

## **Experiment-2**

### **Determination of Crude Protein (Kjeldahl Method)**

#### **Principle:**

A known weight of fish sample is decomposed by digesting with concentrated sulphuric acid in the presence of catalyst mixture to accelerate the process, which results in the formation of ammonium sulphate on addition of known quantity of 40% NaOH and subsequent distillation, ammonia is liberated, which is absorbed in a known volume of 2% boric acid solution. Then formed ammonium borate is titrated against 0.1N H<sub>2</sub>SO<sub>4</sub> using indicator.

#### **Reagents:**

1. Sulphuric acid
2. Catalyst mixture (0.1N H<sub>2</sub>SO<sub>4</sub>)
3. 4% boric acid solution
4. Sodium hydroxide, 40% solution
5. Digestion mixture

#### **Apparatus required:**

- I. Micro kjeldahl digestion and distillation units,
- II. Kjeldahl glass digestion tubes (300 ml capacity)
- III. Conical flasks, 250 ml
- IV. Burette

#### **Procedure:**

- Take approximately 2 gm of sample in a kjeldahl digestion flask
- Add a pinch of digestion mixture (5 parts K<sub>2</sub>SO<sub>4</sub> and 1 part CuSO<sub>4</sub>) and 10 ml concentrated sulphuric acid to the flask
- Keep it above a heating mantle where the temperature and time already settled at 250°C-15 min, 380°C-15 min and 420°C-60 min until the solution turned colorless
- Make the final volume of the sample up to 50 ml by adding distilled water

- Proceed with 2ml of digested sample taking in a distillation flask and add 4 ml of 40% NaOH in to the flask
- Organize the distillation process by micro-kjeldahl distillation apparatus.
- The Distillation process last longs for for 6-7 minutes and the liberated ammonia will absorb into 2% boric acid containing a drop of tashiro's indicator
- The indicator will turn the sample into light greenish colour
- The amount of ammonia liberated will be determined by titrating with 0.1N sulphuric acid
- Crude protein content of the sample may calculated by multiplying total nitrogen content with the conversion factor 6.25 and expressed as a percentage.

**Calculation:**

$$\text{Total nitrogen (\%, wet weight)} = \frac{14 \times N \times X \times 100}{1000 \times V \times W} \times 100$$

Hence, % protein = % total nitrogen 6.25 (conversion factor)

Where, N = normality of H<sub>2</sub>SO<sub>4</sub>

X = ml of standard H<sub>2</sub>SO<sub>4</sub> required for titration of samples

V = aliquot (ml) of digested extract taken for distillation

W =weight (g) of sample