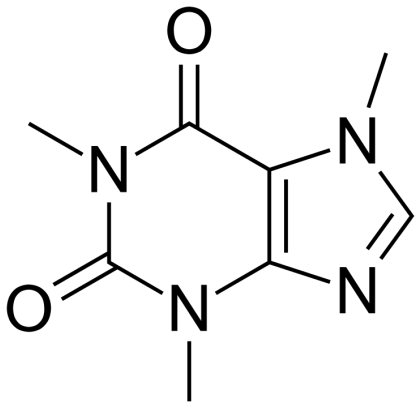
BCH 445- Biochemistry of Nutrition [Practical]

Determination of Caffeine Content in Tea

Caffeine

- Coffee is the most popular beverage after water and is consumed worldwide in daily amounts of approximately 1.6 billion cups and is also a major source of caffeine for most populations.
- Caffeine is a naturally occurring, central nervous system (CNS) stimulant and is the most widely taken psychoactive stimulant in the world.
- Caffeine, the common name for 1,3,7-trimethylxanthine.
- It belongs to a group of methylxanthine.



Sources of caffeine

- **Caffeine** is a chemical that is found naturally in the leaves and seeds of various plants.
- Natural sources of caffeine include coffee beans, cocoa beans, kola nuts, tea leaves and fruits of more than 60 plants.

- Tea leaves contains 1.5% to 3.5% caffeine.
- Roasted coffee beans contain 0.75% to 1.5% caffeine.
- Cocoa bean contains 0.03% to 1.7% caffeine.

- Caffeine can be added to **energy drinks** and some **carbonated drinks** and **drug products.**
- Various **carbonated beverages** contain caffeine in the amount 30 to 60 mg per 355 ml (a can).

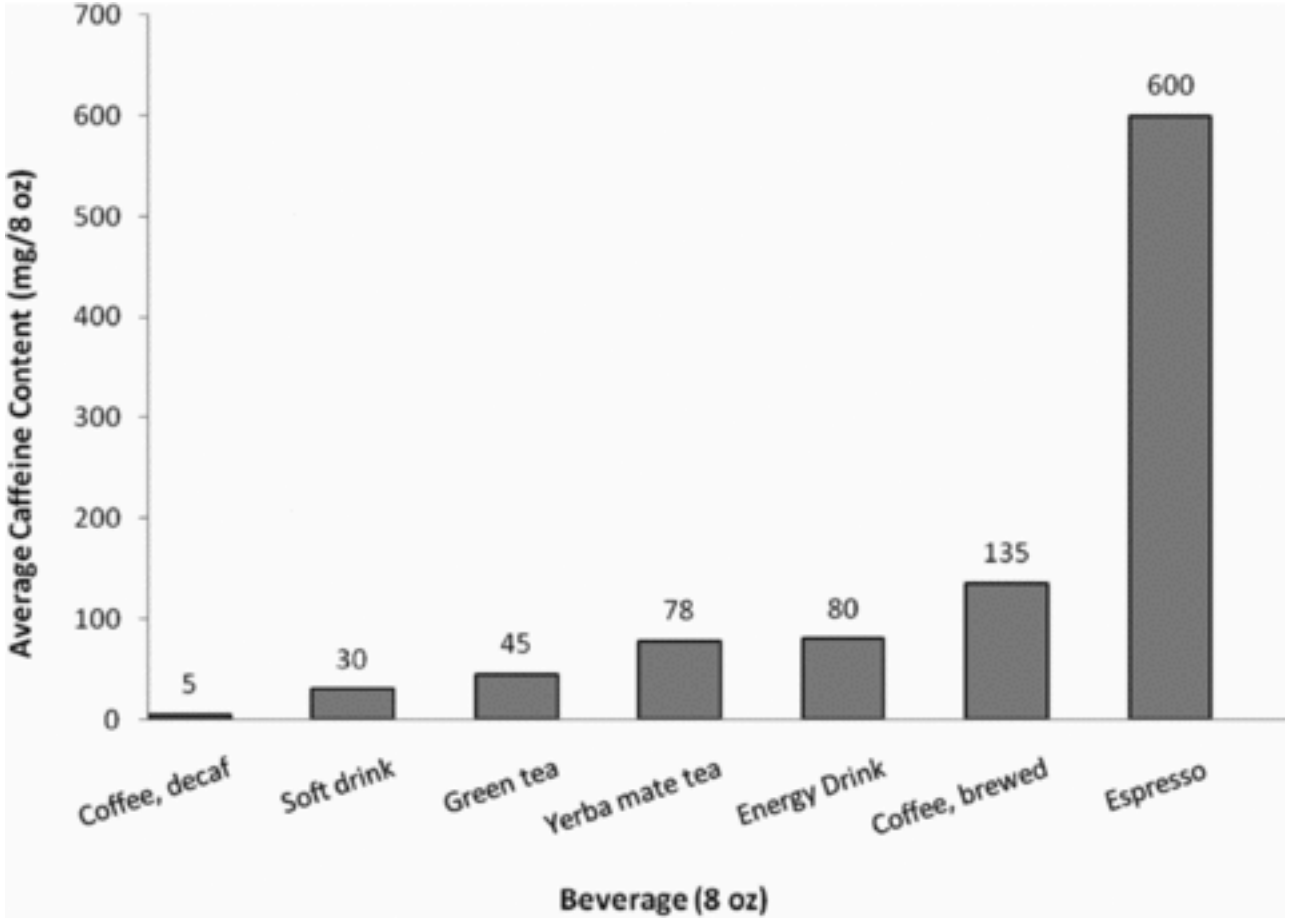


Figure 1. Caffeine content comparison in a variety of beverages.

Effect of caffeine

- Caffeine's main effect on your body is to make you feel more awake and alert for a while, but it can also cause problems.
- Many studies confirm caffeine's (if it consumed properly) ability to **enhance mood** and, **exercise performance**, the speed at which **information is processed**, **awareness**, **attention**, and **reaction time**.
- Non-proper consuming of caffeine leads to **anxiety**, **insomnia**, **facial flushing**, **increased urination**, **muscle twitches** or **tremors**, **irritability**, elevated or irregular **heart rate**, **GI upset.**
- In massive doses, caffeine is **lethal**.
- A fatal dose of caffeine is more than 10 grams (about 170 mg/kg body weight).

Effects of caffeine include:

1- An increase in heart rate

2- Constriction of blood vessels (↑ **BP**)

3- Relaxed air passages to improve breathing

4- Ease of muscle contraction

Mechanism of action

- Adenosine is a central nervous system neuromodulator that can act as neurotransmitter which has specific receptors.
- When adenosine binds to its receptors, neural activity slows down, and you feel sleepy.

  ➔Adenosine thus facilitates sleep and dilates the blood vessels (**opposite action**) .

- Caffeine acts as an adenosine-receptor antagonist.

This means that it binds to these same receptors, but without reducing neural activity.

- **Fewer receptors are thus available** to the natural "braking" action of adenosine, and neural activity therefore speeds up.
- **Caffeine** also causes the pituitary gland to secrete hormones that in turn cause the adrenal glands to produce more adrenalin so it increases your attention level and gives your entire system an extra burst of energy.

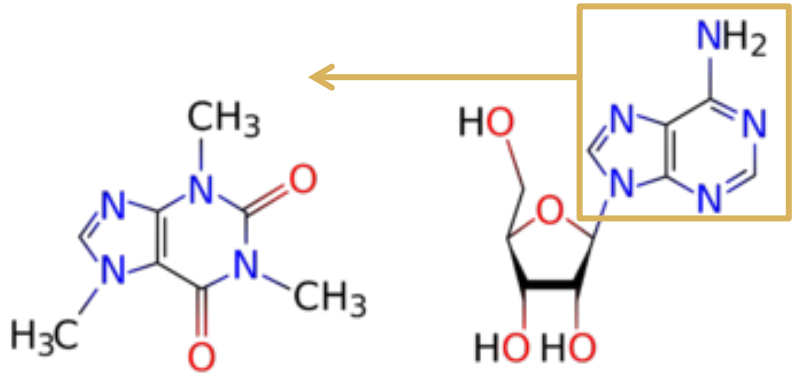
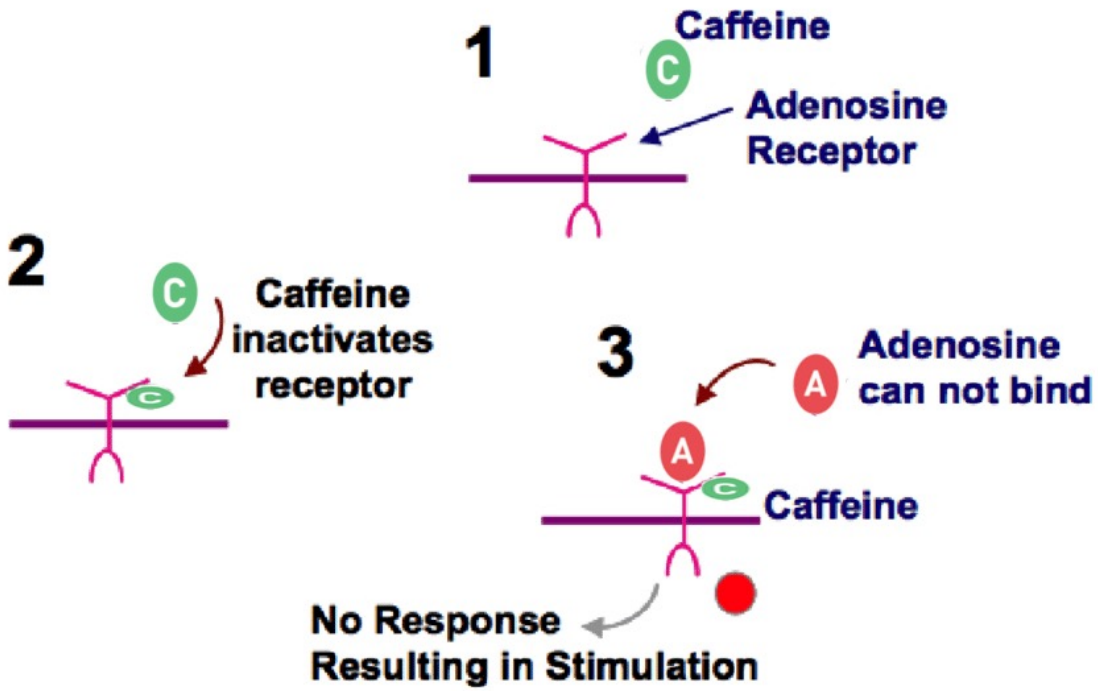
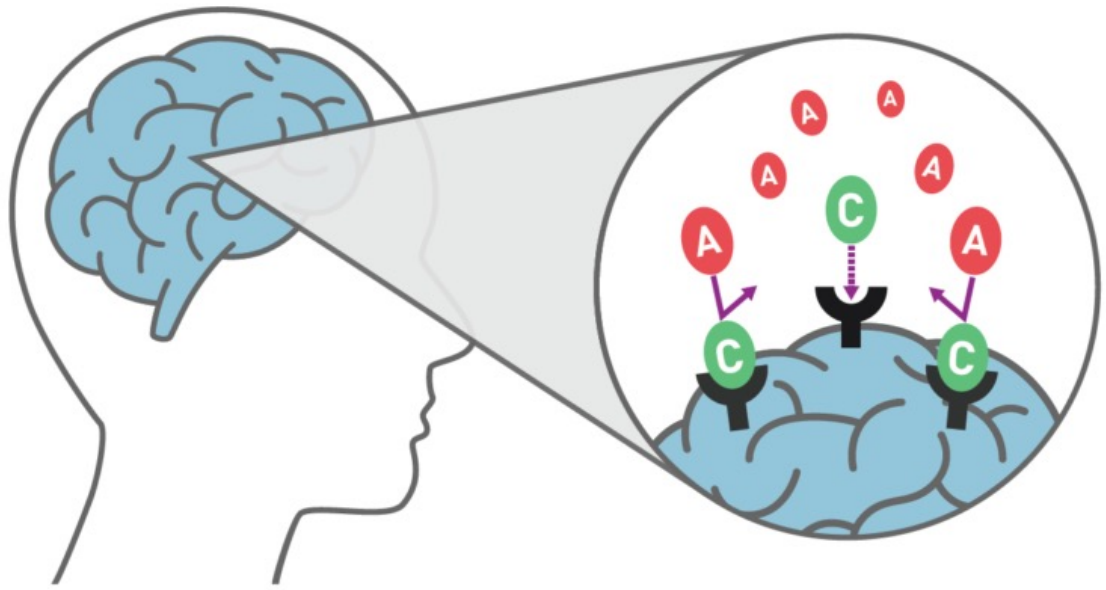


Figure 2. Chemical structures of caffeine and adenosine

Mechanism of action





Inhibitory effect

Adenosine → ↑ GABA → ↓ wakefulness neurons

Excitatory effect

Caffeine → ↑ dopamine → ↑ adrenaline

Practical Part

Objective:

- Determination of caffeine content in tea and soft drink using **direct absorption** of caffeine at 270 nm.

Principle

- Even though caffeine is soluble in water, it is more soluble in chloroform.

➔Therefore, caffeine can be extracted by chloroform from the aqueous mixture using Liquid-liquid extraction involves the distribution of a substance between two immiscible liquid phases.

- Caffeine absorb light at 270 nm directly.

Note:

This method will give a general estimation of caffeine concentration, it will not give an accurate concentration of caffeine in the sample.

When handling chloroform, safety goggles, face mask and gloves should be worn.

Method

First: Sample preparation:

1. 10 ml of (soft drink samples or hot water extract of tea samples) is taken in separating funnels, and 10 ml of chloroform was added to each sample.
2. The separating funnel should be shaken vigorously for 5 min while shaking, open the cover from time to time to release any pressure within the funnel. Be sure funnel is pointing away from you before opening.
3. The solutions then allowed to separate for 10 min at room temperature.
4. Only the lower chloroform layer will be collected for further analysis in a test tube or flask.
5. This chloroform layer will be diluted with pure chloroform (as shown in the table) appropriately to read absorbance.
6. Absorbance at 270 nm against pure chloroform as blank.

Chloroform layer
(which must be collected)

* Each group will either have green tea or black tea sample.

Method

Second: Preparation of caffeine standard :

Tubes	Caffeine standard (100µg/ml)	Sample	Chloroform
S1	0.1	--	2.9
S2	0.2	--	2.8
S3	0.3	--	2.7
S4	0.4	--	2.6
S5	0.5	--	2.5
S6	0.6	--	2.4
S7	0.7	--	2.3
S8	0.8	--	2.2
Sample	--	(try different dilutions)	

Results

Tubes	Absorbance at 270 nm	Caffeine concentration µg/ml
S1		
S2		
S3		
S4		
S5		
S6		
S7		
S8		
Tea sample		

- Plot absorbance against protein concentration (standard curve)
- Determine the caffeine concentration in the sample from the standard curve
- Calculate the concentration of caffeine in (µg/ml)

Calculations

Caffeine concentration (µg/ml)= Conc. from curve x 10 x dilution factor

Homework

- Mention 3 health benefits of caffeine
- What are the withdrawal symptoms of caffeine?