

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

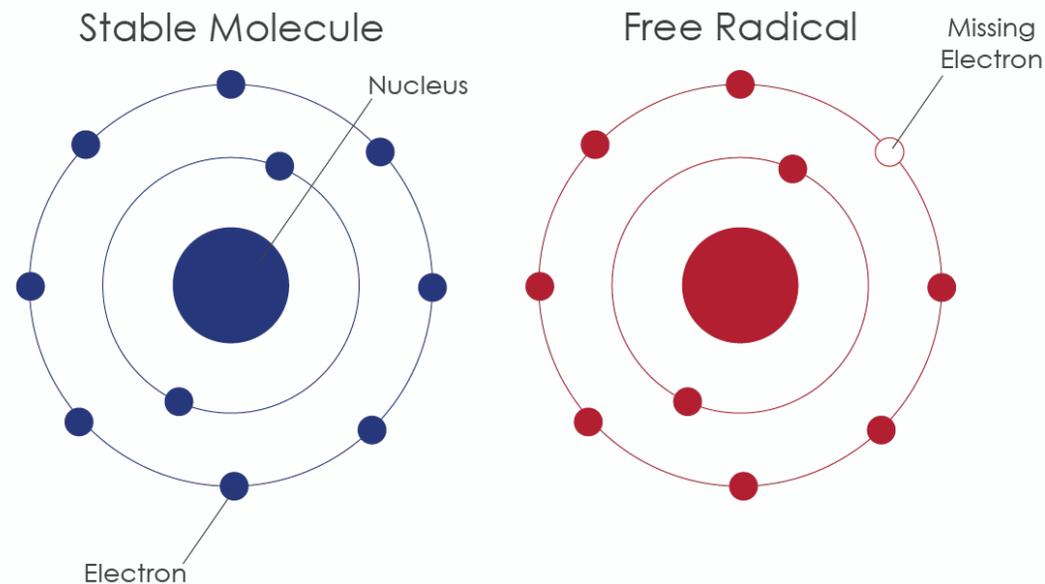
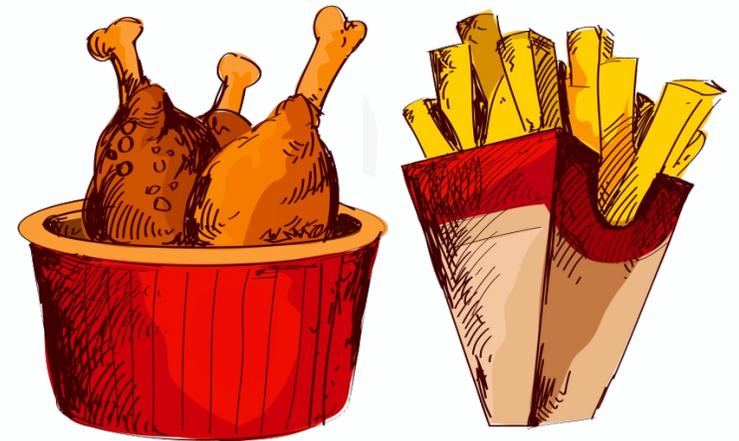


Figure 1. Illustration of a free radical molecule

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. **HOW?**



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.

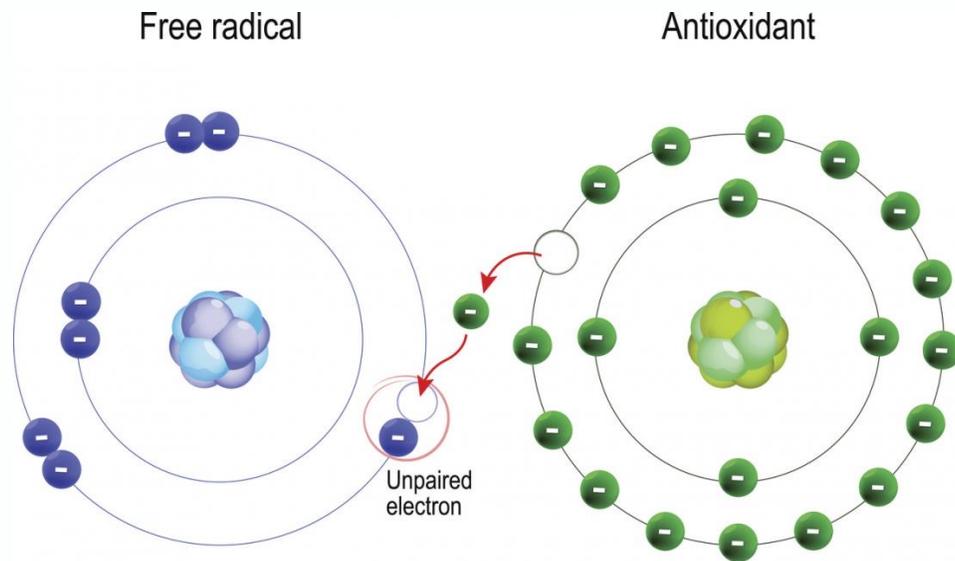


Figure 2. Mechanism of action of antioxidants

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**, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other auto-immune diseases besides ageing.

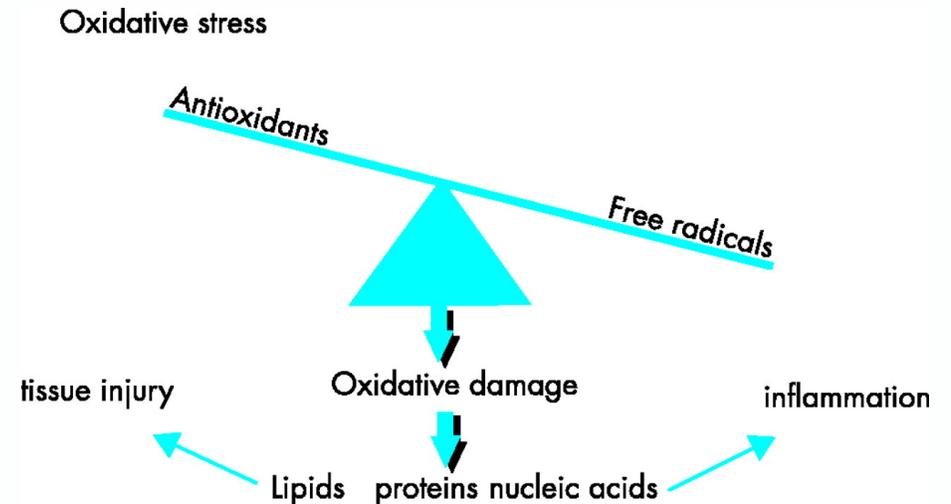


Figure 3. Harmful effects of oxidative stress

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
- Phenolic compounds include **flavonoids**, **phenolic acids**, **tannins**, **stilbenes**, and **lignans**.
- Studies have shown that consumption of food rich in phenolics can **slow the progression of various debilitating diseases**.

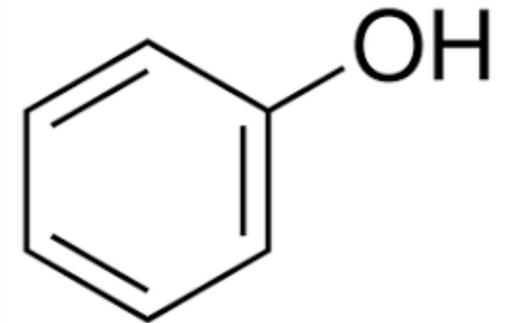


Figure 4. Chemical structure of phenol

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.

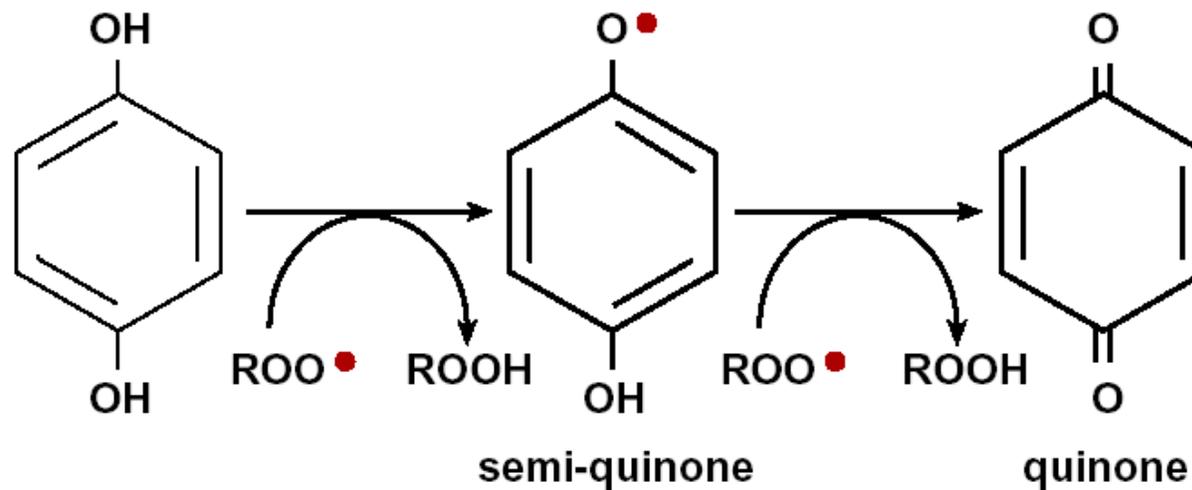


Figure 5. Phenolic antioxidant mechanism

Practical Part

Objective:

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



When handling Folin-Ciocalteu reagent, **safety goggles, face mask and gloves** should be worn.

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 absorbs light at 650nm.

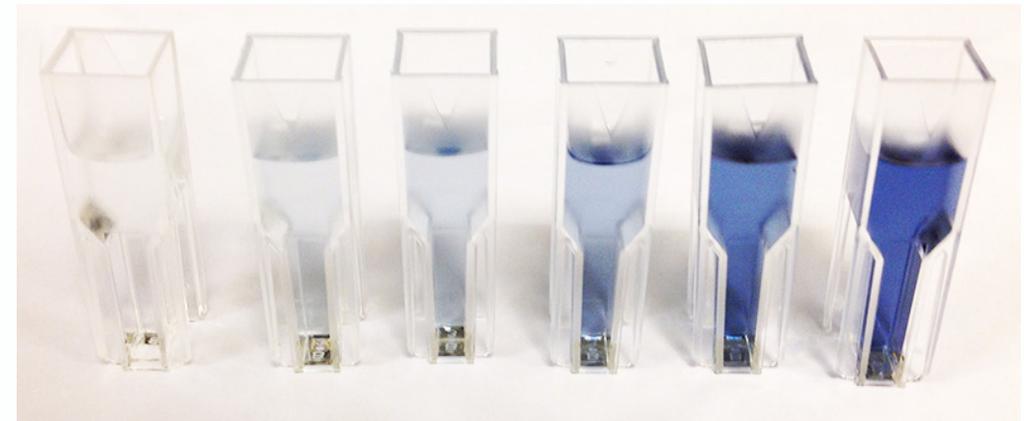
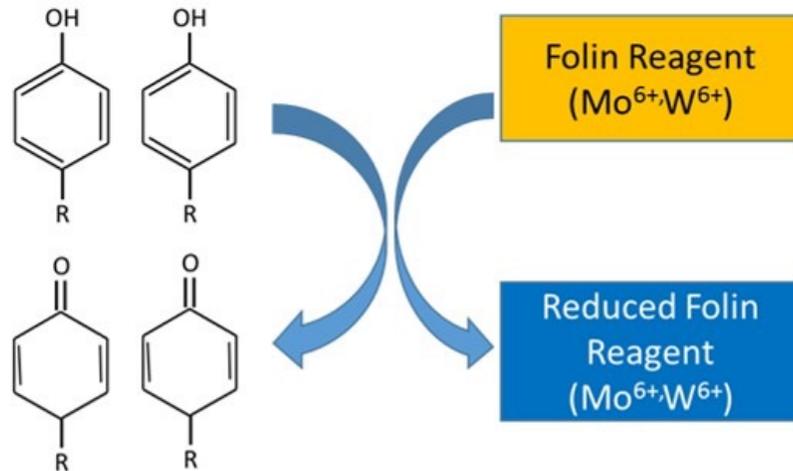


Figure 6. Principle of Folin-Ciocalteu assay

Principle cont.

- **Catechol** is used to produce a standard curve and final phenolic concentrations in the sample express as mg phenols/100 g material
- 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**

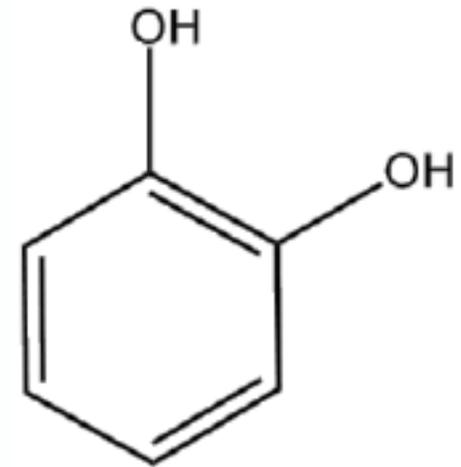


Figure 7. Chemical structure of catechol

Method

Tubes	Catechol standard 5mg/100ml	Sample	Dist. H ₂ O (ml)	Folin- Ciocalteu reagent (ml)	Na ₂ CO ₃ (ml)
1	0.2	--	3.8	0.5 ml	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	Wait 3 min	
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** (mg/dl)**

A (mg/dl) X 1 dl → ...**B**...

B (*mg*) → 2 grams

? → 100 grams

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