

## **Aim - Estimation of Total Volatile Basic Nitrogen (TVBN)**

The estimation of total volatile nitrogenous bases has been generally made use of in routine analysis for the chemical assessment of the degree of spoilage in fish samples. The measurement of this index of spoilage furnishes a reasonably accurate and rapid method for the determination of the keeping quality of cured fish products. It measures the content of trimethylamine, dimethylamine, ammonia and other basic nitrogenous compounds associated with the spoilage of fish, where ammonia comprises a major proportion of the TVBN value.

### **Principle:**

Meat extract is treated with a strong alkali and the volatile base is distilled or diffused over into standard acid or boric acid. The volatile bases are first separated by precipitating the proteins using trichloroacetic acid (TCA) solution. The filtered solution (TCA extract), which contains the volatile substances, is treated with a strong alkali ( $K_2CO_3$ ). The liberated ammonia is trapped by the standard  $H_2SO_4$  solution and the quantity absorbed is determined by titrating of the remaining acid with the standard NaOH.

### **Materials:**

- 10% Trichloroacetic acid (TCA) solution
- Conway's micro-diffusion unit
- Mortar and pestle
- Paraffin wax and Vaseline in 1:2 ratio
- 0.02 (N)  $H_2SO_4$
- Saturated Potassium carbonate solution: 50g  $K_2CO_3$  in 100ml distilled water by heating.
- Toshiro's indicator
- NaOH: 0.02N

### **Method:**

- Take 10g fish muscle and macerate by adding 20 ml of 10% TCA using mortar and pestle.
- Filter the content through filter paper in 100 ml volumetric flask.
- Repeat the extraction process twice or thrice to make the volume to 100 ml (TCA extract).

- Apply a mixture of melted paraffin wax and Vaseline this on the rims of cups.
- Add 1 ml of 0.02(N)  $H_2SO_4$  into the inner chamber of each cup of previously wash and dried Conway cups.
- Place lid over the Conway cup covering part of the outer chamber and complete inner chamber.
- Add 1 ml of TCA extract in to the outer chamber followed by 1ml of saturated  $K_2CO_3$  solution.
- Close the unit with the lid and mix the contents by gently rotating the unit
- Leave the unit overnight for reaction (it can be kept inside an incubator at  $37^\circ C$  for 3 hrs).
- Then, titrate the excess acid, left in inner chamber against 0.02(N) NaOH and drop of Tashiro's indiactor.
- Run a blank simultaneously with 10% TCA solution instead of TCA extract. .
- Sample preparation should be done as fast as possible, since ammonia is highly volatile).

**Calculation:**

TVBN (mg%) =

Where,

N = Normality of  $H_2SO_4$

A = ml of standard NaOH required for titration of samples

B = ml of standard NaOH required for blank

W = Weight of the sample