Lab (3): Column chromatography of Carotenoids

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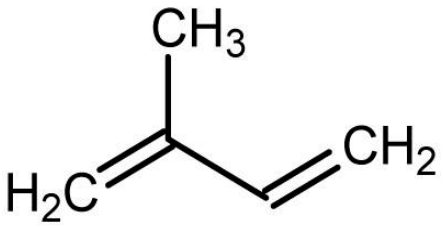
Background

- Carotenoids are organic, fat-soluble pigments that are naturally occurs in plants and some other photosynthetic organisms (bacteria, algae, fungi)
- Animals are unable to synthesize carotenoids, they only obtain them from diet.
- These compounds are responsible for the red, yellow, and orange color of fruits and vegetables.
- There are over 600 different carotenoids known, they are split into two classes, **xanthophylls** and **carotenes**.

Structure

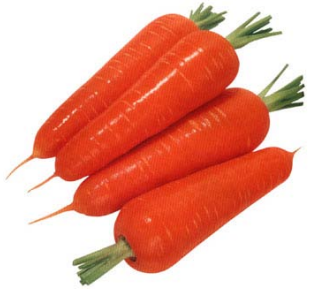
- Structurally carotenoids belongs to category of compounds called **tetraterpenes**
- Terepene is repeating units of Isopren $(C_5H_8)_n$

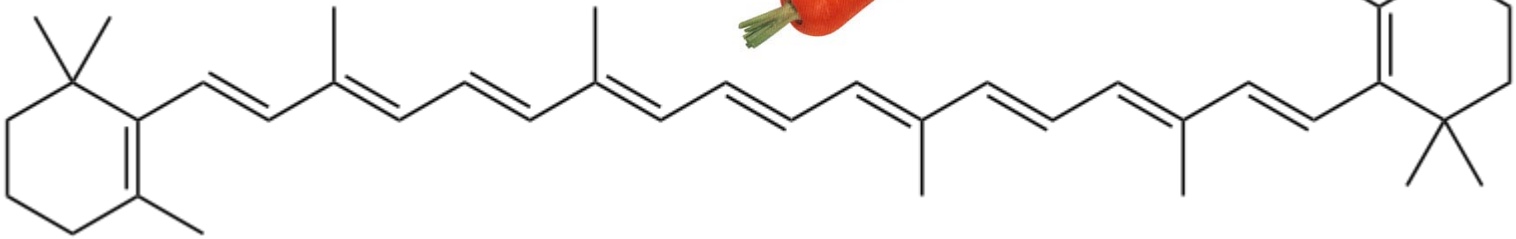


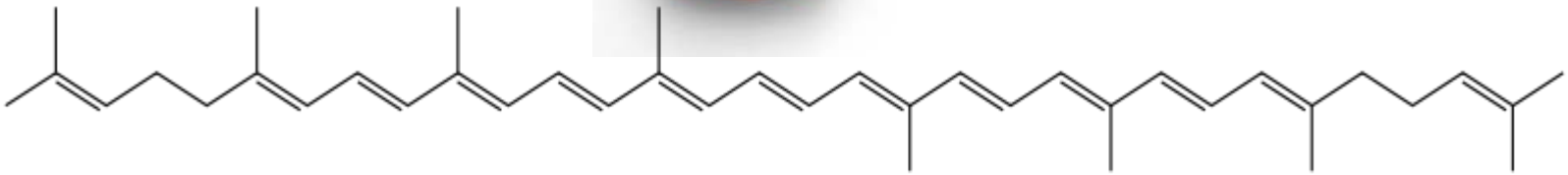
Isopren C_5H_8

- **Tetraterpenes** = 8 isoprene units = 40-C ($C_{40}H_{64}$)
- The chain may be terminated by cyclic end-groups (rings) complemented with oxygen-containing functional groups.

B-carotene







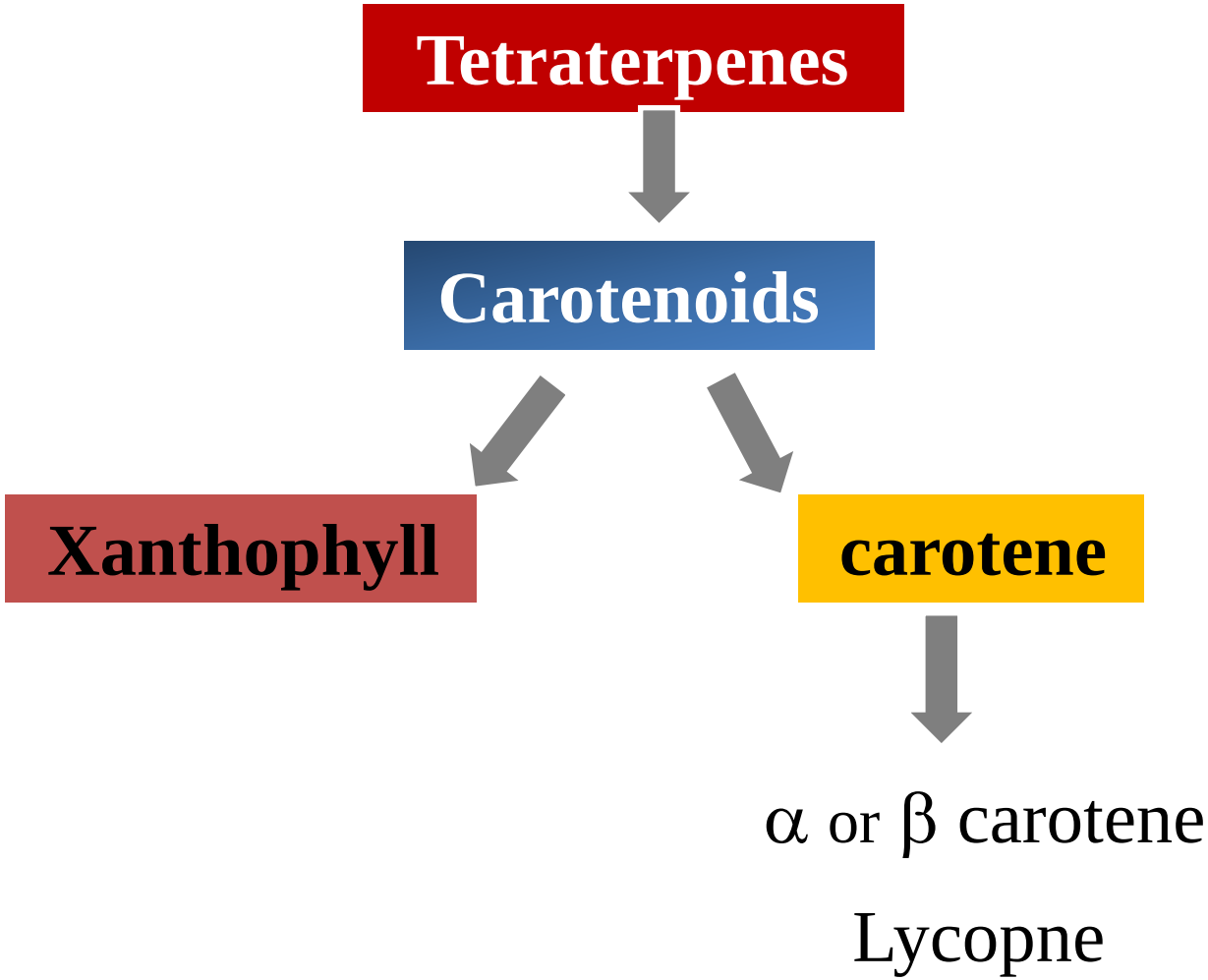
Lycopene

Two major class of carotenoids:

- **Xanthophylls:** if contains oxygen (Lutein)
- **Carotenes:** unoxygenated Carotenes (only carbon and hydrogen), example (α-carotene, β-carotene, lycopene)

The commonest type of carotene:

- β-carotene: is orange or yellow pigment found in carrots and citrus fruits
- Lycopene: is a red pigment found in peppers and tomatoes


Tetraterpenes
Carotenoids
Xanthophyll
carotene
α or β carotene
Lycopne

BIOLOGICAL IMPOTENT OF CAROTEIONDS

- Carotenes act as a precursor of vitamin A or (retinol) which plays an important role in vision
  - ❖ Only α-carotene and β-carotene are converted to significant amounts of vitamin A in the body
  - ❖ β-carotene is the most plentiful carotenoid found in fruits and vegetables.
- β-carotene acts as antioxidant (protects against cancer, heart disease)
- Lycopene is also acts as antioxidant (stop free radical production, lower the risk of prostate cancer)
  - ❖ The best food source of lycopene are processed tomato products such as ketchup, tomato paste, and tomato juice.

Experiment:
Isolation of Carotenoids

Aims:

- Isolate and identify lycopene and β-carotene from two foods rich in them, tomato paste and carrots.

- Analyze the fractions by TLC to determine if the fraction contains more than one component.

- Identification: identify maximum wavelength of both lycopene and β-carotene because both of them absorb light in the UV and visible range,

TECHNIQUES USED

PART I:
Dehydration and Extraction by organic solvents

PART II:
Separation by Column chromatography

PART III:
TLC analysis of sample fractions

PART IV:
UV/Vis spectroscopy

PART I: Dehydration and Extraction

- In this part you will extract pigments from tomato paste and carrots, using ethanol and chloroform as an organic solvents.

Procedure

- in a small beaker:

  3 g tomato paste or 9 g carrots + 30 ml ethanol (95%)

  (Stir the suspension for at least 5 min).

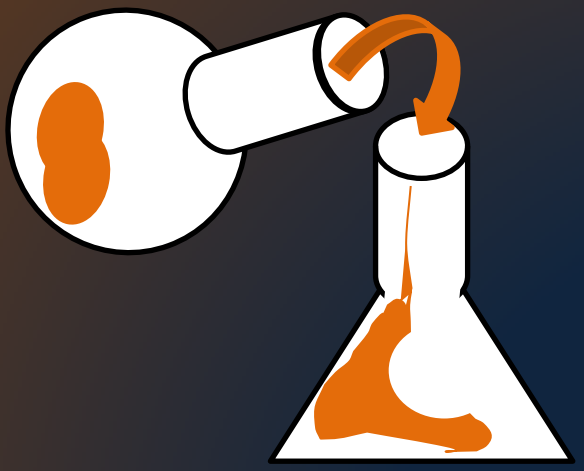
- by using filter paper and funnel:

  filter, discard all the solvent
  and collect the solid material

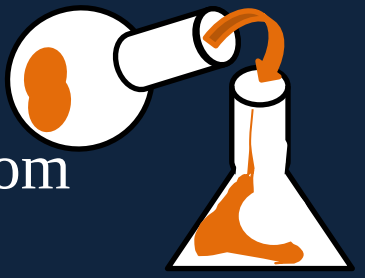
- transfer solid residue to a round-bottom flask
  add 15 ml chloroform.
  fit the flask with a condenser and refluxed
  at 40°C for 4 min.

- Decantation step: collect the liquid in new flask, the solid material is left in the round flask.

"Decantation: is separation of liquid from solid slowly"



- Repeat the extraction procedure for the solid residue, that was left in the round flask, two more times with 15 ml chloroform each time.

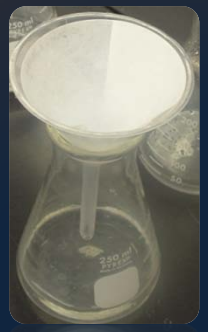


- Combine the organic solvent extracts (liquid part) from each extraction step in a flask and discard the solid.

- Pour the combined organic solvent extracts into a separation funnel, add 20 ml of saturated NaCl solution, shake 5 min.

NaCl solution (help in layer separation)

- prepare funnel with filter paper and add 2 spatulas of anhydrous Na_2SO_4.



- collect the lower layer of the separating funnel by let them pass through the filtering funnel. (Chloroform is more dense than water

(anhydrous Na_2SO_4 removes any water from the colored extract).

- Evaporate the colored extract on a hot plate under the hood until about 1 ml of solvent remains in beaker. Do not allow the solvent to completely evaporate.

- Warp the flask with foil to avoid light oxidation, label the flask with your name and place it in a beaker. Store it in the freezer until the next lab.