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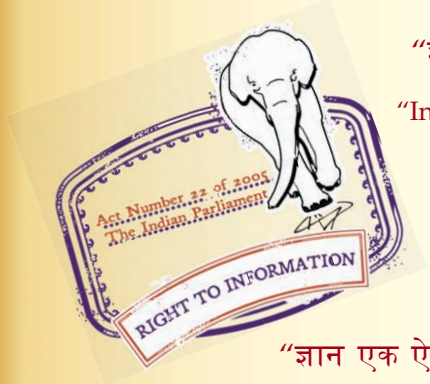

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IS : 7219 - 1973
(Reaffirmed 2005)

Indian Standard

METHOD FOR DETERMINATION OF PROTEIN IN FOODS AND FEEDS

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METHOD FOR DETERMINATION OF PROTEIN IN FOODS AND FEEDS

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Indian Standard

METHOD FOR DETERMINATION OF PROTEIN IN FOODS AND FEEDS

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 26 November 1973, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Protein is estimated by multiplying total nitrogen content with an appropriate conversion factor. The conversion factor for calculating total protein from nitrogen is generally taken to be 6·25. This factor is in fact a statistically determined average on the assumption that all proteins contain 16 percent nitrogen. The actual conversion factors for most of the foods and feeds have been worked out. For instance the conversion factor for milk is 6·38, for wheat and wheat products 5·70, for whole egg powder 6·68, etc. A comprehensive list of conversion factors is given in Table 1.

0.3 Since protein estimation is based on total nitrogen, it is necessary to standardize the procedure most suited to foods and feeds to get concordant results. This standard is based on ISO/TC 34 N 692 'Determination of total nitrogen protein in food and feeds by Kjeldahl method' issued by the International Organization for Standardization and prescribes full details for the determination of nitrogen by Kjeldahl method. This standard is also aligned with IS : 5194-1969*.

0.3.1 Nitrogen conversion factors given in Table 1 are largely based on Appendix II-B of ALINORM 74/26 issued by the Codex Alimentarius Commission, Rome. To this, some additions have been made from the following publications:

Official methods of analysis. Association of Official Analytical Chemists. Ed 11. 1970.

PEARSON (D). The chemical analysis of foods. Ed 6. 1970.

0.4 In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960†.

*Method for determination of nitrogen—Kjeldahl method.

†Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes Kjeldahl method for the determination of total nitrogen content and specifies the factors used in converting nitrogen to protein for various foods and feeds.

2. DEFINITION

2.1 Total protein, by the Kjeldahl method, is defined as the amount of nitrogen experimentally found and multiplied by an appropriate conversion factor (*see* Table 1).

3. PRINCIPLE

3.1 The sample is oxidized in the presence of sulphuric acid and nitrogenous compounds are converted into ammonium sulphate. Mercury is added to the digestion mixture as a catalyst and alkali sulphate (*see* **6.3**) as a boiling-point elevator. Ammonia is liberated by adding an excess of alkali and is quantitatively distilled into a measured volume of standard hydrochloric or sulphuric acid. The acid not neutralized by ammonia is back-titrated with standard alkali.

4. QUALITY OF REAGENTS

4.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1960*) shall be employed in the test.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

5. APPARATUS

5.1 For Digestion — Use 500- to 800-ml Kjeldahl flasks. Conduct digestion over a heating device adjusted to bring 250 ml water at 25°C to rolling boil in approximately 5 minutes. To test heaters, preheat for 10 minutes, if gas, or for 30 minutes if electric. Add 3 to 4 boiling chips or glass beads to prevent superheating.

5.2 For Distillation — Fit the flask with a rubber stopper through which passes the lower end of an efficient scrubber trap or bulb to prevent mechanical carryover of alkali during distillation. Connect the upper end of the trap to a condenser by rubber or glass tubing. Immerse the trap outlet of the condenser in such a way as to ensure complete absorption of ammonia distilled over into acid in a 500-ml Erlenmeyer flask (*see* Fig. 1).

6. REAGENTS

6.1 Concentrated Sulphuric Acid — 93 to 98 percent by mass, nitrogen-free.

*Specification for water, distilled quality (*revised*).

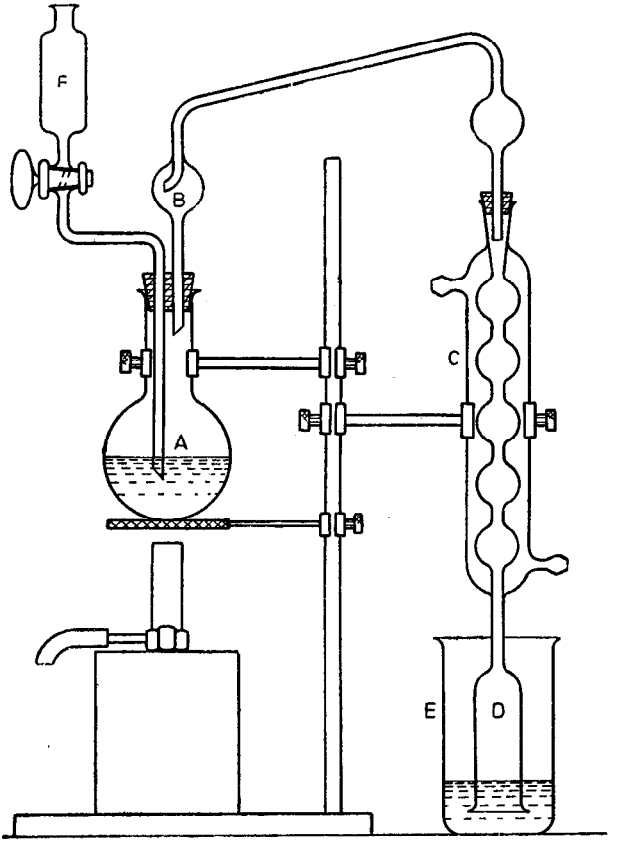

FIG. 1 DISTILLATION ASSEMBLY

6.2 Mercuric Oxide or Metallic Mercury — nitrogen-free.

6.3 Potassium Sulphate or Anhydrous Sodium Sulphate — nitrogen-free.

6.4 Zinc Granules

6.5 Sulphite or Thiosulphate Solution — Dissolve 40 g potassium sulphide or 80 g hydrated sodium thiosulphate in 1 litre distilled water.

6.6 Sodium Hydroxide — pellets, flakes or solution; nitrogen free. For solution, dissolve about 450 g solid sodium hydroxide in distilled water, cool, and dilute to 1 litre. The specific gravity should be at least 1·36 at 20°C.

6.7 Hydrochloric or Sulphuric Acid, Standard Solution — 0·1 or 0·5 N. Standardize against primary standard and against sodium hydroxide standard solution (*see* **6.8**).

6.8 Sodium Hydroxide Standard Solution — 0·1 N. Standardize against primary standard and against standard acid solution (*see* **6.7**).

6.9 Methyl Red Indicator — Dissolve 1 g methyl red in 200 ml alcohol.

7. SAMPLING

7.1 Prepare a representative and homogeneous sample as appropriate for the specific product to be analysed.

8. PROCEDURE

8.1 Digestion — Accurately weigh 0·7 to 2·2 g of the sample into the digestion flask. Add 0·7 g mercury oxide or 0·65 g mercury and 15 g powdered potassium sulphate or anhydrous sodium sulphate, and 25 ml sulphuric acid (*see* **6.1**). Ratio of salt to acid (*m/v*) should be approximately 1:1 at the end of digestion for proper temperature control. Digestion may be incomplete at a lower ratio and nitrogen may be lost at a higher ratio.

8.1.1 Each gram of fat consumes 10 ml and each gram of carbohydrate 4 ml sulphuric acid during digestion. Place the flask in an inclined position on a heater and heat gently until foaming ceases (*see* also **5.1**). A small amount of paraffin or silicon antifoam may be added to reduce foaming. Boil vigorously until the solution becomes clear and then continue boiling it for 1 to 2 hours.

8.2 Distillation — Cool, add about 200 ml distilled water, and in order to avoid complex formation, add 25 ml of the sulphide or thiosulphate solution. Mix to precipitate the mercury. Add a few zinc granules to prevent bumping, incline flask, and add without agitation 25 g of sodium hydroxide as solid or equivalent as solution, to make solution strongly alkaline (the thiosulphate or sulphide solution may be mixed with the sodium hydroxide solution before addition to the flask). Immediately connect flask to distillation bulb or trap on condenser, and, with tip of the condenser immersed in a measured quantity standard acid (usually 50 ml, 0·5 N or an appropriate quantity of 0·1 N) in the receiver, rotate flask to mix the contents thoroughly; then heat immediately until all ammonia has distilled over (at least 150 ml distillate). Lower the receiver before stopping distillation and wash tip of condenser with distilled water. Back-titrate excess acid with standard 0·1 N sodium hydroxide, using methyl red as indicator. Correct for blank determination in reagents.

8.3 Blank — Conduct determinations using all reagents and 2 g of sugar.

9. CALCULATION, EXPRESSION AND INTERPRETATION OF RESULTS

9.1 Methods of Calculation and Formulae

9.1.1 *Calculation of Nitrogen Content*

Nitrogen content (N) in g $=(a-0{\cdot}2b)-(c-0{\cdot}2d)\times 0{\cdot}007$

where

a = volume in ml 0·5 N acid measured for main distillation,

b = volume in ml 0·1 N alkali used for back-titrating a,

c = volume in ml 0·5 N acid measured for blank distillation, and

d = volume in ml 0·1 N alkali used for back-titrating c.

OR

Nitrogen content (N) in g $=(A-B)-(C-D)\times 0{\cdot}001\ 4$

where

A = volume in ml 0·1 N acid measured for main distillation,

B = volume in ml 0·1 N alkali used for back-titrating A,

C = volume in ml 0·1 N acid measured for blank distillation, and

D = volume in ml 0·1 N alkali used for back-titrating C.

9.1.2 *Calculation of Total Protein*

$$\text{Protein, percent by mass} = \frac{N\times 100\times \text{Conversion factor}}{W}$$

where

N = mass of nitrogen content in g of original sample, and

W = mass of sample in g.

9.2 Reporting — Report nitrogen to nearest 0·01 percent and protein to nearest 0·05 percent.

9.3 Precision of Method — Duplicate determinations of the nitrogen should agree within 0·05 percent nitrogen.

TABLE 1 FACTORS USED IN CONVERTING NITROGEN TO PROTEIN

(*Clauses* 0.2, 0.3.1 *and* 2.1)

Sl No.	Food and Feedingstuff	Conversion Factor for Protein	Correction Factor for Conversion of Reporting Protein to Crude Protein
(1)	(2)	(3)	(4)
i)	*Wheat* (*hard, medium or soft*):		
a)	Whole meal or flour or bulgur	5·83	1·07
b)	Flour, medium or low extraction	5·70	1·10
c)	Macaroni, spaghetti, vermicelli wheat paste, bread	5·70	1·10
d)	Bran	6·31	0·99
ii)	*Maize*:	6·39	—
	Maize gluten, maize germ oilcake	6·25	—
iii)	*Rye*:		
a)	Whole meal, dark flour	5·83	1·07
b)	Flour, medium extraction		
c)	Flour, light or low extraction		
iv)	*Rice*:		
a)	Husked or brown (only hulls removed)		
b)	Home pounded, undermilled, parboiled, milled, white	5·95	1·05
c)	Rice polish, rice bran	6·25	—
v)	*Barley*:		
	Whole seed, except hulls and groats, pearled, light or dark	5·83	1·07
vi)	*Oatmeal, rolled oats*:	5·83	1·07
vii)	*Pulses*:		
	MUNG, TUR, URAD, Gram, *MASOOR, BESAN*	6·25	—
viii)	Cashew	5·30	—
ix)	Almond	5·18	1·21
x)	Brazil nut	5·46	1·14
xi)	Coconuts (outer husk removed), old ripe, in shell; young under ripe, in shell	5·30	1·18
xii)	Chestnut, fresh or dry		
xiii)	Other tree nuts		

(*Continued*)

TABLE 1 FACTORS USED IN CONVERTING NITROGEN TO PROTEIN — *Contd*

Sl No.	Food and Feedingstuff	Conversion Factor for Protein	Correction Factor for Conversion of Reporting Protein to Crude Protein
(1)	(2)	(3)	(4)
xiv)	*Oil seeds, oilcakes and edible flours*:		
a)	Groundnut	5·46	1·14
b)	Sesame, safflower, sun flower	5·30	1·18
c)	Castor, cottonseed, linseed		
d)	Soyabean (seeds, flour or products)	5·71	1·09
xv)	*Milk and milk products*:		
	Milk, all species, fresh or dry, cheese, hard or soft, whey cheese, malted milk, condensed milk	6·38	0·98
xvi)	*Oils and fats*:		
a)	Margarine (either vegetable or animal)	6·38	0·98
b)	Butter		
xvii)	*Poultry and poultry products*:		
a)	Egg white	6·70	—
b)	Egg yolk	6·62	—
c)	Egg whole, egg powder	6·68	—
d)	Chicken essence	6·68	—
xviii)	*Meat and meat products*:		
	Meat (mutton, beef)	5·80	—
xix)	*Gelatin*	5·55	—
xx)	*Other products*:		
a)	Dried yeast	6·25	—
b)	Beer		
c)	Wine		
d)	Tea		
e)	Cocoa		
f)	Coffee		
g)	Malt		
h)	Guar meal		
j)	*TUR* husk		
k)	Cottonseed oilcake		
m)	Coconut oilcake		

(*Continued*)

TABLE 1 FACTORS USED IN CONVERTING NITROGEN TO PROTEIN — *Contd*

Sl No.	Food and Feedingstuff	Conversion Factor for Protein	Correction Factor for Coversion of Reporting Protein to Crude Protein
(1)	(2)	(3)	(4)
xx)	*Other products — Contd*		
n)	Cottonseed flour	6·25	—
p)	Groundnut flour		
q)	Groundnut oilcake		
r)	Linseed oilcake		
s)	Sesamum oilcake		
t)	Mustard and rape seed		
u)	Nigerseed oilcake (meal)		
v)	Gram husk		
w)	Gram *CHUNI*		
y)	Tapioca spent pulp, as feed		
z)	Tapioca chips, as feed		

NOTE 1 — Problem will arise where a food is a mixture of wheat cereal, soya and milk-derived protein. It is, therefore, recommended that where the food is composed of more than, for example, 90 percent by mass of either wheat cereal, soya or milk-derived ingredients, the protein factors shall be 5·7, 6·25 and 6·38 respectively. It is further recommended that where the food comprises, for example, 80 percent by mass of either wheat cereal, soya or milk or mixture thereof, the protein factor employed shall in all cases be 6·25. It is also recommended that where a manufacturer knows precisely the amount of each wheat cereal, soya or milk-derived ingredients in his formulation, protein factor employed shall be derived proportionately using the wheat cereal, soya and milk factors of 5·7, 6·25 and 6·38 respectively.

NOTE 2 — The conversion factor adopted for computing protein from nitrogen values should be included in the test report.

INDIAN STANDARDS
ON
FOOD HYGIENE, SAMPLING AND ANALYSIS

IS:

2491-1972 Code for hygienic conditions for food processing units (*first revision*)
5059-1969 Code for hygienic conditions for large scale biscuit manufacturing units and bakery units
5398-1969 Methods for estimation of thiamine (vitamin B_1) in foodstuffs
5399-1969 Methods for estimation of riboflavin (vitamin B_2) in foodstuffs
5400-1969 Methods for estimation of nicotinic acid (niacin) foodstuffs
5835-1970 Method for estimation of vitamin D in foodstuffs
5837-1970 Code for hygienic conditions for soft drinks manufacturing units
5838-1970 Method for estimation of vitamin C in foodstuffs
5839-1970 Code for hygienic conditions for manufacture, storage and sale of ice-creams
5886-1970 Method for estimation of carotenes and vitamin A (retinol) in foodstuffs
6540-1972 Code for hygienic conditions for manufacture and handling of ice for human consumption
6541-1972 Code for hygienic conditions for establishment and maintenance of midday school meal programmes
6542-1972 Code for hygienic conditions for fruit and vegetable canning units
6968-1973 Code for hygienic conditions for *PAN* (betel) stalls and vendors
6969-1973 Code for hygienic conditions for handling and sale of refrigerated drinking water
7003-1973 Code for hygienic conditions for sago (*SABOODANA*) manufacturing units
7005-1973 Code for hygienic conditions for production, processing, transportation and distribution of milk
7234-1974 Method for estimation of folic acid in foodstuffs
7235-1974 Method for estimation of tocopherols (vitamin E) in foodstuffs

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