



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.
- 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 under- goes

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 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.

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 NH_3 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):

- Ruhemann's purple is also yielded, but it quickly loses 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	--	0.2	0.4	0.6	0.8	1	-----
Sample	--	--	--	--	--	--	(try different dilutions)
H ₂ O	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.

RESULT

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

CALCULATION

The result you got from the curve x 1 dl =X.....

X-----→ grams

?-----→ 1000 grams

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