**Estimation of Tryptophan**

**Principle**

Tryptophan is one of the limiting amino acids in food grains. Tryptophan and p-dimethyl amino benzaldehyde react in acid medium to form a condensation product. This condensation product is oxidized by sodium nitrite to yield a blue color, which can be measured at 590 nm.

**Required materials and instruments:**

1. Glass tubes (15 ml) and rack
2. Pipettes
3. Measuring cylinders
4. Plastic cuvettes
5. Balance
6. Spectrophotometer

**Reagents**

1. 21.4 N H2SO4 solution (make from 37 N stock in fume hood with caution).
2. 2 N H2SO4 solution.
3. 0.045% NaNO2 solution: Dissolve 0.045 g NaNO2 in 100 ml distilled water.
4. Ehrlich reagent: p-dimethyl amino benzaldehyde (make in the H2SO4 solution as a master mix).
5. Standard tryptophan solution (1 mg per ml):

Dissolve 1 mg of DL-tryptophan in 1 ml of distilled water (make a 1:10 dilution from the 1 mg/mL stock to get 0.1 mg/mL).

**Procedure**

1. Weigh 15–20 mg of fine powdered defatted flour and dissolve in water at 1 mg/mL.
2. Add 9 ml of the H2SO4 and p-dimethyl amino benzaldehyde mixture (Ehrlich reagent) to both standards and sample according to the table below. Keep the tubes in dark for 20 h. Remove the tubes from dark and add 0.1 ml of 0.045% NaNO2 solution to each tube. Mix properly and gently, keep it for half an hour at room temperature and measure the absorbance of blue color developed at 590 nm.

**Standard Curve**

Standard curve of tryptophan is prepared by using different concentrations (10–100 mg/ml) of standard tryptophan solution. Adjust the volume to 1 ml by adding distilled water, add 9.4 ml H2SO4 (21.4 N) very slowly, mix the content gently and further proceed as per the steps described above.

Tryptophan (μg/g) = μg of tryptophan from standard curve X 1000

weight of the sample(mg)

**Precautions**

1. Sulphuric acid should be added slowly by the sides of the flasks.

2. After the addition of sulphuric acid into the sample, keep it in dark.

Preparation of Ehrlich reagent:

|  |  |  |
| --- | --- | --- |
|  | 10 ml (for 1 tube) | 100 ml (for 20 tubes) |
| 23.5 N H2SO4 | 8 ml | 160 ml |
| 2N H2SO4 | 1 ml | 20 ml |
| H2O | 1ml | 20 ml |
| p- DAB | 30 mg | 600 mg (0.6g) |

**Preparation of H2SO4**

Bottle is 37 N H2SO4

23.5 N – 127 ml of H2SO4 + 73 ml of H2O

2N H2SO4 – 5.4 ml +94.6 ml of H2O

Stock standard tryptophan: 100 μg/ml

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Concentration (μg) | Volume of stock (ml) | H2O (ml) | Ehrlich reagent (ml) |  | NaNO2 (0.045%) ml |  | OD 590 nm |
|  | Blank | - | 1 |  |  |  |  |  |
| 1 | 10 | 0.1 | 0.9 |  |  |  |  |  |
| 2 | 20 | 0.2 | 0.8 |  |  |  |  |  |
| 3 | 40 | 0.4 | 0.6 | 9 ml | 24 h in dark |  | 45 min |  |
| 4 | 50 | 0.5 | 0.5 |  |  |  |  |  |
| 5 | 100 | 1.0 | - |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  | Sample 1 | 0.5 | 0.5 |  |  |  |  |  |
|  | Sample 1’ | 1.0 | - |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Concentration (μg) | Volume of stock (ml) | H2O (ml) | H2SO4 (ml) |  | NaNO2 (0.045%) ml |  | OD 590 nm | OD 590 nm (24h) |
|  | Blank | - | 1 |  |  |  |  |  |  |
| 1 | 10 | 0.1 | 0.9 |  |  |  |  | 0.052,  0.027 | 0.068 |
| 2 | 20 | 0.2 | 0.8 |  |  |  |  | 0.082,  0.078 | 0.139 |
| 3 | 40 | 0.4 | 0.6 | 9 ml | 24 h in dark |  | 45 min | 0.191  0.188 | 0.273 |
| 4 | 50 | 0.5 | 0.5 |  |  |  |  | 0.224  0.279 | 0.654 |
| 5 | 100 | 1.0 | - |  |  |  |  | 0.567 |  |
|  |  |  |  |  |  |  |  |  |  |
|  | Sample 1 | 0.5 | 0.5 |  |  |  |  | 0.045 | 0.076 |
|  | Sample 1’ | 1.0 | - |  |  |  |  | 0.110 | 0.151 |