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Lipid Peroxidation

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The aged seeds and viability of seeds are affected by many factors during seed production and during storage. The rate of ageing of seeds depends on the ability of the seed to resist the degradative changes and the defence mechanism that differs in species. The degree of seed damage is influenced by duration of storage or ageing period, storage condition, type of storage, species character, chemical composition of oilseed. Auto-oxidation of lipids and increase the polyunsaturated fatty acids during storage accelerates the damage of seeds. The frequent vigour and viability tests to be done to the stored seeds under unknown or adverse conditions of storage. As we know that vigour of seeds declines initially than viability, which can be known as a measure of seed accumulated damage as viability declines. It's a fact that seed is a living organism where the biological processes will occur and damages the seed and decreases its quality, especially in oilseeds.

Introduction

The seeds are stored for using it adverse weather conditions. As the seed has life, all the metabolic activities will occur. The speed of seed aging process depends on the ability of the seed to resist degradation changes and defence mechanisms, which are species-specific (Gupta and Aneja, 2004; Sisman and Delibas, 2004). In seeds during storage, a physiological and physicochemical changes will occur and is termed as aging (Silva *et al*., 2005; Sisman, 2005). The storage conditions of seeds depends on the specific species it loses a degree of greater or lesser the viability (Mohammadi *et al*., 2011). So, seed storage has a marked effect on seed viability. As a solution to this it should in the field to harvest. A viable seed germinates under both favourable and stress conditions.

Harvested seed quality is maintained by the storage and its conditions. The main external factors causing seed damage during storage are the temperature, relative air humidity and oxygen. Possibility to regulate these factors makes the basis for longer seed storage. Seed with low viability dies first.

Seed rich in lipids has limited longevity due to its specific chemical composition. During storage of oily species declining trend of total oil content and seed germination can be observed. A fatty acid composition is the most important factor which determines oils susceptibility to oxidation (Morello et al., 2004). Quality parameters of seed such as oil content, fatty acid composition and protein content are significantly influenced by storage conditions and time (Ghasemnezhad and Honermeier 2007). For example, sunflower seed storage demands special care due to high oil content which can easily provoke processes that can lead to loss of germination and viability.

Lipid Peroxidation

Lipid peroxidation is a free-radical chain process leading to the deterioration of polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogen atoms.

- Most frequently cited cause of seed deterioration
- Free radicals cause profound membrane damage and loss of membrane integrity.

Generation of free radicals enzymatically or by autoxidation

- Singlet oxygen (O_2)
- Superoxide anion (O_2^-)
- Hydrogen peroxide (H_2O_2)
- Hydroxl radicals (OH^-)
  - ✓ Free radicals are suspected of attacking chromosomal DNA.
  - ✓ Potential targets are purine and pyrimidine bases.
  - ✓ Modifications in the strand lead to increased propensity to genetic mutations.
  - ✓ Occurs in all seeds, but more in oil seeds.

Relation of moisture content of seed and auto oxidation and degradation

Below 6%	Auto Oxidation is primary cause of seed deterioration
Between 6-14%	Lipid Peroxidation is minimum Because sufficient water is available act as a buffer against autoxidatively
Above 14%	Lipid Peroxidation stimulated by the activity of hydrolytic oxidative enzymes such generated free radical attack as lipoxygenase

Lipid peroxidation occurs by

1. Auto-oxidation : Auto Oxidation by free radical reaction. Autoxidation may be primary cause of seed deterioration at moisture content below 6%.
2. Enzyme action Above 14 % moisture content, lipid peroxidation may again be stimulated by the activity of hydrolytic oxidative enzymes such as lipoxygenase

Lipid peroxidation initiates a chain of events that leads to loss of membrane integrity, changes protein structure and chromosomal DNA modifications

Definition:

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. As with any radical reaction, the reaction consists of three major steps:

Initiation, propagation, and termination. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs)..

1. **Initiation** is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH· and HOO·, which combines with a hydrogen atom to make water and a fatty acid radical.
2. **Propagation:** The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way.
3. **Termination:** When a radical reacts with a non-radical, it always produces another radical, which is why the process is called a "chain reaction mechanism". The radical reaction stops when two radicals react and produce a nonradical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision

of two radicals. Living organisms have different molecules that speed up termination by neutralizing free radicals and, therefore, protecting the cell membrane. One important antioxidant is vitamin E. Another important antioxidant is vitamin C. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

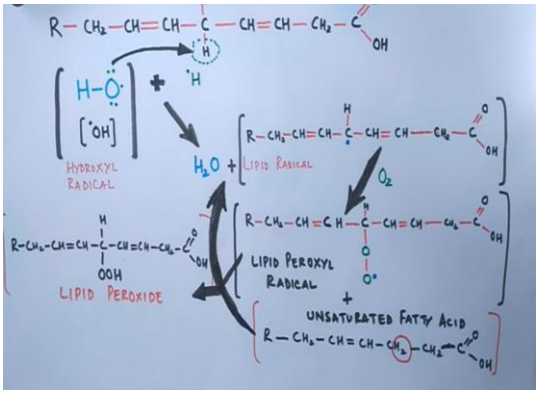


Figure 1 Steps involved in Lipid Peroxidation

The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4- hydroxynonenal (HNE), the second one being known also as "second messenger of free radicals" and major bioactive marker of lipid peroxidation, due to its numerous biological activities resembling activities of reactive oxygen species.

Enzyme Action : Lipoxygenase

Lipoxygenases (LOXs)

- Non-heme iron containing dioxygenases. Widely distributed in plants and animals.
- Catalyze polyunsaturated fatty acid to yield unsaturated fatty acid hydro-peroxides.

Initiates the synthesis of a group of acyclic or cyclic compounds called oxylipins-products of fatty acid oxidation with diverse functions in the plant cells.

LOX activity in membranes can be reduced by

- Reducing the quantity of polyunsaturated fatty acids in seeds through breeding
- Short time heat treatments (Peanut- 79 ^{0}C/ 90s; 60 ^{0}C/ 10min)
- Inhibitors of LOX [U28938 (300µg/mg protein), Nordihydroguaiaretic acid (500 µM), 4-Nitrocatechol (600 µM) and Eicosa-5,8,11,14-tetraynoic acid (200 µM)]
- Temperature of storage
- Substrate concentration and pH.

Damage of mitochondrial membrane due to lipid peroxidation leads

- To reduces respiration capacity.
- Reduced ATP production leading to reduced energy availability for the breakdown of food reserves.
- Reduce the supply of ATP to the growing points during germination.

How to measure lipid peroxidation?

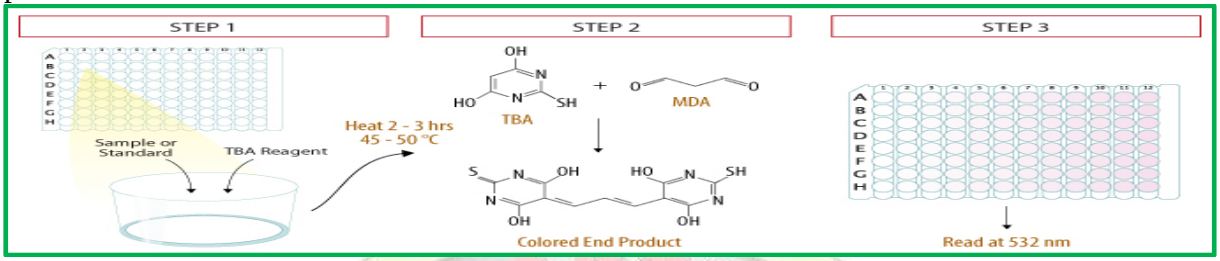
1. Direct detection of free radicals: **Electron spin resonance (ESR)**
2. Detection of primary and secondary products of free radical damage: Estimation of Hydroperoxides, Conjugated dienes, Malondialdehyde, Hexaldehyde *etc*……
3. Loss of substrates: There is preferrential deletion of PUFA(Linoleic and Linolenic acids)
4. Monitoring changes in antioxidant levels: Tocopherols, Ascorbate, Glutathione
5. Effect of antioxidant treatments.

Methods of estimating Lipid peroxidation

Component	Method
4-hydroxynonel	HPLC
Isoprostanes	HPLC, ELISA
Exhaled gases	GC
Lipid DNA adducts	Fluroscence

TBARS Assay

2-ThioBarbituric Acid Reactive Substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress. TBARS assay values are usually reported in malonaldehyde (malondialdehyde, MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides. The TBARS assay is a well-recognized, established method for quantifying these lipid peroxides, although it has been criticized for its reactivity towards other compounds other than MDA. This kit offers the researcher a straightforward, reproducible and consistent method for analyzing urine for lipid peroxidation products.



Lipid hydroperoxides

Iodometric method

Iodometric method for lipid hydroperoxides determination is one of the oldest methods and is still used to determine lipid peroxide number. The method can be applied to extracts of biological samples without present the oxidizing agents. The possible interfering factors are especially the presence of oxygen, hydrogen peroxide and protein peroxides, which are able to oxidize iodide.

Principle of this method is based on the ability of lipid hydroperoxides to oxidize iodide (I-) to iodine (I2), which further reacts with unreacted iodide (I-) to triiodide anion (I3-) and can be determined spectrophotometrically at 290 or 360 nm

Modification of the iodometric method using commercially available reagent used for the determination of lipid (hydro)peroxides spectrophotometrically at 365 nm

Ferrous oxidation in xylenol orange

Lipid hydroperoxides (LHPO) were determined spectrophotometrically based on their reaction with an excess of Fe^{2+} at low pH in the presence of the dye xylenol orange. Triphenylphosphine-mediated hydroxide formation was used to authenticate the signal generated by the hydroperoxides.

(1) $Fe^{2+} + LOOH + H^{+} \rightarrow Fe^{3+} + H_2O + LO^{\bullet}$

(2) $LO^{\bullet} +$ xylenol orange $+ H^{+} \rightarrow LOH +$ xylenol orange$^{\bullet}$

(3) Xylenol orange$^{\bullet} + Fe^{2+} \rightarrow$ xylenol orange $+ Fe^{3+}$

(4) $LO^{\bullet} + Fe^{2+} + H^{+} \rightarrow Fe^{3+} + LOH$

(5) $Fe^{3+} +$ xylenol orange $\rightarrow$ blue – violet complex (560 nm)

Minimization of lipid peroxidation

- Lipid modification – breeders can modify the Poly unsaturated to the Mono unsaturated or saturated fatty acids by modifying the gene combinations
- Regulation of oxygen pressure
- Antioxidant treatments
- Hydration/dehydration treatments

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