# BCH 445- Biochemistry of Nutrition [Practical]

## 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 amylase, 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.

| Component | Average |
|---|---|
| Carbohydrates | 82.4g |
| Fructose | 38.5g |
| Glucose | 31g |
| Sucrose | 1g |
| Other Sugars | 11.7g |
| Dietary fiber | 0.2g |
| Fat | 0g |
| Protein | 0.3g |
| Water | 17.1g |
| Riboflavin (Vit. B2) | 0.038mg |
| Niacin (Vit. B3) | 0.121mg |
| Pantothenic acid (Vit. B5) | 0.068mg |
| Pyridoxine (Vit. B6) | 0.024mg |

**Figure 1.** Nutrient components of honey per 100 g

# Proline in honey

- There are approximately 26 free amino acids in honey.
- The **major** amino acid is proline (up to 80%).
- 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

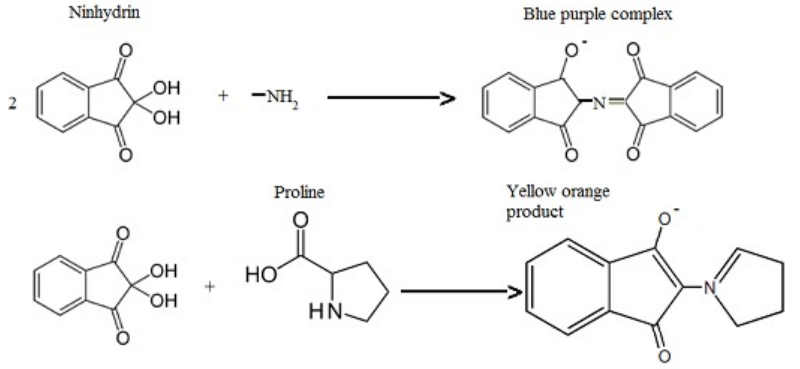
- The proline content in honey is related to the degree of nectar processing by the bees.
- Also, proline content in some cases used as indicator for sugar adulteration.
- It was proposed that **natural honey** should have a proline content of more than 180mg/kg.
- A **lower proline** content could mean 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

## Objective:

- To determine proline concentration in Honey sample.

# 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 $NH_3$ 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 cyclized N-group is not released.
  - Pause and Think **imino acids ?**

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

When handling formic acid, safety goggles, face mask and gloves should be worn.

# Method

| | B | 1 | 2 | 3 | 4 | 5 | S1 |
|---|---|---|---|---|---|---|---|
| Standard | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1 | -- |
| Sample | -- | -- | -- | -- | -- | -- | 1 |
| $H_2O$ | 1 | 0.8 | 0.6 | 0.4 | 0.2 | 0 | |
| Formic acid | 0.5 ml | | | | | | |
| Ninhydrin | 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 color 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 200mg/dl |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| Sample | | |

## Calculations

- **The result you got from the curve**= A (mg/dl)
  - Conc. of proline= A mg/dl x 1 dl= A (mg)
  - A (mg) ➔ 10 grams
  - ? ➔ 1000 grams (1Kg)

  **Note:** *(preparation of our sample: 10g of honey in100ml water)*
- **The proline content=** ----------mg/Kg
- For proline the 180 mg/kg minimum value is internationally accepted

## Homework

What are the different techniques for adulterants detection in honey? Name "4"