

*Indian Standard*

**SPECIFICATION FOR EDIBLE GROUNDNUT FLOUR (EXPPELLER PRESSED)**

*(First Revision)*

**0. FOREWORD**

**0.1** This Indian Standard (First Revision) was adopted by the Indian Standards Institution on 30 October 1975, after the draft finalized by the Nutrition Sectional Committee had been approved by the Agricultural and Food Products Division Council.

**0.2** Edible groundnut flour is well recognized as a rich source of dietary protein. Its properties, such as taste, odour and colour have led to its use in many food products like biscuits, BALAHAR and other snack foods.

**0.3** This Indian Standard was first published in 1968. Consequent upon improvement in the quality of the edible groundnut flour produced commercially, it was felt that the standard required to be modified. Accordingly, 1975 revision was issued. In this revision the requirement for available lysine has been excluded, limit for aflatoxin reduced and description of raw material preparation elaborated.

**0.4** One of the toxicants which is usually present in edible groundnut protein products is aflatoxin, produced by a fungus (*Aspergillus flavus*). It is now well-established that aflatoxin is harmful to human beings when ingested even in minute quantities. It, therefore, becomes imperative that the edible flour is produced under strictly controlled hygienic conditions. Under optimum conditions of growth, harvesting, drying, the toxin content can be almost negligible. At the same time several methods have been developed for detoxification of the aflatoxin present in edible groundnut flour, by treatment with chemicals like ammonia, hydrogen peroxide and certain oxidizing agents. Simultaneously, these treatments bring about a lowering of the nutritive value of the edible groundnut protein products through destruction of the sulphur amino acids. Therefore, safe methods of making edible groundnut protein products of good nutritive value and low aflatoxin content are either by treating groundnut pods in the field to avoid fungal contamination, or by manually removing fungus-affected kernels before processing the remainder, or by using groundnut naturally resistant to fungus. At present, manual removal of fungus-affected kernel is adopted for commercial production of edible groundnut flour, and products with an aflatoxin content well below the prescribed limit are regularly obtained.

**0.5** A separate Indian Standard (IS 1875 : 1975\*) for solvent extracted (variety of groundnut flour) has also been formulated.

**0.6** Due to increasing export market demand of the product, new technological developments and to meet other standard norms of same and similar product , This standard is again being revised in 2022. This standard has been formulated in close collaboration with the Protein Foods and Nutrition Development Association of India. While formulating this standard due consideration has been given to Food safety Standards and other international standards to upgrade the quality and meet norms for export market

**0.7** For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of test or analysis, shall be rounded off in accordance with IS 2 : 1960. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

## 1. SCOPE

1.1 This standard prescribes the requirements, and the methods of sampling and test for edible groundnut flour (expeller pressed).

## 2. REQUIREMENTS

2.1 **Raw Material** - Expeller pressed edible groundnut flour shall be made by expelling in a screw press fresh, clean degermed groundnut kernels which have been decuticled after mild roasting. In order to reduce the proportion of immature, shrivelled and mouldy kernels which could carry high levels of aflatoxin, the kernels shall be graded as per Table 1 and selected as follows, either by visual inspection, inspection under ultraviolet light, electronic sorting or by other means. (Pls see Annex K). The kernels shall be free from insect or fungal infestation.

Sr No	Factor	Requirement		
		Grade 1	Grade 2	Grade 3
1	Damaged kernels % by mass max	0.5	1.0	1.0
2	Other defects % by mass Max	0.5	1.0	1.0
3	Split and broken kernels % by mass max	1.0	2.0	3.0
4	Foreign matter % by mass Max	0.1	0.2	0.3
5	Total tolerance for factors 1,2,3& 4	2.0	3.0	4.0
6	Moisture % by mass Max	7.0	7.0	7.0

2.2 **Description** - Expeller pressed edible groundnut flour shall be whitish to light brown in colour, uniform in composition and shall be free from insect or fungal infestation, objectionable odour and rancid taste. It shall not contain added flavouring and colouring agents or any other extraneous matter. Permitted colouring and flavouring additives permitted under the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 may be added depending on end use.

2.2.1 Expeller pressed edible groundnut flour shall be free from castor husk or MAHUA oilcake when tested according to the methods prescribed in IS 7874 : Part 1 : 1975

### 2.3 Particle Size -

Unless otherwise specified, the material shall be of such fineness that mutually accepted by the customer .

**2.4 Premises** - Place where manufacture, packing and storage of the material is done, and the equipment employed, shall be maintained under hygienic conditions (*see* IS 2491 : 2013). Code of practice for control of aflatoxin in groundnut: Part 2 plant storage and processing flour and oil as per IS 9071 : Part 2:1979 may be implemented at the premises.

**2.5** Edible groundnut flour (expeller pressed) shall also comply with the requirements given in Table 1.

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‡Method of sampling and test for animal feeds and feeding stuffs: Part 1 General method

**TABLE 1 REQUIREMENTS FOR EDIBLE GROUNDNUT FLOUR  
(EXPELLER PRESSED)**

Sl. No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST
(1)	(2)	(3)	(4)
i)	Moisture, percent by mass, Max	7.0	Annexure A
ii)	Protein (N X 6.25) (on dry basis), percent by mass, Min	45.0	Annexure B
iii)	Total ash (on dry basis), percent by mass, Max	4.5	Annexure C
iv)	Acid-insoluble ash (on dry basis), percent by mass, Max	0.35	Annexure D
v)	Fat ( on dry basis), percent by mass, Max	9.0	Annexure E
vi)	Acid value of extracted fat, Max	6.0	G
vi)	Crude fibre (on dry basis), percent by mass, Max	5.0	Annexure F
vii)	Free Fatty Acids (FFA) content, % by mass max.	4.0	IS 548 (Part 1/Sec 2) : 2021

**2.6 Bacteriological Requirements** - The edible groundnut flour (expeller pressed) shall be tested periodically to comply with the requirements given in Table 2.

**TABLE 2 BACTERIOLOGICAL REQUIREMENTS FOR EDIBLE GROUNDNUT FLOUR (EXPELLER PRESSED)**

*(Clause 2.6)*

Sl. No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST, REF TO
(1)	(2)	(3)	(4)
i)	Total bacterial count per g, Max	10 000	IS 5402 : Part 1 : 2021
ii)	Coliform bacterial count per G, Max	10	IS 5401 : Part 1 : 2012
iii)	Salmonella bacteria	Nil	IS 5887 : Part 3 : 1999
iv)	Ecoli, per 10 g max	Absent	IS 16067-1 (2013)
v)	Staphylococcus, per g max	Absent	IS 5887 : Part 8 : Sec 1 : 2002
vi)	Yeast and Mould, per g max	10000	IS 5403:1999

**2.7 Contaminants**

**2.7.1 Pesticide residues:** The edible groundnut flour (expeller pressed) shall comply with Table 4

**2.7.2 Heavy Metals:** The edible groundnut flour (expeller pressed) shall comply with Table 4

**2.7.3 Mycotoxin-** The edible groundnut flour (expeller pressed) shall comply with Table 5

**TABLE 4 REQUIREMENTS FOR MAXIMUM RESIDUAL LEVEL OF AGROCHEMICALS AND HEAVY METALS.**

Sr No	Parameter	Requirement	Test method
1	Lead	0.1 ppm	Any international Test method
2	Arsenic	0.1 ppm	Any international Test method
3	Epoxyconazole	0.05*	IS 13830:1993 or any international test method

\* Maximum Residue Limit fixed at Limit of Quantification (LOQ)

**TABLE 5- REQUIREMENTS FOR MYCOTOXINS**

Sr No	Mycotoxin	Requirement	Test method
1	Total Aflatoxin, $\mu$ g/kg, Max	20	IS 16287:2015
2	Aflatoxin B1, $\mu$ g/kg, Max	5	IS 16287:2015

### 3. PACKING AND MARKING

**3.1 Packing** - The edible groundnut flour (expeller pressed) shall be packaged in food grade materials as agreed between manufacturer and customer , that will safeguard hygienic, nutritional, technological and organoleptic qualities of the product. Each package shall be securely closed and sealed.

**3.2 Marking** - The following particulars shall be marked or labelled on each container:

- a) Name of the material,
- b) Name and address of the manufacturer,
- c) Batch or code number,
- d) Net mass, and
- e) Date of manufacture.
- f) Expiry date
- g) Country of manufacture
- h) Food additives if any added
- i) Storage instructions

### 3.3 BIS Certification Marking

The product may also be marked with Standard Mark.

**3.3.1** The use of the Standard Mark is governed by the provisions of the Bureau of Indian Standards Act, 2016 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

### 4. SAMPLING

**4.1** Representative samples of the material shall be drawn and tested for conformity to this standard as prescribed in IS 4115 :1967.

### 5. TESTS

**5.1** Tests shall be carried out as prescribed in 2.2 and 2.3, and Tables 1, 2, 3 and 4

**5.2 Quality of Reagents** - Unless specified otherwise, pure chemicals and distilled water ( see IS 1070 : 1992 ) shall be employed in tests.

## ANNEXURE A

(Clause 2.3)

### DETERMINATION OF PARTICLE SIZE

#### A-1. TEST SIEVES

A-1.1 Make a nest of two test sieves, the upper being 710 micron IS Sieve and the lower 500 micron IS Sieve provided with a cover and receiver.

NOTE – In case the prescribed IS Sieves are not available, the following corresponding sieves, which have their apertures within the limits specified for the prescribed one, may be used:

IS Sieve	Corresponding Sieve
710 micron	BS Test Sieve 22, ASTM Sieve 25 or Tyler Test Sieve 24
500 micron	BS Test Sieve 50, ASTM Sieve 35 or Tyler Test Sieve 52

#### A-2. PROCEDURE

A-2.1 Weigh accurately about 100 g of the material and transfer to the upper sieve. Fit the upper sieve with the cover, place the nest of sieves in a suitable mechanical sieve shaker and sieve the material continuously for five minutes. Stop sieving, remove the nest of sieves and transfer the residue on each sieve separately to two tared weighing dishes using a brush. Weigh each dish.

#### A-3. CALCULATION

A-3.1 Calculate the percentage of the material retained on the respective sieves as follows:

a) Material retained on 710 micron

$$\text{IS Sieve, percent by mass} = \frac{100 M_1}{M}$$

where

$M_1$  = mass in g of the material retained on 710 micron IS Sieve, and

$M$  = mass in g of the material taken for the test.

b) Material retained on 500 micron

$$\text{IS Sieve, percent by mass} = \frac{100 M_2}{M}$$

where

$M_2$  = mass in g of the material retained on 500-micron IS Sieve, and  
 $M$  = mass in g of the material taken for the test.

## ANNEXURE A

[Table 1, Item (i) ]

### DETERMINATION OF MOISTURE

#### B-1. PROCEDURE

**B-1.1** Weigh accurately about 10 g of the material in a dish made of porcelain, silica or platinum, previously dried in an air-oven and weighed. Place the dish in an air-oven maintained at  $105 \pm 2^\circ\text{C}$  for five hours. Cool the dish in a desiccator and weigh with the lid on. Heat again at  $105 \pm 2^\circ\text{C}$  in the air-oven for 30 minutes. Cool the" dish in the desiccator and weigh. Repeat this process of heating for 30 minutes, cooling and weighing till the difference in mass between two successive weighings is less than one milligram. Note the lowest mass.

NOTE - Preserve the dish containing this dried material for determination of total ash (*see* D-1.1).

#### B-2. CALCULATION

**B-2.1** Calculate the moisture as follows:

$$\text{Moisture, percent by mass} = \frac{100(M_1 - M_2)}{M_1 - M}$$

where

$M_1$  = mass in g of the dish with the material before drying,

$M_2$  = mass in g of the dish with the material after drying, and

$M$  = mass in g of the empty dish.



## ANNEXURE B

[Table 1, Item (ii)]

### DETERMINATION OF PROTEIN

#### C-1. APPARATUS

C-1.1 A recommended apparatus, as assembled, is shown in Fig. 1.

C-1.1.1 *Description* - The assembly consists of a round-bottom flask A of 1000-ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube B. The other end of the bulb tube B is connected to the condenser C which is attached by means of a rubber tube to a dip tube D which dips into a known quantity of standard sulphuric acid contained in a beaker E of 250-ml capacity.

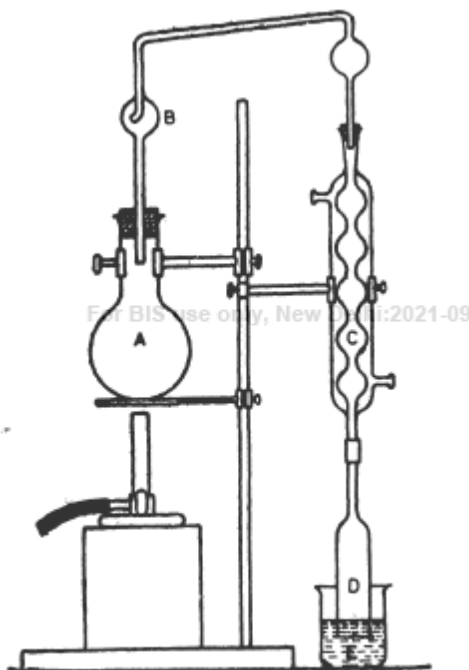


FIG. 1 APPARATUS FOR DETERMINATION OF PROTEIN

C-1.2 Kjeldahl Flask - of capacity 500 ml.

#### C-2. REAGENTS

C-2.1 Anhydrous Sodium Sulphate

C-2.2 Copper Sulphate

C-2.3 Concentrated Sulphuric Acid - sp gr 1.84.

**C-2.4 Sodium Hydroxide Solution** - Dissolve about 225 g of sodium hydroxide in 500 ml of water.

**C-2.5 Standard Sulphuric Acid** – 0.1 N.

**C-2.6 Methyl Red Indicator Solution** - Dissolve 1 g of methyl red in 200 ml of rectified spirit (95 percent by volume).

**C-2.7 Standard Sodium Hydroxide Solution** – 0.1 N.

### **C-3. PROCEDURE**

**C-3.1** Transfer carefully about 0.25 g of the material, accurately weighed, to the Kjeldahl flask, taking precaution to see that particles of the material do not stick on to the neck of the flask. Add about 10 g of anhydrous sodium sulphate, about 0.2 to g of copper sulphate and 20 ml of concentrated sulphuric acid. Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase the heat until the acid boils vigorously and digest for 30 minutes after the mixture becomes clear and pale green or colourless. Cool the contents of the flask. Transfer it quantitatively to the round-bottom flask with water, the total quantity of water used being about 200 ml, Add with shaking a few pieces of pumice stone to prevent bumping.

Add about 50 ml of sodium hydroxide solution (which is sufficient to make the, solution alkaline) carefully through the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid layer. Assemble the apparatus as shown in Fig. 1, taking care that the dip tube extends below the surface of known quantity of standard sulphuric acid contained in the beaker. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Shut off the burner and immediately detach the flask from the condenser. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker.

When all the washings have drained into the beaker, add two or three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution.

**C-3.1** Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

### **C-4. CALCULATION**

**C-4.1** Calculate protein from the following formula:

$$\text{Protein (on dry basis) ,percent by mass} = \frac{875 (B-A)N}{M(100-m)}$$

where

B = volume in ml of the standard sodium hydroxide solution used to neutralize the acid in the blank determination;

A = volume in ml of the standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material;

N = normality of the standard sodium hydroxide solution;

M = mass in g of the material taken for the test; and

m = moisture, percent by mass, in the material (*see B-2.1*).

## ANNEXURE C

[Table 1, Item (iii) ]

### DETERMINATION OF TOTAL ASH

#### D.1. PROCEDURE

**D.1.** Ignite the dried material in the dish (8-1.1) with the flame of a suitable burner for about one hour. Complete the ignition by keeping in a muffle furnace at 550 to 600°0 until grey ash results. 0001 in a desiccator and weigh. Repeat the process of igniting, cooling and weighing at half-hour intervals until the difference in mass between two successive weighings is less than one milligram. Note the lowest mass.

NOTE - Preserve the dish containing this ash for the determination of acid insoluble ash (**E-2.1**).

#### D.2. CALCULATION

**D-2.1** Calculate total ash as follows:

$$\text{Total ash (on dry basis), percent by mass} = \frac{100(M_2 - M)}{M_1 - M}$$

where

M<sub>2</sub> = mass in g of the dish with the ash,

M = mass in g of the empty dish? and

M<sub>1</sub> = mass in g of the dish with the dried material taken for the test ( M<sub>2</sub> under **B-2.1**).

## ANNEXURE D

[Table 1, Item (iv)]

### DETERMINATION OF ACID INSOLUBLE ASH

#### E-1. REAGENT

**E-1.1 Dilute Hydrochloric Acid** - approximately 5 N, prepared from concentrated hydrochloric acid (see IS 265: 2021).

#### E-2. PROCEDURE

**E-2.1** Add 25 ml of dilute hydrochloric acid to the ash contained in the dish (**D-1.1**), cover with a watch-glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through a Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid. Return the filter and the residue to the dish. Keep it in an electric air-oven maintained at  $135 \pm 2^\circ\text{C}$  for about 3 hours. Ignite in a muffle furnace at 550 to  $600^\circ\text{C}$  for one hour. Cool the dish in a desiccator and weigh. Repeat the process of igniting in the muffle furnace, cooling and weighing at half-hour intervals until the difference in mass between two successive weighings is less than one milligram. Note the lowest mass.

#### E-3. CALCULATION

**E-3.1** Calculate the ash content from the following formula:

$$\text{Acid-insoluble ash (on dry basis), percent by mass} = \frac{100(M_2 - M)}{M_1 - M}$$

Where

$M_2$  = mass in g of the dish with the acid-insoluble ash,

$M$  = mass in g of the empty dish, and

$M_1$  = mass in g of the dish with the dried material ( $M_2$  under **B-2.1**).

## ANNEXURE E

[Table 1, Item (v) ]

### DETERMINATION OF FAT

#### F-1. APPARATUS

##### F-1.1 Soxhlet Extraction Apparatus

#### F-2. SOLVENT

##### F-2.1 Ethyl Ether, or Petroleum Ether - distilling below 65°C.

#### F-3. PROCEDURE

**F-3.1** Transfer about 10g of the material accurately weighed to a suitable thimble and extract with the solvent in a Soxhlet extraction apparatus for about 16 hours. Dry the extract contained in the Soxhlet flask, whose empty mass has been previously determined at 95 to 100°C for 30 minutes. Cool in a desiccator and weigh. Continue drying and weighing alternately at 30-minute intervals until the loss in mass between two successive weighings is not more than one milligram. Record the lowest mass.

NOTE - Preserve the material in the thimble for estimation of crude fibre (*see* Annexure H).

#### F-4. CALCULATION

**F-4.1** Calculate the fat as follows:

$$\text{Fat, percent by mass} = \frac{100(M_1 - M_2)}{M}$$

where

$M_1$  = mass in g of the Soxhlet flask with the extracted fat;

$M_2$  = mass in g of the empty Soxhlet flask, clean and dry; and

$M$  = mass in g of the material taken for the test.

## ANNEXURE F

[Table 1, Item (vi)]

### DETERMINATION OF ACID VALUE

#### G-1. APPARATUS

##### G-1.1 Soxhlet Fat Extraction Apparatus

#### G-2. REAGENTS

G-2.1 Petroleum Ether – distilling below 65°C.

G-2.2 Benzene-Alcohol-Phenolphthalein Solution—Add 0.4 g of phenolphthalein to one litre of benzene and one litre of ethyl alcohol to form 0.02 percent solution.

G-2.3 Standard Potassium Hydroxide Solution—0.2 N, carbon dioxide free.

G-2.4 Standard Potassium Permanganate Solution—0.01 percent.

#### G-3. PROCEDURE

G-3.1 Extract  $10.00 \pm 0.01$  g of the sample taken in a thimble with petroleum ether for about 16 hours in a Soxhlet extraction apparatus. Completely evaporate the solvent from extraction flask (weighed previously) on a steam bath, cool and weigh the Soxhlet flask with the residue and from the difference of the mass find out the mass of fat obtained from the material. Dissolve the residue in the extraction flask with 50 ml of the benzene-alcohol-phenolphthalein solution, Titrate the dissolved extract with standard potassium hydroxide solution to distinct pink colour, or in the case of yellow solution to orange pink colour. If emulsion is formed during titration, dispel by adding second 50 ml portion of the benzene-alcohol-phenolphthalein solution. The end-point should match colour of the solution made by adding 2.5 ml of standard potassium permanganate solution to 50 ml of potassium dichromate solution of proper strength to match colour of original solution being titrated. (Add 0.5 percent potassium dichromate solution dropwise to 50 ml of water until the colour matches. Then add 2.5 ml of standard potassium permanganate solution).

G-3.2 Make a blank titration on 50 ml of the benzene-alcohol-phenolphthalein solution and subtract this value from the titration value of the sample, If the additional 50 ml portion of the benzene-alcohol-phenolphthalein solution is added, double the blank titration.

#### G-4. CALCULATION

G-4.1 Calculate the acid value from the following formula:

$$\text{Acid value (as oleic acid)} = \frac{56.4 V f}{M}$$

where

$Y$  = volume in ml of standard potassium hydroxide solution used,

$N$  = normality of standard potassium hydroxide solution, and

$M$  = mass in g of the material taken for the test.

## ANNEXURE F

[Table 1, Item (vii) ]

### DETERMINATION OF CRUDE FIBRE

#### H-1. REAGENTS

**H-1.1 Dilute Sulphuric Acid** – 1.25 percent (m/v), accurately prepared.

**H-1.2 Sodium Hydroxide Solution** – 1.25 percent (m/v), accurately prepared.

**H-1.3 Ethyl Alcohol** - 95 percent by volume.

**H-1.4 Petroleum Ether** - distilling below 65°C.

#### H-2. PROCEDURE

**H-2.1** Dry to constant mass about 5 g of the material on an electric oven at  $105 \pm 1^\circ\text{C}$ . Weigh accurately about 2.5 g of the dried material on a thimble, extract it with petroleum ether free of fat on a Soxhlet apparatus and transfer it to a one-litre flask. Take 200 ml of dilute sulphuric acid in a beaker and bring to the boil. Transfer the whole of the boiling acid to the flask and immediately connect the flask with a water-cooled reflux condenser and heat, so that the contents of the flask begin to boil within one minute, Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen (about 18 threads to a centimetre) held in a funnel, and wash with boiling water until the washings are no longer acid to litmus. Bring to the boil 200 ml of sodium hydroxide solution under a reflux condenser, Wash the residue on the linen into the flask with the boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with boiling water, and transfer to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15 ml of ethyl alcohol. Dry the Gooch crucible and contents at  $105 \pm 2^\circ\text{C}$  in an air-oven to constant mass, Cool and weigh. Incinerate the contents of the Gooch crucible in an electric muffle furnace at  $600 \pm 2^\circ\text{C}$  until all the carbonaceous matter is burnt. Cool the Gooch crucible containing the ash in a desiccator and weigh.

### H-3. CALCULATION

H-3.1 Calculate the crude fibre as given below:

$$\text{Crude fibre (on dry basis), percent by mass} = \frac{100(M_1 - M_2)}{M}$$

where

$M_1$  = mass in g of the Gooch crucible and contents before ashing;

$M_2$  = mass in g of the Gooch crucible and contents after ashing; and

$M$  = mass in g of the dried material taken for the test.



## ANNEXURE G

{Table 1, Item (viii)}

### DETERMINATION OF AFLATOXIN

#### J-1. PRINCIPLE OF THE METHOD

**J-1.1** The method involves direct extraction of a water-wetted sample by chloroform with the use of silica gel column for defatting and clean up for obtaining clean quantitative extracts of aflatoxin from 50 g of groundnut product samples for thin layer chromatography. The low level of interference permits the determination and detection of as little as 1 µg of aflatoxin per kilogram of groundnut product sample.

#### J-2. APPARATUS

##### J-2.1 Disc Mill

**J-2.2 Wrist Action Shaker**—having a capacity of 1 400 to 1 600 rev/min.

**J-2.3 Chromatographic Column**—of silica gel.

**J-2.4 Funnel**—having a diameter of 150 mm to fit fluted filter paper. Buchner funnel of 320 mm in diameter to fit Whatman No. 1 or equivalent filter paper may also be used.

##### J-2.5 Rotary Evaporator with Continuous Feed

##### J-2.6 Thin-Layer Chromatographic Apparatus

**J-2.6.1 Glass Plates**—200 × 200 mm.

**J-2.6.2 Applicator**

**J-2.6.3 Mounting Board**

**J-2.6.4 Spotting Template**

**J-2.6.5 Microsyringe**—10 µl.

**J-2.6.6 Desiccating Storage Cabinet**

**J-2.6.7 Storage Rack**

**J-2.6.8 Long Wave 15-Watt Ultraviolet Lamp or Chromato-View Cabinet**—equipped with one or two 15-watt lamps.

#### J-3. REAGENTS

**J-3.1 Silica Gel**—for chromatography 0.15 to 0.2 mm.

**J-3.2 Chloroform**

**J-3.3 Hexane**

**J-3.4 Diethyl Ether**

**J-3.5 Methyl Alcohol**

**J-3.6 Acetone**

**J-3.7 Sodium Sulphate (Anhydrous)**

**J-3.8 Bolling Chips**

**J-3.9 Aflatoxin Standard Solution**—Prepare from pure crystalline aflatoxine solutions containing 0.5 µg of aflatoxin B<sub>1</sub> and 0.4 µg of aflatoxin G<sub>1</sub> per millilitre of chloroform. If pure crystalline aflatoxin is not available, use extracts of known aflatoxin content. Concentrations of 0.5 to 1.5 µg of B<sub>1</sub> and 0.3 to 1.0 µg of G<sub>1</sub> per millilitre are satisfactory. Keep exposure to light at minimum. Store at freezing temperature in glass stopper volumetric flask. Place in a closed jar containing a small amount of chloroform which should be replaced periodically. Chloroform in outside jar eliminates concentration of standard solution by evaporation and the jar prevents contamination with condensing water as standard solution is warm to room temperature each time it is used.

## **J-4. PROCEDURE**

**J-4.1 Preparation of the Silica Gel Plate**—Weigh 30 g of silica gel into a 300 ml glass stoppered Erlenmeyer flask. Add 60 ml of distilled water. Shake vigorously for not more than a minute and pour into the applicator. Immediately coat five 200 × 200 mm glass plates with 0.25 mm thickness of silica gel suspension, and let the plates rest undisturbed until gelled. Dry the coated plates for at least 2 hours at 80°C and store in the desiccating cabinet with active desiccant until just before use.

**J-4.1 Preparation of the Sample**—Groundnut butters and ground nut meals need no preparation for extraction unless they contain large particles, in which case some type of milling operation should be used to reduce the particle size. A hammer mill or rotary cutter is used for meals. Samples of raw and roasted groundnut should be ground to a paste before extraction. For this operation and for chunky type groundnut butter, a disc type mill is used.

**J-4.3 Extraction**—Weigh a 50-g sample (groundnut butter, groundnut meal, or finely ground groundnuts) into a 500-ml glass stoppered Erlenmeyer flask. Add 25 ml of distilled water and mix with a spatula until visually uniform. Add 250 ml of chloroform and tape the stopper in place. Shake for 30 minutes on a wrist-action shaker and filter through a fluted filter paper. Collect the first 50 ml portion of clear chloroform. This is the sample extract. Prepare the silica gel column

by placing a ball of glass wool loosely in the bottom of a 22 × 300 mm chromatographic column and add 5 g of anhydrous sodium sulphate to give an even base for the silica gel. Add chloroform until the column is about two-thirds full; then add 10 g of silica gel (0.05 to 0.20 mm) with stirring to prevent air entrapment. Let silica gel settle and slowly add 15 of anhydrous sodium sulphate. Draw off chloroform to the top of the silica gel. Add the sample extract to the column and elute at a flow rate of 10 to 20 ml per minute with 150 ml of hexane followed by 150 ml of anhydrous diethyl ether. Finally, elute the aflatoxin with 150 ml of methyl alcohol or chloroform (3/97, v/v). Collect this fraction from the time the methyl alcohol or chloroform was added until the flow stops in a vial, evaporate to near dryness and cap it.

**J-4.3.1** The sample extract may now be prepared in the appropriate manner for quantitative assay.

**J-4.4 Qualitative Determination** – For preparing sample extract, uncap the vial containing dry sample extract. Add to it 500 µl of chloroform, and reseal with polyethylene stopper. Puncture polyethylene stopper to accommodate the needle of 10 µl syringe. Spot as rapidly as possible in subdued incandescent light 2, 5 and 10 µl on imaginary line 4 cm from bottom edge of thin layer chromatography plate. In the presence of large quantities of extraneous material, 10 µl of the extract may not be used. Reserve the vial for quantitative analysis. On the same plate, spot 1, 3 and 5 µl standard aflatoxins solution. Also spot 5 µl of standard solution containing aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, to show that the four aflatoxins are properly resolved under conditions used. Draw line across the plate 2 to 3 cm from top edge as stop for solvent front. Also draw lines 0.5 cm from each side edge. Immediately insert plate into unlined and unequilibrated tank containing acetone/chloroform (1/9), v/v) as the developing system. (Position the plate to expose coated surface to maximum tank volume and seal.) Withdraw the plate from tank when solvent front reaches stop line, 12 to 14 cm above origin. Let solvent evaporate and illuminate plate from below by placing it flat, coated side up, on longwave ultraviolet lamp in darkened room. Alternatively, view plate in chromate view cabinet or illuminated from above. (If illumination requires looking directly at lamps, protect eyes with ultraviolet absorbing filter, such as Eastman 2A filter). Observe pattern of 4 fluorescent spots of qualitative standard. In the order of decreasing R<sub>f</sub> these are aflatoxins B<sub>1</sub>-B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. Note small colour difference, bluish fluorescence of 'B' contrasted with slightly green 'G' aflatoxins. Examine patterns from sample for fluorescent spots having identical R<sub>f</sub> and similar appearances to those of standards. From this preliminary plate, estimate suitable dilution for quantitative thin layer chromatography analysis. Take into account a quantity of the extract used for preliminary thin layer chromatography in final calculations.

**NOTE** – If four clearly identifiable spots are not visible in the qualitative standard, repeat chromatography, correcting or adjusting conditions to obtain proper resolution.

**J-4.5 Quantitative Determination** – Spot successively 3.5, 5, and two 6.5 µl portions of sample extract. All spots should be of the same size and shall not be more than 0.5 cm in diameter. On the same plate spot 3.5, 5, 6.5 µl of B<sub>1</sub> and G<sub>1</sub> standards, As internal standard spot 5 µl of B<sub>1</sub> and G<sub>1</sub>

on top of one of the two 6.5 µl portions of sample. Also spot 5 µl of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> as qualitative standard. Proceed as given under qualitative analysis (see J-4.4).

## J-5. CALCULATIONS

**J-5.1 Interpretation of the Chromatogram**—Examine the pattern from the sample spot containing internal standard for aflatoxin B<sub>1</sub> and G<sub>1</sub> spots. R<sub>f</sub> values of B<sub>1</sub> and G<sub>1</sub> used as internal standard should be the same as, or may differ only very slightly from those of respective standard aflatoxin spots. (Since spots from sample extract are compared directly with standard aflatoxin on the same plate, magnitudes of R<sub>f</sub> are unimportant. These vary from plate to plate). Compare sample patterns with that containing internal standard. Fluorescent spots in sample thought to be B<sub>1</sub> or G<sub>1</sub> should have R<sub>f</sub> values identical to end colour and similar to be B<sub>1</sub> or G<sub>1</sub> used as internal standard. Identify unknown spot of sample as B<sub>1</sub> or G<sub>1</sub> only when unknown spot and internal standard spots are superimposed. Spot from sample and internal standard combined should be more intense than either sample or standard alone. Compare sample pattern with qualitative standard to determine if B<sub>2</sub> and G<sub>2</sub> are present. Compare fluorescent intensities of B<sub>1</sub> spot of the sample with those of the standard spots and determine which of the sample portions matches one of the standard. Ultraviolet light may be attenuated by moving the plate away from lamp so that any particular pair of spots be compared at extinction. Interpolate, if sample spot Intensity is found to be between those of two standard spots. If spots of smallest portion of sample are too intense to match standards, dilute sample and rechromatograph. Compare G<sub>1</sub> spots in the same manner. Assume that B<sub>1</sub> and B<sub>2</sub> have same fluorescent intensity to mass relationships and compare B<sub>2</sub> spots of sample with B<sub>1</sub> standard spots to make quantitative estimate of B<sub>2</sub>. Likewise, assume G<sub>2</sub> has the same fluorescent intensity to mass relationship as G<sub>1</sub> and compare G<sub>2</sub> spot of the sample with G<sub>1</sub> spot of the standard.

**J-5.1.1 Calculation**—Calculate aflatoxin from the following formula:

$$\text{Aflatoxin B}_1 \text{ in } \mu\text{g/kg} = S \times Y \times \frac{V}{X} \times M$$

where

S = µl of aflatoxin B<sub>1</sub> standard equal to unknown;

Y = concentration of aflatoxin B<sub>1</sub> standard µg/ml;

V = volume in µl of final dilution of sample extract;

X = µl of sample extract spotted giving fluorescent intensity equal to S, the aflatoxin B<sub>1</sub> standard; and

M = mass in g of sample applied to silica column.

**NOTE**—For 10 g of sample extract 50 ml of aqueous methyl alcohol extract is used. If final extract dilution does not represent 10 g, calculate correct sample mass and substitute.

**J-5.1.2** Calculate aflatoxin  $G_1$  as given above for aflatoxin  $B_1$ .

**J-5.1.3** Calculate aflatoxin  $B_2$  as ' $\mu\text{g}$  aflatoxin  $B_2$  per kilogram based on fluorescence of aflatoxin  $B_1$ '.

**J-5.1.4** Calculate aflatoxin  $G_2$  as ' $\mu\text{g}$  aflatoxin  $G_2$  g per kilogram based on fluorescence of aflatoxin  $G_1$ '.

## **ANNEXURE G**

### **Sampling and determination of defects**

K-1. The representative sample shall be reduced by riffle type sample divider to obtain a portion weighing 1 000 g to 1 500 g. Sub-samples weighing approximately 200 g shall be drawn from the representative sample by use of a riffle divider.

K-2 Bags of groundnuts, which are not of the sample quality as the bulk of the consignment, shall be graded separately.

K-3 Foreign matter shall be separated by hand picking of such material from the sub-sample, weighing the picked material and expressing the weight as a percentage.

K-4 The percentage of damaged groundnuts shall be determined by hand picking from the subsample, weighing such groundnuts and expressing the weight as a percentage.

K-5 The percentage of splits shall be determined by hand separation of the splits from the subsample, weighing splits and expressing the weight as a percentage.

K-6 Other defective groundnuts, which are not, splits broken or damaged shall be weighed and expressed as percentage of total sample.

K-7 The percentage of total defective groundnuts is the sum of splits and broken, damaged and other defectives. The percentage of splits shall be determined by hand separation of the splits from the sub-sample, weighing these splits and expressing the weight as a percentage.

K-8 The percentage of groundnuts of other varieties shall be determined by picking such grains from the sub-sample, weighing these groundnuts and expressing the weight as a percentage.