

# 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  $\alpha$ -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.

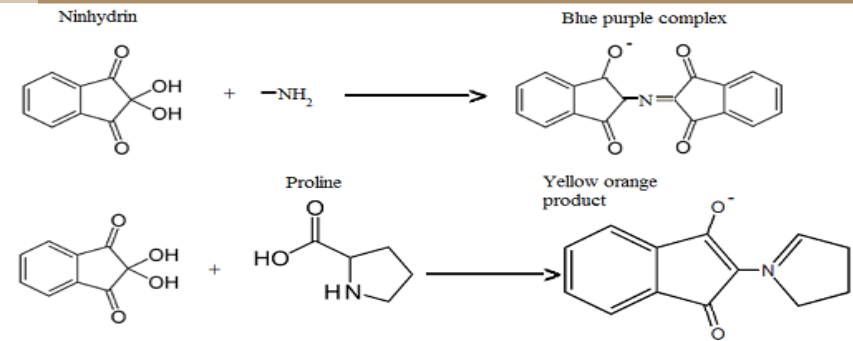
## At neutral pH:

It destroys each primary  $\alpha$ -amino acid and also reacts with the released  $\text{NH}_3$  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>B</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>S1</b>
Standard (200mg/dl)	--	0.2	0.4	0.6	0.8	1	-----
H <sub>2</sub> O	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						
<ul style="list-style-type: none"> <li>• Mix thoroughly after each addition .</li> <li>• Boiling water bath for 10 min and then allow to cool at room temperature for 5 min.               <ul style="list-style-type: none"> <li>• (a deep red color should develop).</li> <li>• <u>Add 10 ml of 2-propanol-water solution (1:1) to each tube</u> <ul style="list-style-type: none"> <li>• <u>Mix well using Vortex</u></li> <li>• Measure the absorbance at 520 nm.</li> </ul> </li> </ul> </li> </ul>							

# 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 in 100ml water)

**X**-----→ 12 grams

?-----→ 1000 grams

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