# Estimation of Total phenolic content in different plants
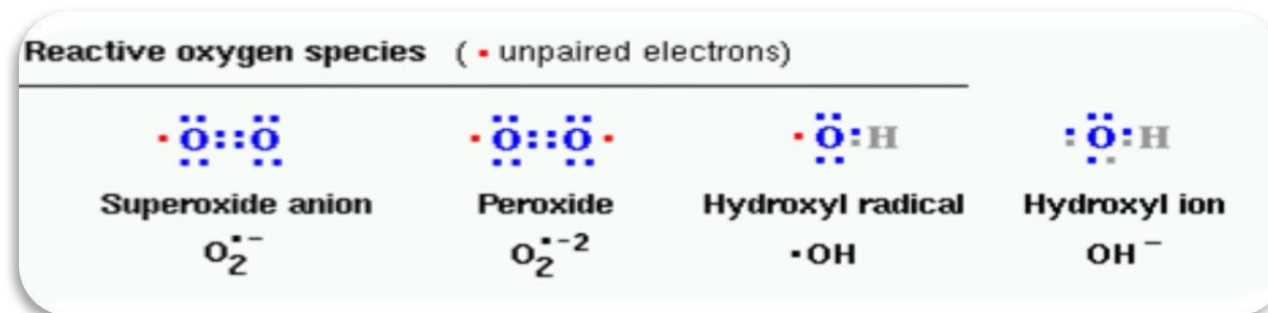
# Free radicals:

- Free radicals are those particles and molecules that cause damage to the body's cells and essential fatty acids by their ready reactivity and oxidizing ability.
- This characteristic is defined by their unpaired electron.

Reactive oxygen species ( • unpaired electrons)

| Superoxide anion | Peroxide | Hydroxyl radical | Hydroxyl ion |
|---|---|---|---|
| $O_2^{•-}$ | $O_2^{•-2}$ | •OH | OH⁻ |

- These free radical molecules are released during the normal metabolic process of oxidation.

# Free radicals:

- Free radicals come from a wide variety of sources but <u>mainly our diet.</u>

- The biggest source of ingested free radicals is probably fried foods and heated cooking oils, e.g. potato crisps/chips, french fries, onion rings etc. (fried in vegetable oils which oxidises readily into free radicals).

# Oxidative stress:

- Oxidative stress is an **imbalanced state** where excessive quantities of reactive oxygen and/or nitrogen species **over come** <u>endogenous antioxidant capacity</u>, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids.
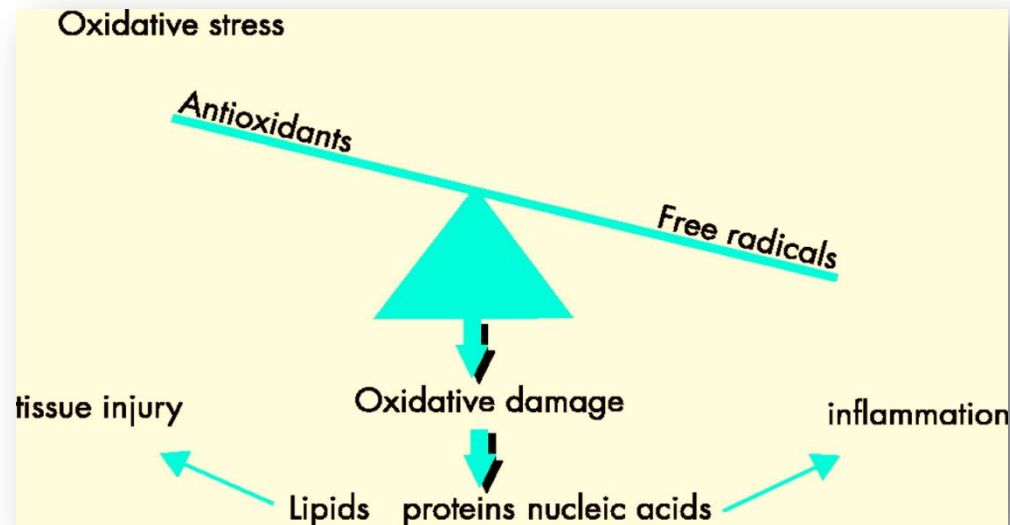
- Oxidative stress involve in the development of chronic degenerative diseases including coronary heart disease, cancer and aging.
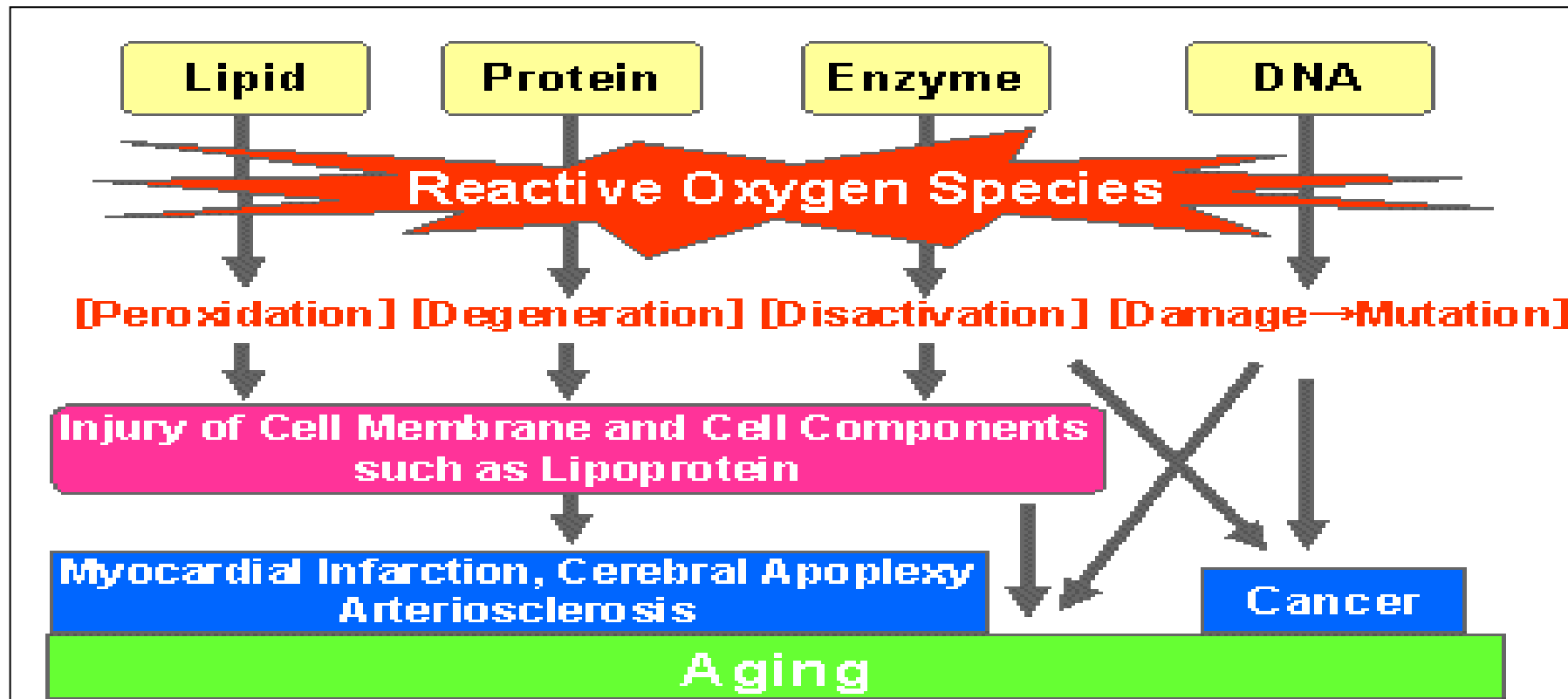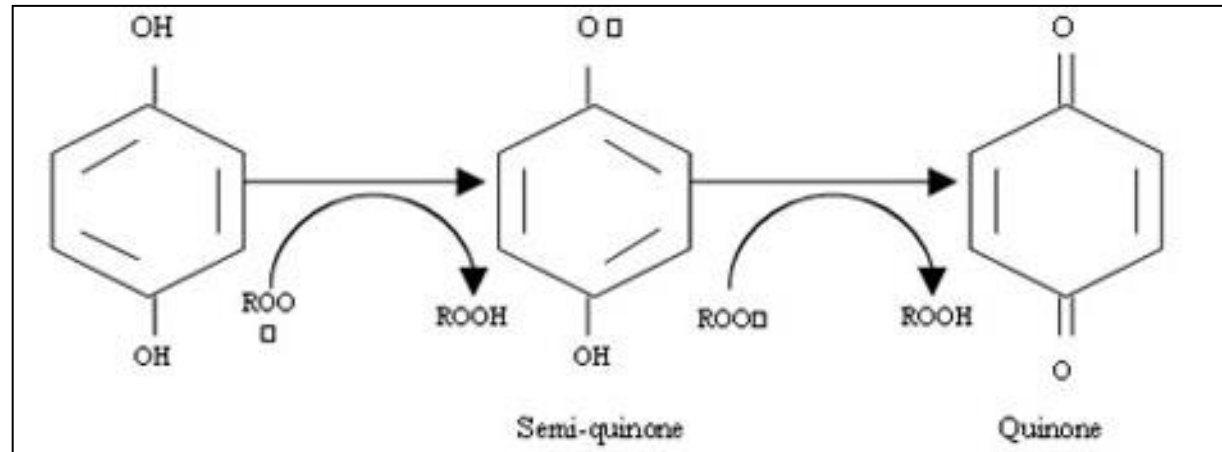
# Antioxidants:

- **Antioxidants are defined as** compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress.

- Fruits and vegetables contain a wide variety of free-radical scavenging molecules, including phenolic compounds, carotenoids, and vitamins.

# Phenolic compounds:

- Phenolics are compounds possessing <u>one or more aromatic rings with one or more hydroxyl groups.</u>

- Plant phenolic compounds are extremely heterogeneous and may range from simple monomers to very large polymers.

- Studies have shown that consumption of food rich in phenolics can <u>slow the progression of various debilitating diseases.</u>

- Therefore, mostly, the current focus is on the **anti-oxidant action of phenolics**.

*7*

- The antioxidant activity of phenol is mainly related to redox properties.



- **Tea** remains one of the most popular beverages world-wide and contains a variety of phenolic compounds which are potent antioxidants.

# Practical Part

# Objective:

- Determination of total phenolic content in green tea and black tea.

# Principle:

- In this method, we will use a colorimetric method, the Folin-Ciocalteu assay, to quantify the total phenolic content of the samples.

- The oxidation of a phenolate ion from the sample and the reduction of the phosphotungstic-phosphomolybdic reagent which known as Folin-Ciocalteu, the result of this reduction produce a blue complex that absorb light at 650nm.
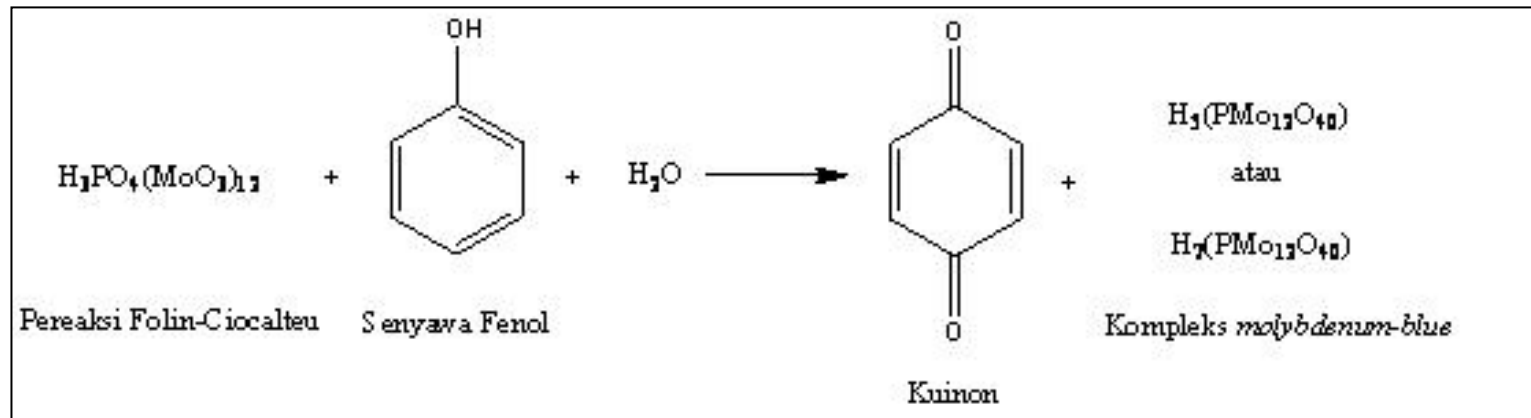
# Principle cont':

- The reaction must take place **under alkaline conditions** in order to <u>aid with the uptake of oxygen by the phenol</u> , which occurs most efficiently near the pka (approximately 10) of the phenol, and this is done by the addition of sodium carbonate.

# Method:

| Tube | Catechol standard 5mg/100ml | Sample (ml) | Dist. H2O (ml) | Folin-Ciocalteu reagent (ml) | | Na2CO3 (ml) |
|---|---|---|---|---|---|---|
| 1 | 0.2 | -- | 3.8 | | | |
| 2 | 0.4 | -- | 3.6 | | | |
| 3 | 0.6 | -- | 3.4 | | | |
| 4 | 0.8 | -- | 3.2 | | | |
| 5 | 1 | -- | 3 | 0.5 ml | Wait 3 min | 2 ml |
| 6 | 1.2 | -- | 2.8 | | | |
| 7 | 1.4 | -- | 2.6 | | | |
| Black tea | -- | 0.1 | 3.8 | | | |
| Green tea | -- | 0.1 | 3.8 | | | |
| Coffee | -- | 0.1 | 3.8 | | | |
| Mint | -- | 0.1 | 3.8 | | | |

# Method:

- Mix thoroughly and measure the absorbance at 650 nm against a reagent blank.

- Prepare a standard curve using different concentrations of catechol.

# Results:

| Tube | Absorbance | Concentration (mg/dl) |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| **Black tea** | | |
| **Green tea** | | |
| **Coffee** | | |
| **Mint** | | |

# Calculations:

- The concentration from the standard curve x dilution factor =……A….

- A x 1 dl = ……B…..

- B ➔ 2 grams
- ? ➔ 100 grams

- **Phenol content=**………..mg/100 g