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 <u>released during the normal metabolic process</u> 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 <u>fried foods and heated cooking oils</u>, e.g. potato crisps/chips, french fries, onion rings etc. (<u>fried in vegetable oils which oxidises readily into free radicals</u>).

Oxidative stress

Oxidative stress is an <u>imbalanced state</u> where excessive quantities of reactive oxygen and/or nitrogen species over come endogenous antioxidant capacity, <u>leading to oxidation of a varieties of</u> <u>biomacromolecules</u>, such as enzymes, proteins, DNA and lipids.

Oxidative stress involve in the development of chronic degenerative **diseases** including coronary heart disease, cancer and aging.







Antioxidant

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

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



Phenolic compounds

ОН

- Phenolics are compounds possessing one or more <u>aromatic rings</u> with one or more <u>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 <u>can slow the progression of various</u> <u>debilitating diseases.</u>

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 <u>antioxidants</u>.



Objective

Determination of total phenolic content in green tea, black tea and ginger.



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 <u>result of this reduction</u> produce a **blue phosph complex**.



- The reaction must take place under **alkaline conditions** in order <u>to 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 10mg/dl	Dist. H2O (ml)	Sample (ml)	Folin- Ciocalteu reagent (ml)		Na2CO3 (ml)
Blank		4				
1	0.2	3.8				
2	0.4	3.6		0.5 ml	Wait	2 ml
3	0.6	3.4			3 min	
4	0.8	3.2				
5	1	3				
6	1.2	2.8				
7	1.4	2.6				
Black tea		3.8	0.2			
Green tea		3.8	0.2			
ginger		3.8	0.2			

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

Result

	Absorbance	Concentration (mg/dl)
1		
2		
3		
4		
5		
6		
7		
Black tea		
Green tea		
ginger		

Calculation

1- The concentration from the standard curve x dilution factor=.....A....(mg/dl)

(preparation of our sample: 2g in 100ml of water)

- 2- A X 1dl =B.....
- 3- B-----→ 1 grams ?-----→ 100 grams

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

Discussion

Discuss your results

H.W

Does coffee contain phenolics and antioxidant property?