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 floral source, climate, 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 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.

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**.

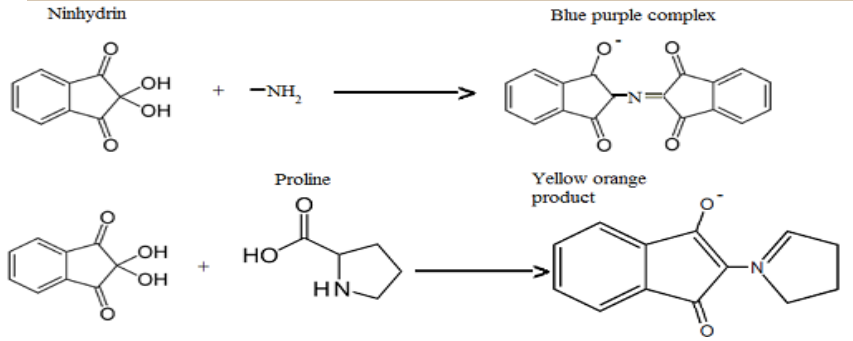
-A **lower proline content** could mean that the honey has been adulterated with sugar.

-However, this value can be higher for certain honeys as the proline content depends on honey types.

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
Proline Standard (200mg/dl)	--	0.2	0.4	0.6	0.8	1	------
H2O	1	0.8	0.6	0.4	0.2	0	------
Sample (12 g in 100ml)	--	--	--	--	--	--	2 ml
Formic acid	0.5 ml						
Ninhydrine	2 ml						

- Mix throughly 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
- 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 (**X**) **mg/dl**

(preparation of our sample: 12g of honey in100ml water)

X----------→ 12 grams

?----------→1000 grams

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