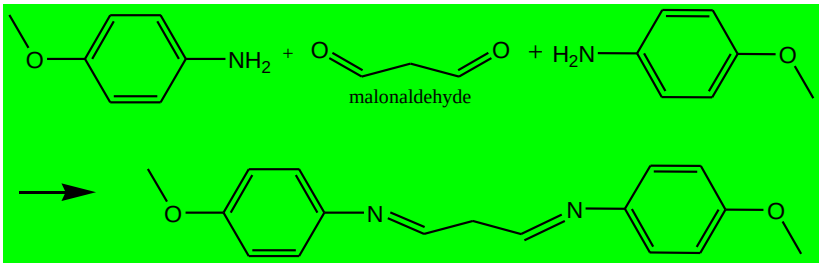
- Titration method to measure content of peroxides (peroxide value, POV, AOCS official method Cd-8-53); Our lab has experience and reagents to do this measurement.



- Thiobarbituric acid (TBA) test to measure secondary oxidation product malonaldehyde (MA) (AOCS official method Cd-19-90) (530nm, pink color); Our lab has experience and reagents to do this measurement.



- Anisidine value(Cd-18-90) to measure secondary oxidation product malonaldehyde (MA) and ketone (amount of aldehydes, 350nm, yellow color); Our lab has experience and reagents to do this measurement.



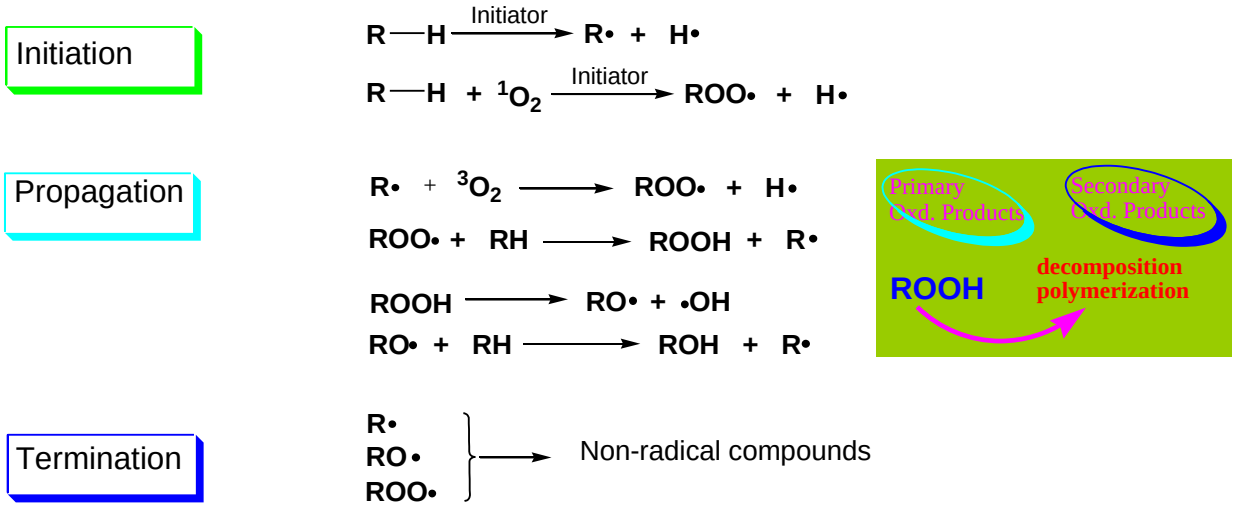


Fig. 1. A general mechanism of lipid oxidation

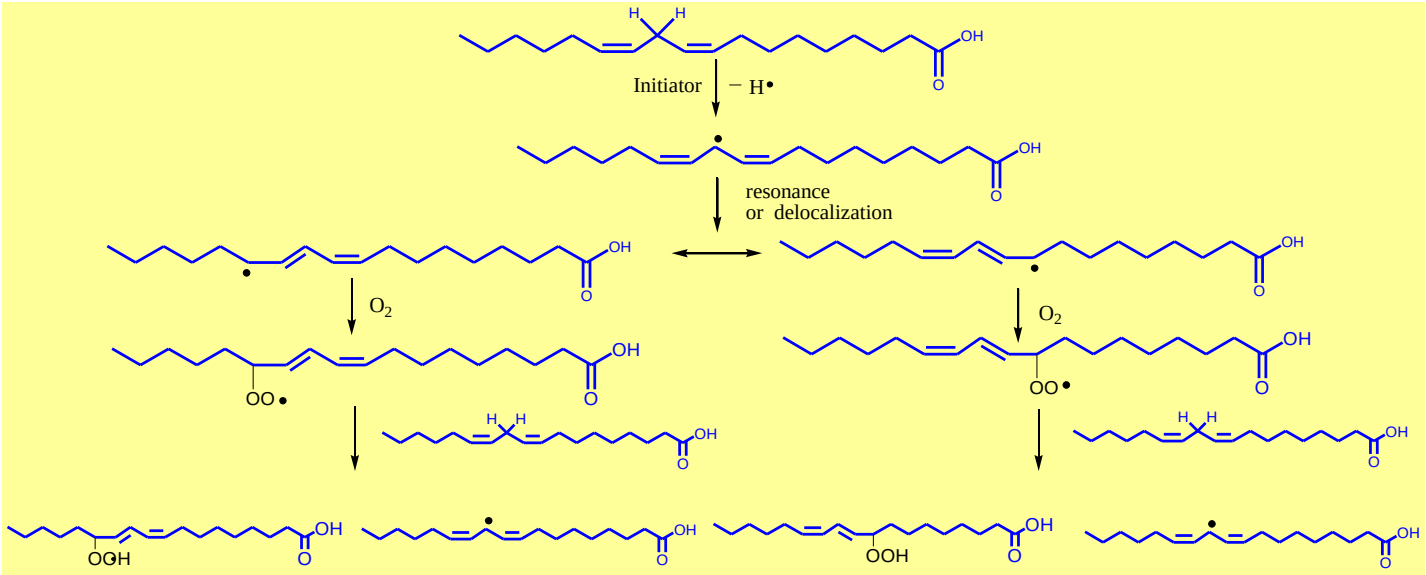


Fig. 2. Mechanism of autooxidation of linoleic acid

Spectrophotometer methods to measure the content of conjugated fatty acid: Oxidation can lead to isomerization of double bonds in polyunsaturated fatty acids to for conjugated diene or triene. The conjugated fatty acids have absorption in 232-234nm. Our lab is equipped with Shimadzu uv-vis spectrophotometer which can be used for this measurement.

Headspace Gas Chromatograph – Mass Spectrometer to measure secondary oxidation products: volatiles : Our lab has Thermo Fisher DSQ GC-MS equipped headspace injection which can be used for idenfication and qualification of secondary oxidation compounds such aldehydes, ketones, etc. (Fig. 3)

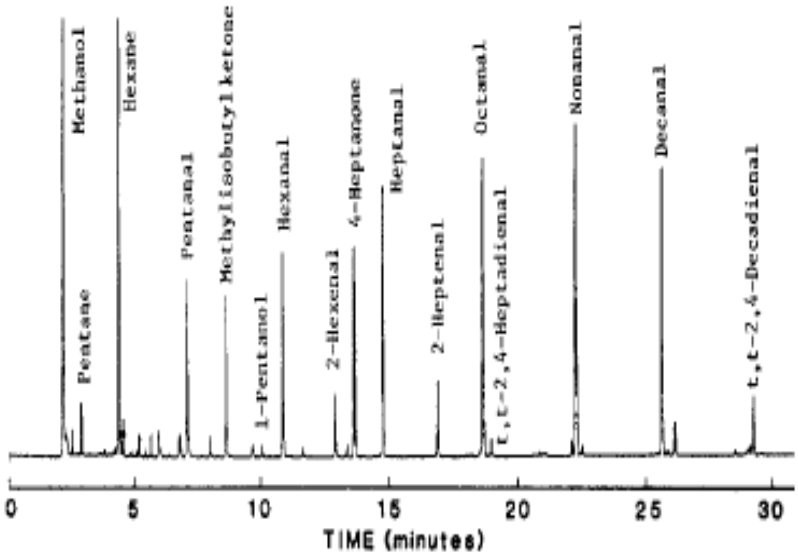


Fig. 3. **Gas chromatogram obtained with DHS-GC analysis** of **volatiles standard (30 ng/5 mL).**

HPLC-PDA to measure unvolatile oxidized products: high column temperature of GC can destroy peroxidized triglycerides, however, HPLC performed at room temperature can detect peroxides based on the polarity difference between oxidized and non-oxidized triglycerides. Our lab has Thermo Fischer HPLC-PDA-ELSD and normal/reversed phase columns, which can be used for quantification of peroxidized triglycerides (Fi. 4.)

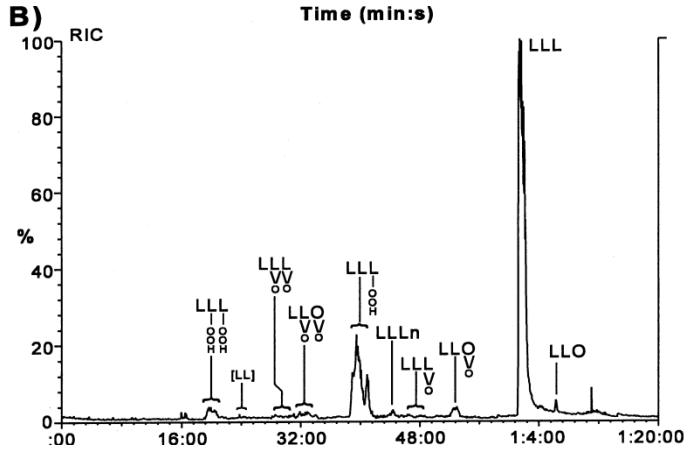


Fig. 4. HPLC chromatogram of autooxidized trilinolein and 1,2-dilinoleoyl-3-oleylglycerol.

Non-invasive emerging methodology to monitor lipid oxidation:

In analysis by all above methods, the sample has to be processed and the progress of oxidation will be interrupted or stopped. However, Confocal Raman spectroscopy and Luminescence imaging are two emerging technologies which are capable to real time monitor progress of oxidation. Our lab are in progress to do the related research through collaboration with Department of Chemistry, Aarhus University.