12. Chemical Methods

Crude protein – Kjeldahl method, boric acid modification

1 Scope

Applicable to wheat and flour mill products only.

2 Apparatus

2.1 Kjeldahl flasks, Pyrex, 800 ml capacity; used for both digestion and distillation (distn).

2.2 Digestion heaters, 600 W (more or less, depending on voltage). Heater unit should boil 250 ml water starting at 25°C in 5 min with hot burners.

2.3 Digestion unit; consists of electric heaters, large lead tube, fume stack (plastic), and suction fan capable of exhausting toxic fumes to outside air.

2.4 Distn unit (see footnote); to consist of Iowa State type connecting bulbs (traps) 36 x 100 mm, Pyrex glass condenser tubes, pure gum-rubber stoppers and tubing, electric heating units (600 W), condenser tubes capable of being kept cool with adequate amts of cool water during distn and with thermo-water control on stills. Upper ends of bulbs or traps connect with high quality rubber tubing to condenser tubes and lower ends with rubber stoppers to 800 ml distn flask. Lower ends of condenser tubes have rubber-connected glass or polyethylene tubes that lead to:

2.5 Receiving bottles or flasks, 300 ml capacity.

2.6 Proper burettes for dispensing (a) conc. H_2SO_4, (b) conc. NaOH, (c) boric acid-indicator soln, and (d) class A burette for dispensing 0.1000 N H_2SO_4. (See 5.1).

3 Reagents

3.1 H_2SO_4, conc., 93-98 %, nitrogen-free.

3.2 Catalyst. Polyethylene packets contg 15 g potassium sulphate, 0.7 g mercuric oxide, and approx. 0.10 g pumice stone. (See 5.2).

3.3 Anti bumping agent. Either zinc metal, 20 mesh, or pumice stone (if pumice is not already combined in catalyst mixt).

3.4 NaOH, pellets or soln, nitrate-free. For soln, dissolve approx. 450 g solid NaOH in 1 L water. (Sp gr of soln should be 1.36 or more.) Since mercury is used as catalyst, add 80 g sodium thiosulphate per L to NaOH soln to ppt mercury.

3.5 Methyl red-methylene blue indicator. Mix 2 parts 0.2 % alc methyl red soln with 1 part 0.2 % alc methylene blue soln. Other indicators may be used satisfactorily. (See 5.3).

3.6 Std H_2SO_4, approx. 0.1 N but accurately stdze (See 5.4).

3.7 Boric acid-methyl red-methylene blue receiver soln. Add 360 g boric acid (crystals) and 48 ml methyl red-methylene blue indicator (reagent 3.5) to 18 L water. (See 5.5).

4 Procedure

4.1 Weigh quickly and accurately 1 g finely ground sample. Place in digestion flask. (Sample may be placed in nitrogen-free paper to prevent clinging to sides of flask.) Add polyethylene packet of catalyst, or equiv., and 25 ml conc. H_2SO_4 to flask (reagent 3.1). Digest till soln is clear and then 30 min longer; remove and cool but do not allow to crystallise.

4.2 Place 300 ml bottle or flask contg 50ml boric acid-methyl red-methylene blue indicator soln (reagent 3.7) under condenser tube with tip of condenser tube immersed under surface of soln. Add to original flask that is cooling 250-30 ml tap water and anti bumping agent, if not previously added. Gently add 50 ml conc. NaOH (reagent 3.4), connect to condenser with tight fitting rubber stopper, and swirl. Boil until all ammonia has dist (at least 150 ml of distillate), and then set receiving bottle down so that condenser tube is completely drained.

4.3 Titrate distillate to neutrality with std 0.1 N H_2SO_4, using burette graduated in 0.1 ml. Read ml of acid used, directly from burette.

4.4 Run blank detn periodically, using all ingredients except sample. Corr burette reading for nitrogen in reagents as shown by blank.

5 Notes

5.1 In routine testing of large no. of samples, use large dispensing burettes for conc. acid and alk and Schellbach automatic zero burets at titration table.

5.2 Precaution: Copper sulphate is recommended as a less hazardous catalyst than either mercury or selenium, or their compounds (see Ref. 4). Adequate exhaust ventilation must be provided in digestion-distn area. With mercury as catalyst and 40 min digestion time, use polyethylene packets contg 9.9 g potassium sulphate, 0.41 g mercuric oxide, 0.08 g copper sulphate, and approx. 0.10 g pumice stone.

5.3 Mixed indicator consisting of 0.75 g methyl red and 0.625 g Guinea green per L or 0.75 g methyl red and 0.5 g methylene blue (Ref. 8) dissolved in 300 ml alc may be used. Any indicator used should have sharp end point and distinct colour change. Use 35 ml to 18 L bottle.

5.4 Rodkey (Ref. 8) has successfully applied tris (hydroxymethyl) aminomethane as a convenient primary std for direct stdn of acid solns.

5.5 For proteins higher than 22 %, use 720 g (4 %) boric acid crystals and 48 ml methyl red-methylene blue indicator to 18 L bottle of water.

5.6 To check entire protein method, digest 0.1 g pure ammonium oxalate (monohydrate) with 2 g pure sugar using regular procedure. Resulting protein should be 11.24 % calcd as follows: $(NH_4)2C_2O_4 \bullet H_2O$ with mol wt of 142.12 contains 28.016 g nitrogen or 19.713 %; 19.713 % x 5.7 : 10(0.1g sample) = 11.25 % protein.

5.7 It is best that Boric Acid modification be used in air-conditioned laboratory (Ref. 5). Ammonia may be lost if contents of receiver flasks exceed 40 °C.

5.8 Use of 0.1253 N H_2SO_4 simplifies calculation by making % protein equal to the ml of 0.1253 N H_2SO_4 used for titrating sample, minus the ml used for titrating blank.

6 Calculation

$$\% \text{ Protein } \frac{(\text{ml std } H_2SO_4 \text{ x N of } H_2SO_4) \text{ x } 1.4007 \text{ x } 5.7}{\text{sample wt (g)}}$$

where: factor for wheat, flour, and bread = 5.7.

7 References

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