# Estimation of proline in Honey

# Honey

- Honey is a naturally sweet and viscous fluid produced by honeybees from the nectar of flowers.

- It is a supersaturated complex natural liquid that contains about 31% glucose, 38% fructose.

- In addition, there is a great variety of **minor components**, including phenolic acids and flavonoids, the enzymes glucose oxidase and catalase, ascorbic acid, carotenoids, organic acids, free amino acids, proteins, and α- tocopherol.

- The actual **composition of honey varies**, depending on many factors such as the flora environmental conditions, and the processing it undergoes .

# Proline In Honey

- Most of amino acids content may be as low as **one fifth of the total**. free amino acids are minor but important component of honey.
- There are approximatly 27 free amino acids in honey.
- The major amino acid is **proline (50-85%)**
- Proline content **varies** in different honeys according to its floral type.

- Also, Proline comes mainly from honey bee during the conversion of nectar into honey which leads to a high variability of the proline content within honeys from the same botanical source.

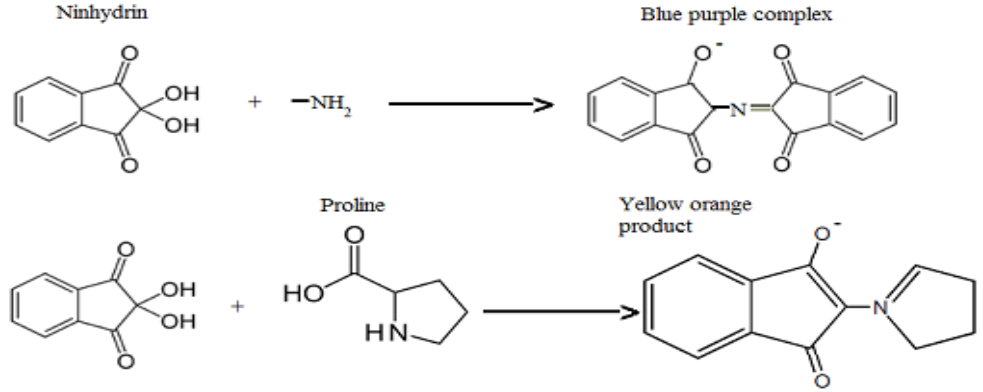
# Proline In Honey

- The proline content in honey is related to the degree of nectar processing by the bees. This makes the honey proline content is **a criterion of honey ripeness** (Together with other factors related to bees, such as saccharide and glucose oxidase activities)
- Also, proline content in some cases **used as indicator for sugar adulteration**.
- It was proposed that natural honey should have a proline content of **more than 180 mg/kg**.
- A **lower proline content** could mean that the honey has been adulterated with sugar.
- However, this value can be higher for certain honeys as the proline content depends on honey t

# Objective

To determine proline concentration in Honey

# Principle




**Ninhydrin is used to assay amino acids.**

**At neutral pH:**

It destroys each primary α-amino acid and also reacts with the released NH3 to form a deep purple chromogen referred to as **Ruhemann's Purple**, which has a maximum absorption at about 570 nm.

The reaction with proline and other imino acids yields a yellow- orange product at neutral pH, as the cyclised N-group is not released.

**At low pH (a pH of approximately 1.0) (The principle of experiment):**

- **Ruhermann's purple** is also yielded, but it quickly looses an amine residue, which results into **colourless derivatives**.

- With **proline**, a **red** water-insoluble reaction stable product is formed.

# Method

| | B | 1 | 2 | 3 | 4 | 5 | S1 |
|---|---|---|---|---|---|---|---|
| Standard (200mg/dl) | -- | 0.2 | 0.4 | 0.6 | 0.8 | 1 | ------ |
| $H_2O$ | 1 | 0.8 | 0.6 | 0.4 | 0.2 | 0 | ------ |
| Sample (12g in 100ml) | -- | -- | -- | -- | -- | -- | 2 ml |
| Formic acid | 0.5 ml | | | | | | |
| Ninhydrine | 2 ml | | | | | | |

- Mix thoroughly after each addition .
- Boiling water bath for 10 min and then allow to cool at room temperature for 5 min. →**(a deep red color should develop).**
- Add 10 ml of 2-propanol-water solution (1:1) to each tube
- Mix well using Vortex
- Measure the absorbance at 520 nm.

# Result

| Tubes | Abs. At 520 nm | Proline concentration mg/dl |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| Sample | | |

- Plot absorbance against protein concentration (standard curve).
- Determine the proline concentration in the sample from the standard curve.
- Calculate the concentration of proline in **(mg/Kg)**

# Calculation

The result you got from the curve is in **mg/dl**

**Knowing preparation of our sample: 12g of honey in100ml water**

The wight of proline in mg **(X)** is obtained by multiplying by **1 dl.** (100 ml = 1dl)

**(X) mg** → 12 grams of honey
? → 1000 grams = Kg

The proline content = ---------- **mg/Kg**