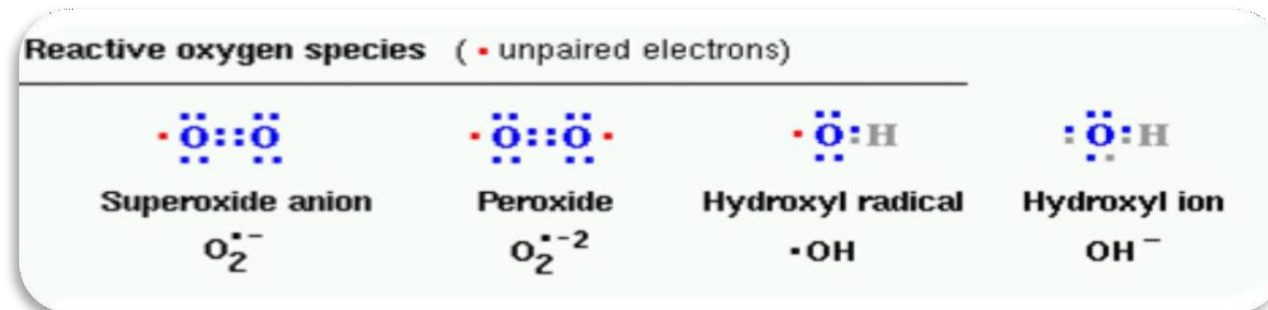


Estimation of Total phenolic content in different plants

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**.



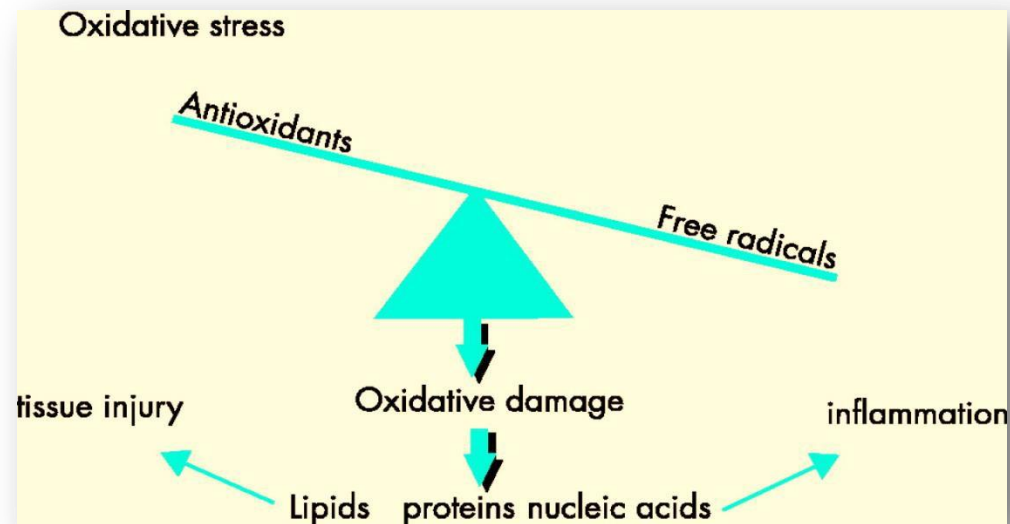
- These free radical molecules are released during the **normal** metabolic process of oxidation.

Free radicals:

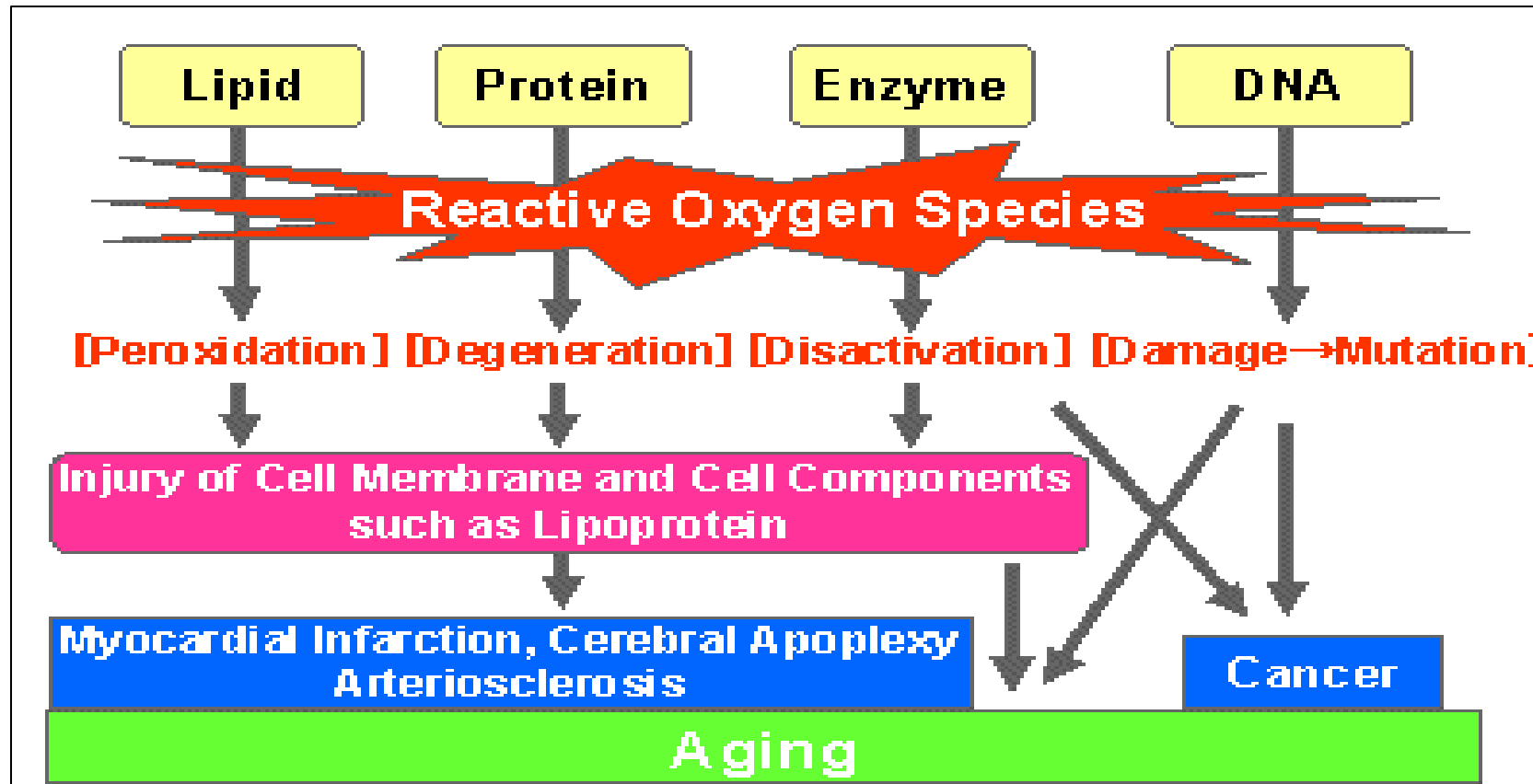
- Free radicals come from a wide variety of sources but mainly our diet.
- The **biggest source of ingested free radicals** is probably fried foods and heated cooking oils, e.g. potato crisps/chips, french fries, onion rings etc. (fried in vegetable oils which oxidises readily into free radicals).

Oxidative stress:

- Oxidative stress is an **imbalanced state** where excessive quantities of reactive oxygen and/or nitrogen species **over come** endogenous antioxidant capacity, 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.



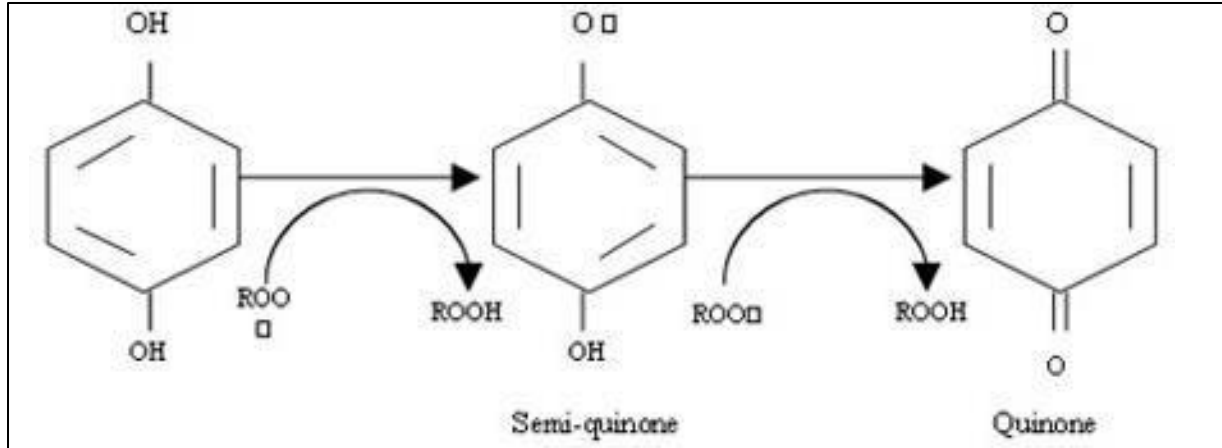
Antioxidants:

- **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 one or more aromatic rings with one or more hydroxyl groups.
 - **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 slow the progression of various debilitating diseases.
-
- Therefore, mostly, the current focus is on the **anti-oxidant action of phenolics**.

- 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

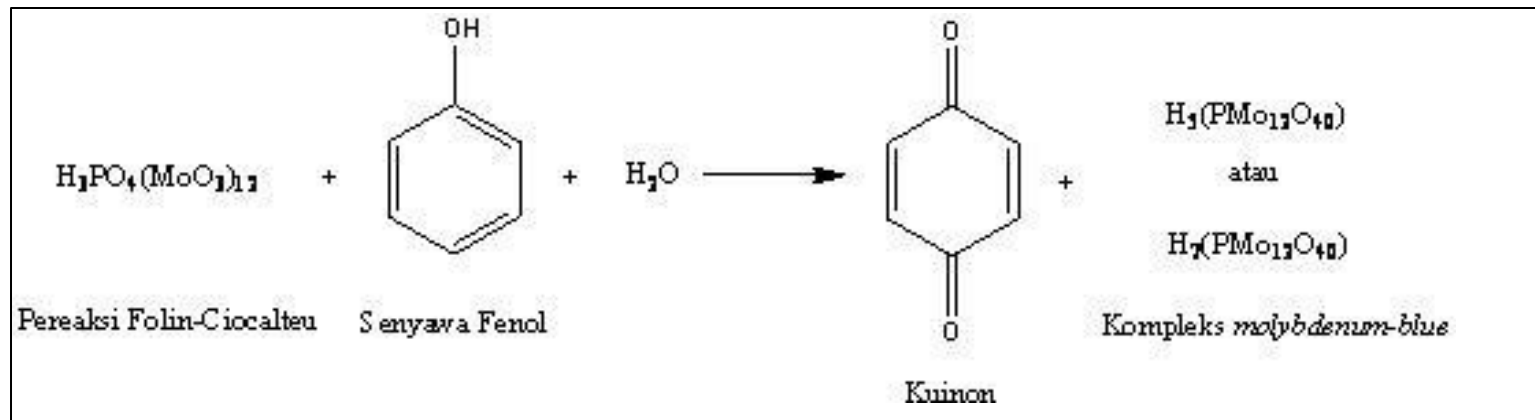
Objective:

- 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 result of in the formation of a **blue complex** that absorb light at 650nm.



Principle cont':

- The reaction must take place **under alkaline conditions** in order to aid with the uptake of oxygen by the phenol, 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)		Na ₂ CO ₃ (ml)
1	0.2	--	3.8	0.5 ml	Wait 3 min	2 ml
2	0.4	--	3.6			
3	0.6	--	3.4			
4	0.8	--	3.2			
5	1	--	3			
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