Estimation of proline In Honey

- Honey:

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

- It is a supersaturated complex natural liquid that contains about 31% glucose, 38% fructose (honey also contains other sugars with lower concentration).

- In addition, there is a great variety of minor components, including phenolic acids and flavonoids, the enzymes glucose oxidase and fructose oxidase, 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: floral source, climate, environmental conditions, and the processing it undergoes.

TABLE 6.2
Nonsugar Honey Components

Major Groups of Compounds	Content
Nitrogen Compounds	
Total proteins (mg/100 g)	50–1000
Free proline (mg/100 g)	20–300
Other free amino acids (mg/100 g)	30–700
Acids (gluconic, citric, lactic, malic, succinic, butyric, propionic, and other) (mg/100g)	10–300
Ash (Mn, Co, Fe, and others) (mg/100 g)	70–900
Essential oils (in fresh honey) (mg/100 g)	30–200
Dyes (carotenoids, anthocyanines, flavones) (μg/100g)	1.5–180
Vitamins and other active substances (mg/100 g)	0–0.1

- 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 approximately 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.

- Importance of 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.

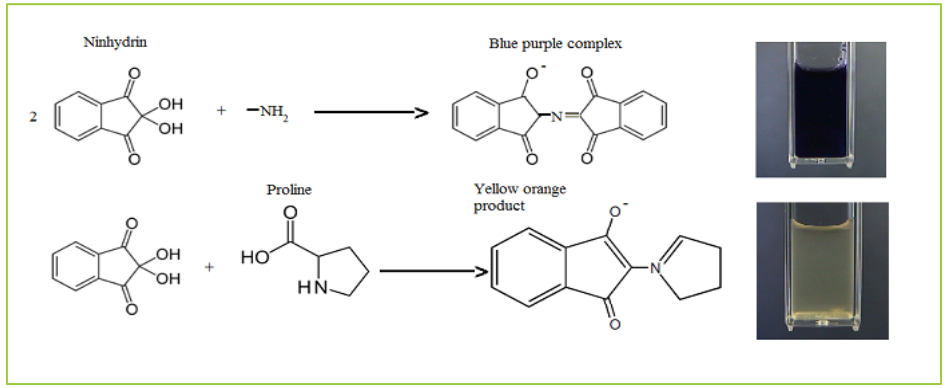
- It was proposed that **natural honey** should have a proline content of more than 180mg/kg.

- 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 types.

Practical Part

- Principle:



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

1. 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.

2. 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 which absorb at 520nm.

Method:

	B	1	2	3	4	5	S1
Standard	--	0.2	0.4	0.6	0.8	1	------
Sample	--	--	--	--	--	--	1
H2O	1	0.8	0.6	0.4	0.2	0	------
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 10 min.
- (a deep red colour should develop).
- Add 10 ml. of the 2-propanol-water solution (1:1) were added to each tube .
- Mix well using Vortex.
- Measure the absorbance at 520 nm.

- Results:

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

- Calculation:

- The result you got from the curve = A mg/dl

- A ➔ 11.7 grams
- ? ➔ 1000 grams (1Kg)

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