11.3 Determination of protein-bound formaldehyde

The methods described below are applicable to any sample of formalin treated protein of feed. The estimated formaldehyde includes both CH_2O which is loosely bound and that which is tightly bound to the protein and which in the literature are often referred to as the "free" and "irreversibly bound" forms. The samples must be ground through a 1 mm screen.

Reagents

- Distilled water
- Anhydrous sodium sulphate
- Phosphoric acid, concentrated
- Chromotopic acid
- Sulphuric acid, 36 N.
- Formaldehyde solution – dilute 0.25 ml of formalin to a litre.

Equipment

- 500 ml Kjeldahl flasks
- Quickfit macro Kjeldahl distillation unit
- Electrical heating unit
- 50 ml volumetric flask
- Spectrophotometer – set at 570 mμ.

Procedure

Distillation: (this two-step distillation procedure takes approximately 1 hour)

1. Accurately weigh 1 g of feed sample (or 5 ml of milk) into a 500 ml Kjeldahl flask.
2. Add 50 ml of distilled water, 2.0 g of anhydrous sodium sulphate and 3 ml of concentrated phosphoric acid and anti-bumping granules.
3. Place the flask on an electrical heating unit and connect it to a condenser through a trap, claisen head and plug funnel.
4. Place a 100 ml stoppered measuring cylinder with a wide neck under the condenser, containing 5 ml of distilled water, and begin the distillation until 40 ml have been collected.
5. Allow the Kjeldahl unit to cool, and then add another 50 ml of distilled water through the plug funnel and continue the distillation until 90 ml have been collected.
6. Make up to 100 ml with distilled water and mix.

Color development

1. Weigh 100 ± 10 mg of chromotopic acid into a 30 ml beaker.
2. Add 1.0 ml aliquot of the distillate.
3. Evaporate the solution to dryness by placing the beaker in an air oven at 100ºC for 30 minutes.
4. When the residue has cooled down to room temperature, 5 ml of 36 N sulphuric acid is added and the resulting solution is heated for 30 minutes in an air oven at 100ºC.
5. After cooling, the solution is diluted to 50 ml with distilled water in a volumetric flask.
6. The absorbance of this solution is measured against a reagent blank at 570 nm using a 1 cm light path cell in a spectrophotometer.

Calibration curve

1. To a series of solutions containing from 0 to 100 µg of CH_2O carefully add into 30 ml beakers 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of a standard formaldehyde solution prepared by diluting 0.25 ml of formalin to a litre (The concentration is checked by titration). Make up to 1 ml with distilled water.
2. Proceed for the color development as for the samples.
3. Prepare a calibration curve by plotting absorbance versus micrograms formaldehyde.
4. By linear regression analysis, determine the slope of the best line fit.

Calculation

$$W = \frac{As}{S}$$

Where,

W = micro grams formaldehyde found
As = absorbance units for sample
S = slope of calibration curve

Reference: Van-Dooren (1975), *J. Sci Fd. Agric*. 26: 1265-1271.