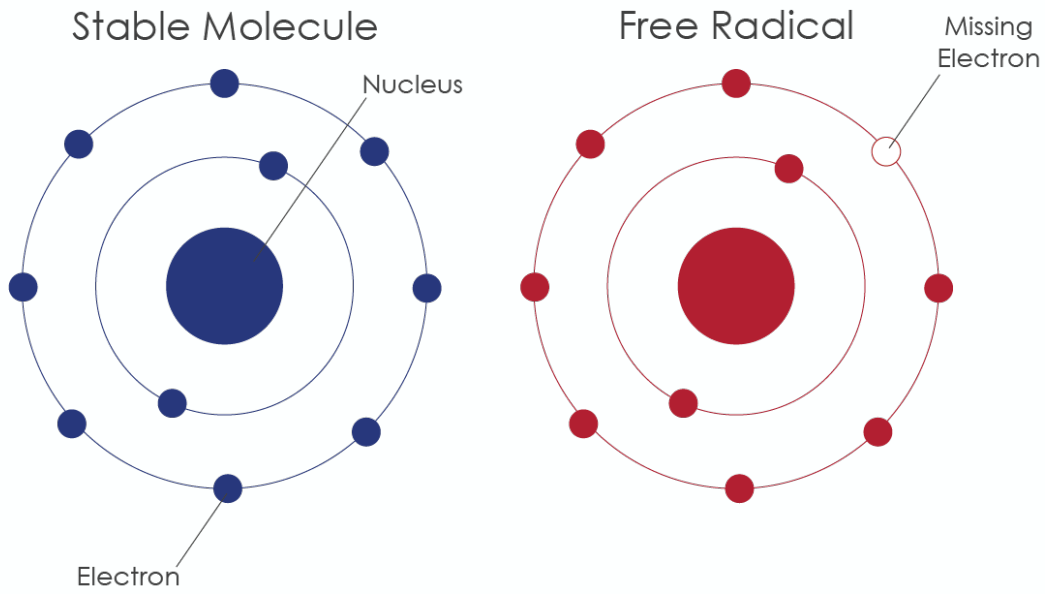
BCH 445- Biochemistry of Nutrition [Practical]

Estimation of Total phenolic content in different plants

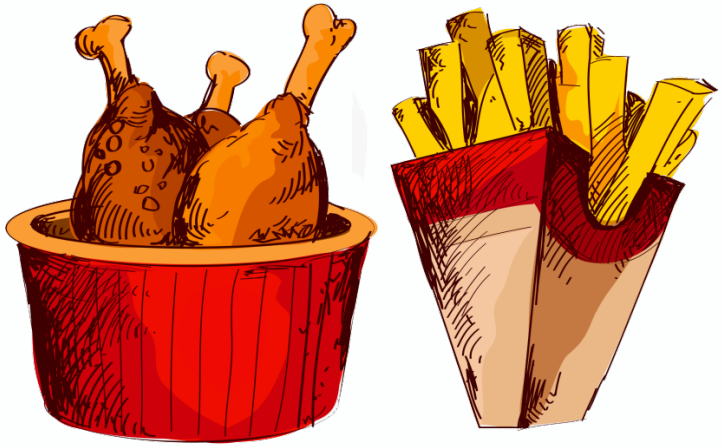
Free radicals

- **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 in an outer orbit.
- Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants.
- These free radical molecules are released during the normal metabolic process of oxidation.



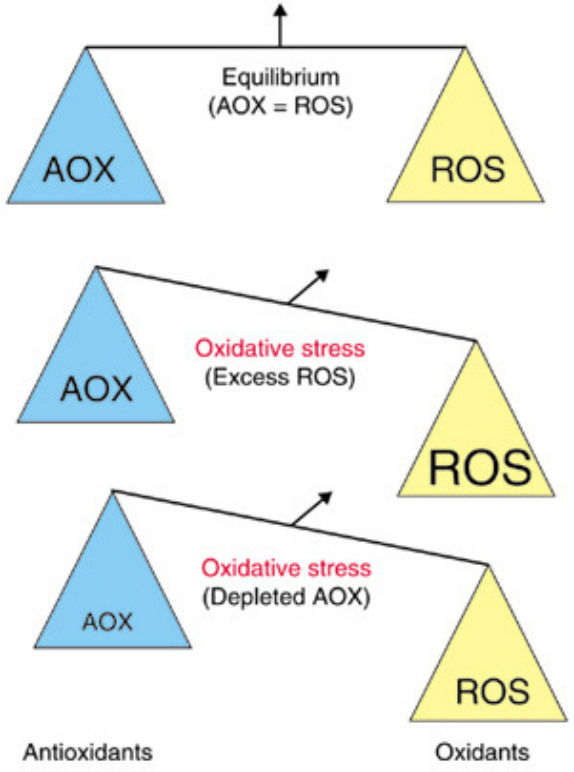
Sources of free radicals

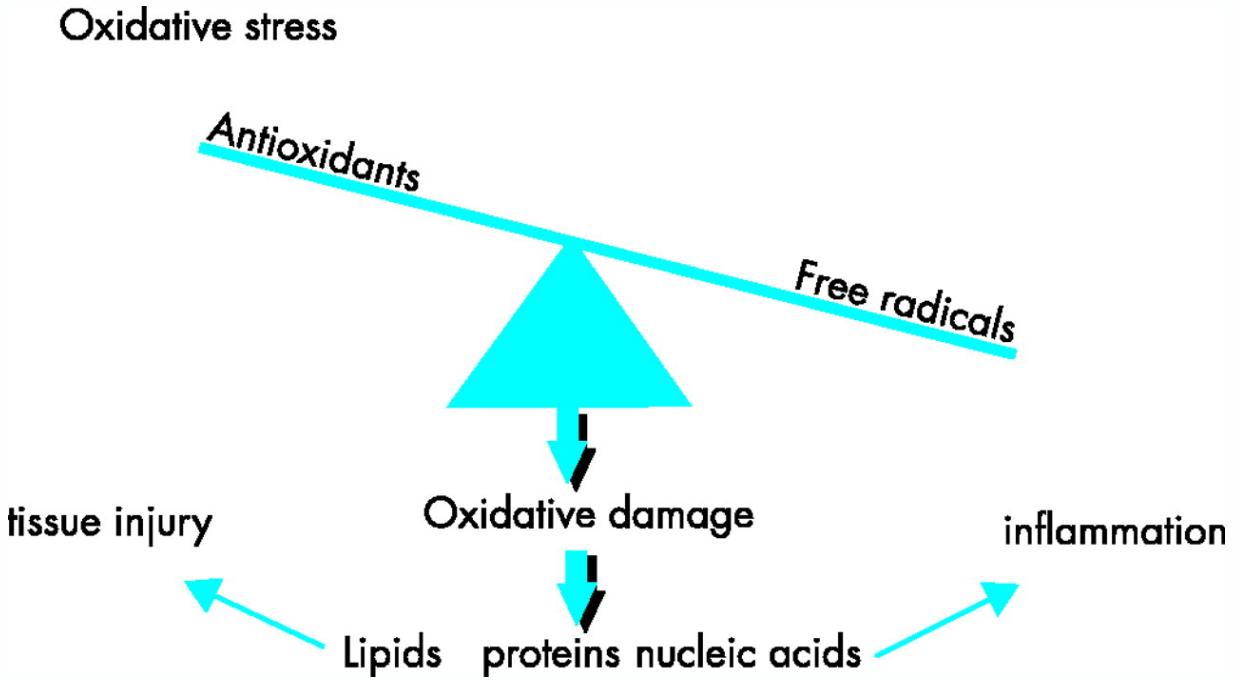
- Free radicals come from a wide variety of sources but mainly our diet.
- The biggest source of ingested free radicals is probably fried foods and heated cooking oils, e.g. potato crisps/chips, French fries, onion rings etc. (fried in vegetable oils which oxidizes readily into free radicals).



Oxidative stress

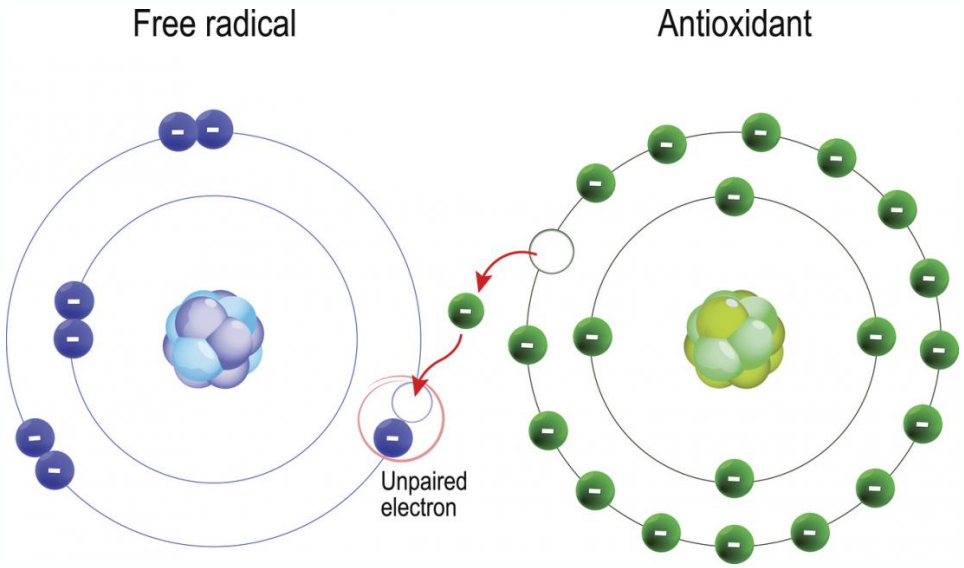
- **Oxidative stress** is an imbalanced state when excessive amounts of free radicals are produced or antioxidant capacity is decreased, leading to oxidation of a varieties of biomacromolecules, such as **enzymes, proteins, DNA and lipids**.
- Oxidative stress involve in the development of chronic diseases including coronary heart disease, cancer, Alzheimer's and aging.





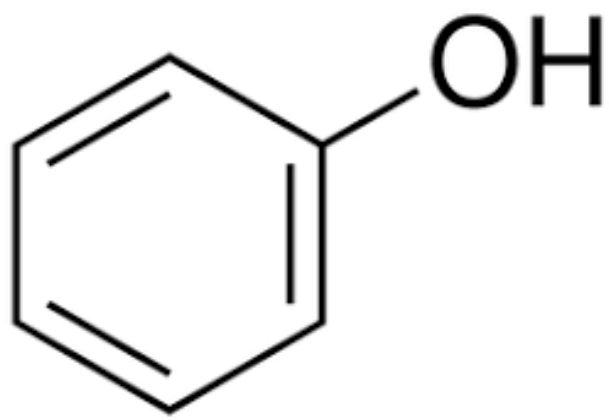
Antioxidants

- **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 A,C and E.



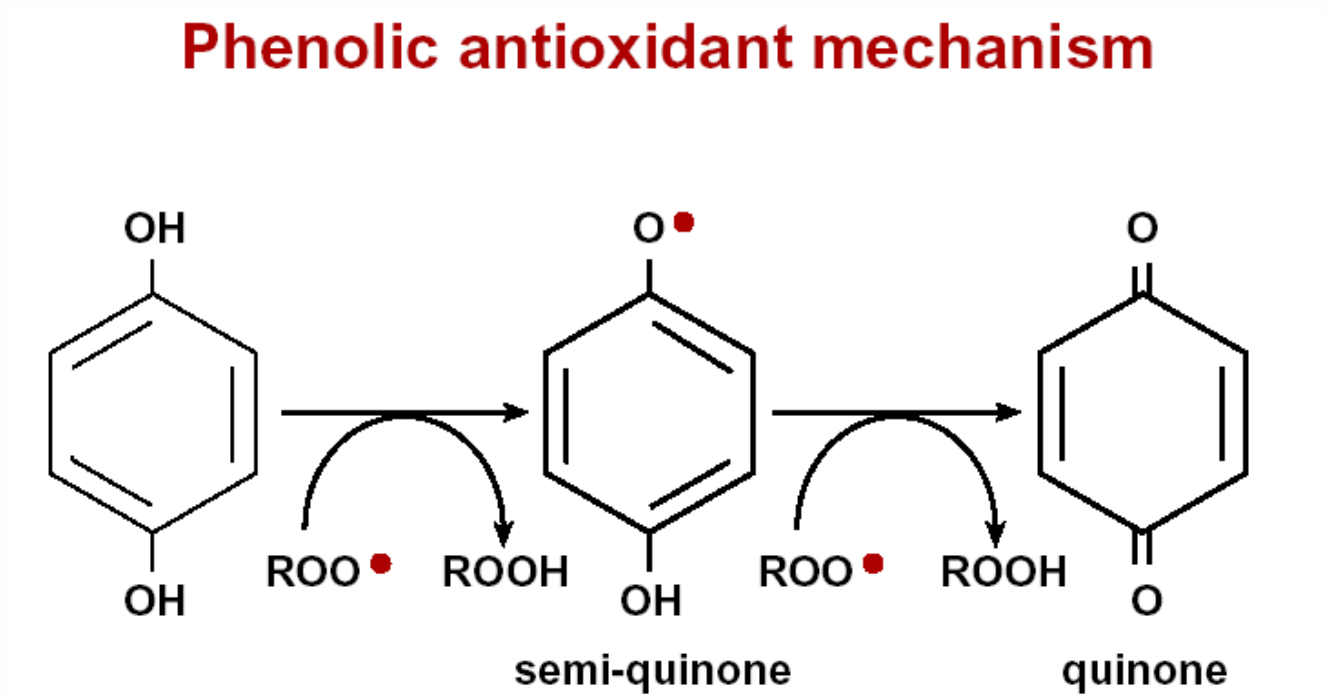
Phenolic compounds

- 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 phenolic molecules to highly polymerized compounds
- Studies have shown that consumption of food rich in phenolics can slow the progression of various debilitating diseases.
- Polyphenols include flavonoids, phenolic acids and tannins.



Phenolic compounds

- 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.



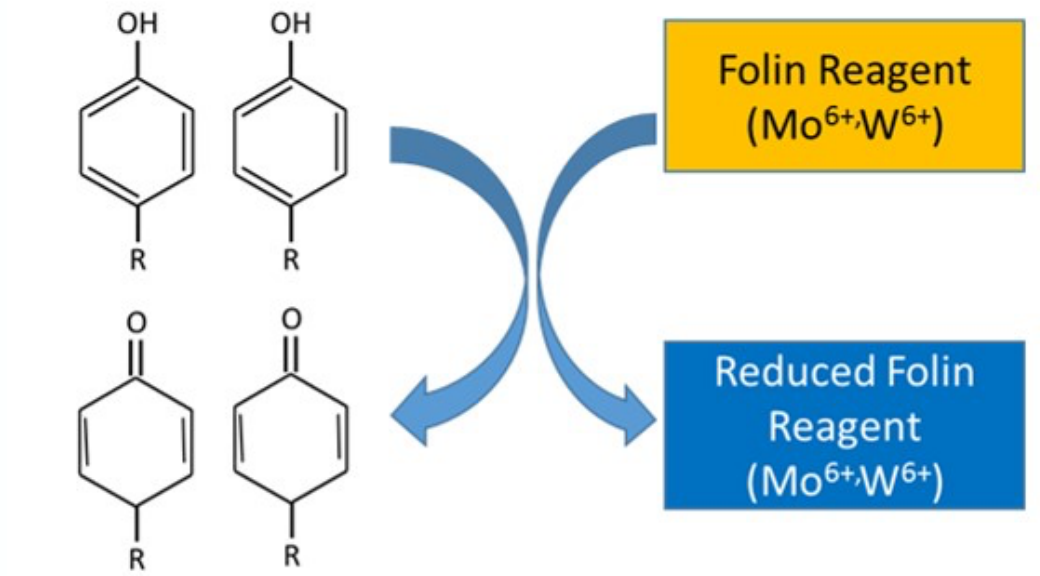
Practical Part

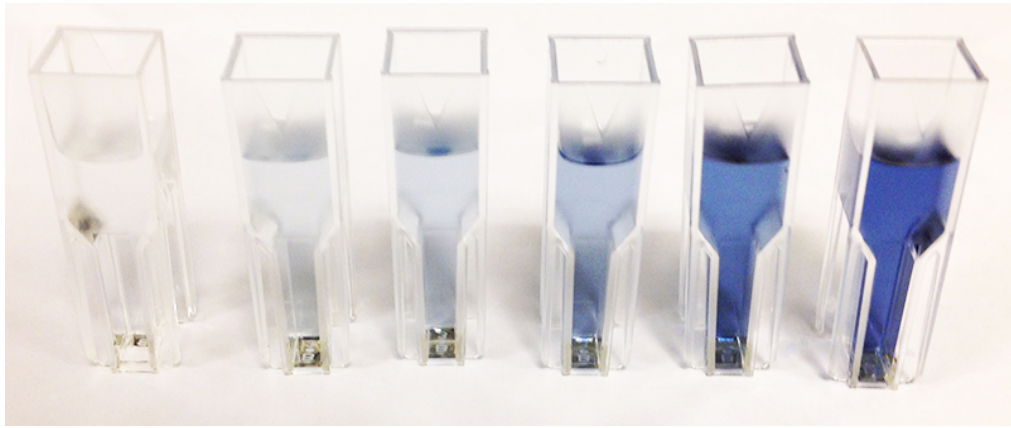
Objective:

- 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 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

Tubes	Catechol standard 5mg/100ml	Sample	Dist. H_2O (ml)	Folin-Ciocalteu reagent (ml)		Na_2CO_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	--	2			
6	1.2	--	2.8			
7	1.4	--	2.6			
Black tea	--	0.1	3.9			
Green tea	--	0.1	3.9			

- Mix thoroughly and measure the absorbance at 650 nm against a reagent blank.
- Prepare a standard curve using different concentrations of catechol

Results

Tubes	Absorbance at 650 nm	Concentration mg/dl
1		
2		
3		
4		
5		
6		
7		
Black tea		
Green tea		

Calculations

- **The result you got from the curve x dilution factor= A**

A X 1 dl ➔ …**B**…

B ➔ 2 grams

? ➔ 100 grams

- **Phenol content=.....mg/100 g**