**Lab (5)**

**Estimation of proline in Honey**

**Method**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **B** | **1** | **2** | **3** | **4** | **5** | **S1** |
| Standard (200mg/dl) | -- | 0.2 | 0.4 | 0.6 | 0.8 | 1 | ------ |
| H2O | 1 | 0.8 | 0.6 | 0.4 | 0.2 | 0 | ------ |
| Sample | -- | -- | -- | -- | -- | -- | 2 ml (12g in 100ml) |
| Formic acid | 0.5 ml | | | | | | |
| Ninhydrine | 1. 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 using Vortex * Measure the absorbance at 520 nm. | | | | | | | |

**Results:**

|  |  |  |
| --- | --- | --- |
| **Proline concentration mg/dl** | **Abs. At 520 nm** | **Tubes** |
|  |  | 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)