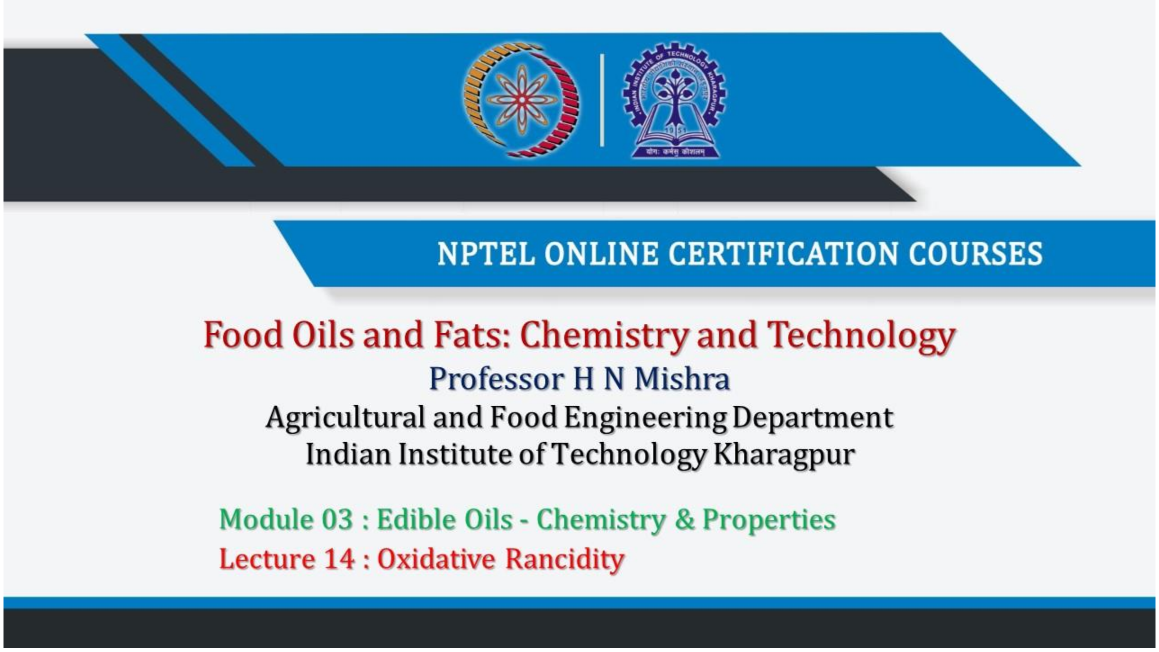
# Food Oils and Fats: Chemistry and Technology
Professor H N Mishra
Agricultural and Food Engineering Department
Indian Institute of Technology Kharagpur
Module 03 : Edible Oils - Chemistry & Properties
Lecture 14 : Oxidative Rancidity



NPTEL ONLINE CERTIFICATION COURSES

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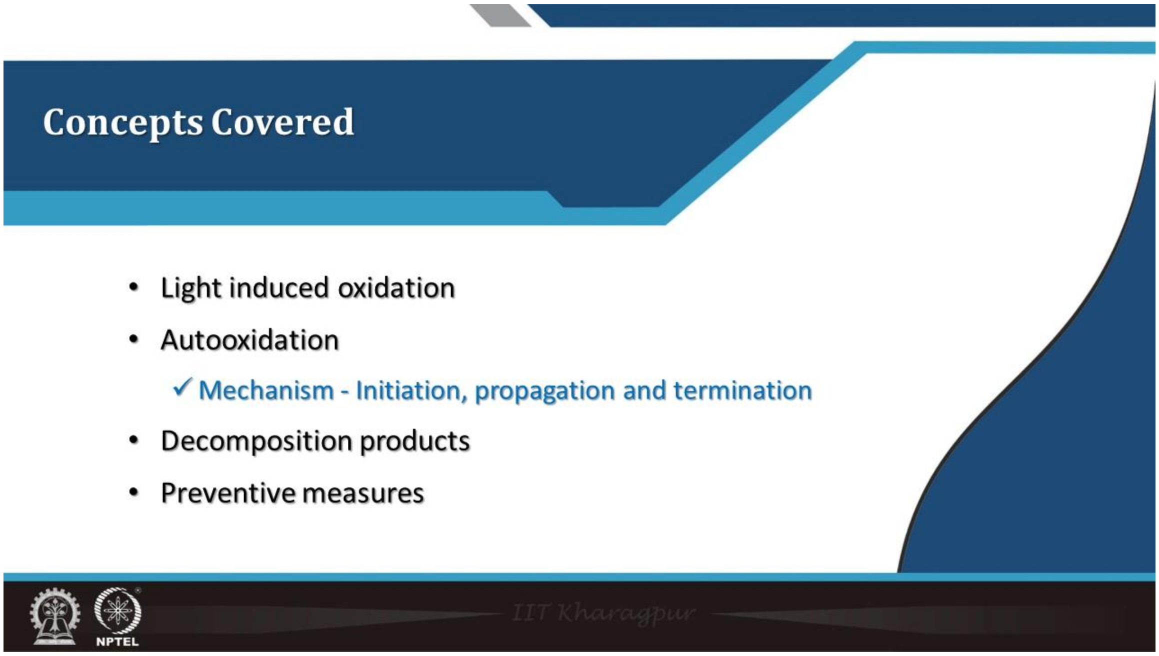
Lecture 14 : Oxidative Rancidity

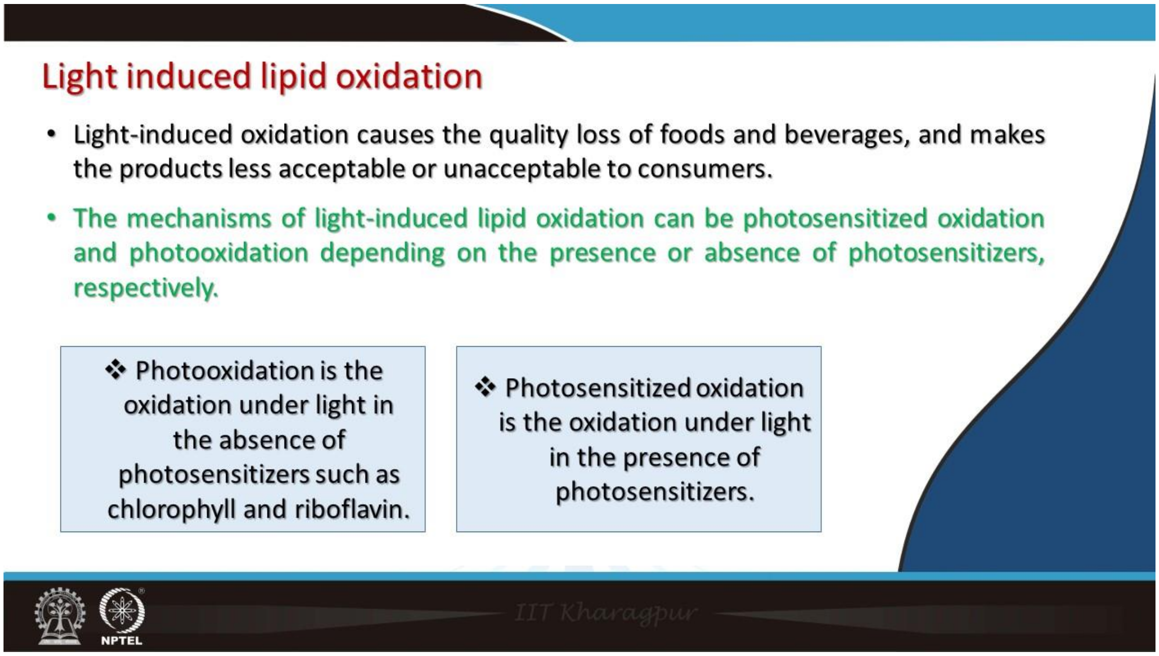
Hello everybody, Namaskar.  Now, we are in the 14th lecture.  In this lecture during the next half an hour or so, we will discuss Oxidative Rancidity.



## Concepts Covered

- Light induced oxidation
- Autooxidation
  - ✓ Mechanism - Initiation, propagation and termination
- Decomposition products
- Preventive measures

In the earlier class, we discussed rancidity and reversion and where oxidation of the oils and fats was seen as a major cause for the rancidity. So, let us see in detail the mechanism of oxidative rancidity and how it can be prevented because, in most oils, this is the major problem that is oxidation and auto-oxidation. So, we will discuss in this class what is lipid-induced oxidation, then finally, the mechanism of auto-oxidation is initiation, propagation, and termination, what the various decomposition products of oil that is auto oxidized oils decomposition products, and how auto-oxidation can be stopped, what the various preventive measures.



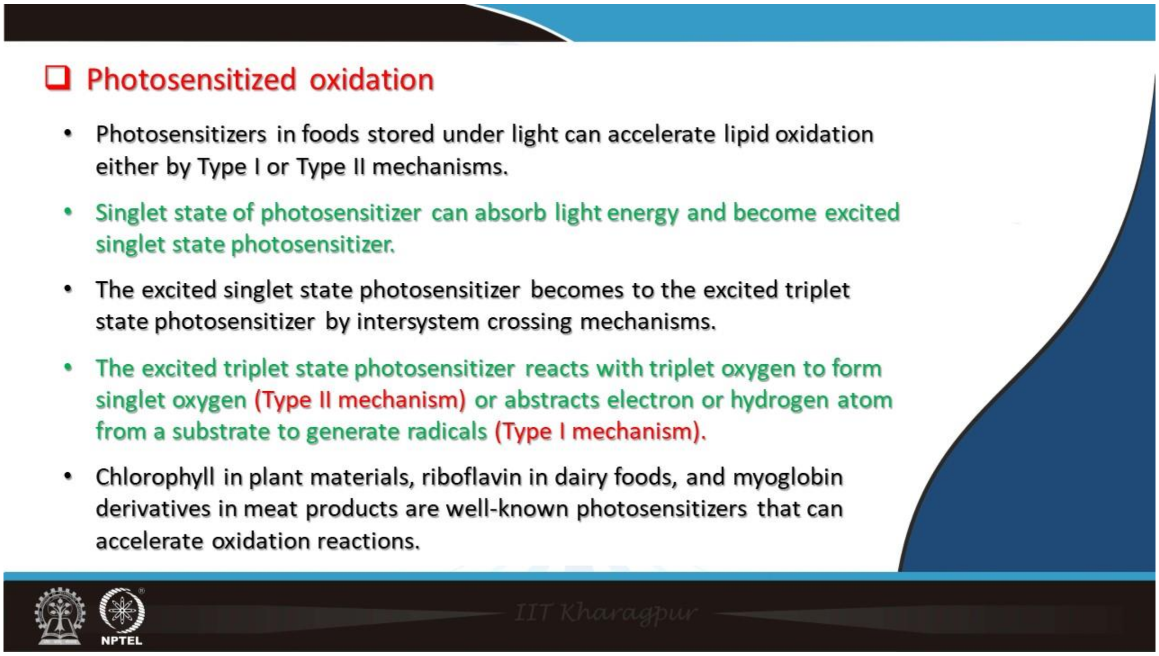
## Light induced lipid oxidation

- Light-induced oxidation causes the quality loss of foods and beverages, and makes the products less acceptable or unacceptable to consumers.
- The mechanisms of light-induced lipid oxidation can be photosensitized oxidation and photooxidation depending on the presence or absence of photosensitizers, respectively.

❖ Photooxidation is the oxidation under light in the absence of photosensitizers such as chlorophyll and riboflavin.

❖ Photosensitized oxidation is the oxidation under light in the presence of photosensitizers.

Let us see what is light-induced lipid oxidation. In the earlier class, there was something about oxidation and reduction, what is the oxidation reaction I told you. So, light-induced oxidation causes the quality loss of foods and beverages and makes the product less acceptable or unacceptable to the consumer. The mechanism of light-induced lipid oxidation can be photosynthesized or photosensitized oxidation and photo-oxidation. It may be either photosensitized oxidation or it may be photo-oxidation depending on the presence or absence of photosensitizers, respectively. So, photo-oxidation is the oxidation under light in the absence of photosensitizers such as chlorophyll and riboflavin etcetera. Whereas, photosensitized oxidation is the oxidation under the light in the presence of photosensitizers.



☐ Photosensitized oxidation

- Photosensitizers in foods stored under light can accelerate lipid oxidation either by Type I or Type II mechanisms.
- Singlet state of photosensitizer can absorb light energy and become excited singlet state photosensitizer.
- The excited singlet state photosensitizer becomes to the excited triplet state photosensitizer by intersystem crossing mechanisms.
- The excited triplet state photosensitizer reacts with triplet oxygen to form singlet oxygen (Type II mechanism) or abstracts electron or hydrogen atom from a substrate to generate radicals (Type I mechanism).
- Chlorophyll in plant materials, riboflavin in dairy foods, and myoglobin derivatives in meat products are well-known photosensitizers that can accelerate oxidation reactions.

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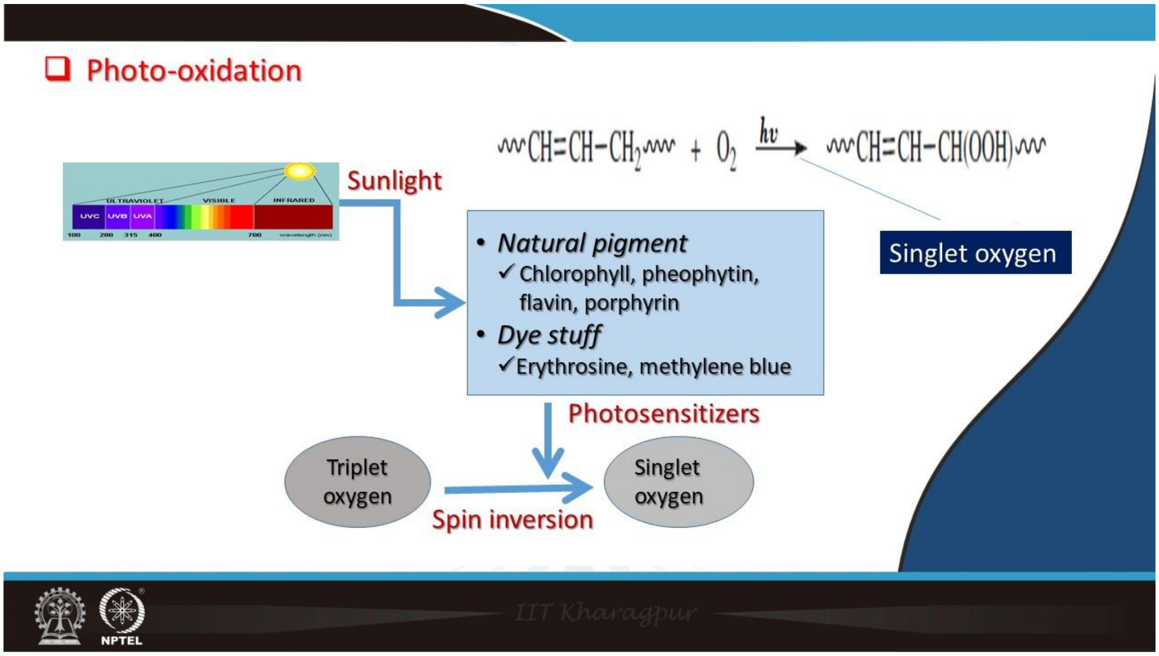


## Photo-oxidation

$$\sim CH=CH-CH_2\sim + O_2 \xrightarrow{h\nu} \sim CH=CH-CH(OOH)\sim$$

Singlet oxygen

Sunlight

- *Natural pigment*
  - ✓ Chlorophyll, pheophytin, flavin, porphyrin
- *Dye stuff*
  - ✓ Erythrosine, methylene blue

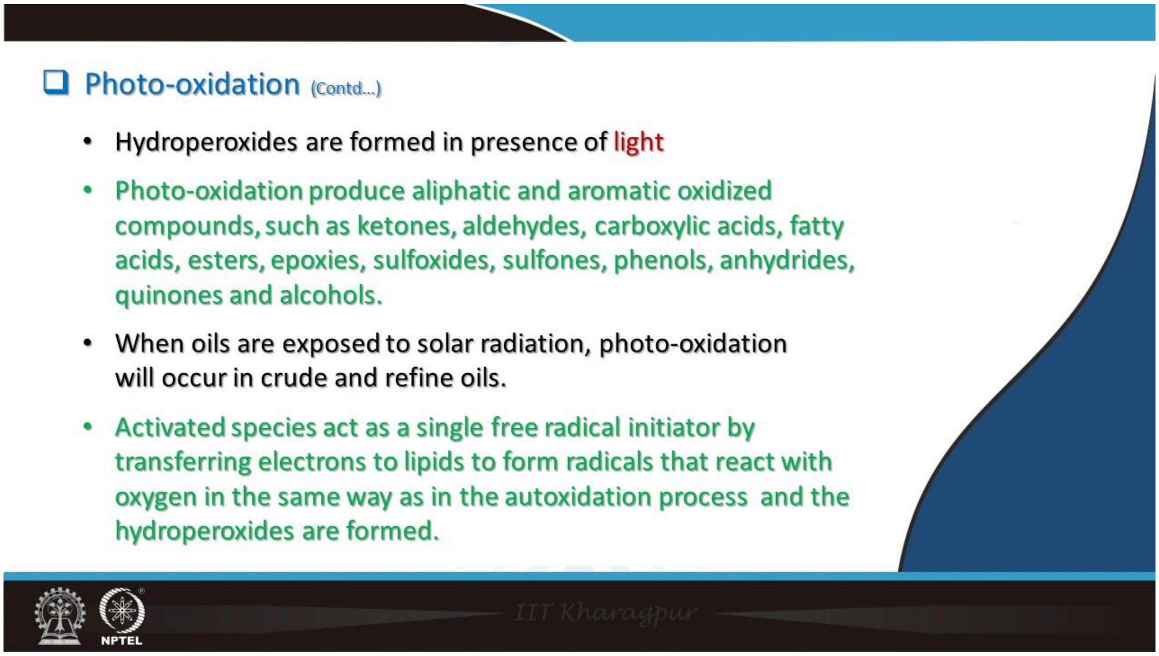
Photosensitizers

Triplet oxygen

Spin inversion

Singlet oxygen

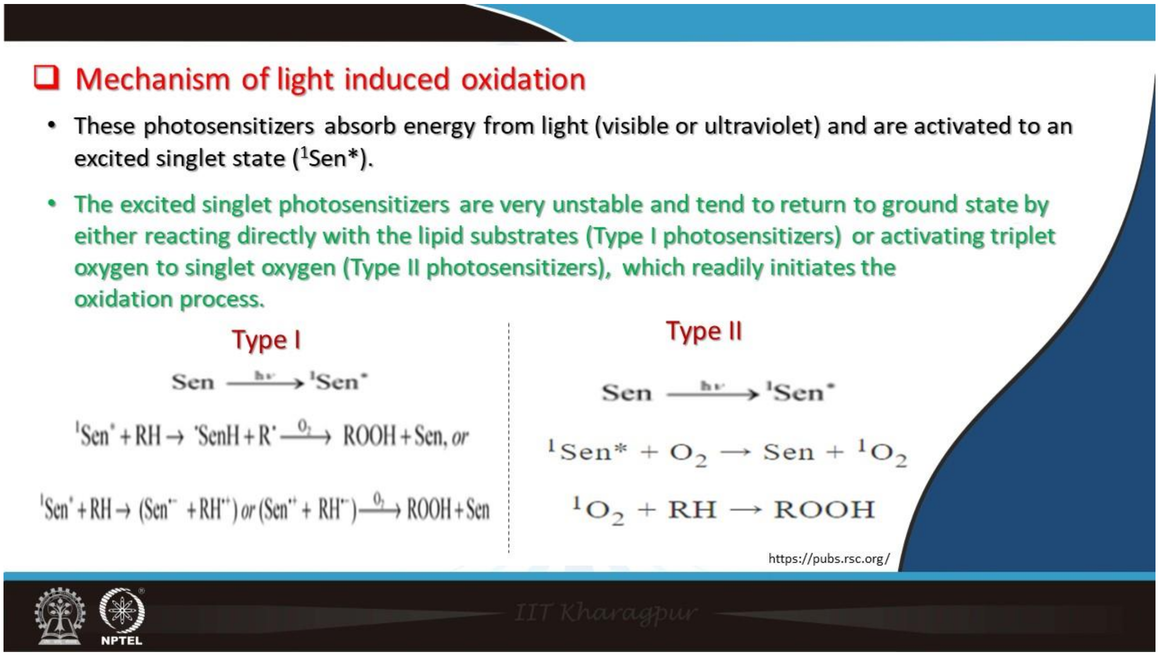
The photosensitized oxidation or photo-oxidation is shown pictorially for better understanding. The sunlight consists of UV and all the radiations shown here and when these lights they falls on the food that has natural pigments or photosensitizer like chlorophyll, pheophytine, flavin, porphyrin or dye stuff like erythrosine, methylene, blue, then these photosensitizer convert into that is the singlet oxygen and triplet oxygen through spin inversion. Singlet oxygen acts on the free fatty acids, particularly in the saturated linkage and the oxidation products are formed.



## Photo-oxidation (Contd...)

- Hydroperoxides are formed in presence of light
- Photo-oxidation produce aliphatic and aromatic oxidized compounds, such as ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxies, sulfoxides, sulfones, phenols, anhydrides, quinones and alcohols.
- When oils are exposed to solar radiation, photo-oxidation will occur in crude and refine oils.
- Activated species act as a single free radical initiator by transferring electrons to lipids to form radicals that react with oxygen in the same way as in the autoxidation process and the hydroperoxides are formed.

Hydroperoxides are formed in the presence of light and these are the result of photo oxidation. Photo-oxidation produces aliphatic and aromatic oxidized compounds, such as ketones, aldehydes, carboxylic acids, fatty acids, esters, epoxies, sulfoxides, sulfones, phenols, anhydrides, quinones, and alcohols. When oils are exposed to solar radiation photo-oxidation will occur in crude and refined oil. And it is in crude oil there may be photosensitizers like chlorophyll etcetera they may get extracted from oil. So, obviously, that is it will be photosensitized which is other mediated photo-oxidation. Photosensitized oxidation and the refined oils where these coloring compounds are removed may be another mechanism. Activated species act as a singlet single free radical initiator by transforming electrons to lipids to form radicals that react with oxygen in the same way as in the auto-oxidation process and the hydroperoxides are formed.



## ❑ Mechanism of light induced oxidation

- These photosensitizers absorb energy from light (visible or ultraviolet) and are activated to an excited singlet state (${}^1$Sen*).
- The excited singlet photosensitizers are very unstable and tend to return to ground state by either reacting directly with the lipid substrates (Type I photosensitizers) or activating triplet oxygen to singlet oxygen (Type II photosensitizers), which readily initiates the oxidation process.

Type I

$$\mathrm{Sen} \xrightarrow{h\nu} {}^1\mathrm{Sen}^*$$

$${}^1\mathrm{Sen}^* + \mathrm{RH} \rightarrow {}^{\cdot}\mathrm{SenH} + \mathrm{R}^{\cdot} \xrightarrow{O_2} \mathrm{ROOH} + \mathrm{Sen},\ \mathit{or}$$

$${}^1\mathrm{Sen}^* + \mathrm{RH} \rightarrow (\mathrm{Sen}^{\cdot -} + \mathrm{RH}^{\cdot +})\ \mathit{or}\ (\mathrm{Sen}^{\cdot +} + \mathrm{RH}^{\cdot -}) \xrightarrow{O_2} \mathrm{ROOH} + \mathrm{Sen}$$

Type II

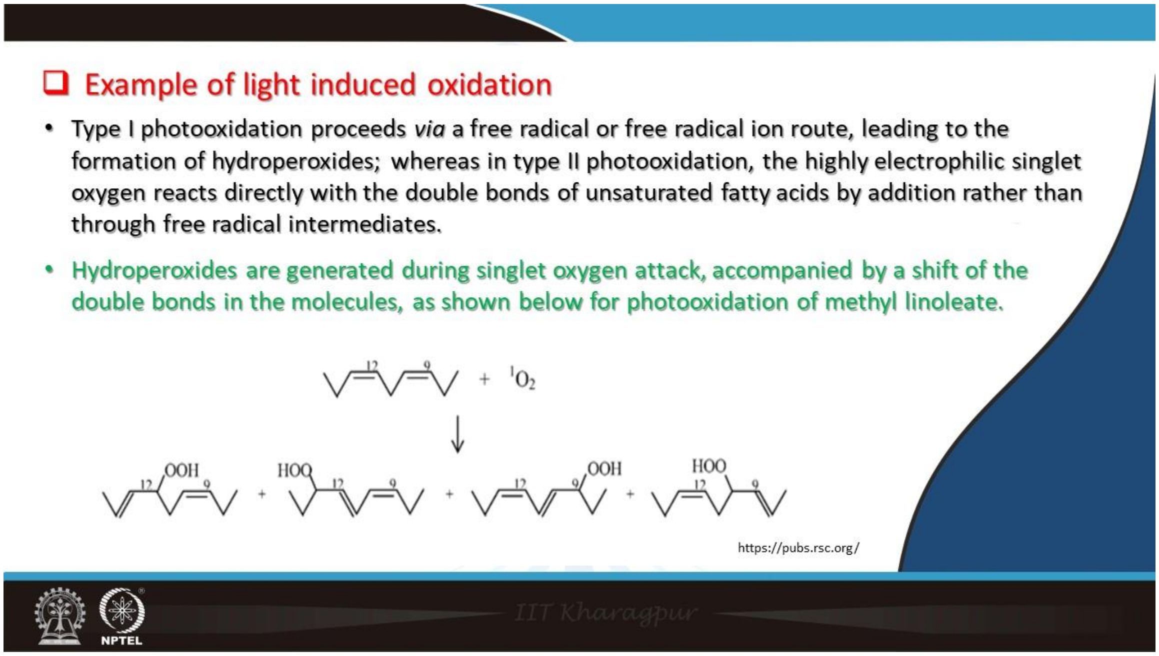
$$\mathrm{Sen} \xrightarrow{h\nu} {}^1\mathrm{Sen}^*$$

$${}^1\mathrm{Sen}^* + \mathrm{O}_2 \rightarrow \mathrm{Sen} + {}^1\mathrm{O}_2$$

$${}^1\mathrm{O}_2 + \mathrm{RH} \rightarrow \mathrm{ROOH}$$

https://pubs.rsc.org/

If you see the mechanism of light-induced oxidation little like the type 1 mechanism or type 2 mechanism that is these photosensitizers absorb energy from light as you have seen in the earlier figure. They absorb energy from light that may be visible light or ultraviolet light and they are activated to an excited singlet state one SN, which is activated singlet state, which you can see this synthesizer absorb the light and get into excited states. So, these excited singlet photosensitizers are very unstable and tend to return to the ground state by either reacting directly to the lipid substrate you can see here directly to the lipid substrate like type 1 mechanism in the photosensitizer alright or that is they activate triplet oxygen to singlet oxygen. You can see here in the second type 2 mechanism which readily initiates the oxidation process and both ways either they directly interact with the lipid substrate or they interact via activating triplet oxygen to singlet oxygen parallel. So, this is both type 1 and type 2 mechanisms.

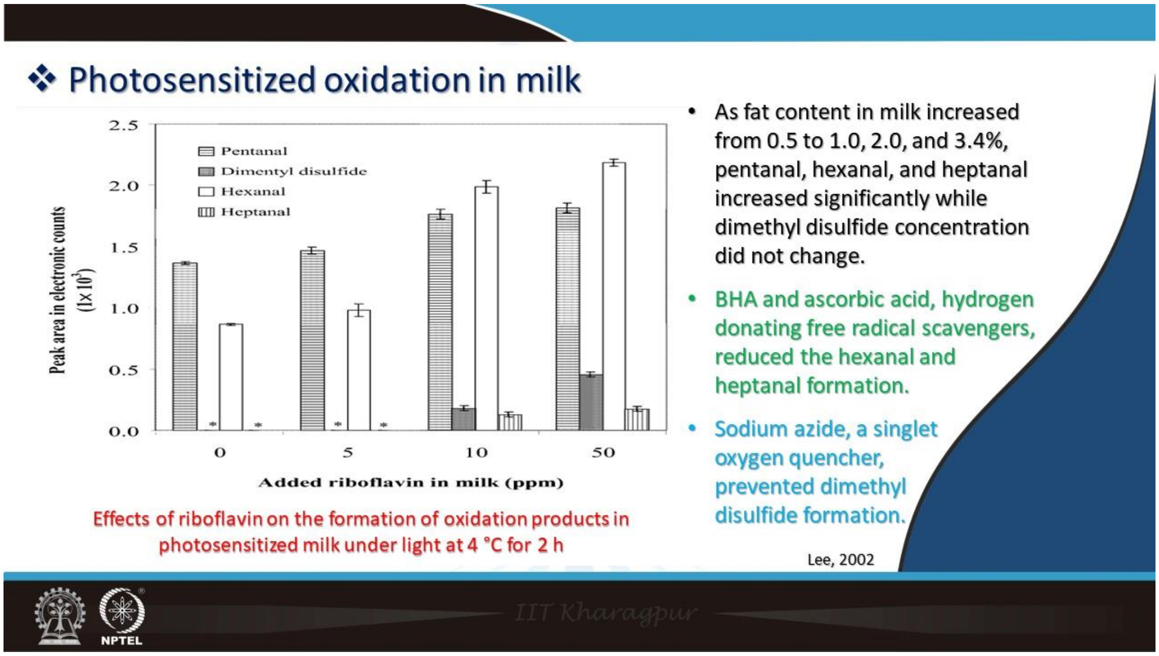


## ❑ Example of light induced oxidation

- Type I photooxidation proceeds *via* a free radical or free radical ion route, leading to the formation of hydroperoxides; whereas in type II photooxidation, the highly electrophilic singlet oxygen reacts directly with the double bonds of unsaturated fatty acids by addition rather than through free radical intermediates.
- Hydroperoxides are generated during singlet oxygen attack, accompanied by a shift of the double bonds in the molecules, as shown below for photooxidation of methyl linoleate.

https://pubs.rsc.org/

Let us take one example of light-induced oxidation, type 1 photo-oxidation proceeds via a free radical or free radical iron route free radical chain mechanism leading to the formation of hydroperoxide; whereas, type 2 photo-oxidation, the highly electrophilic singlet oxygen reacts directly with the double bonds of unsaturated fatty acids by the addition rather than through free radical intermediates. That is here oxygen directly adds to the saturation position in the bond. Hydroperoxide is generated during a singlet oxygen attack which is accompanied by a shift of the double bond in the molecules as shown below for photooxidation of methyl linoleate. You can see here methyl enolate that O2 9 and 12 here the unsaturated linkages that here at such points oxygen adds here and it becomes that hydroperoxides.



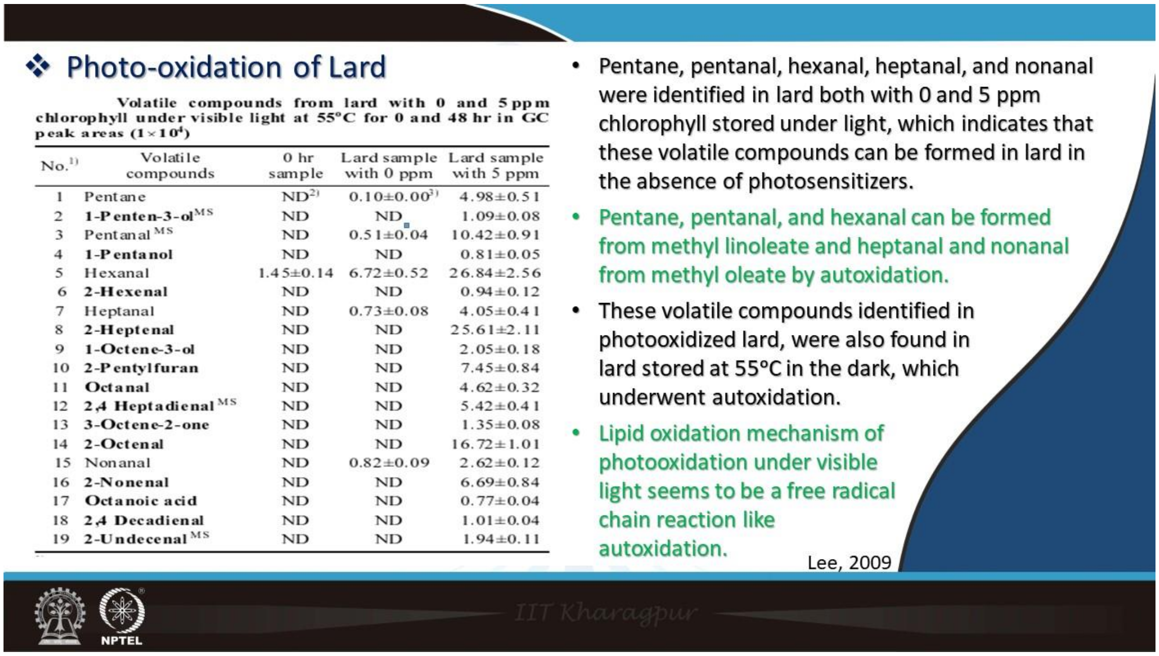
## ❖ Photosensitized oxidation in milk

- As fat content in milk increased from 0.5 to 1.0, 2.0, and 3.4%, pentanal, hexanal, and heptanal increased significantly while dimethyl disulfide concentration did not change.
- BHA and ascorbic acid, hydrogen donating free radical scavengers, reduced the hexanal and heptanal formation.
- Sodium azide, a singlet oxygen quencher, prevented dimethyl disulfide formation.

Effects of riboflavin on the formation of oxidation products in photosensitized milk under light at 4 °C for 2 h

Lee, 2002

Then the photosynthesized oxidation in milk let us see a case study, where riboflavin in the milk is shown in this bar diagram. The peak area in the electronic columns and the columns show the products like pentanols, dimethyl disulfide, hexanols, and heptanols that are in this product formation. It can be seen here that the figure shows the effect of riboflavin on the formation of oxidation product in milk under light at 24 degrees Celsius for 2 hours. So, it can be seen that as the fat content in the milk increases pentanol, hexanol, and heptanol increase significantly while the dimethyl disulfide concentration does not change, it remains almost the same. BHA that is butylated hydroxy anisole and ascorbic acid hydrogen donating free radical scavengers reduce the hexanol and heptanol formation. Sodium azide, a singlet oxygen quencher prevented dimethyl disulfide formation in milk.



## Photo-oxidation of Lard

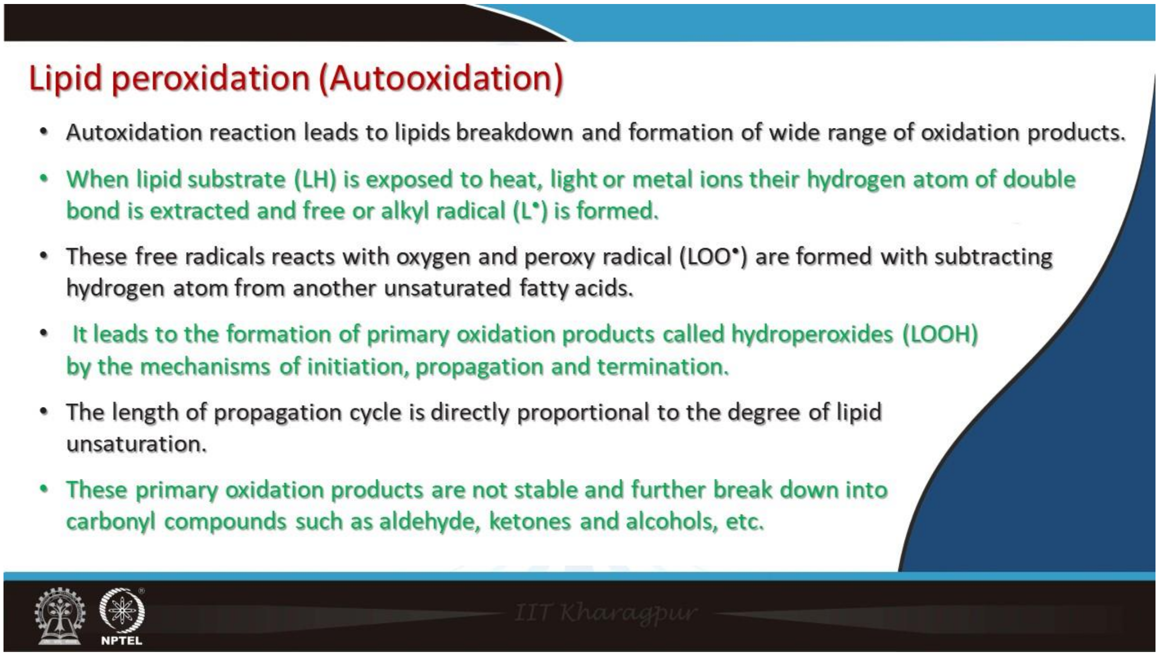
**Volatile compounds from lard with 0 and 5 ppm chlorophyll under visible light at 55°C for 0 and 48 hr in GC peak areas ($1 \times 10^4$)**

| No.[1] | Volatile compounds | 0 hr sample | Lard sample with 0 ppm | Lard sample with 5 ppm |
|---|---|---|---|---|
| 1 | Pentane | ND[2] | 0.10±0.00[3] | 4.98±0.51 |
| 2 | **1-Penten-3-ol**$^{MS}$ | ND | ND | 1.09±0.08 |
| 3 | Pentanal$^{MS}$ | ND | 0.51±0.04 | 10.42±0.91 |
| 4 | **1-Pentanol** | ND | ND | 0.81±0.05 |
| 5 | Hexanal | 1.45±0.14 | 6.72±0.52 | 26.84±2.56 |
| 6 | **2-Hexenal** | ND | ND | 0.94±0.12 |
| 7 | Heptanal | ND | 0.73±0.08 | 4.05±0.41 |
| 8 | **2-Heptenal** | ND | ND | 25.61±2.11 |
| 9 | **1-Octene-3-ol** | ND | ND | 2.05±0.18 |
| 10 | **2-Pentylfuran** | ND | ND | 7.45±0.84 |
| 11 | **Octanal** | ND | ND | 4.62±0.32 |
| 12 | **2,4 Heptadienal**$^{MS}$ | ND | ND | 5.42±0.41 |
| 13 | **3-Octene-2-one** | ND | ND | 1.35±0.08 |
| 14 | **2-Octenal** | ND | ND | 16.72±1.01 |
| 15 | Nonanal | ND | 0.82±0.09 | 2.62±0.12 |
| 16 | **2-Nonenal** | ND | ND | 6.69±0.84 |
| 17 | **Octanoic acid** | ND | ND | 0.77±0.04 |
| 18 | **2,4 Decadienal** | ND | ND | 1.01±0.04 |
| 19 | **2-Undecenal**$^{MS}$ | ND | ND | 1.94±0.11 |

- Pentane, pentanal, hexanal, heptanal, and nonanal were identified in lard both with 0 and 5 ppm chlorophyll stored under light, which indicates that these volatile compounds can be formed in lard in the absence of photosensitizers.
- Pentane, pentanal, and hexanal can be formed from methyl linoleate and heptanal and nonanal from methyl oleate by autoxidation.
- These volatile compounds identified in photooxidized lard, were also found in lard stored at 55°C in the dark, which underwent autoxidation.
- Lipid oxidation mechanism of photooxidation under visible light seems to be a free radical chain reaction like autoxidation.

Lee, 2009

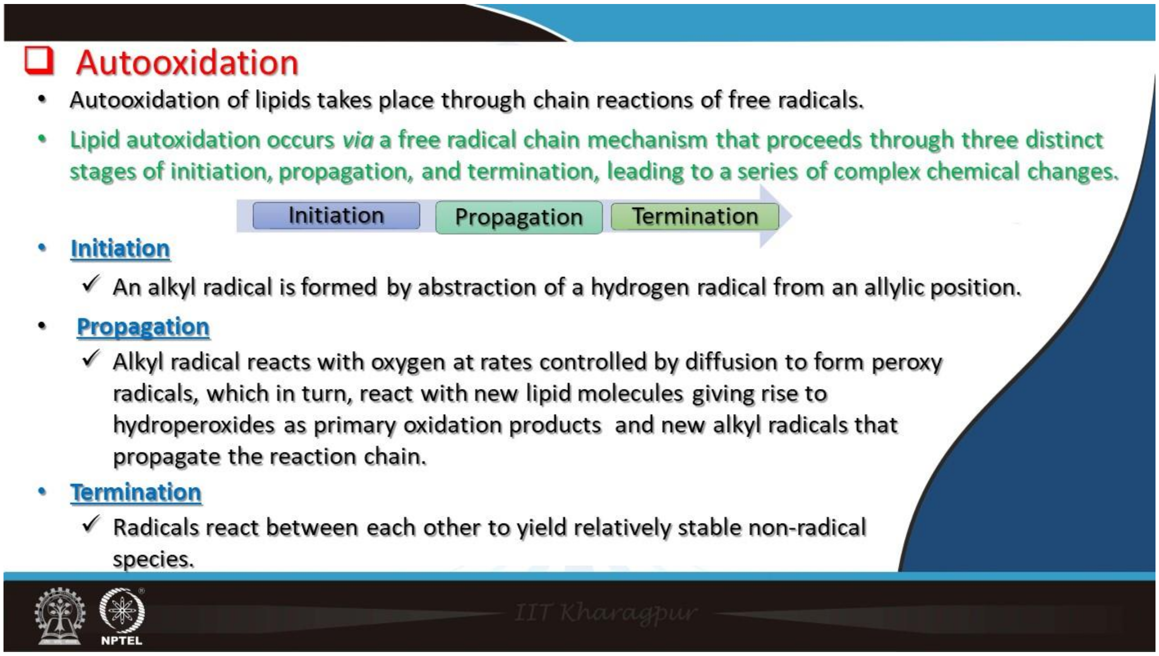
Then photo-oxidation of lard you can see here in the table that there are various volatile compounds from lard with 0 and 5 ppm chlorophyll under visible light at 55 degrees Celsius for 0 and 48 hours in the GC peak area. These are the various compounds etcetera which are detected. ND means not detected. Pentane, pentanal, hexanal, heptanal, and nonanal were identified in lard both with 0 and 5 ppm chlorophyll stored under light, which indicates that these volatile compounds can be formed in lard in the absence of photosensitizers. Pentane, pentanal, and hexanal can be formed from methyl linoleate and heptanal and nonanal from methyl oleate by autoxidation. These volatile compounds identified in photo oxidized lard were also found in lard stored at 55°C in the dark, which underwent autoxidation. Lipid oxidation mechanism of photooxidation under visible light seems to be a free radical chain reaction like autoxidation.



## Lipid peroxidation (Autooxidation)

- Autoxidation reaction leads to lipids breakdown and formation of wide range of oxidation products.
- When lipid substrate (LH) is exposed to heat, light or metal ions their hydrogen atom of double bond is extracted and free or alkyl radical ($L^{\bullet}$) is formed.
- These free radicals reacts with oxygen and peroxy radical ($LOO^{\bullet}$) are formed with subtracting hydrogen atom from another unsaturated fatty acids.
- It leads to the formation of primary oxidation products called hydroperoxides (LOOH) by the mechanisms of initiation, propagation and termination.
- The length of propagation cycle is directly proportional to the degree of lipid unsaturation.
- These primary oxidation products are not stable and further break down into carbonyl compounds such as aldehyde, ketones and alcohols, etc.

Then let us see the study of lipid peroxidation which is more commonly known as auto-oxidation. So, the auto-oxidation reaction leads to lipid breakdown and the formation of a wide range of oxidation products and we discussed in the last class also briefly about this. Autoxidation reaction leads to lipids breakdown and formation of a wide range of oxidation products. When lipid substrate (LH) is exposed to heat, light, or metal ions the hydrogen atom of the double bond is extracted, and free or alkyl radical (L•) is formed. These free radicals react with oxygen and peroxy radicals (LOO•) are formed by subtracting a hydrogen atom from another unsaturated fatty acid. It leads to the formation of primary oxidation products called hydroperoxides (LOOH) by the mechanisms of initiation, propagation, and termination. The length of the propagation cycle is directly proportional to the degree of lipid unsaturation. These primary oxidation products are not stable and further break down into carbonyl compounds such as aldehydes, ketones alcohols, etc.



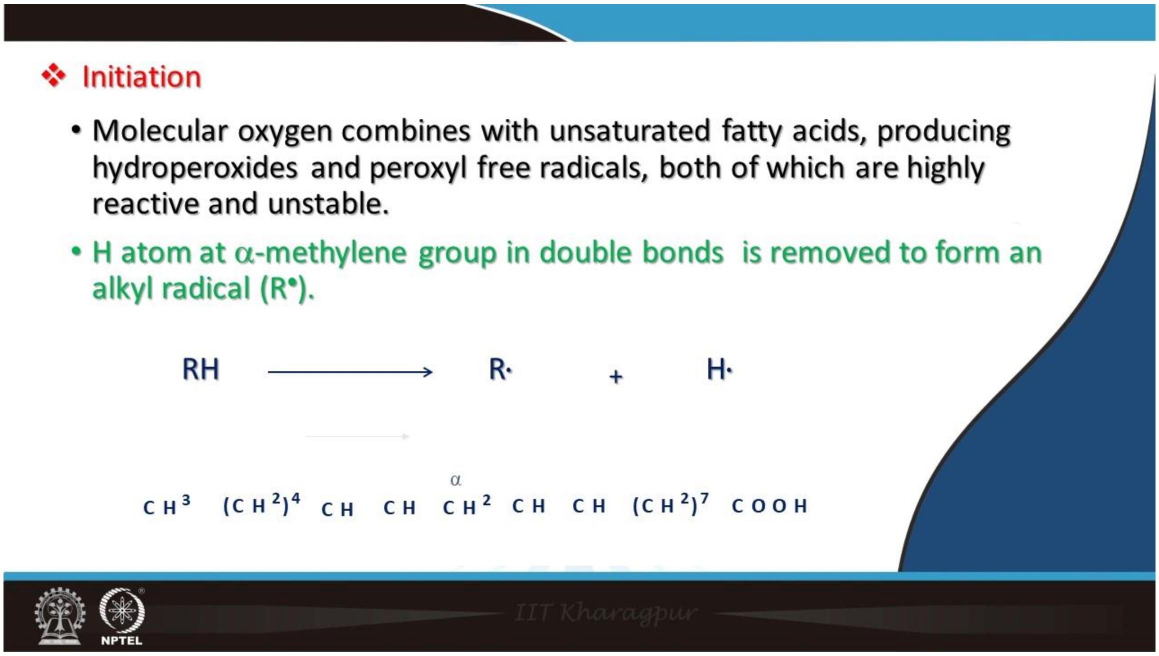
## ❑ Autooxidation

- Autooxidation of lipids takes place through chain reactions of free radicals.
- Lipid autoxidation occurs *via* a free radical chain mechanism that proceeds through three distinct stages of initiation, propagation, and termination, leading to a series of complex chemical changes.

Initiation → Propagation → Termination

- **Initiation**
  - ✓ An alkyl radical is formed by abstraction of a hydrogen radical from an allylic position.
- **Propagation**
  - ✓ Alkyl radical reacts with oxygen at rates controlled by diffusion to form peroxy radicals, which in turn, react with new lipid molecules giving rise to hydroperoxides as primary oxidation products and new alkyl radicals that propagate the reaction chain.
- **Termination**
  - ✓ Radicals react between each other to yield relatively stable non-radical species.

So, I told you that the oxidation of lipids takes place through a chain reaction mechanism which is called the free radical chain reaction mechanism. Lipid auto-oxidation occurs via a free radical chain mechanism that proceeds through three distinct stages, these are called initiation, propagation, and termination, and which ultimately leads to a series of chemical changes in the oil. So, the initiation as I told you is an alkyl radical formed by abstraction of a hydrogen radical from an allylic position. Then alkyl radical reacts with oxygen at rates controlled by diffusion to form peroxy radicals which in turn react with new lipid molecules giving rise to the hydroperoxide as the primary oxidation product and new alkyl radicals that propagate the reaction chain. And then finally, in the termination stage radicals interact or react with each other to yield relatively stable non-radical products.



**Initiation**

- Molecular oxygen combines with unsaturated fatty acids, producing hydroperoxides and peroxyl free radicals, both of which are highly reactive and unstable.
- H atom at $\alpha$-methylene group in double bonds is removed to form an alkyl radical ($R^\bullet$).

$$RH \longrightarrow R\cdot + H\cdot$$

$$CH^{3}\ \ (CH^{2})^{4}\ \ CH\ \ CH\ \ \overset{\alpha}{CH^{2}}\ \ CH\ \ CH\ \ (CH^{2})^{7}\ \ COOH$$

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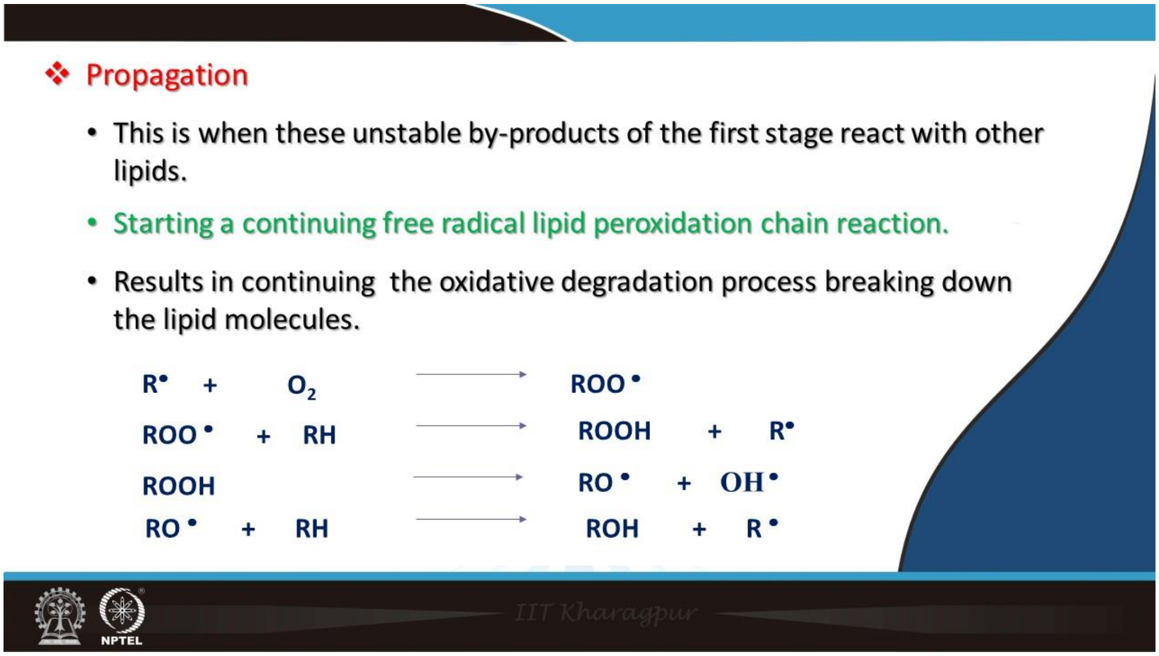
Let us see a little more detail about these processes in the initiation as you can see molecular oxygen combines with unsaturated fatty acid-producing hydroperoxides and peroxyl free radicals, both of which are highly reactive and unstable molecules. The hydrogen atom at the methylene group in double bonds is removed to form an alkyl free radical or free radical that is here in this is the fatty acids C-OH is single bond C-H and this C-H and C-H this is here these are the link with the double bond. The single bond C-H2 then methyl group is called alpha group here and then the C-H double bond here. So, these two double bonds are here. So, the 7, 8, 9, position has a double bond here, then 11 and 12. So, in the unsaturated position at the alpha-methyl group that is the hydrogen atom, double bonds are removed and you get this alkyl free radical.



## ❖ Propagation

- This is when these unstable by-products of the first stage react with other lipids.
- Starting a continuing free radical lipid peroxidation chain reaction.
- Results in continuing the oxidative degradation process breaking down the lipid molecules.

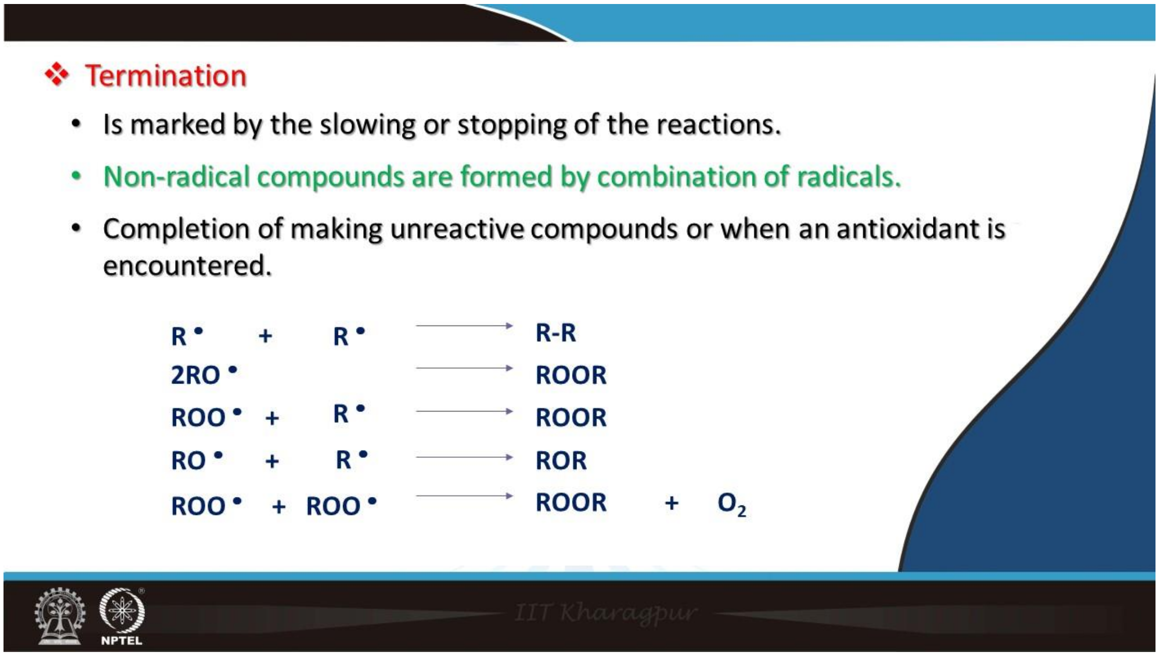
$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

$$ROOH \longrightarrow RO^{\bullet} + OH^{\bullet}$$

$$RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet}$$

Then propagation this is when these unstable byproducts of first stage react with other lipids continuing the free radical lipid peroxidation chain reaction and it results in the continuing the oxidative degradation process breaking down the lipid molecules like you can see in alkyl free radical which is formed in the initiation step. It may react with another oxygen molecule may form ROO free radicals like peroxy free radicals then RO plus R-H is called topoxides and R free radical R-OH may further break down into RO and OH and RO plus R-OH is RO radical here and R-H is a new lipid molecule. It gives R-OH and alkyl R free radicals. So, this process continues means there is more and more it is once it is initiated if it is not controlled the process will go on.

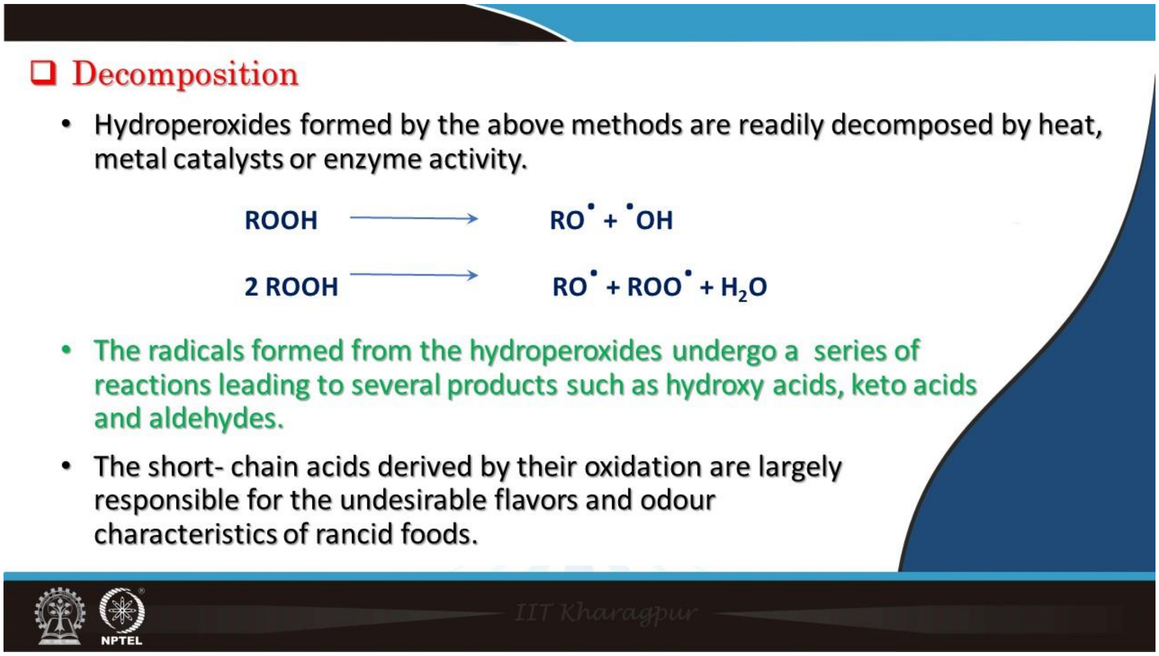


### ❖ Termination

- Is marked by the slowing or stopping of the reactions.
- Non-radical compounds are formed by combination of radicals.
- Completion of making unreactive compounds or when an antioxidant is encountered.

$$R^\bullet + R^\bullet \longrightarrow R\text{-}R$$
$$2RO^\bullet \longrightarrow ROOR$$
$$ROO^\bullet + R^\bullet \longrightarrow ROOR$$
$$RO^\bullet + R^\bullet \longrightarrow ROR$$
$$ROO^\bullet + ROO^\bullet \longrightarrow ROOR + O_2$$

Then finally, it leads to the termination it is marked by the slowing or stopping of the reaction radical compounds are formed by the combination of the radicals and it is the completion of making unreactive compounds or when an antioxidant is encountered. You can see here two alkyl free radicals R and R combined with another new alkyl R-R. Two RO free radicals may give R-OR, R-OO plus R may give R-OO-R. R is an alkyl group O is oxygen RO free radical and R radical may give non-radical products like RO-R. Then RO-O, RO-O these two are the radicals combined giving RO-OR plus O2, and oxygen is released. So, this is the termination reaction.



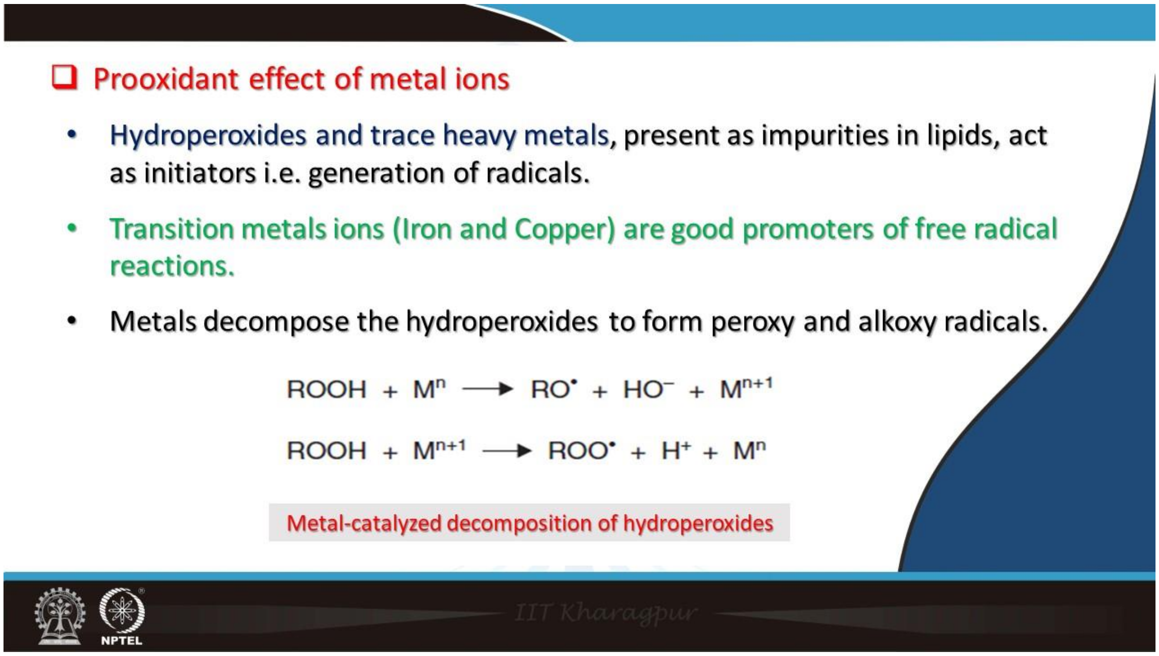
### ❑ Decomposition

- Hydroperoxides formed by the above methods are readily decomposed by heat, metal catalysts or enzyme activity.

$$ROOH \longrightarrow RO^\bullet + {}^\bullet OH$$
$$2\,ROOH \longrightarrow RO^\bullet + ROO^\bullet + H_2O$$

- The radicals formed from the hydroperoxides undergo a series of reactions leading to several products such as hydroxy acids, keto acids and aldehydes.
- The short- chain acids derived by their oxidation are largely responsible for the undesirable flavors and odour characteristics of rancid foods.

Then finally, comes the decomposition that is hydroperoxide which is formed by these above methods are readily decomposed by heat, metal catalyst, or enzyme activity. For example, RO-OH which was formed in the termination reaction further decomposed to RO plus OH, 2RO-OH, and RO plus ROO plus H2O. These radicals formed from the hydroperoxide undergo a series of reactions leading to several product formations such as hydroxy acid, keto acids, aldehydes, and many other carbonyl compounds. The short-chain acids derived by oxidation are largely responsible for the undesirable flavor and other characteristics of the rancid oil.



❑ Prooxidant effect of metal ions

- Hydroperoxides and trace heavy metals, present as impurities in lipids, act as initiators i.e. generation of radicals.
- Transition metals ions (Iron and Copper) are good promoters of free radical reactions.
- Metals decompose the hydroperoxides to form peroxy and alkoxy radicals.

$$\mathrm{ROOH} + \mathrm{M}^{n} \longrightarrow \mathrm{RO}^{\bullet} + \mathrm{HO}^{-} + \mathrm{M}^{n+1}$$

$$\mathrm{ROOH} + \mathrm{M}^{n+1} \longrightarrow \mathrm{ROO}^{\bullet} + \mathrm{H}^{+} + \mathrm{M}^{n}$$

Metal-catalyzed decomposition of hydroperoxides

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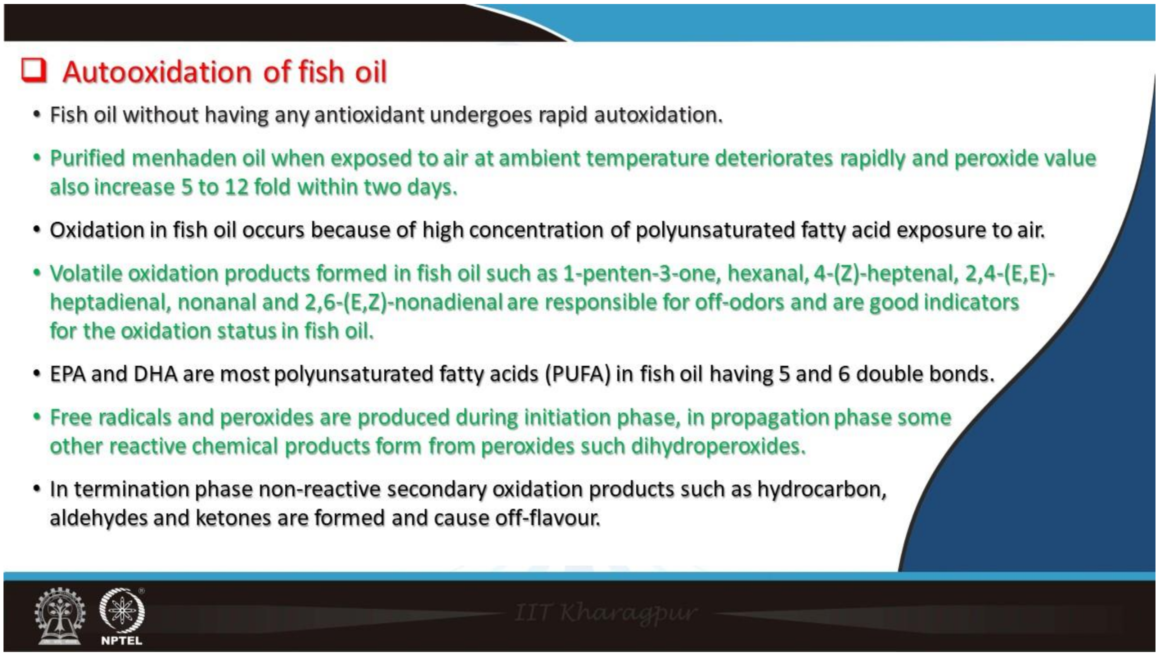
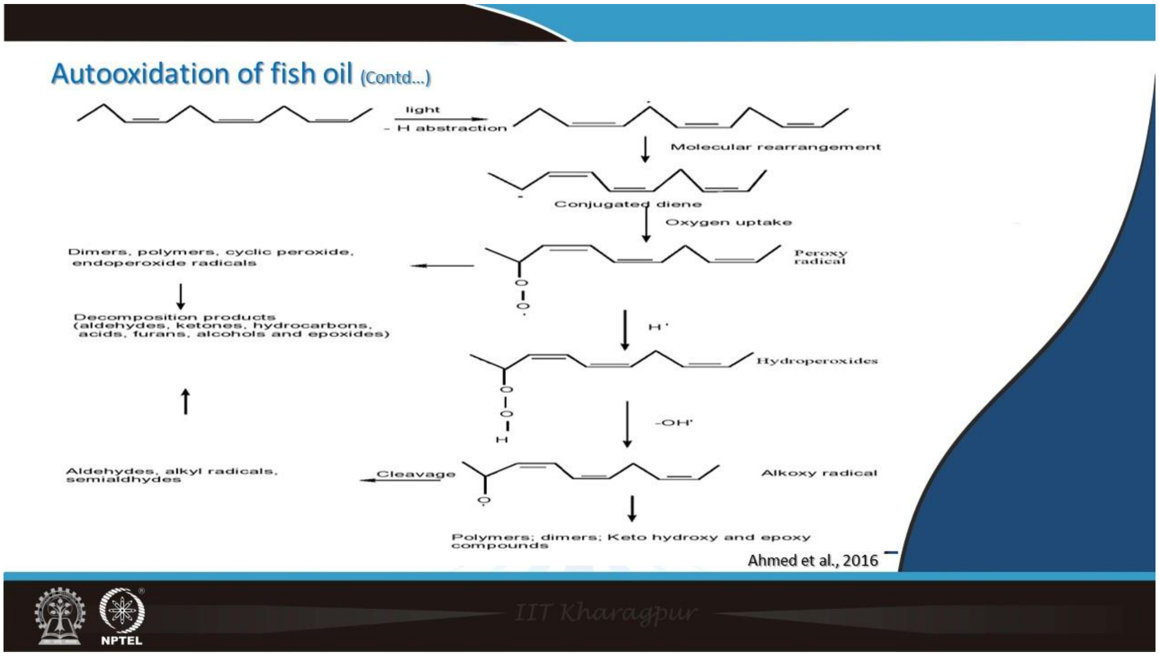
So, this auto-oxidation is enhanced by certain factors like metal ion. So, this acts as a pro-oxidant that is the metal ions and trace heavy metals present as impurities in the lipids they act as initiators, like generation of radicals. Transition metals or transition metal ions like ions of iron and copper are good promoters of free radical reactions that decompose the hydroperoxide to form peroxy and alkoxy radicals like ROOH and the metal ions produce RO plus OH ions plus Mn plus 1, ROOH and Mn plus 1 then produce ROO plus H plus ions and Mn. So, this is metal-catalyzed decomposition of hydroperoxides.



## Autooxidation of fish oil

- Fish oil without having any antioxidant undergoes rapid autoxidation.
- Purified menhaden oil when exposed to air at ambient temperature deteriorates rapidly and peroxide value also increase 5 to 12 fold within two days.
- Oxidation in fish oil occurs because of high concentration of polyunsaturated fatty acid exposure to air.
- Volatile oxidation products formed in fish oil such as 1-penten-3-one, hexanal, 4-(Z)-heptenal, 2,4-(E,E)-heptadienal, nonanal and 2,6-(E,Z)-nonadienal are responsible for off-odors and are good indicators for the oxidation status in fish oil.
- EPA and DHA are most polyunsaturated fatty acids (PUFA) in fish oil having 5 and 6 double bonds.
- Free radicals and peroxides are produced during initiation phase, in propagation phase some other reactive chemical products form from peroxides such dihydroperoxides.
- In termination phase non-reactive secondary oxidation products such as hydrocarbon, aldehydes and ketones are formed and cause off-flavour.

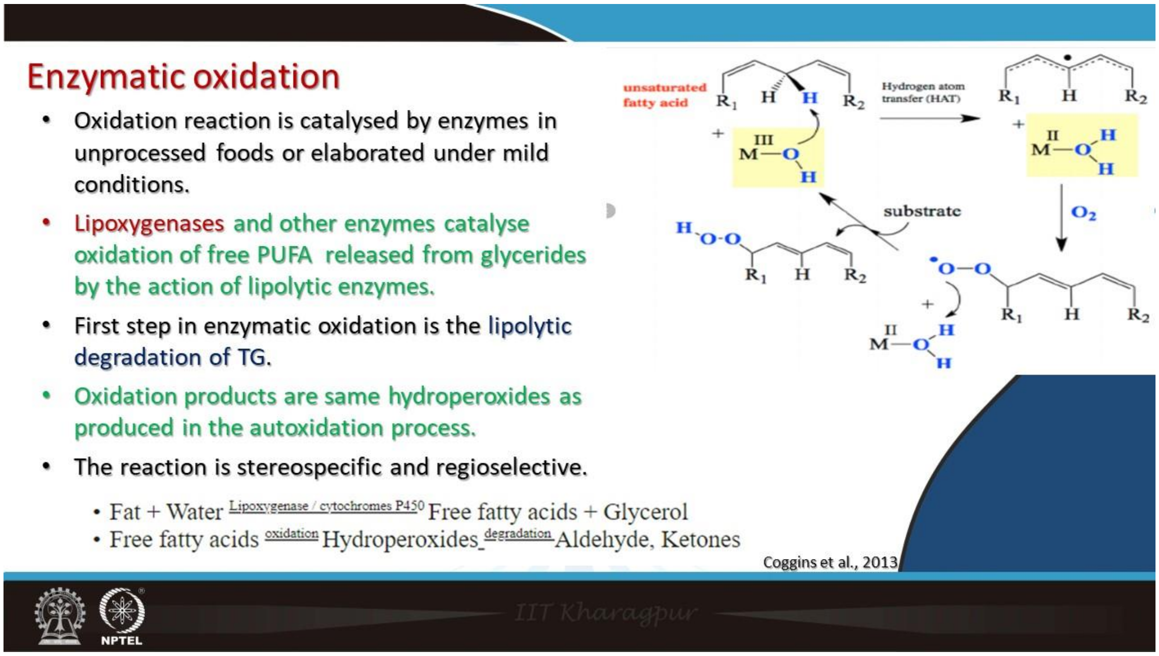
Fish oil without having any antioxidants undergoes rapid autoxidation. Purified menhaden oil when exposed to air at ambient temperature deteriorates rapidly and peroxide value also increases 5 to 12-fold within two days. Oxidation in fish oil occurs because of high concentrations of polyunsaturated fatty acid exposure to air. volatile oxidation products formed in fish oil such as 1-penten-3-one, hexanal, 4-(Z)-heptenal, 2,4-(E,E)-heptadienal, nonanal and 2,6-(E,Z)-nonadienal are responsible for off-odors and are good indicators for the oxidation status in fish oil. EPA and DHA are the most polyunsaturated fatty acids (PUFA) in fish oil having 5 and 6 double bonds. Free radicals and peroxides are produced during the initiation phase, and in the propagation phase, some other reactive chemical products form from peroxides such as dihydroperoxides. In the termination phase, non-reactive secondary oxidation products such as hydrocarbon, aldehydes, and ketones are formed and cause off-flavor.



## Autooxidation of fish oil (Contd...)

Ahmed et al., 2016

The oxidation of fish oil is shown that is this that is the unsaturated fatty acids when it comes in the light etcetera there is a hydrogen abstraction and then followed by molecular rearrangement. Then it further takes oxygen uptake then peroxy radical is formed and these peroxy radicals then take hydrogen that is hydroperoxides are formed and then hydroperoxide further gets alkoxy radicals like polymers or dimers keto hydroxy or epoxy compound and it cleaves into aldehyde, alkyl radicals etcetera. This peroxy radical that dimers polycyclic peroxides, endoproteins, and radicals decomposes into decomposition products like aldehydes, ketones, and hydrocarbons which gives off flavor.

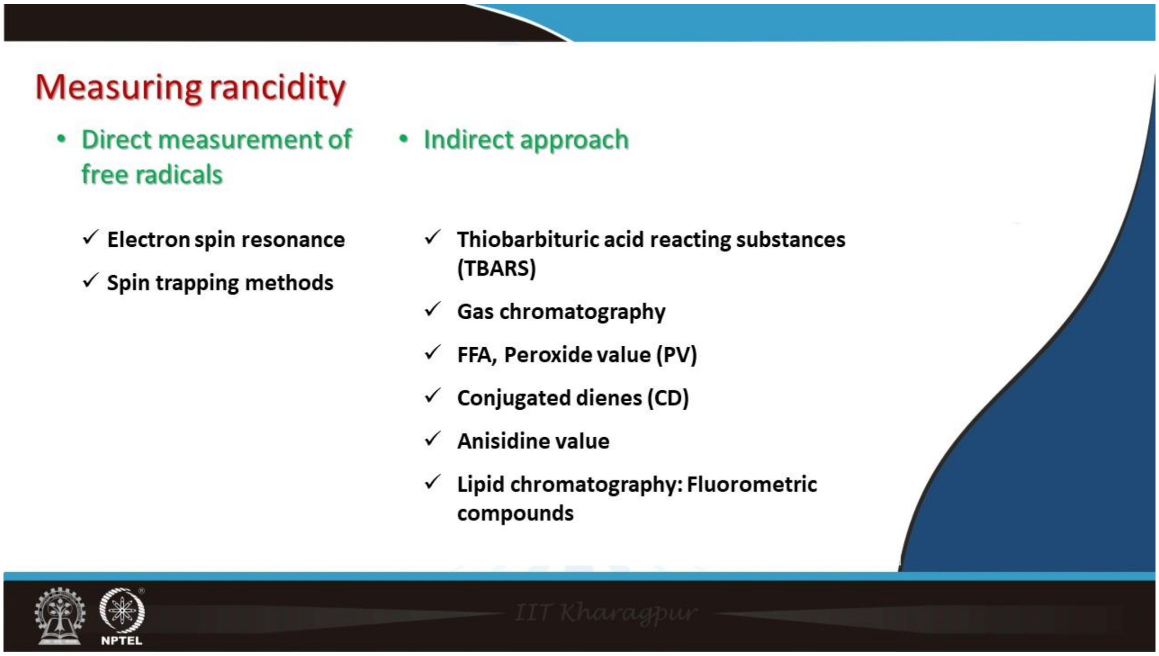


## Enzymatic oxidation

- Oxidation reaction is catalysed by enzymes in unprocessed foods or elaborated under mild conditions.
- Lipoxygenases and other enzymes catalyse oxidation of free PUFA released from glycerides by the action of lipolytic enzymes.
- First step in enzymatic oxidation is the lipolytic degradation of TG.
- Oxidation products are same hydroperoxides as produced in the autoxidation process.
- The reaction is stereospecific and regioselective.
  - Fat + Water $\xrightarrow{\text{Lipoxygenase / cytochromes P450}}$ Free fatty acids + Glycerol
  - Free fatty acids $\xrightarrow{\text{oxidation}}$ Hydroperoxides $\xrightarrow{\text{degradation}}$ Aldehyde, Ketones

Coggins et al., 2013

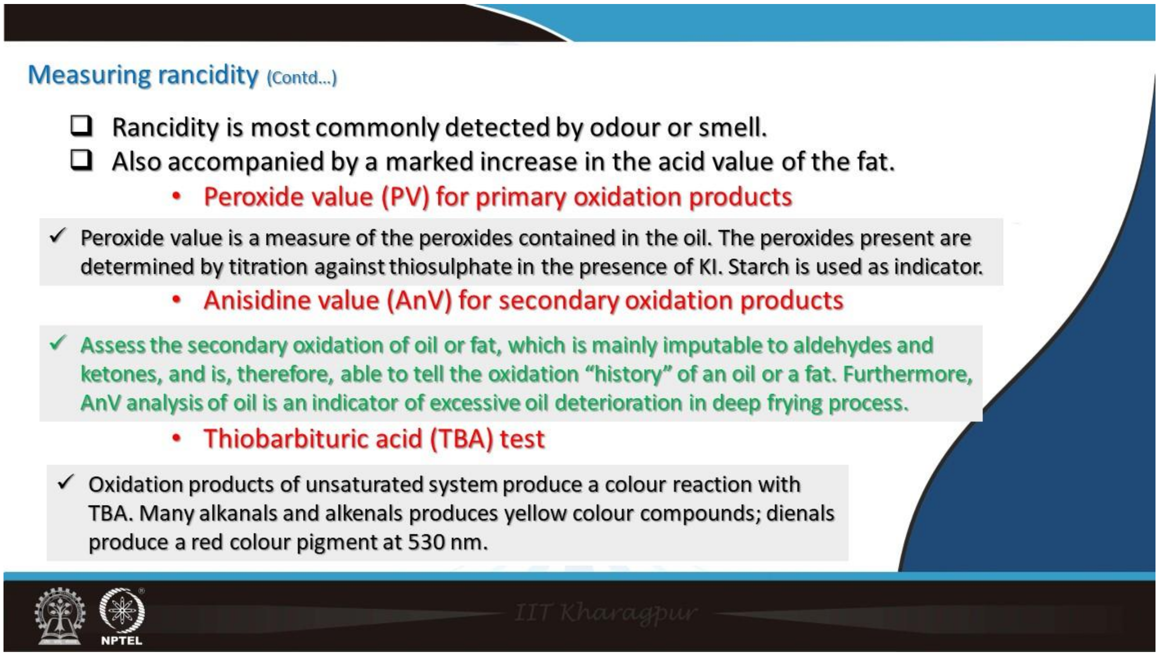
Another important oxidation is the oxidation reaction that is catalyzed by the enzyme, known as enzymatic oxidation. oxidation reaction is catalyzed by enzymes in unprocessed foods or elaborated under mild conditions. So, the lipoxygenase and other enzymes catalyze the oxidation of free polyunsaturated fatty acids released from the glycerides by the action of lipolytic enzymes like lipases, etc., and then the lipolysis causes the removal or generation of free fatty acids, and then these lipoxygenases act on the unsaturated or polyunsaturated fatty acids and auto-oxidation occurs.  So, the first step in enzymatic oxidation is the lipolytic degradation of triglycerides.  Oxidation products are the same hydroperoxides as produced in the autooxidation process. The reaction is stereospecific and regioselective. The fat and water in the presence of lipoxygenase or cytochromes P450, give free fatty acids and glycerol and these free fatty acids oxidize into hydroperoxide and then finally, they degrade into aldehyde ketones, etc.



## Measuring rancidity

- Direct measurement of free radicals
  - ✓ Electron spin resonance
  - ✓ Spin trapping methods
- Indirect approach
  - ✓ Thiobarbituric acid reacting substances (TBARS)
  - ✓ Gas chromatography
  - ✓ FFA, Peroxide value (PV)
  - ✓ Conjugated dienes (CD)
  - ✓ Anisidine value
  - ✓ Lipid chromatography: Fluorometric compounds

The developed rancidity in oil could be measured by two methods direct measurement of free radicals or indirect approach.  So, in the direct measurement of free radicals, one can use electron spin resonance or spin trapping methods, where the free radicals formed can be measured directly.  In the indirect approach, thiobarbital acid reacting substances etcetera can be used and these thiobarbital acids are formed in the oil during autooxidation or during the oxidation process, and these compounds are measured by either gas chromatography or by other methods.  Then free fatty acids peroxide value conjugated dienes, anisidine value, and lipid chromography like fluorometric compounds could be used to assess the development of rancidity in the fats or oils.



## Measuring rancidity (Contd...)

- ☐ Rancidity is most commonly detected by odour or smell.
- ☐ Also accompanied by a marked increase in the acid value of the fat.
  - Peroxide value (PV) for primary oxidation products

✓ Peroxide value is a measure of the peroxides contained in the oil. The peroxides present are determined by titration against thiosulphate in the presence of KI. Starch is used as indicator.

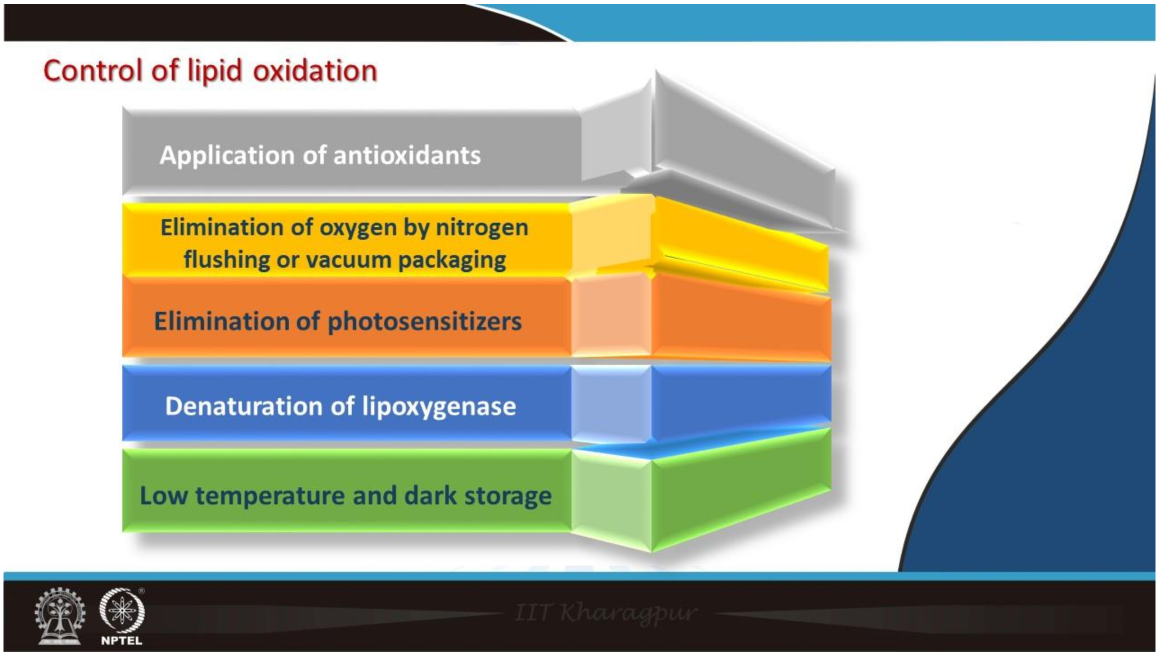
  - Anisidine value (AnV) for secondary oxidation products

✓ Assess the secondary oxidation of oil or fat, which is mainly imputable to aldehydes and ketones, and is, therefore, able to tell the oxidation “history” of an oil or a fat. Furthermore, AnV analysis of oil is an indicator of excessive oil deterioration in deep frying process.

  - Thiobarbituric acid (TBA) test

✓ Oxidation products of unsaturated system produce a colour reaction with TBA. Many alkanals and alkenals produces yellow colour compounds; dienals produce a red colour pigment at 530 nm.

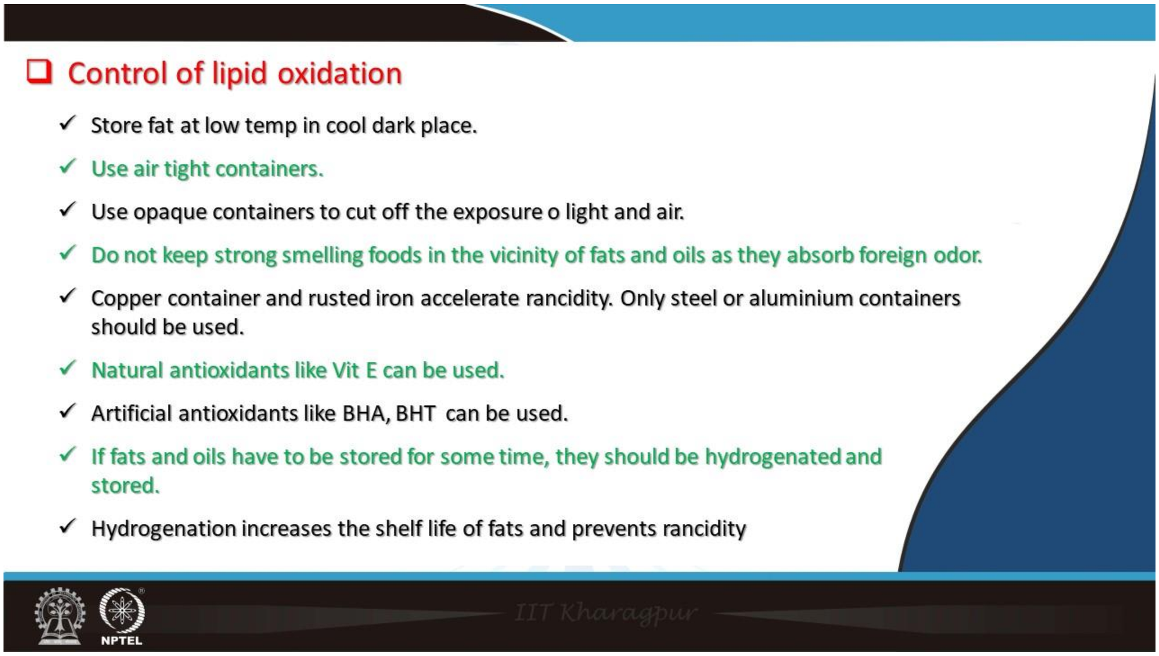
Rancidity is most commonly detected by odor or smell which is another common method. So, odor or smell one can sense the rancidity in oil and there is an electronic nose etcetera one can sense or by other methods, one can use. Also, the odor and smell are accompanied by a marked increase in the acid value of the fat, like peroxide value, anisidine value, and thiobarbituric acid test. The peroxide value is used for the primary oxidation products and as discussed in earlier classes peroxide value is a measure of the peroxides contained in the oil. The peroxide present is determined by titration against thiosulfate in the presence of potassium iodide and starch is used as an indicator. Then anisidine value is used as an indicator of the secondary oxidation product that accesses the secondary oxidation of oil or fat, which is mainly imputable to aldehydes and ketones and is therefore, able to tell the oxidation history of an oil or a fat. Furthermore, anisidine value analysis of oil is an indicator of excessive oil deterioration in the deep frying process. Then the thiobarbituric acid test is based on the reaction that oxidation products of the unsaturated system produce a color reaction with TBA. Many alkanals and alkenals produce yellow color compounds, and dienals produce a red color pigment at 530 nanometers and these indicate the development of rancidity in the oil.



## Control of lipid oxidation

- Application of antioxidants
- Elimination of oxygen by nitrogen flushing or vacuum packaging
- Elimination of photosensitizers
- Denaturation of lipoxygenase
- Low temperature and dark storage

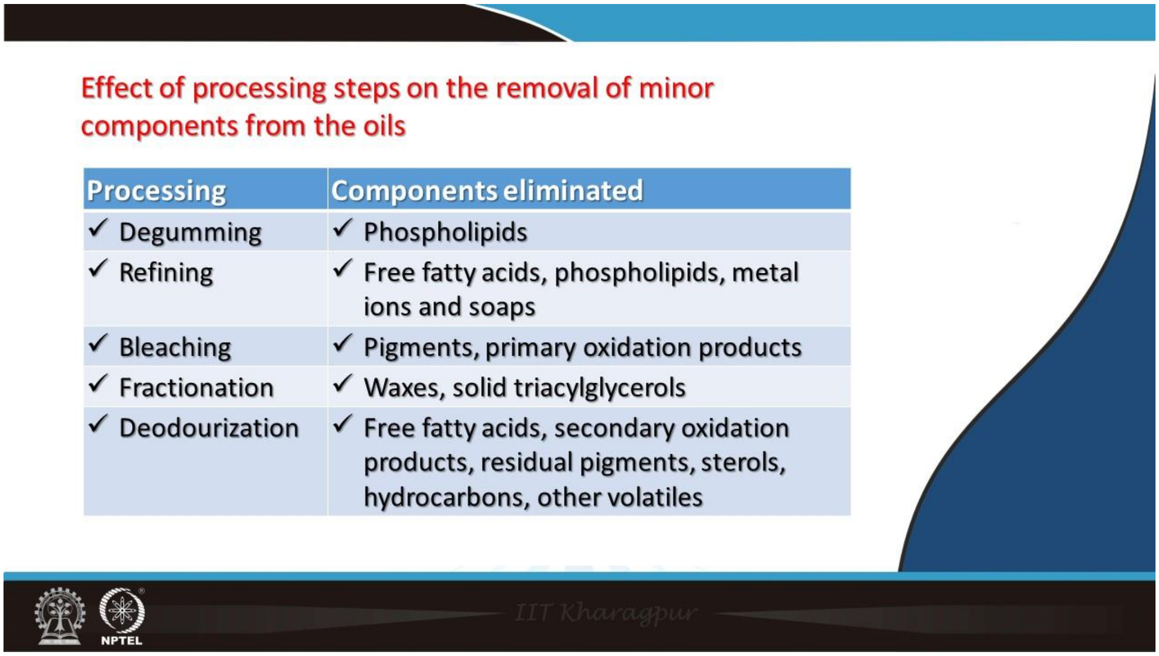
There are different ways for controlling this lipid oxidation process and they can be controlled by the addition of an antioxidant. Then eliminate oxygen by nitrogen flushing or by vacuum packaging that is we eliminate the contact of the oil with the oxygen and this can be especially related to the nitrogen packaging. Elimination of the photosensitizer like coloring pigments etcetera can be reduced by the bleaching step of the refining process or by other methods. Then denaturation of the lipoxygenase enzyme or lipase enzymes which might be there in an extraction process, thus before extraction, some sort of treatments will be given to the oil seeds. So, these enzymes which are responsible for either hydrolytic rancidity or oxidative rancidity can be eliminated.



## ❑ Control of lipid oxidation

- ✓ Store fat at low temp in cool dark place.
- ✓ Use air tight containers.
- ✓ Use opaque containers to cut off the exposure o light and air.
- ✓ Do not keep strong smelling foods in the vicinity of fats and oils as they absorb foreign odor.
- ✓ Copper container and rusted iron accelerate rancidity. Only steel or aluminium containers should be used.
- ✓ Natural antioxidants like Vit E can be used.
- ✓ Artificial antioxidants like BHA, BHT can be used.
- ✓ If fats and oils have to be stored for some time, they should be hydrogenated and stored.
- ✓ Hydrogenation increases the shelf life of fats and prevents rancidity

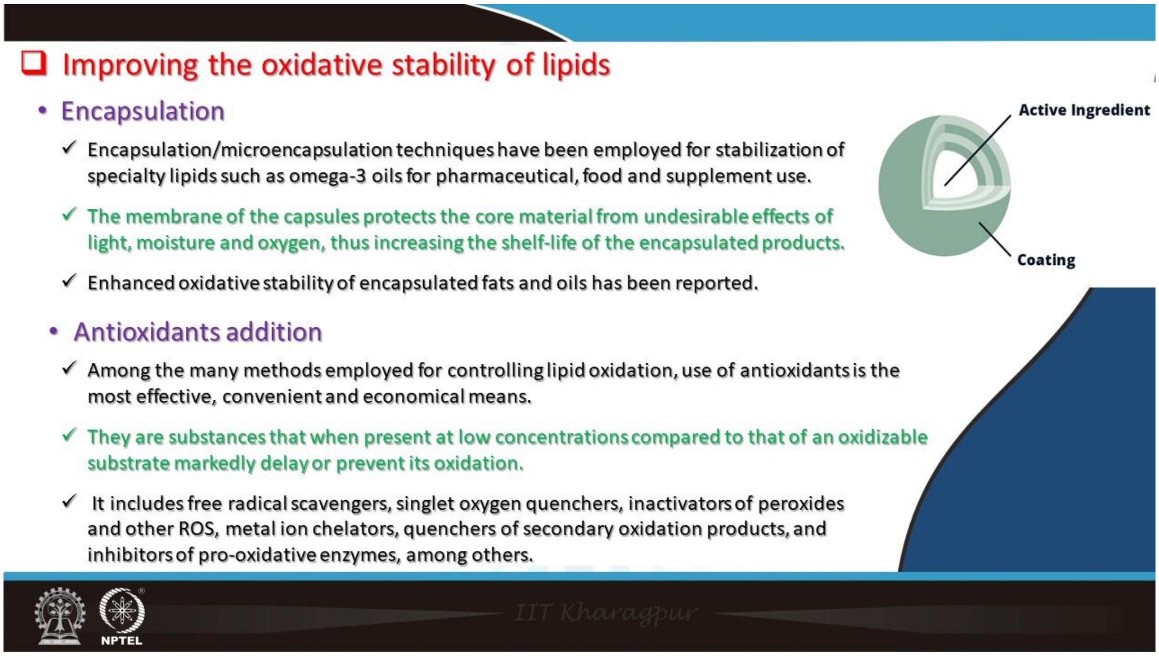
Low temperature and dark storage help the controlling lipid oxidation. For fat at low temperatures in cool dark places use airtight containers, use opaque containers to cut off the exposure to light and air. Do not keep strong-smelling foods in the vicinity of the fat and oil absorb foreign order easily. Copper containers and rusted iron accelerate rancidity. Only steel or aluminum containers should be used. Natural antioxidants like vitamin E can be used. Artificial antioxidants like BHA, BHT etcetera are also used. If fats and oils have to be stored for some time they should be better to be hydrogenated and then stored because these hydrogenated fats or solid fats are less prone to oxidative rancidity. Hydrogenation increases the shelf life of fats and prevents oxidative rancidity.



## Effect of processing steps on the removal of minor components from the oils

| Processing | Components eliminated |
|---|---|
| ✓ Degumming | ✓ Phospholipids |
| ✓ Refining | ✓ Free fatty acids, phospholipids, metal ions and soaps |
| ✓ Bleaching | ✓ Pigments, primary oxidation products |
| ✓ Fractionation | ✓ Waxes, solid triacylglycerols |
| ✓ Deodourization | ✓ Free fatty acids, secondary oxidation products, residual pigments, sterols, hydrocarbons, other volatiles |

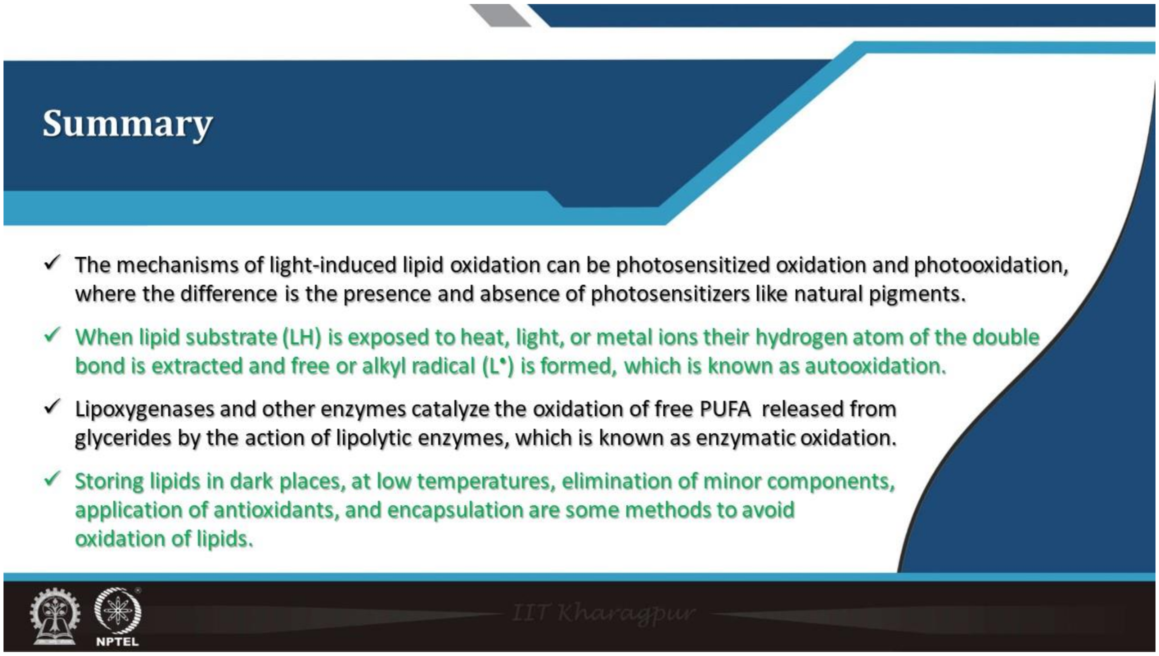
Here I have just tried to give you the effect of the processing step on the removal of minor components from the oils. Degumming removes phospholipids and in refining we remove free fatty acids phospholipids, metal ions, and soaps. In the bleaching, it removes pigment, and primary oxidation products, and in fractionation waxes, solid triacylglycerols are removed in the deodorization process that is free fatty acid, secondary oxidation products, residual pigments, sterols, hydrocarbons, and other compounds are removed and all these removals of all these compounds helps in preventing the oil from oxidation or hydrolysis and therefore, it increases the shelf life of the oil.



❑ Improving the oxidative stability of lipids

- Encapsulation
  - ✓ Encapsulation/microencapsulation techniques have been employed for stabilization of specialty lipids such as omega-3 oils for pharmaceutical, food and supplement use.
  - ✓ The membrane of the capsules protects the core material from undesirable effects of light, moisture and oxygen, thus increasing the shelf-life of the encapsulated products.
  - ✓ Enhanced oxidative stability of encapsulated fats and oils has been reported.
- Antioxidants addition
  - ✓ Among the many methods employed for controlling lipid oxidation, use of antioxidants is the most effective, convenient and economical means.
  - ✓ They are substances that when present at low concentrations compared to that of an oxidizable substrate markedly delay or prevent its oxidation.
  - ✓ It includes free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other ROS, metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes, among others.

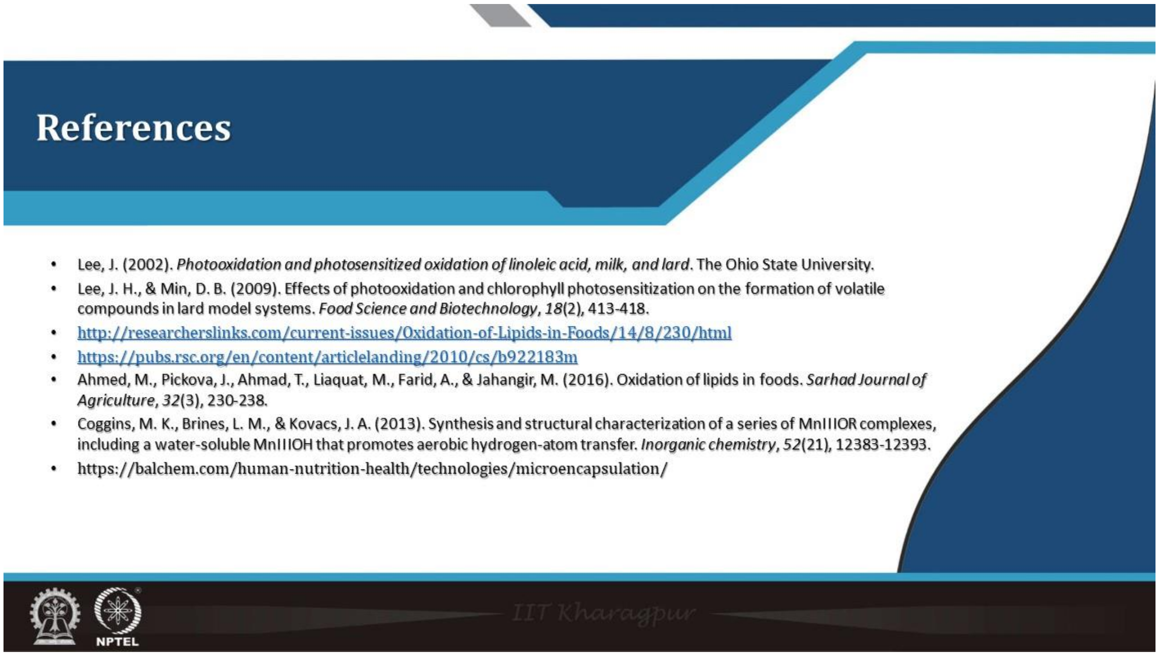
Then there are other ways to improve the oxidative stability of the oil. Number one is the encapsulation. Nowadays either encapsulation or microencapsulation techniques have been employed for the stabilization of specialty lipids such as omega-3 oils for pharmaceutical applications, food, supplements, and other uses. Here a membrane is created and the active ingredient like oil is encapsulated under a membrane. This membrane of the capsule protects the core material from undesirable effects of light, moisture, and oxygen, and thus increases the shelf life of the encapsulated products. Enhanced oxidative stability of the encapsulated fats and oil has been reported. Antioxidant addition is one of the main methods employed for controlling lipid oxidation. The use of antioxidants is more effective and it is more commonly used in fact, whatever edible oil etcetera which we get in the market most of them antioxidants are added. This antioxidant addition is reported to be a convenient and economical means for preventing the oxidation of oil. These antioxidants are substances that when present at low concentrations compared to that of an oxidizable substrate markedly delay or prevent its oxidation. It includes free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other radical oxygen scavengers, metal ion chelators, quenchers of secondary oxidation products, and inhibitors of prooxidative enzymes among others. The antioxidants commonly which are used are BHA, BHT, and propyl gallate these are the tertiary with TBHQ these are the synthetic antioxidants whereas, the vitamin E tocopherol etcetera are natural antioxidants.



## Summary

- ✓ The mechanisms of light-induced lipid oxidation can be photosensitized oxidation and photooxidation, where the difference is the presence and absence of photosensitizers like natural pigments.
- ✓ When lipid substrate (LH) is exposed to heat, light, or metal ions their hydrogen atom of the double bond is extracted and free or alkyl radical (L•) is formed, which is known as autooxidation.
- ✓ Lipoxygenases and other enzymes catalyze the oxidation of free PUFA released from glycerides by the action of lipolytic enzymes, which is known as enzymatic oxidation.
- ✓ Storing lipids in dark places, at low temperatures, elimination of minor components, application of antioxidants, and encapsulation are some methods to avoid oxidation of lipids.

So, finally, I would like to summarize this lecture. The mechanisms of light-induced lipid oxidation can be photosensitized oxidation and photooxidation, where the difference is the presence and absence of photosensitizers like natural pigments. When lipid substrate (LH) is exposed to heat, light, or metal ions the hydrogen atom of the double bond is extracted and free or alkyl radical (L•) is formed, which is known as autooxidation. Lipoxygenases and other enzymes catalyze the oxidation of free PUFA released from glycerides by the action of lipolytic enzymes, which is known as enzymatic oxidation. Storing lipids in dark places, at low temperatures, elimination of minor components, application of antioxidants, and encapsulation are some methods to avoid oxidation of lipids.



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These are the references that are used in this lecture. Thank you very much for your patience here. Thank you.