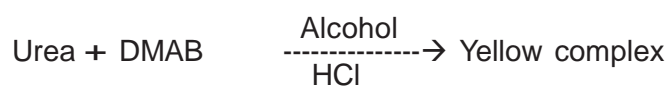


7.16 Estimation of urea in milk-1,4-p-dimethylaminobenzaldehyde (DMAB) method

Principle

This method is based on the principle that urea forms a yellow complex with p-dimethyl aminobenzaldehyde in a low acidic solution at room temperature. The intensity of yellow color is measured at 425 nm.



Reagents

- Trichloro acetic acid (TCA) 24 per cent w/v: Dissolve 24 g of TCA in distilled water and make the total volume to 100 ml.
- Phosphate buffer (pH 7.0): Dissolve anhydrous potassium di-hydrogen orthophosphate (3.403 g) and anhydrous di-potassium mono hydrogen orthophosphate (4.355 g) in distilled water and make the volume to one litre.
- Diluting reagent: Mix equal volumes of 24 per cent (w/v) TCA and phosphate buffer (pH 7.0) to make the diluting reagent.
- p-dimethylaminobenzaldehyde (DMAB) reagent (1.6 per cent w/v): Dissolve p-dimethylaminobenzaldehyde (1.6 per cent w/v) in ethyl alcohol containing 10 per cent (v/v) concentrated hydrochloride acid.
- Standard urea solution (1 mg/ml): Dissolve 100 mg of urea (AR grade) in phosphate buffer (pH 7.0) and make up the volume to 100 ml.

Procedure

1. Ten ml of well mixed sample of milk is mixed with 10 ml of TCA (24 per cent w/v) to precipitate the protein and filtered through Whatman No. 42 filter paper.
2. Add five ml of filtrate with 5 ml of 1.6 per cent DMAB reagent to develop the yellow colour in a test tube.
3. Prepare reagent blank by taking 5 ml of diluting reagent and treating it with 5 ml of DMAB reagent.
4. Measure the optical density of yellow colour of the sample at 425 nm in a spectrophotometer against reagent blank.
5. From standard curve, as described below, the amount of urea in milk is calculated.

Preparation of standard curve

Prepare standard urea solution (1 mg/ml) in phosphate buffer (pH 7.0). Take different concentrations of urea solution ranging from 0.1 to 2.0 mg separately in different test tubes and the total volume made to 5 ml with diluting reagent solution. Add 5 ml of 1.6% DMAB reagent to each test tube to develop the colour. Measure the optical density of the yellow colour thus obtained at 425 nm and plot against concentration.

Reference: Indian standard methods of test dairy industry. Part II. Chemical analysis of milk. IS: 1479 (Part II). Indian Standard Institution, New Delhi.