PROTEIN DETERMINATION BY BIURET METHOD (MODIFIED BY UMEMOTO)

LIM P. Y.

INTRODUCTION

This method is applicable to extracted liquid fish protein aliquots (See A-5 Section IV, 2 and 3) with a protein concentration of between 0.1 to 0.5 mg N/ml.

The method is based on the reaction of Cu^{++} with peptides in alkaline solution to yield a purple Cu^{++} — peptide complex that has a peak of absorption at 545 nm.

Some fish protein fractions contain interfering substances which cause turbidity to the sample solution when the sample is left to stand for attainment of chemical equilibration (for full colour development). These substances include tris — (hydroxymethyl) methylamine used as buffering reagent during the extraction of fish protein and sucrose & sorbitol used as cryoprotective reagent in minced fish flesh during frozen storage. Other interfering chemicals are ammonium sulphate, mercapto-ethanol, Triton X-100 etc. Therefore this method is not suitable for samples containing the above interfering substances.

I APPARATUS

Bulb pipette, 5 ml
Quickfit test tube with stopper, 25 ml
Test tube shaker
Spectrophotometer
Magnetic stirrer
Beaker 250 ml

II REAGENTS

a) Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$)

b) Sodium hydroxide (NaOH)

c) Glycerine

d) Reagent A.

Dissolve 8 g NaOH in 40 ml distilled water. Add the NaOH solution to 30 ml distilled water containing 0.2 g glycerine. Dissolve 0.4 g $CuSO_4 \cdot 5H_2O$ in 30 ml distilled water, add this solution slowly to the above mixture solution with continuous agitation to prevent precipitation. This solution should not be kept in refrigerator for more than 2 months.

e) Reagent B.

Dissolve 8 g NaOH in 80 ml distilled water. Weigh 0.2 g glycerine and dissolve it in 20 ml distilled water. Mix these two solutions and keep in refrigerator and it should not be kept for more than 2 months.

III PROCEDURE

A. PREPARATION OF CALIBRATION CURVE

1. The Bovin Serum Albumin or myofibrillar protein extract from fish can be used as working solution for the preparation of calibration curve.

   a) Bovin Serum Albumin

   Stock solution: Dissolve about 400 mg albumin in distilled water and dilute to 50 ml (about 8 mg/ml)

   Working solution: Pipette 5 ml of stock solution into 1000 ml volumetric flask and dilute with distilled water (about 0.4 mg/ml).

   b) Myofibrillar protein extract

   It is preferably to use the myofibrillar protein extract obtained from the same group of fish for the preparation of calibration curve.

   The protein extract has to be digested following Kjeldahl method and the nitrogen is to be determined accordingly. The following formula is for the calculation of nitrogen concentration in fish protein extract (see B-1 formula (1)):

   N content (mgN/ml) = $(b - a) \times 0.1 \times 14.00 \times 1/n$

   b: sample titration value (ml)

   a: blank titration value (ml)

   n: ml of extract used in digestion

   Based on the concentration of nitrogen protein in the fish protein extract, appropriate dilution can be made using KCl-phosphate buffered solution for the preparation of calibration curve.

   The concentration of the diluted fish protein extract should fall in beteween 0.1 to 0.5 mgN/ml.

2. Preparation of protein extract for spectrophotometric reading.

   Prepare 2 sets of 6 test tubes, each containing 5, 4, 3, 2, 1 and 0 ml (blank) of Bovin serum albumin working solution or fish myofibrillar protein extract and 0, 1, 2, 3, 4 and 5 ml of KCl-phosphate buffered solution respectively. Pipette 5 ml each Reagent A to one set of test tubes and pipette 5 ml each Reagent B to the other set of test tubes. Shake well and leave to stand 2 hrs at room temperature (26°C). Set up the spectrophotometer as specified by the manufacturer, adjust the wavelength to 545 nm and read the absorbance of the solution relative to the reagent blank (contains only KCl-phosphate buffered solution).

3. Calculation & calibration curve

   Calculate the solutions' absorbance containing various concentration of protein solutions.

   $Absorbance_{545nm} = (O.D._A - Blank_A) - (O.D._B - Blank_B)$

   $O.D._A$ and $O.D._B$ = optical density of sample solutions with Reagent A and B, respectively.

   $Blank_A$ and $Blank_B$ = optical density of blank solutions with Reagent A and B, respectively.

Plot the absorbance values of the protein solutions versus the concentrations of the protein solutions to obtain the calibration curve.

B. DETERMINATION OF PROTEIN CONCENTRATION OF UNKNOWN SAMPLE (FISH MYOFIBRILLAR PROTEIN EXTRACT)

Pipette 5 ml protein sample and 5 ml Reagent A into a test tube. To another test tube add 5 ml protein sample and 5 ml Reagent B. Also prepare another 2 test tubes each containing 5 ml KCl-phosphate buffered solution and 5 ml Reagent A and B, respectively. Shake well and leave to stand at room temperature for 2 hrs. Set up the spectrophotometer, adjust the wavelength to 545 nm, and read the absorbance of the solution relative to the reagent blank.

IV CALCULATIONS

Based on the calibration curve, express the result in mgN/ml. Convert the value to equivalent meat wt. and express as mgN/100 g sample, if required.

N.B. Experiments have shown that the results are relatively reliable as compared to results obtained by Kjeldahls' method for fish myofibrillar protein extract in the concentration range of 0.1-0.5 mgN/ml.

REFERENCE

Umemoto, S. (1966)
A modified method for estimation of fish muscle protein by Biuret method. Bull. Jap. Soc. Sci. Fish. 32:427-435.