

Aim - Estimation of Free α -Amino Nitrogen (AAN)

Principle: Amino acids of fish tissues occur in a free states in addition to intact protein. The protein is precipitated by adding 10% TCA solution leaving free amino acids in the extract. When the extract is treated with copper salt like copper phosphate, cupric chloride or copper sulphate it can solubilise amino acids in copper amino-complex compound which has a dark blue colour depending upon the concentration of amino acids. Insoluble form of copper is preferable and for this purpose the pH of the extract is to be increased to alkali side of the pH around 8.5 to 10.0 which is done by an alkali using thymolphthalein indicator.

Materials:

- Cupric chloride (CuCl_2): 27.3g of cupric chloride in 1 litre of distilled water.
- Tri-sodium phosphate (Na_3PO_4): 65.5 g of disodium hydrogen phosphate (Na_2HPO_4) is dissolved in 500 ml of distilled water and 7.2f of NaOH is added to it and then dissolved by stirring. The solution is made upto 1 litre.
- Borate buffer: 57.2g of sodium borate is dissolved in 1500 ml of distilled water and 100 ml of normal HCl is added to it and their volume is made upto 2 litres.
- Thymolphthalein: 0.25g of thymolphthalein in 100ml of 50% ethyl alcohol.
- N/100 sodium thiosulphate solution: 0.248g of $\text{Na}_2\text{S}_2\text{O}_3$ is dissolved in 100 ml of distilled water. This solution is standardized by using standard potassium dichromate solution (iodometry).
- 1N NaOH : 4g of sodium hydroxide is dissolved in 100ml of distilled water.
- Starch indicator: 1g of starch powder is added to 100ml boiling water and boiled for 1 minute.
- Glacial acetic acid.
- Potassium iodide (AR grade).

Method:

- Measure 20 ml of TCA extract into a 100ml volumetric flask and add few drops of thymolphthalein indicator.

- Make the extract alkaline by adding 1N NaOH, till a distinct blue colour appears.
- Mix CuCl₂: tri-sodium phosphate: borate buffer = 1:2:2 and mix well.
- Add 60 ml of this suspension to alkaline solution in volumetric flask and make the volume upto 100 ml with distilled water
- Shake it and allow it to stand for 30 minutes and then filter.
- Pipette out 10 ml of the filtrate to a conical flask to which 0.5 ml of glacial acetic acid (CH₃COOH) and about 1g of potassium iodide.
- Titrate the liberated iodine against N/100 sodium thiosulphate using starch as indicator.
- Add few drops of starch solution when yellow solution of iodine becomes faint yellow.
- Continue the titration till blue colour completely disappears.

Calculation:

1 ml of N/100 Na₂S₂O₃ = 0.28mg of α-amino nitrogen.

α-amino nitrogen (mg%) =

Where,

A = Total volume of TCA extract

B = Vol. Of TCA extract taken for estimation

C = Filtered extract taken from titration

D = Vol. Of Na₂S₂O₃ used in titration

W = Weight of fish sample